BANANA SAP, A RESOURCE MATERIAL FOR BIOETHANOL PRODUCTION AND OTHER VALUE ADDED PRODUCTS

A thesis *submitted in the fulfilment of the requirement*

for the award of degree of

DOCTOR OF PHILOSOPHY

IN

BIOTECHNOLOGY

Submitted By

GEETIKA GUPTA

(Registration No. 901400005)

THAPAR INSTITUTE
OF ENGINEERING & TECHNOLOGY
(Deemed to be University)

Under Supervision of

Dr. M Sudhakara Reddy	**Dr. Manoj Baranwal**	**Dr. Sanjai Saxena**
(Professor)	**(Professor)**	**(Professor)**

Department of Biotechnology

Thapar Institute of Engineering and Technology

PATIALA – 147004 PUNJAB (INDIA)

September 2022

CERTIFICATE

This is to certify that the thesis entitled "**Banana sap, a resource material for bioethanol production and other value added products**" submitted by Ms. Geetika Gupta (Roll no: 901400005) in fulfilment of the requirement for the award of the degree of **Doctor of Philosophy** in Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, Punjab, India is a record of candidate's own independent and original research work carried out by her under our supervision and guidance. The matter embodied in this thesis has not been submitted for the award of any other degree or certificate in this or any other University or Institute.

(Dr. M Sudhakara Reddy)
Supervisor
Professor and Head
Department of Biotechnology
Thapar Institue of Engineering and Technology
Patiala - 147004

(Dr. Manoj Baranwal)
Supervisor
Professor
Department of Biotechnology
Thapar Institue of Engineering and Technology
Patiala - 147004

(Dr. Sanjai Saxena)
Supervisor
Professor
Department of Biotechnology
Thapar Institue of Engineering and Technology
Patiala - 147004

CANDIDATE'S DECLARATION

I, hereby declare that the work which is being presented in this thesis entitled **"Banana sap, a resource material for bioethanol production and other value added products "** submitted by me for the award of *Degree of Doctor of Philosophy*, in the Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, Punjab, India is true and original record of my own independent and original research work carried out under the supervisions of **Dr. M. Sudhakara Reddy, Professor, Dr. Manoj Baranwal , Professor and Dr. Sanjai Saxena, Professor, Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, India.** The matter embodied in this thesis has not been submitted in part or full to any other University or Institute for the award of any degree in India or abroad.

Date: Sep 12, 2022

Place: Patiala

Geetika Gupta

(Geetika Gupta)

Dedicated to my parents,

for raising me to believe that nothing is impossible

to my husband and Salasaram

for making everything possible……..

ACKNOWLEDGEMENTS

God helps those who help themselves by being there with us and blessing us at all times provided we dare to dream and dare to achieve those dreams. First of all I thank the God almighty for giving me a breakthrough whenever I broke down and blessed me with whatever I have ever achieved. I feel my faith in Divine Power had a profound impact on my ability to complete my thesis.

I take this opportunity to thank my supervisors ***Prof. M S Reddy, Prof. Manoj Baranwal*** *and* ***Prof. Sanjai Saxena*** *for providing me an opportunity to work under their guidance and supervision, assisting with all kinds of support, inspiration, constructive criticism, valuable suggestions, and comments throughout the PhD work.*

I am profoundly obliged to ***Sh. R R Vederah, Chairman****, Thapar Institute of Engineering and Technology, Patiala for his good wishes and motivation. It was his support which made me thrive for excellence and nothing less. His constant support helped me to sail through this tedious journey. He taught me another aspect of life that "goodness can never be defied and good human beings can never be denied".*

I wish to express my thanks to ***Prof. Prakash Gopalan, Director****, Thapar Institute of Engineering and Technology, Patiala for providing infrastructure and helping me towards the completion of my research work.*

I extend my sincere word of thanks to ***Prof. Rafat Siddique****, Dean (Research and Sponsored Projects), Thapar Institute of Engineering and Technology, Patiala for his encouragement and support during my research work in the institute.*

I feel lacunae of words to express my deepest gratitude to ***Prof. (Mrs). Seema Bawa****, Department of Computer Science and Engineering, Thapar Institute of Engineering and Technology, Patiala. Though I will never find words to tell what I owe to her, and if I start doing, I would not know where to stop…thanks for everything Ma'am...To me, professionally and personally, you are "perfection personified".*

I wish to express my thanks to ***Prof. S S Bhatia,*** *Controller of Examination Former Dean Academic Affairs, Thapar Institute of Engineering and Technology, Patiala for always been there to help me at every single step, motivating me, guiding me and encouraging me to take up new challenges every day and tackle them with determination and optimistic attitude. I really feel privileged to be associated with a person like him during my life. His association with this endeavor of mine will remain a beacon light to me throughout my life.*

A sincere token of gratitude to ***Dr. Gurbinder Singh,*** *Registrar, Thapar Institue of Engineering and Technology, Patiala for his valuable support that made me consistent performer.*

I would like to thank my doctoral committee members ***Dr. Siddharth Sharma, Prof. Amjad Ali*** *and* ***Prof. Anil Kumar*** *for their encouragement, suggestions during the course of my study.*

Most of the work would have been incomplete without the sincere support, guidance, constructive suggestions and cooperation of ***Prof. Manmohan Chhibber****, School of Chemistry and Biochemistry and* ***Dr. Anita Rajor,*** *School of Energy and Environment, Thapar Institute of Engineering and Technology, Patiala.*

I am grateful to ***Prof. S.K Kaushik,*** *Ex-Head, Department of Civil Engineering, IIT, Roorkee whose blessings and support have always encouraged me to perform to the best of my abilities.*

The research was possible due to the support of the ***Department of Science and Technology****,* ***Government of India, New Delhi*** *for providing me financial support to accomplish my work under the sponsored project DST/SSTP/Haryana/345. I appreciate the financial help received from Thapar University, Patiala in lieu of Teaching Associate (TA).*

Most of the results described in this thesis would not have been possible without the help of Sophisticated Analytical Instrument Facility (SAIF) at Indian Institute of Technology (IIT) Bombay, Mumbai and Punjab Biotechnology Incubator (PBTI) Mohali.

I take the opportunity to acknowledge ***Institute of Microbial Technology (IMTECH)****, Chandigarh for providing the Saccharomyces cerevisiae strains (MTCC 170 and MTCC180), and* ***Sukhjit Starch and Chemical Limited****, Phagwara and* ***Patiala Distillers and Manufacturers Limited****, Patiala, for providing the Corn Steep Liquor(CSL) and Spent Wash (SW), respectively.*

My warm thanks are due to all the faculty members and my lab mates of the Department of Biotechnology and School of Energy and Environment, Thapar Institute of Engineering and Technology, Patiala for enlightening me with their knowledge and inputs.

I would like to thank Lab technicians of the Department of Biotechnology and School of Energy and Environment, Thapar Institute of Engineering and Technology, Patiala for their constant help and cooperation. I am also thankful to ***Mr. Vijay Verma*** *for his help and suggestion in financial matters which enables smooth running of this research project.*

Some friends are never too busy to give us a hand whenever they are needed. I want to make a special mention for the friends who showed faith in me and my intellect even when I felt like digging hole and crawling into one because I didn't have faith in myself. I could not have asked for more than what I got from you all. I express my sincere love and affection towards all those benevolent souls and true friends ***Sejal di, Usha, Anu, Kamal di, Aditi di, Rupal*** *and* ***Rohini ma'am****.*

Where emotions are involved, words cease to mean: words don't seem adequate to express my deepest feeling for the love, care, affection, concern and support rendered by ***my parents****,* ***siblings*** *and* ***in-laws*** *who have always showered unconditional love on me, encouraged and supported me in every aspect. They formed a part of my vision and taught me the good things that really matter in life. I would like to mention the care and affection that I received from* ***Dr. Maneek Kumar*** *(sir) Director, Steam and*

*Quality, DIT University, Dehradun and **Mrs. Aarti** (ma'am). They always infused me with hopes in the admist of my tough times. I am deeply grateful to them for the times and emotions I shared with them.*

*For any successful accomplishment, the support and faith of near and dear ones is a must. I am totally indebted to **my loving husband** for his love, patience, understanding and endurance during the whole tenure of the work. When my words ebbed and enthusiasm waned, it was my **SALASARAM** who helped me getting out of it, a feat that can never be paid back in feelings or words. To them, I owe my wonderful today and dream-filled future.*

Last but not the least, I wish to acknowledge all those, whose names have not figured here, but who helped me in any form during the period of my research work.

OM JAI SHREE RAM...

Date: Sep 12, 2022

Place: Patiala

Geetika Gupta

(Geetika Gupta)

ABSTRACT

Bananas (*Mus*a spp.) are commonly grown in tropical and subtropical nations, and each hectare of a banana plantation generates approximately 220 tons of biomass waste. About 4-5 m^3 of sap are produced from one tonne of dried banana pseudostem, due to its high chemical oxygen demand (COD) and biological oxygen demand (BOD). Banana pseudostem contains nearly 90% of moisture. In this study, the potential of utilizing banana sap from pseudostems as a feedstock for ethanol production has been investigated. Furthermore, antibacterial, antioxidant, and anticancer activities of banana sap and crude extracts were examined, followed by LCMS analysis. Concentrated banana sap was combined with other industrial by-products such as corn steep liquor (CSL), spent wash (SW), and yeast extract (YE) for ethanol production followed by acid and alkali hydrolysis to enhance the sugar levels in the sap. Fermentation is carried out using the MTCC170 and MTCC180 strains of *Saccharomyces cerevisiae*. Concentrated banana sap augmented with 25% SW (v/v) and MTCC170 produces 16-fold higher amount of ethanol (2.5 g L^{-1}) than banana sap alone. Alkali-hydrolyzed banana sap supplemented with 25% SW contains high ethanol than the control. These findings indicate that banana sap, when combined with other industrial by-products, can be employed as a sustainable source to produce ethanol. The antibacterial potential of oxidised and un-oxidized banana sap against a microbial test panel comprising of gram-positive and gram-negative bacteria, and *Candida albicans*, by *in vitro* microbroth dilution method was evaluated. The minimum inhibitory concentration (MIC) of unoxidized banana sap exhibited a potent anti-bacterial activity which ranged from 15.625 to 62.5 mg/mL. By using the DPPH technique, the *in vitro* radical scavenging activity of dichloromethane (DCM) and ethyl acetate extract (EA) of banana sap showed 54.62 ± 1.09% and 79 ± 1.05% of antioxidant activity at the concentration of 1 mg/mL, respectively. Human breast cancer cell line proliferation (MCF-7) was inhibited to the greatest extent by the DCM extract of banana sap at a concentration-dependent decrease in a proliferative index, indicating a cytotoxic effect. The IC_{50} values were calculated and found to be 34.15±8.75 µg/mL. Cytotoxic effect of the banana sap extract increased significantly with increasing concentration, but the EA extract showed inconsistent results, revealing no cell growth inhibition. Additionally, LCMS investigations identified the presence of bioactive components as dihydrorescinnamine, epimedin A, and rescinnamine derivative in DCM and EA extract of banana sap. The findings of the present research indicated that banana sap is a potential source of bioactive substances with useful antibacterial, antioxidant, and anticancer activities.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt
ANOVA	Analysis of variance
APHA	American public health association
APOX	Ascorbate peroxidase
ATCC	American type culture collection
BOD	Biological oxygen demand
CAA	Chlorogenic acid
CAT	Catalase
CO_2	Carbon dioxide
COD	Chemical oxygen demand
CS	Chitosan
CSL	Corn steep liquor
$CuSO_4.5H_2O$	Copper sulfate pentahydrate
DCM	Dichloromethane
DMEM	Dulbecco's modifed eagle medium
DMSO	Dimethyl sulfoxide
DNS	3,5- dinitrosalicyclic acid
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
EA	Ethyl acetate
EDTA	Ethylenediamine tetraacetic acid
ELISA	Enzyme linked immuno sorbent assay
et al	And others
Etc	And other things
FBS	Fetal bovine serum
FRAP	Ferric reducing antioxidant power
GC-MS	Gas chromatography mass spectroscopy
HCl	Hydrochloric acid
HIAA	Hydroxy indole acetic Acid
HPLC	High performance liquid chromatography
IC_{50}	Half maximal inhibitory concentration

IMTECH	Institute of microbial technology
LCMS	Liquid chromatography mass spectrometry
LPO	Lipid peroxide
MH	Muller hinton
MIC	Minimum inhibitory concentration
$MnSO_4$	Manganese sulphate
MTCC	Microbial type culture collection
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Na_2CO_3	Sodium carbonate
$Na_2S_2O_5$	Sodium metabisulfite
NaCl	Sodium chloride
NADPH	Nicotinamide adenine dinucleotide phosphate
Na-K	Sodium potassium
NaOH	Sodium hydroxide
NCCS	National centre for cell sciences
OD	Optical density
OHRLCMS	Orbitrap liquid chromatography mass spectroscopy
PBS	Phosphate buffered saline
PBTI	Punjab biotechnology incubator
PEG	Polyethylene glycol
POX	Peroxidase
Rpm	Revolutions per minute
RT	Retention time
SD	Standard deviation
SDA	Sabouraud dextrose agar
SOD	Superoxide dismustase
SSF	Simultaneous saccharification and fermentation
SW	Spent wash
TAA	Total antioxidant activity
TS	Total solid
TTC	2, 3, 5-triphenyl tetrazolium chloride
YE	Yeast extract
YEPD	Yeast extract peptone dextrose

LIST OF SYMBOLS

α	Alpha
β	Beta
℃	Degree celsius
ω	Omega
g	Gram
gL^{-1}	Gram per litre
$gL^{-1}h^{-1}$	Gram per litre per hour
>	Greater than
Hrs	Hour(s)
IU/mL	International Unit per milliliter
Kcal	Kilo calories
Kg	Kilogram
<	Lesser than
L	Liter
l/kg/day	Liter per kilogram per day
m/z	Mass to Charge ratio
µg	Microgram
µg/mL	Microgram per milliliter
µL	Microliter
µm	Micrometer
mg/kg	Milligram per kilogram
mg/mL	Milligram per milliliter
mg	Milligram(s)
mL/g	Milliliter per gram
mL/L	Milliliter per liter
mL	Milliliter(s)
mM	Millimolar(s)
min	Minute(s)
nm	Nanometer
N	Normal
%	Percentage
±	Plus minus
pH	Potential of hydrogen
v/v	Volume by volume
v/w	Volume by weight

CHAPTER -1
INTRODUCTION

1.1 Introduction

Energy usage has significantly increased due to the rising global population (Angulo-Mosquera et al. 2021; Voca and Ribic 2020). Rising energy demand is projected to pose problems for supply sustainability as resources are disseminated globally. Energy is an integral part of all economies, and ensuring a steady energy supply is vital for long-term economic development and national defense (Sivaram and Saha 2018). As per the reports released by BP Statistical Review of World Energy in 2021, despite the chaos in 2020, the usage of renewable energy, particularly wind and solar energy, kept expanding tremendously. There are unsettling indications that the decrease in carbon emissions brought on by coronavirus disease (COVID -19) since 2020 may be short-lived as the global economy recovers and lockdowns are released. The problem is reducing emissions steadily and comparably yearly while eliminating the impact on our daily lives and activities. Demand for renewable energy increased by 9.7%, slower than the ten-year average. Still, on an energy-per-unit-of-demand basis, it grew at a rate comparable to 2017, 2018, and 2019 (BP Statistical Review of World Energy 2021). From Covid-19 lows in 2021, biofuel demand rose to levels similar to those in 2019, and we anticipate growth to increase by 5% in 2022 and 3% in 2023 (Renewable Energy Market Update Outlook 2022 and 2023). Statistical reports by India Energy Agency published in the World Energy Outlook 2021 revealed that oil consumption has more than doubled in the transportation sector since 2000. Diminishing oil supplies, concerns over global warming from greenhouse gas emissions, and a desire to support domestic rural economy have stimulated the search for environmentally friendly fuel alternatives (IEA 2021).

The sky-rocketing levels of fossil fuel consumption have resulted in a potential imbalance between the availability and consumption of non-renewable resources. Thus the search for alternative fuel forms has been the recent trend globally. In this regard, potential biofuels such as ethanol and butanol have emerged as crucial alternative resources for liquid fuel. However, research on significantly improving biofuels production has been expanding for ecological and economic reasons,

particularly for its use as a substitute to petroleum-based fuels (Okolei et al. 2021; Prasad et al. 2007).

Biofuels act as an alternative that possesses the potential to replace the traditional fuel sources and thereby safeguard the remaining fossil fuel reserves. Moreover, the use of biofuels would also reduce the increased dependency on fossil energy sources. Using biofuels can mitigate greenhouse gas emissions and lower the impact of CO_2, enabling the atmosphere to be sustainable. The production of biofuels introduces additional market prospects, aids in expanding agricultural goods, and therefore results in extra income for farmers, improving their socioeconomic situation (Datta et al. 2019). Biofuels are liquid or gaseous fuels from natural resources such as plant matter and residues, municipal garbage, and agricultural and forestry by-products. However, the type of materials used and the methods adopted for the production process determine the type of biofuels produced. Biofuels are broadly classified as bio-diesel and bio-ethanol and then further subdivided into conventional or advanced fuels (Hirani et al. 2018).

ProAlcool launched in the middle of the 1970s amid the first oil crisis and the issue of fluctuating sugar costs on the global market. The government increased the number of ordinary vehicles fuelled with a combination of ethanol (24%) and gasoline (76%). Since then, the automobile sector has grown into alcohol-fuelled vehicles that reached their maximum in the middle of the 1980s (Soccol et al. 2005).

From the early 1980s, there was a demand in the United States to promote the development and usage of biofuels, notably maize-based bioethanol. This mainly aimed to stabilize the farming industry amid a period of agricultural product overproduction. E85, a fuel mixture consisting of 85% bioethanol and 15% gasoline, is one example of a bioethanol fuel combination that can be utilised in vehicles designed specifically for it. Consequently, the quantity of bioethanol consumed in the US from E85 is only about 1% (Jull et al. 2007). In order to promote the development of E85 mix gasoline and other alternative transportation fuels, the US Congress has established a number of legislative mandates and incentives. The Energy Policy Act of 2005 (EPAct 2005) at the National level is one of the most significant steps (Hoekman 2009). Corn ethanol contributes significantly to energy security and the agricultural economy while simultaneously serving to reduce carbon emissions in the transportation industry in the United States. In 2019, ethanol contributed more than

10% of the US fuel industry (Lee at al. 2021). Bioethanol has generated considerable global, national, and regional interest. By 2023–2024, India has set the audacious goal of mixing 20% ethanol into gasoline, up from the present level of 9% (Business Standard Report 2021).

Materials such as fibers, molasses, fruits, and others rich in sugar content, thereby facilitating the process of fermentation, are used for the synthesis of bioethanol (Bušić et al. 2018). Depending upon the source (feed) used to produce bioethanol, the various raw materials are divided into four categories (Azad et al. 2015). The first-generation of bioethanol is obtained from food crops such as potatoes, sugarcane (Brazil), maize (USA), oilseeds, sunflower, and rapeseed, while the second-generation is derived from the lignocellulosic biomass (Binod et al. 2019). Algal biomass with high carbon content, such as microalgae, is used to make third-generation bioethanol (Patle et al. 2021). Since algae-based bioethanol production is an emerging tool, substantial challenges must be overcome before biofuels can be industrialized and generated on a large scale. Some of the most challenging concerns include a comprehensive understanding of the microalgae carbohydrate process, harvesting biomass, low production, production of adequate amounts of algal biomass, and high operational expenses of microalgae biomass bioreactors. As the fourth-generation biofuel, genetically modified microorganisms such as yeast, microalgae, fungus, and cyanobacteria are used as raw resources to produce sustainable bioethanol (Saha et al. 2019). Fourth-generation biofuels are still in the early stages of development since it is not economically feasible to produce such feedstocks on a large scale due to the risks that growing genetically modified bacteria poses to human health and the environment, as well as because of ethical and legal issues, low biomass yield, and expensive manufacturing processes. Lignocellulosic biomass, which typically requires agricultural and forest residues as feedstocks, has drawn significant attention among the various generations for the production of bioethanol, mainly because it is not involved in the food vs. fuel debate that is connected with first-generation biofuel feedstocks (Morales et al. 2021). Biofuels from agro-wastes, in particular, have been a focus of ongoing research. India has one of the world's best agricultural economies, with agriculture providing a sustainable income to 75% of the population (Singh and Sawarkar, 2020). Thus, India generates more than 350 million tonnes of agro-waste each year (Singh et al. 2020).

Additionally, the National Policy on Biofuels-2018 of the Government of India places a high emphasis on the production of biofuels from agro-waste biomass (Kirti et al. 2022). In turn, effective agricultural residue usage has received further validation for bioethanol production.

Unlike Europe and USA, all other countries cannot afford to utilize cereal grains for bioethanol production (Ingale et al. 2014). Instead, abundantly available renewable sources can be utilized. Such resources mainly comprise energy-rich carbohydrates that microbes can easily convert into biofuels. Presently, bioethanol has gained immense popularity and is produced on an industrial scale (Rosales-Calderon and Arantes 2019). The pretreatment of lignocelluloses is essential to the enzymatic hydrolysis of cellulose. In recent years, a considerable discussion has focused on alternative vegetable fibers sources, alternatives to wood as a raw materials, paper and pulp applications. Banana (*Musa acuminata*), a monocotyledonous annual herbaceous plant, can be a potential crop for these applications (Mohapatra et al. 2010).

The banana is a popular tropical fruit all around the world. It belongs to the family Musaceae and comprises several hybrid varieties in the genus *Musa*. The four sections of the genus Musa are *Eumusa, Rhodochlamys, Australimusa,* and *Callimusa*. The section with the greatest geographic reach is *Eumusa,* followed by *Australimusa.* The other two possess ornamental properties (Pereira and Maraschin 2015).

Banana is a highly nutritious fruit abundant in protein, vitamins, and carbohydrates. In bananas, the protein content is initially noted to be 1-2.5% whereas with ripening, the protein content increases to 3.8-4.2%. Moreover, bananas are abundant source of fat and contain polyunsaturated fatty acids, mainly linoleic and α-linolenic acid. The antioxidant properties of bananas are largely attributed to high levels of flavonoids and phenolic compounds (Akaniwor and Sodje 2005).

Plant extracts containing antimicrobial and antioxidant properties are significant in therapeutic applications (Sarma et al. 2021). The banana plant is amongst the most beneficial plants owing to the multidisciplinary uses of the diverse plant parts including blossom, leaves, stem, and pulp. Some of them possess medicinal properties (Imam and Akter 2011). Among the different bioactive compounds, banana peels contain a significant amount of flavonoids, tannins, phlobatannins, alkaloids,

glycosides, and terpenoids exhibiting antidiabetic, antioxidant, anti-inflammatory, and antibacterial properties (Hikal et al. 2022). Kumar et al. (2014) investigated antioxidant and antimicrobial properties of banana sap extracted from *Musa acuminata* pseudo-stem. The study revealed the presence of antioxidants, carbohydrates, protein, and phenolic compounds in the banana pseudo-stem methanolic and ethanolic extracts.

Further, potent antimicrobial activity against several strains of the bacteria *Escherichia coli, Streptococcus faecalis, and Klebsiella sp., from* the pseudo-stem sap was observed. A similar study by Ighodaro (2012) revealed the potential of banana peel extract obtained from *M. paradisiaca* against human pathogenic bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa, a*nd *Proteus mirabilis.* A comparative study was conducted by Chabuck et al. (2013) to analyze the efficacy of banana peel extract as an antimicrobial agent against Gram-positive (*Streptococcus pyogenes* and *Staphylococcus aureus*), Gram-negative (*Enterobacter aerogens, Moraxella catarrhalis*, and *Escherichia coli*) and yeast (*Candida albicans*) microorganisms. Results revealed the potent antibacterial activity against *Staphylococcus aureus* and *Moraxella catarrhalis* followed by *Streptococcus pyogenes, Klebsiella pneumonia* and *Enterobacter aerogens.*

The high levels of dopamine further add to the antioxidative properties of bananas. Banana peel and pulp are more effective at scavenging free radicals than catechin and other strong antioxidants (Kanazawa and Sakakibara 2000).

Banana is a rich source of secondary metabolites, protecting against physical stresses and harmful effects of bioactive compounds. Pothavorn et al. (2010), in their investigation, revealed the presence of several aromatic and phenolic compounds in different banana varieties. Some of the significant compounds obtained from banana species such as *Musa laterita, Musa balbisiana, Musa ornate, and Musa acuminate* include dopamine, apigenin glycosides, kaempferol-3-O-rutinoside, myricetin glycoside, naringenin glycosides, and N-acetyl serotonin.

Anhwange (2008), in his studies, mentioned that although the banana has originated in Southern Asia tropical regions, production has now extended to different areas of the world. As per the reports of FAOSTAT (2013), the banana has been identified as one of the most popular fruit after apple and orange. It was estimated that banana

contributes upto 16.8% of the fruit production in the world, whereas apple and orange's combined contribution is 11.4%. Banana production has increased gradually over the past 20 years, from around 46 million tonnes in 1993 to about 105 million tonnes in 2013. Asia continues to be the continent that produces the most bananas, accounting for about 57.3% of global production. It is followed by the America and Africa. The two regions with the lowest production, Oceania and Europe, contribute for 0.3% of global production (FAOSTAT 2013). According to FAOSTAT 2019, India produced 30.5 million tonnes of bananas, representing 26.02% of the total global. India, Brazil, China, Indonesia, and Ecuador are the top five producers, accounting for 53.94% of the global output.

Studies by Graefe et al. (2011) revealed that many countries export bananas. However, it has been found that in many cases, around 20–40% of the banana does not meet the standards for exports or get perished. For instance, in Brazil, it was reported that every ton of bananas produced about 160 kg of stems, 3 tons of pseudo-stem, 440 kg of skins and 480 kg of leaves. So, researchers were interested in the judicious use of these agro-wastes after crop harvest (Zhang et al. 2010; Fernandes et al. 2013). Further, Fernandes et al. (2013) reported that less than 10% of available biomass as agro-waste, which amounts to 440 million tons, is potentially used for several purposes that could benefit the people. Moreover, commercial applications for such residues would not only help the farmers to earn extra money but also help to reduce environmental pollution.

Besides being used as fuel, banana peels and stems have other beneficial applications. Banana peels are also used as a biosorbent. In this regard, Reddy et al. (2015) treated 100 ml of a contaminated water sample with 0.05 g of 106 µm size fraction banana peel powder. Biosorbents help in water treatment processes and facilitate the nitrate removal during the drinking water. The study results revealed that the banana peels possessed a removal efficiency of nearly 80%.

An ethnomedicinal survey related to the flowers of *Musa* sp. revealed that these flowers could be used in treating various diseases. In this regard, Singh (1986) mentioned in his studies the use of *Musa* flowers to alleviate menorrhagia, and dysentery. The flower extracts were evaluated to possess therapeutic properties for

illnesses such as diabetes mellitus (Pari and Maheswari 1999), and malaria (Bagavan et al. 2011).

Other applications of bananas include manufacturing paper and textile materials (Balda et al. 2021). In the Philippines, banana pseudo-stem has also been utilized for synthesizing tissue paper and paper boards (Vigneshwaran et al. 2015). Moreover, banana is a bio-remediation agent used for water purification. Banana fibers were used to make handicrafts, paper bags, tea bags, high-quality fabric material, ropes, and papers for printing currency notes. Multi-dimensional uses of banana agro wastes thus make it a fruit of immense research scope (Subagyo and Chafidz 2018).

Research has revealed the composite nature of banana fibers (Hariprasad et al. 2013). Cellulose nanofiber is regarded as the prominent green materials because of its many intrinsic advantages, including its biocompatibility, high abundance, mechanical ability, and renewability (Trache et al. 2020). The price of filler in the plastic industry can be reduced by using cellulosic fibers, particularly banana fiber. Additionally, combining a banana with glass fiber in the form of fabric will yield the requisite high tensile strength (Pothan et al. 2005).

Recent environmental concerns in European nations have highlighted the significance of natural fibers as reinforcing fillers in polymer composites. Natural fibers are preferred over synthetic fibers because they are more affordable, produce with less tool wear, have low densities, are biodegradable, and are environmentally friendly (Raksakulpiwat et al. 2005). Due to this, banana fibers act as an alternative for their fiber potential.

Banana (*Musa sapientum*) has also been identified as a source of enzyme production. Due to the large-scale production of bananas around the world demonstrates that fruit stalk is widely accessible in banana cultivation fields and markets. Although it doesn't have any substantial industrial or commercial uses, it builds up in the agro-industrial yards and causes serious environmental issues. Krishna (1995) identified that this stalk, with a reported cellulose content of 23.85%, is easily accessible in tropical and subtropical countries and is a major source of cellulose. This agro-industrial waste can be employed in the solid-state fermentation for α-amylase production employing *Bacillus subtilis* by (Krishna and Chandrasekaran 1996).

In their study, Kumar et al. (2012) highlighted the medicinal uses of bananas. They reported that bananas can be used for treating gastric and intestinal disorders. Furthermore, the study also suggested the potential benefits of the young leaves of the banana plant in skin irritations. Medicinal properties of the leaves, roots, ashes of peels and seed mucilage were also reported in some cultures. Additionally, studies have shown that weaning meal blends, including bananas, help babies grow in size and proportion, which makes them strong and healthy (Chitra 2015). Traditionally, dried peels of ripe bananas have been utilized as herbal medicines for cold, cough, and gastritis, ripe pulp for dysentery, and pseudo-stem exudates for pinworm infection (Borborah et al. 2016). In order to treat burns locally and reduce pain and swelling, banana peels can be wrapped around the burn (Pereira et al. 2015).

Any chemical necessary for developing dye (color) in a particular substance is a mordant. Using banana pseudo-stem sap as a mordant produced both efficient and inexpensive outcomes. Tannins, abundant in sap, are the primary chemical ingredient responsible for the sap's ability to color (Barhanpurkar et al. 2015). Moreover, the dye industry uses the pseudo-stem sap of bananas as a mordant (Mohiuddin et al. 2014). The study of Ammayappan (2004) showed that sap extracted from the pseudo-stem of bananas contains 2-3% dye. This dye imparts vanilla cream color without mordanting and with mordants gave different color only in pale and light shade on silk fabric. Hence, it revealed the potential application of the pseudo-stem of bananas for producing natural dye that could be used in silk.

Banana by-products have potential of applications including food production, food wrapping, and paper production (Mohapatra et al. 2010; Padam et al. 2014). The pseudo-stem carries the bunch of bananas dies after harvesting the fruit from the plant (Kumar et al. 2014). Thus banana stems, leaves, and peels account for an enormous amount of biowaste in the environment. Banana waste contains a lot of biodegradable substances including high biological oxygen demand (BOD) and chemical oxygen demand (COD). If these wastes are not treated and controlled, their uncontrolled disposal will damage the environment by releasing harmful toxins and methane (Waldron 2009). As a result, adequate planning, administration, and usage of these food processing industries are required.

1.2 Research problem

Banana is a staple food that is consumed all around the world. FAOSTAT 2018 estimates that 115.7 million tonnes of fruit are produced worldwide each year. With a global production share of 26.7% and a yearly yield of about 30.81 million tonnes from an area of 0.88 million hectares, India is the world's top banana producer. Unlike many other fruits, in the case of bananas, the peelings contribute 35% of the fruit weight. Thus estimations reveal that banana consumption generates approximately 36 million tonnes of banana peel. Several researchers have predicted the potentiality of these agro-waste peels in producing essential items (Vu et al. 2017). However, Schieber et al. (2001) reported that most of these peels were either wasted or utilized in land filling. The potential benefits thus remained unexplored. Further, a considerable amount of agro-waste is generated after the harvest of bananas from the plantation, which poses a severe threat to the environment. Hence based on this premise, the primary idea is to explore banana sap as a source for bioethanol production and bioactive constituents.

1.3 Research gap

Banana is a major cash crop of the country that yields a massive amount of agro-waste yearly. These wastes are usually discarded, leading to increased levels of environmental pollution. An estimated 220 tonnes of waste are produced per acre of banana cultivation. About 190 million tons of biomass wastes from banana farms are produced by India alone (Balda et al. 2021). According to the FAOSTAT 2018 estimate, approximately 5.72 million hectares of land worldwide are under cultivation, generating 1.243 billion tonnes of waste. The majority of the banana plant's organic waste is pseudostem. This waste is either burned or dumped in shallow waters or lakes due to a lack of understanding and resources, which results in the emission of greenhouse gases that pose a significant danger to the ecosystem. This makes it necessary to properly utilize banana biomass refuse, particularly in nations like India, which is the world's top consumer of banana fruit. This study aims to identify the beneficial aspects of banana sap that can be explored to ensure judicious use of these fruit agro-wastes. In the past, several researchers have identified several positive applications of the banana peels, stems, and leaves in producing various items such as ethanol, textiles, papers, enzymes, and many more. This study specifically

aims to identify the role of banana sap in bioethanol production and other value-added products of economic importance.

Only preliminary information is available on using banana sap as a bioenergy source. There is no significant research done on the utilization of sap as biofuel. Banana sap has very nutritive and medicinal value (Feriotti and Iguti 2012). Banana sap contains substantial amounts of carbohydrates, proteins, and fiber, and it could act as a suitable substrate for producing value-added products such as bioethanol and other medicinal vital compounds. The current study aims to expand the utilization of banana sap, a low-cost underutilized agricultural waste, to develop a cost-effective process for producing bioethanol and some other value-added products.

1.4 Conceptual framework

The banana sap can be utilized as a sustainable option for renewable energy production using carbohydrates, sugar, starch, pectin, proteins, lipids, etc., of juice. Thus, improving the conversion efficiency of waste to commercially valuable products would be a significant step toward reducing the environmental impact.

1.5 Hypothesis

Overall the sap has high phenolic and aromatic compounds and less sugar and nitrogen compounds required for effective fermentation, which could show poor conversion of sap to ethanol. The banana sap utilization for biofuel production has been studied to a limited extent due to poor conversion rates of sap to biofuel. However, if one can enhance the efficiency of conversion of sap, it would be very effective in producing the required biofuel.

1.6 Aims and Objectives

The primary objective of this study is to evaluate bioethanol production using banana sap as a source of material, aiding further scope of research in this field. The idea is to examine the effectiveness of banana sap in producing bioethanol and its efficacy in terms of currently used market strategies in manufacturing bioethanol from another source of materials. An attempt has also been made to evaluate the bioactive properties of banana sap under *in vitro* conditions.

CHAPTER -2
REVIEW OF LITERATURE

Banana is one of the most popular tropical fruits across large number of geographies in the world. It is widely cultivated across 130 countries, including the Caribbean and subtropics regions. Edible bananas are derived from two distinct genera, *Australi musa* and *Eumusa*. *Musa acuminata* is the most widely produced edible banana (Banana market review by FAO 2017). Approximately 70 species and 300–500 various cultivars constitute the genus Musa (Häkkinen 2009; Maduwanthi and Marapana 2019). India produces the most bananas globally, at over 30 million tonnes annually, accounting for about 116 million tonnes of the total world production (FAO 2019). The banana fruit is high in nutrients and is easily digestible. It is rich in vitamins, carbohydrates, proteins, minerals and phenolic compounds. There are various other parts of the banana plant, which includes stem, banana peel, banana leaf, sheath, banana inflorescence, pseudo stem, pith and male bud. The various plant parts have been effectively utilized for the production of ethanol, dye, and biogas, respectively. The banana plant parts have also been used as effective vermicompost (Padam et al. 2014). Many studies exist on bioethanol production from banana stem, peel and decomposing fruit. The high phenolic content of banana pith has made it an effective mordant for preparing dyes. However, there is not much data on the usage of sap for bioethanol production (Mohapatra et al. 2010). Banana is one of the largest herbaceous plants that grow worldwide. Usually, the plant grows from 6-8m in length (Kumar et al. 2014). The plants consist of rhizomes, pseudo-stem, flowers and leaves. The inflorescence consists of female flowers which develop into fruits. Banana has been cultivated for a long time. Traditionally it is used as a food source, fiber, and medicinal applications (Kumar et al. 2012). The various plant parts (digestible and indigestible) of bananas have been used in different industries are mentioned in Figures 2.1 and 2.2 (Gupta et al. 2022).

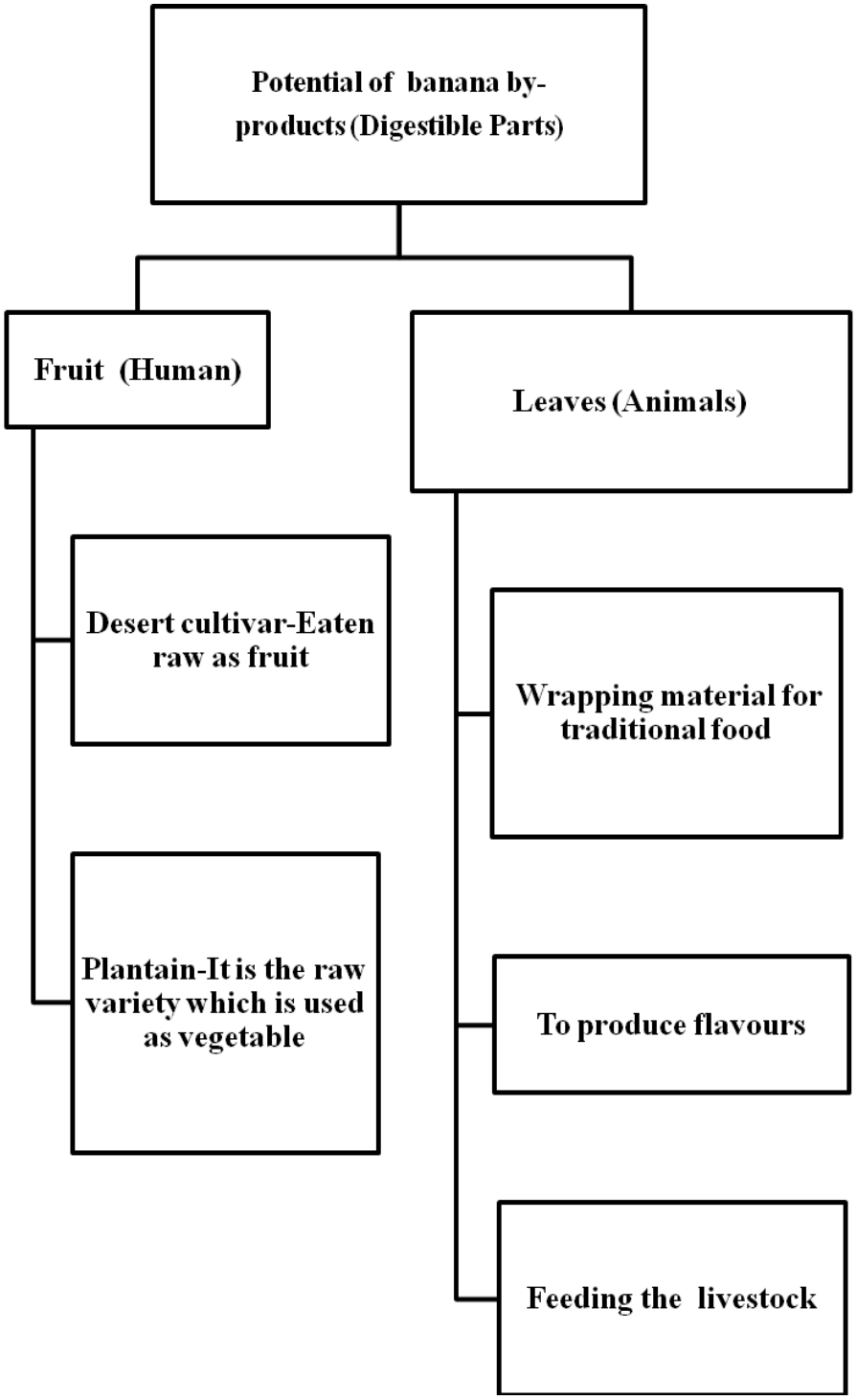


Figure 2.1: Banana by-products (digestible parts) as a potential raw material in different industries (Gupta et al. 2022)

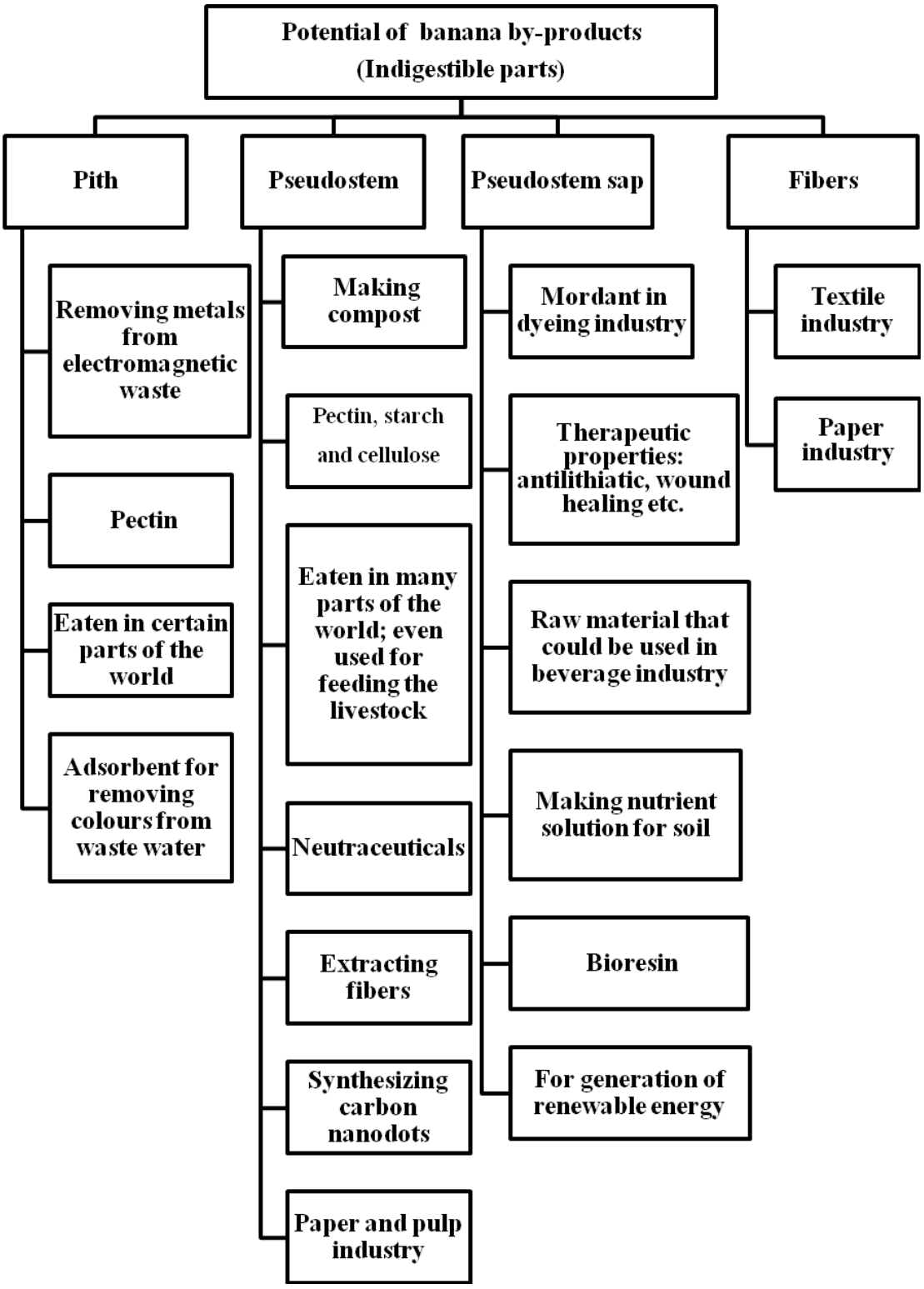


Figure 2.2: Banana by-products (indigestible parts) as a potential raw material in different industries (Gupta et al. 2022)

Banana has been used effectively for bioethanol production. Several groups have studied the use of various plant parts for production of bioethanol. Ingale et al. (2014) studied the usage of banana pseudo-stem for ethanol production. The pseudo-stem was processed by fungal strains or by alkaline treatment. The treated material was subjected to fermentation with *Saccharomyces* for ethanol production. The pretreatment of banana pseudo-stem enhanced bioethanol production. Hammond et al. (1996) showed that enzymatic hydrolysis of whole banana fruit, peel and pulp showed effective bioethanol production.

Many research groups have effectively studied biogas production. In an anaerobic digester, biogas was produced successfully under anaerobic conditions. Biogas can be produced from any accessible bio-waste. Banana processing generates millions of million tons of waste, which, if left untreated, produces toxic gases during decomposition. A single hectare of banana crop yields up to 220 tons of bio-waste, which comprises both leaves and pseudo-stem. Lignocellulose is the main component of the waste. Divyabharathi et al. (2017) studied biogas generation from different bio-waste generated from the banana plantation. Anaerobic digestion of waste was carried out using cow dung as a source for fermentation. Batch fermentation of various bio-wastes showed that, on average, about 0.35 l/kg/ day of biogas was generated. The methane content was highest with 75% banana peel and 25% cow dung mix. Of the various banana waste tested for biogas production, banana peel gave the highest biogas generation.

Sidhu and Zafar (2018) studied the potential of bioactive compounds in banana fruits and revealed their health significance. Their study has shown that banana rhizome, bracts, pulp, pseudo-stem and peel are rich in polyphenols, anthocyanins, and carotenoids. These compounds have a very high antioxidant capacity as evaluated by inhibiting free radicals by various assays. They also have several other applications, as enumerated in the Table 2.1 (Gupta et al. 2022).

Table 2.1: Bioactive compounds obtained from banana by-products (Gupta et al. 2022)

Banana by-product	Bioactive compound	Applications	References
Banana sap	Lupeol, Ferulic Acid, Vanillic Acid, Trans-Cinnamic Acid, p-Hydroxybenzoic Acid, p-Coumaric Acid, Rutin, Catechin/Epicatechin, Chlorogenic Acid, Gallic Acid, Caffeic Acid And Nicotiflorin	Antidiabetic activity	Nguyen et al. 2017
Banana peel	Myricetin, Quercetin, Kaempferol, Rhamnetin	Strong antioxidant and metal chelating properities	Manthey et al. 2016
Banana pulp	Ferulic acid, Synapic acid	Inhibiting LDL oxidation	Tsamo et al. 2015
Banana peel	Isorhamnetin, Kaempferol, Quericitein, Myricetin and Methylmyricetin	Treatment of inflammation, allergy, viral and cancer	Tsamo et al. 2015
Banana flowers	Rutin, Quericetin	Treatment of gastrointestinal diseases	China et al. 2011
Banana sap	Apigenin Glycosides, Myricetin Glycoside,Myricetin-3-O-Rutinoside, Naringenin Glycosides, Kaempferol-3-O-Rutinoside, Quericitin-3-O-Rutinoside, Dopamine And N-Acetyl Serotonin	Inhibition of cancer cell lines, Neurodegenerative disorders, anti-estrogen, antinflammation, antivenom, capillaries strengthening	Pothavorn et al. 2010
Banana bract	Anthocyanin	Antioxidant activity	Kitdamrongsont et al. 2008

2.1. An insight on origin and mass production of banana sap at Global level

The global production of bananas is around 102028.17 thousand tons, of which India accounts for around 30% of the production. India, China, Philippines, Ecuador and Brazil are other significant producers (Apeda 2010).

The state with the largest production of banana cultivation is Andhra Pradesh, Maharashtra, Gujarat and Tamil Nadu shown in Figure 2.3 (Apeda 2010; 2015). The various commercial varieties of bananas available include Grand Naine, Robusta, Dwarf cavendish, Red Banana and Nendran. Global exports of bananas have shown a 6% increase in 2017 compared to 2016. An estimated 18.1 million tonnes have been exported globally (Banana market review published by FAO 2017).

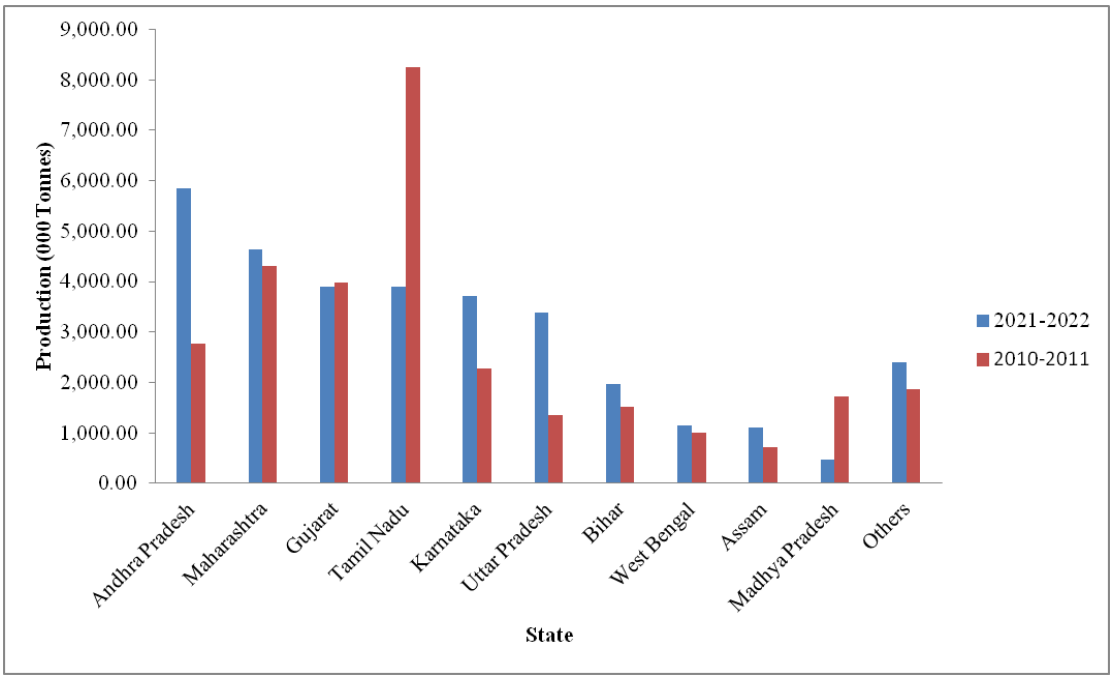


Figure 2.3: State wise production of banana in Indian subcontinent (Apeda 2010; 2015)

India is the major exporter of bananas globally. The country has exported 45,574 metric tons of bananas (Apeda 2010). The net value of export corresponds to 9154 lacs from 2011-2012 (Apeda 2010). The bulk of bananas exported is to Middle Eastern countries, which account for more than 50% of bananas exported. In 2018, banana production reached around 115.74 million tonnes globally, with Southern Asia contributing 32.14 million tonnes and Thailand producing 1.05 million tonnes,

respectively (FAO 2020). The Outlook predicts that by 2029, the global banana output would increase by 1.5% annually to 132.6 Mt. Banana consumption is predicted to become saturated in most areas due to population expansion. However, rapid financial development in several emerging nations, particularly India and China, is expected to encourage altering health and nutrition perspectives and development in banana consumption beyond population increase. As a result, Asia is predicted to remain the world's biggest producer, with a worldwide market share of 51.8 % (OECD-FAO Agricultural Outlook 2021-2030).

2.2 Banana sap and its concomitant products

2.2.1 Nutrition from banana and its associated products

Banana has a tremendous nutritional value and contains approximately 74% moisture in them. Carbohydrates constitute the major component and comprise up to 24%. They are relatively less enriched in proteins and fat. Fiber constitutes up to 2.5% (Asif and Kaur 2018). Banana pulp comprises of 72% moisture, 2.9 % total fiber, 1.28 % ash, 1.4 % protein, 2.4 % glucose, 14.4 % sucrose, 8.5 % ascorbic acid and 2.1 % fructose (Forster et al. 2003). Banana is an excellent source of potassium. A single banana contains about 23% of potassium. Vitamins can be found in abundance in bananas such as A, B6, C, and D. Therefore, eating bananas contributes to keeping good health. The fruit pulp and peel of triploid banana types demonstrated great nutritional content. The moisture content in peel and pulp is around 83% and 70%, respectively (Mohapatra et al. 2010). β carotene is in a concentration of up to 55.68 µg/100g of fruit. Pulp has a very high total sugar content, up to 40% of which is sugar. The two main sugar constituents are cellulose and sucrose, followed by glucose and fructose (Mohapatra et al. 2010). Besides potassium, other nutrients such as dietary sodium, magnesium, calcium and phosphorous are found in significant amounts in fruit. Minerals such as iron, zinc, manganese, boron, and copper are found in fruit and pulp. Bananas are rich in phenols and flavonoids. The taste of unripe bananas is mainly due to its phenolic components. The fruit has excellent medicinal properties (Mohapatra et al. 2010). The fruit has mild laxative activity and is used to cure constipation, diarrhoea and intestinal lesions. Extracts of pseudo-stem are believed to help in dissolving kidney stones and in intestinal disorders. Flowers of banana are used to treat dysentery, ulcers and bronchitis. Due to its unique properties,

banana sap is used to treat intestinal disorders, leprosy, insect bites and hemorrhages (Kumar et al. 2012). The peel and pulp have significant antimicrobial activity. The roots are utilize to treat digestive disorders (Kumar et al. 2012). Banana by-products showed various therapeutic applications which are presented in Table 2.2 (Gupta et al. 2022).

Table 2.2: Therapeutic applications of banana by-products (Gupta et al. 2022)

Banana by-products	**Therapeutic applications**	**References**
Banana sap	Antioxidant and Antimicrobial	Kumar et al. 2014
Banana sap	Wound Healing activity	Weremfo et al. 2011
Green Plantain banana fruit	Wound healing activity	Agarwal et al. 2009
Banana meal	Antioxidant activity	Yin et al. 2008
Banana sap	Hyperglycemic effect	Singh et al. 2007
Banana sap	Antilithiatic	Tewtrakul and Subhadhirasakul 2007
Banana (peel, flesh) and ripe banana (peel, flesh)	Anti-allergic activity	Tewtrakul and Subhadhirasakul 2007
Banana pulp	Hypocholesterolaemic activity	Horigome et al. 2002
Green banana	Antidiarrhoeal activity	Rabbani et al. 2001

2.2.2 Bioactive compounds

Banana fruit and various plant components, including stem, rhizome, flower and leaf have bioactive molecules. They include many molecules, such as phenols, terpenes, carotenes, steroids which have a wide variety of bioactivity (Padam et al. 2014). Banana (plantain cultivars) pulp and peel have been shown to include a variety of hydroxycinnamic acids such as ferulic acid-hexoside, caffeic acid-hexoside, sinapic acid-hexoside, ferulic acid, sinapic acid, ferulic acid-dihexoside, hydroxycinnamic acid derivative and flavonols such as quercetin-deoxyhexose-hexoside-hexoside, myricetin-deoxyhexose-hexoside, quercetin-deoxyhexose-hexoside, methylmyricetin-deoxyhexose-hexoside, kaempferol-deoxyhexose-hexoside, kaempferol-3-O-rutinoside, isorhamnetin-hexoside, epicatechin (Tsamo et al. 2015). Fatty acids, sterols, α-tocopherol, and β-sitosterol are the important lipophilic components present in banana pulp have been demonstrated in studies to be helpful in the treatment of chronic illnesses, including cancer and cardiovascular disease (Vilela et al. 2014). Moreover, these lipophilic compounds are not limited to the pulp. However, they are also plentiful in the unripe peel of several banana varieties, which contains bioactive compounds such as saturated fatty acids, unsaturated fatty acids, diacids, ω-hydroxyacids, aromatic compounds, long chain aliphatic alcohols, sterols and tocopherols with high-value phytoconstituents (Villaverde et al. 2013). By using GC-MS analysis, potential bioactive components such as hexadecanoic acid ethyl ester, estragole, epicatechin, p-coumaric acid ethyl ester, gallocatechin, 1,2 benzenedicarboxylic acid mono (2-ethylhexyl) ester, vitamin E and β-tocopherol were identified in banana peel (Waghmare and Kurhade 2014). The banana peels were reported to produce various volatile aromatic chemicals, such as isoamyl alcohol, butyrates, and isovalerates, as well as elemicin, which add potential to the banana-processing food industry (Ji and Srzednicki 2013). Due to the presence of many phenolic components, such as catechol, chlorogenic acid (CAA), epicatechin, kaempferol 3-O-sophoroside, rhamnopyranoside, quercetin, rutin, and apigenin-6-C-glucoside-7-Oglucoside, dried banana pulp powder reduces different inflammatory indicators and oxidative damage and act as one of the promising nutritional candidate (Kumari et al. 2020). GC-MS study of the banana flower reported the 22 bioactive compounds, primarily fatty acids, steroids, and long chain aliphatic compounds

responsible for the antioxidant and cytotoxic applications (Revadigar et al. 2017). Apiforol, a flavanol found in banana seeds, reported to have anti-diabetic properties (Gopalan et al. 2019). The banana rhizome also comprises several phenolic chemicals, including tannic acid, ferulic acid, cinnamic acid, gentisic acid, catechol, gallic acid, protocatechuic acid, and caffeic acid which could be utilized as a potential natural antioxidant source in pharmaceutical and food industries (Kandasamy and Aradhya 2014).

2.2.3 Banana and its biological activities

Banana sap has hemostatic potential. Klotoe et al. (2012) reported the effect of sap in the bleeding treatment. The studies performed show that the sap lowers the clotting time and has no impact on the clotting factors. However, the reduction in clotting time is based on the effective protein network, which results in bleeding control.

Banana by-product extracts have been shown in several investigations to have specific antibacterial action against a broad range of bacteria including *B. subtilis, E coli, Klebsiella, Micrococcus, S. aureus, Salmonella, P. aeruginosa* and *V. cholerae*, represented in Table 2.3 (Gupta et al. 2022). Moreover, several banana components have been investigated for anti-proliferative/anticancer potential on the malignant cell lines shown in Table 2. 4 (Gupta et al. 2022).

Hossain et al. (2011) studied the antibacterial, antioxidant and antidiarrheal activities of the banana seed. Methanolic banana seeds extracts were tested for its bio-activity on a dose of 100-200 mg/kg body weight. The extracts exhibited potent antidiarrhoeal and antioxidant effects in mice. It also exhibited antibacterial potential against gram-positive and gram-negative bacteria.

Shanmuga and Subramanian (2011) studied the hypoglycemic effect of banana flower extracts in a diabetic rat model. Dried ethanolic extracts of the flower were tested on diabetic Wistar rats. When given for 30 days at 200 mg/kg body weight, oral flower extracts drastically reduced diabetic phenotype in rats. Phytochemical screening showed the presence of alkaloids, polyphenols, glycosides, terpenoids, and saponins as components in the flower extract.

Jahan et al. (2010) reported the effect of various banana inflorescence extracts on the growth of microbes. Chloroform, ethanol and aqueous inflorescence extracts were

tested for antibacterial activity. The extracts were blended in chitosan (CS)-Polyethylene Glycol (PEG) films and used to check for activity. Ethanol extract showed activity against all the tested microorganisms.

Dahham et al. (2015) examined the antioxidant and anticancer activity of the banana peel extracts. Extracts made in n-hexane, ethanol and water were compared for bioactivity. The hexane extracts showed the highest antioxidant and anti-proliferative potential against colon cancer cell line HCT 116.

Borges et al. (2005) documented the neutralizing effects of banana juice on various snake venoms. Banana juice obtained from pseudo-stem was used to check for interaction with various snake venom proteins. Phospholipase, myotoxic and hemorrhagic activities of venoms was highly reduced when mixed with the juice. The venom lethality in mice was highly reduced upon mixing with banana juice extracted from pseudo- stem.

Divya et al. (2016) explored the potential of the banana flower extracts against anti-inflammatory, anti-obesity and antioxidant activities. The freeze-dried aqueous extracts of the flower were used for the assessment of lipid peroxidation inhibition, pancreatic lipase and anti-inflammatory activity. The study observed significant total phenol content responsible for anti-inflammatory, antioxidant and anti-obesity potential associated with aqueous banana flower extracts.

Oneyma et al. (2016) examined the antimicrobial potential of banana pseudo-stem extracts. Water (aqueous), methanol and ethanolic extracts were prepared from pseudo-stem and used for anti-microbial analysis. Phytochemical analysis showed that pseudo-stem extracts were rich in phenols, tannins, oxalates and cardiac glycosides. Aqueous extracts showed good antibacterial action when compared to methanolic and ethanolic extracts. However, antifungal activity was not found in any of the extracts.

Rabbani et al. (2001) analyzed the antidiarrhoeal effect of green bananas. The study was done on human volunteers in Bangladesh. Children suffering from diarrhea were treated with green bananas. Analysis of effect was done for 14 days. The study showed that using green bananas was an effective home management method for curing the diarrhea in children.

Yin et al. (2008) evaluated the effect of single banana meals on oxidative stress in the plasma of healthy individuals. Lipid and lipid peroxide (LPO) levels were estimated

before and after the banana meal in healthy individuals. The study found that banana meal helped lower oxidative stress and reduced lipid peroxidation.

Table 2.3: Applications of banana by-products based on antimicrobial activities (Gupta et al. 2022)

Type of banana by-product	Activity	Applications	References
Banana leaf extracted with petroleum ether, chloroform and ethanol solvents	Chloroform and ethanol showed inhibition against all the tested bacteria (*E.coli, B. subtilis, P.aeruginosa* and *S.aureus)* whereas petroleum ether did not show any activity	Antispetic and disinfectant formulations	Naikwade et al. 2014
Banana peels extracted with ethanolic extract and aqueous extract	Ethanolic extract showed inhibition against all the tested bacteria (*B. subtilis, E. coli, P. aeruginosa* and *S. aureus Micrococcus*, *Klebsiella and Salmonella)* whereas aqueous extract does not show activityagainst *Salmonella, S.aureus* and *Micrococcus*	Medicinal plant with antimicrobial activity	Ehiowemwenguan et al. 2014
Banana sap extracted with aqueous, methanol and ethanol solvents	Only *Streptococcus* showed activity against all the solvents out of the tested bacteria	Preparation of antibacterial formulations in pharmaceutical industries	Kumar et al. 2014
Banana inflorescence extracted with aqueous, chloroform, methanolic and ethanolic solvents	*V.parahemolyticus* showed no inhibition in case of aqueous and chloroform extract , chloroform extract showed activity against *B.cereus* and *S.aureus* whereas methanol and ethanol extracts showed activity against all the tested bacteria *B.cereus, S.aureu,, V.parahemolyticus* and *L.monocytogenes*	Bacteriostatic as banana inflorescence is a source of natural antibacterials	Padam et al. 2012
Banana pulp, seed, peel extracted with methanolic solvent	Pulp and peel showed activity against all the bacteria *S. aureus, B. subtilis, E. coli, and P.aeruginosa, Shigella, Vibrio & Salmonella* whereas seed showed no activity	Treatment of dysentery and diarrhoea	Zafar et al. 2011

Table 2.4: Applications of banana by-products based on anticancer activities (Gupta et al. 2022)

Type of banana by-product	Effects	Applications	References
Banana peel, pulp extracted with hexane, water and ethanol	Hexane extracts of peel and pulp showed high cytotoxicity against HCT-116 and MCF-7 whereas water and ethanol extracts showed least activity with HCT-116 and MCF-7 cell lines	Pulp and peel has biological activities lead to therapeutic applications	Dahham et al. 2015
Banana peel extracted with ethanol solvent	Peel showed cytotoxic activity against MCF-7 cell line	Banana peel as a source of antitumor and anticancer agent	El-Zawawy 2015
Banana flower extracted with ethanol solvent	Flowers exhibited good cytotoxic effect with both HeLa and CHO cell lines	Natural source for the development of an anticancer lead molecule	Nadumane and Timsina 2014
Banana peel, pulp extracted with mixture of ethanol-water	Pulp and peel showed anticancer activity against A549, MCF-7, HepG2 and HT-29 cell lines	Prevention and treatment of different cancers in different ways	Li et al. 2013

2.2.4 Purview on food industry for banana and its by-products

2.2.4.1 Ethanol production

Ethanol production has been done by several researchers from various banana plant parts. Banana peel usage has been the subject of numerous investigations, while the bioethanol production from banana pulp, whole bananas, and rotten bananas has received less attention. The highest yield of 84% has been observed for banana pseudo-stem fermentation (Ingale et al. 2014). A yield of 45% has been observed for banana peel by Gebregergs et al. (2016). A summary of bioethanol production with banana by-products has been shown in the Table 2.5 (Gupta et al. 2022).

Guerrero et al. (2018) studied bioethanol production from banana pseudo-stem and rachis. Acid hydrolysis was the first step used for processing the raw material. The acid hydrolysate was subjected to simultaneous saccharification followed by fermentation. Enzymatic techniques were used for saccharification and fungal strain such as *Saccharomyces cerevisiae* was employed in fermentation. The pseudo-stem produced 112 L per ton, while the rachis produced 103 L per ton. Moreover, for pseudo-stem simultaneous saccharification and fermentation (SSF) gave better yields.

Kusumiyati et al. (2018) also demonstrated that simultaneous saccharification and fermentation (SSF) gave higher ethanol yields using the banana stem as raw material. The pseudo-stem was subjected to acid or alkaline hydrolysis at 121ºC for 30 min. The hydrolyzed material was subjected to SSF using strains of *Aspergillus*, *Trichoderma* and *Zymomonas* in fixed ratios. The study observed that acid pretreatment showed better results when compared to alkaline pretreatment.

Arrendonodo et al. (2009) reviewed the utilization of banana by-products for bioethanol production. They observed that acid treatment of banana fruit helps in providing hydrolysed sugars that are easily fermented. The lignocellulosic material undergoes a very high rate of hydrolysis in acidic conditions resulting in good availability of glucose that could be fermented. The resulting supernatant from hydrolysate upon fermentation with yeast gives high ethanol content post-fermentation.

Table 2.5: Ethanol production with different parts of banana by-products (Gupta et al. 2022)

Source of fermentation	Amount of ethanol	References
Whole banana	7.45 %	Itelima et al. 2013
	0.009%	Hammond et al. 1996
	0.009%	Hammond et al. 1996
Banana peel	2.1%	Palacios et al. 2017
	45%	Gebregergs et al. 2016
	3.56%	Waghmere et al. 2016
	6.54%	Singh et al. 2014
	1.309%	Nyandiga et al. 2014
	0.16%	Patel et al. 2012
	3.6 -5.8%	Brooks AA 2008
Banana sap	0.014-0.3%	Gupta et al. 2019
Banana fruit mash	7%	Jaggesar and Fraser 2016
Rotten banana	0.113%	Thancharoen 2015
Banana pseudo stem	84%	Ingale et al. 2014
Chiku and banana peels	2.66% - 78.9%	Chaudhary et al. 2014
Banana stem hydrolysate	0.2%	Thakur et al. 2013
Plantain peel	20%	Itelima et al. 2013
Banana pulp	28.45%	Arumugam and Manikandan 2011
	0.008%	Hammond et al. 1996

2.2.4.2 Source of antioxidants

Banana stem, blossom, fruit, sap, pulp, leaf, peel, and pseudostem have all been demonstrated to exhibit antioxidant activity represented in Table 2.6 (Gupta et al. 2022). Singh et al. (2016) studied the potential of total polyphenol and flavonoid of locally available fruits. Flavonoids and polyphenols help to maximize antioxidant action. Pomegranate had the highest polyphenol content and sapodilla had the lowest. Pomegranate had the highest flavonoid concentration, while kinnow had the lowest. The peel, in all cases, had significant activity than the pulp. Banana had intermediate

phenol and flavonoid content. Dopamine and catecholamines are abundant in banana agro-waste (such as the peel and pulp), which inhibit β-carotene bleaching in the carotene model, reduce lipid peroxidation, and scavenge free radicals (González-Montelongo et al. 2010; Toh et al. 2016). According to research, banana flesh contains potential bioactive antioxidant compounds that act as defenses against free radicals and mediate oxidative cell damage (Qamar and Shaikh 2018). Banana rhizomes comprise the strong antioxidants 4-epicyclomusalenone, protocatechuic acid, gentisic acid, cycloeucalenol acetate, and chlorogenic acid (Kandasamy et al. 2014).

Table 2.6: Antioxidant activities of banana by-products (Gupta et al. 2022)

Type of banana by-product	**Activity**	**Applications**	**References**
Banana sap extracted with methanol and ethanol solvents	Strong antioxidant	Preparation of formulations in pharmaceutical industries	Kumar et al. 2014
Banana leaf extracted with hexane, ethyl acetate and methanol solvents	Inactivate lipid free radicals	Helps to understand the use of *Musa* species in traditional medicine as an antioxidant agent	Karuppiah and Mustafffa 2013
Banana pulp, seed and peel extracted with hexane, ethyl acetate and ethanol solvents	Free radical terminators	Potential source of bioactive compounds	Jain et al. 2011
Banana pseudostem and rhizome juices	Electron transfer or hydrogen donors	Functional beverage	Saravanan and Aradhya 2011b
Banana pseudo stem and flower extracted with chloroform, acetone and methanol solvents	Prevent lipid oxidation via chain breaking reaction	Nutraceutical for the replacement of synthetic antioxidants	Loganayaki et al. 2010
Banana fruit extracted with aqueous, methanol, ethanol and acetone extracts	To scavenge free radicals either by lipid peroxidation or chelating metal ions	Fruits contains antioxidant	Alothman et al. 2009

2.2.4.3 Production of biogas

Pisutpaisal (2014) studied the biomethane production from banana peel. The peel was ground to fine pieces and then used for anaerobic digestion. Experiments were set up in batch reactors with total solid (TS) content varying from 2-10% wv^{-1}. Maximum methane production was seen in 7.5% total solid content. The production rate was 5.3 mL h^{-1}.

Banana pseudo-stem fiber has been used biogas production. Pei et al. (2014) utilized a novel alkaline pretreatment technique for utilizing banana pseudo-stem fiber for biogas production. The main parameters that controlled biogas production included the alkaline pretreatment time, concentration of alkali used, temperature of pretreatment and length of pseudo-stem fiber. Highest concentrations of 463 mL/g volatile solids were seen with alkaline pretreatment method.

Anaerobic digestion of banana waste includes four distinct stages. They are hydrolysis of waste, generation of acid, generation of methane and generation of acetone. The various fermentative bacteria present in cow dung perform each of these steps which results in effective generation of biogas (Divyabharathi et al. 2017).

Co-digestion of banana sap (stem juice), both alone and in combination, was found to be extremely effective for biomethanation. In order to enhance the biomethanation process, the effects of recycling the digestate from the biogas plant were also examined (Tripathi et al. 2020).

2.2.5 Health and therapeutic benefits of banana and its by-products

Banana has very high levels of potassium in them. Due to such high levels of potassium it prevents high blood pressure and in preventing stroke in individuals. The presence of high quantities of fibers in banana helps in regulating the bowel movement. Due to regulation of bowel activity banana has an important role in controlling diarrhoea. Extracts of bananas help in controlling Alzheimer's due to the significant levels of antioxidants (Kumar et al. 2012). According to Lustikasiwi et al. (2021) banana peels contain tryptophan, an amino acid that is underutilized for the protection of dementia but plays a major role in the mechanism. Serotonin and kynurenine are precursors of tryptophan. 5-hydroxyindoleacetate or 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of serotonin, has the ability to break down amyloid- β(Aβ) oligomers, a 36–43 amino acid peptide group derived from the

amyloid precursor protein seen in Alzheimer's patients. Banana sap and banana fruit have beneficial effects on the kidney and prevent kidney stone formation. Bananas contain high levels of tryptophan. Ayurveda uses extracts from several banana plant parts, including the pseudo-stem, flower, roots and pith, to treat ailments of the gut and kidney (Kumar et al. 2014).

Researchers looked at the feasibility of blending plantain stem juice with fruit juice to increase the nutritional content of the sap. The results demonstrated that adding banana juice to grapefruit juice increases the nutritional content and antioxidant activity. This will provide a unique outlook using the juice from plantain stems. Consequently, this will result in the low-cost blended average that consumers demand (Ravi et al. 2011). Bananas have some prebiotic properties, including the presence of resistant starch, cellulose, hemicelluloses, and lignin, which account for 60–80% of their total carbohydrates and are hence indigestible (Sarawong et al. 2014; Cordoba et al. 2018). Probiotic bacteria feed on some of the indigestible carbohydrates found in bananas (e.g. *Lactobacilli* spp.). Probiotic bacteria may break down such carbohydrates to form short-chain fatty acids during fermentation, which helps the probiotic proliferate (Buranawit and Laenoi 2015; Budhisatria et al. 2017). The prebiotic qualities of the banana's carbohydrate content included the ability to hold water and oil as well as antioxidant potential, resistance to trypsin and α-amylase, and the capacity to promote the growth of *Lactobacilli* while preventing the formation of pathogenic bacteria. As a result, these bananas may be employed as a prebiotic source in nutraceutical food products (Powthong et al. 2020).

2.2.6 Merchandise of banana and its by-products

The fiber present in the pseudo-stem has an outstanding applicative value. The fiber has applications in the handicraft industry, including making ropes, twines and mats (Vigneswaran et al. 2015). Green bananas are sliced and used to make chips. Ripe banana fruits are used to make puree in ice creams, cakes and bread making process. Banana leaves are used for making baskets, mats and plates. Banana foliage and pseudo stems are used as cattle feed. Banana peels are dried and used to make flour. The banana flower is edible and is used extensively in cooking (Singh et al. 2018). Presently, banana peels are used in several food categories such as fermentation of dough, jelly, and Indian flatbread to improve health (Al-Sahlany and Al-musafer

2020; Kurhade et al. 2016). In order to substitute wheat flour (between 6.5 and 30%) in the making of noodles and pasta, unripe and ripe banana peels have been combined with the pulp (Castelo-Branco et al. 2017). Additionally, the peel has been used in several other culinary preparations, including jellies, fish patties, and mayonnaise (Kraithong and Issara 2021). Interestingly, banana blossom has also been used in chocolate production. Sharmila (2015) supplemented cocoa powder with a banana blossom at 10%, 20%, and 30% levels and discovered that the product had the best level of approval when incorporating with 20% of banana blossoms. Surprisingly, banana flower has occasionally been mixed with chicken flesh in the production of meat to make shredded banana blossom-chicken meat (Novidiyanto et al. 2020). Banana inflorescences due to the presence of starch and flavour have been combined with potato flour to make banana blossom nugget product which was accepted by the consumers (Wahab et al. 2020).

2.3 Other industrial applications of banana sap and its by-products

Banana pseudo-stems produce fibers that are used to make ropes, baskets, and other goods with additional value. The central core of the stem can be utilized to produce pickles, sweets, and soft beverages. In the textile industry, banana pseudo-stem sap serves as a mordant to bind dyes. It is also used as an organic liquid fertilizer (Subagyo and Chafidz 2018). The processed banana pseudo-stem has been a good absorber of heavy metals such as lead. Leaves and pseudo-stem of bananas are perfect substrates for the growth of mushrooms. Paper production has been done from banana pseudo-stems (Padam et al. 2014). Banana agro-waste can be used as a capping agent in green nanoparticle synthesis, which utilizes them in place of traditional chemical procedures to develop nanoparticles as a potential method of recycling banana by-products (Ibrahim 2015; Mostafa 2021). Banana agro-waste was successfully used to produce nanoparticles such as silver (Ibrahim 2015; Dang et al. 2017), gold (Liu et al. 2020), and titanium (Hameed et al. 2019) that have inhibitory effects on migration, tumour growth, and possible antibacterial properties. Cellulose nanofiber is regarded to be among the most prominent green-created materials due to its helpful qualities like mechanical capabilities, high abundance, biocompatibility and renewability (Trache et al. 2020). Cellulose nanofibers isolated from banana peel and bracts demonstrated improved thermal properties (Tibolla et al. 2018; Harini et al. 2019).

CHAPTER -3
MATERIALS AND METHODS

3.1 Sample collection

Banana adult stems (Grand Naine) were obtained from Thapar Institute of Engineering and Technology Campus, Patiala. The stems were crushed and passed through a filter (pore size 2.5 µm) to remove solid particles. The sap was further stored at 4ºC till further use.

3.2 Preliminary test of banana sap

3.2.1 Chemical characterization of banana sap

For the chemical characterization of banana sap, the various parameters like pH, Chemical Oxygen Demand (mg/L), Biological Oxygen Demand (mg/L), Chemical Oxygen Demand: Biological Oxygen Demand (COD: BOD), organic carbon (%), reducing sugars (mg/mL) and proteins (mg/mL) were examined.

3.2.1.1 pH

The pH of the banana sap was determined according to the standard method APHA 2012 (4500-H^+).

3.2.1.2 Chemical oxygen demand (COD)

COD was determined by using APHA 2012 (5220). To determine COD, a pinch of mercuric sulfate was dissolved with 5 mL of sulphuric acid in a reflux tube, and after that, 5 mL of standard potassium dichromate was added and mixed. 10 mL of a diluted sample (banana sap) was added and mixed properly. Finally, 10 mL of sulphuric acid was added. Tubes were connected to condensers and refluxed at 150 ± 2ºC for 2 hrs. The samples were cooled. Subsequently, it was titrated by using standard ferrous ammonium sulphate. The indicator ferroin was used, and the end point ranged from green-blue to wine red.

3.2.1.3 Biological oxygen demand (BOD)

BOD was estimated using APHA 2012 (5210). To 2 L of dilution water, 2 mL/L of all four nutrients, namely magnesium sulfate, phosphate buffer, ferric chloride, and calcium chloride were prepared. 2 mL/L of seed (organic water) was added, and to this, 5 mL banana sap sample and 1993 mL dilution water were added. Water was added into BOD bottles from aspirator bottles, the sample was allowed to overflow, and then it was stoppered. Five bottles were filled for one sample, three bottles were incubated for five days at 20°C, and two bottles were analyzed for dissolved oxygen

(DO) by Winkler's method (1888). After incubation (five days), bottles were tested for DO concentration.

3.2.1.4 Dissolved oxygen estimation

Dissolved oxygen (DO) was calciulated by Winkler's method (1888). Sap sample was added in 300 mL bottle, 1mL $MnSO_4$ solution, 1mL alkali-iodide-azide solution was added, and the brown precipitate was mixed by shaking them up and down. The bottle was allowed to settle down for 2-3 min, and to this, 2 mL of conc. H_2SO_4 was added. It was titrated with 0.025 N sodium thiosulfate. Starch was used as an indicator, and the end point is blue to colorless.

3.2.1.5 Percentage organic carbon

Organic carbon was estimated using Walkley-Black (1934). 0.1 g of banana sap was taken, and 10 mL of dichromate was added. Concentrated sulphuric acid (20 mL) was slowly added. Afterwards, it was incubated for 30 min. Subsequently, 200 mL of water and 10 mL of phosphoric acid were added. It was titrated by using ferrous sulphate. Diphenyl amine was used as an indicator. The end point was brown to green.

3.2.1.6 Reducing sugars

Reducing sugars were analyzed by using DNS (3,5-dinitrosalicyclic acid) method given by Miller (1959).

The glucose stock used was 1 mg/mL.

Different concentrations of glucose were prepared to range from 0.2 to 1 mg/mL. The volume was made to 1 mL. To this, 3 mL of DNS was added and subsequently placed for 10 min in a boiling water bath. Finally, OD was taken at 540 nm.

3.2.1.7 Proteins

Proteins in the banana sap were estimated by Folin and Lowry method (Lowry et al. 1951)

The BSA stock used was 1 mg/mL.

Reagent A: 20 g Na_2CO_3 and 4 g NaOH were added to distilled water to make 1000 mL

Reagent B: 1g $CuSO_4.5H_2O$, 2 g of Na-K tartarate, and distilled water was added to make 1000 mL

Reagent C: Mixed these in 100 mL of Reagent A and 2 mL of Reagent B.

The standard solutions were prepared in concentrations in the range of 0.05, 0.1, 0.15, 0.2, 0.25, 0.30, 0.35, and 0.4 mg/mL. 5mL of solution C was added and mixed well with a taken sample of BSA. Subsequently, it was incubated at room temperature for 10 min. 0.5mL of Folin's reagent was added, followed by mixing and incubation at room temperature for 30 min. OD was taken at 660 nm.

3.2.2 Phytochemical screening of banana sap

Phytochemical testing involves screening of sap for the presence of compounds like alkaloids, glycosides, tannins, and saponins. Several phytochemical experiments were performed to screen the qualitative profile of the banana sap.

3.2.2.1 Total carbohydrates estimation (Molisch's test)

2 mL of banana sap and a few drops of alcoholic α-naphthol were mixed to test the presence of carbohydrates. After mixing, a few drops of concentrated sulphuric acid were added through the sides of test tubes without mixing to form a layer. A purple to violet color ring appeared at the junction, which confirmed the presence of carbohydrates (Sawhney et al. 2011).

3.2.2.2 Total amino acids estimation (Ninhydrin test)

To confirm proteins, 2 mL of banana sap was added to the few drops of alcoholic ninhydrin solution and heated to boil in a boiling water bath for 10 min. The violet color indicated the presence of amino acids (Sawhney et al. 2011).

3.2.2.3 Total proteins estimation (Biuret test)

To test the presence of total proteins in the banana sap, 2 mL of banana sap was taken, and 2mL (1:1) biuret reagent was added to it. The appearance of violet color revealed the presence of proteins (Khandelwal 2000).

3.2.2.4 Total triterpenoids estimation (Salkowoski's test)

The presence of triterpenoids was tested by mixing a few drops of concentrated sulphuric acid with the 2 mL of banana sap was taken. The appearance of a yellow-colored lower layer revealed the presence of triterpenoids (Ayoola et al. 2008).

3.2.2.5 Total fats and fixed oils estimation (Saponification test)

The saponification test was performed for the presence of total fats and fixed oils. To the 2 mL of banana sap, a few drops of 0.5 N potassium hydroxide and a drop of phenolphthalein were added. After that, it was heated in a water bath for 1 hr. The soapy bubbles formation indicated the presence of fats and fixed oils (Oloyede 2005).

3.2.2.6 Total alkaloids estimation

To test the presence of alkaloids in the banana sap Wagner test was performed, and for this, 2 mL of banana sap was taken, and a few drops of Wagner's reagent was added. The appearance of reddish brown precipitate, confirmed the presence of alkaloids (Sawhney et al. 2011).

3.2.2.7 Total cellulose estimation

To investigate the cellulose in the banana sap, 2 mL of banana sap was taken, and it was mixed with 0.1M iodine solution. After that, sulphuric acid was added. The blue-violet color indicated the presence of cellulose (Khandelwal 2000).

3.2.2.8 Total flavonoids estimation

To confirm the flavonoids in the banana sap, 2 mL of banana sap was taken, and a few magnesium turnings were added. After that, drop wise concentrated hydrochloric acid was added. The presence of flavonoids was revealed by the appearance of a pink/magenta color within two min, which could be further extracted with butanol (Aynehchi et al. 1981).

3.2.2.9 Total tannins and phenolic compounds estimation

To examine the tannins and phenolics in the banana sap, 2 mL of banana sap was taken, and drops of ferric chloride solution was added (Sawhney et al. 2011).

3.2.2.10 Total glycosides estimation (Killer-Killiani test for deoxy sugars)

To confirm the presence of glycosides in the banana sap, 2 mL of banana sap was taken, and to this 1 mL glacial acetic acid, a few drops of concentrated sulphuric acid (diphenylamine reagent), and one drop of 5% ferric chloride were added. The appearance of blue spots/coloration confirmed the presence of glycosides (Narasimhan et al. 1982).

3.3 Conversion of banana sap into bioethanol

3.3.1 Biological Materials

The strains MTCC 170 and MTCC 180 of *Saccharomyces cerevisiae* were obtained from the Institute of Microbial Technology (IMTECH) in Chandigarh, India, and used in this investigation. YEPD medium was used to sustain these cultures (Appendix). Fresh banana stems of the Grand Naine cultivar were obtained from the Thapar Institute of Engineering and Technology Campus in Patiala. In order to eliminate

solid particles, the stems were crushed and passed through a filter. Using a rota evaporator, the clarified liquid was collected. Subsequently, it was concentrated ten times, and then kept at 4°C. Corn steep liquor (CSL) was obtained from Sukhjit starch and chemicals limited, Phagwara, Punjab, India. Spent wash procured from Patiala distillers and manufactures limited, Mann village, Patiala, India, and yeast extract from (Himedia, Mumbai).

3.3.2 Analysis of samples

The standard protocols APHA (2012) were used to analyze concentrated banana sap, spent wash, and corn steep liquor for phosphorus (4500-PB/5), nitrogen (4500-NH_3 B and C), total solids (2540 B), total dissolved solids (2540 C), total suspended solids (2540 D), biological oxygen demand (5210), and chemical oxygen demand (5220).

3.3.3 Hydrolysis of banana sap

Concentrated banana sap was acid hydrolyzed by Hernandez-Sales et al. (2009). Concentrated banana sap was combined with varying amounts of hydrochloric acid (HCl) to obtain a final concentration of 0.5, 1.0, and 1.5 N for optimization. After optimization, 1.0 N HCl was used for further experiments. The acid hydrolyzed sample was autoclaved at 121˚ C at 15 min. Subsequently, it was cooled and pH was neutralized. For alkali hydrolysis, concentrated banana sap was combined with 0.5, 1.0, and 1.5 N NaOH solutions. 1.0 N NaOH was optimized and treated with banana sap for further studies. The pretreated banana sap was autoclaved at 121ºC at 15 min. Afterwards, it was cooled, and pH was neutralized.

3.3.4 Optimization conditions for ethanol production

Concentrated banana sap augmented with 0.3% yeast extract was used for ethanol production by inoculating the two yeast strains *Saccharomyces cerevisiae* (MTCC 170 and MTCC 180). The samples were estimated for ethanol production at different time intervals, i.e., from 1, 2, 3, 4, and 5 days.

3.3.5 Fermentation conditions

The acid and alkali hydrolysed samples supplemented with spent wash (SW), corn steep liquor (CSL), and yeast extract (YE) mixed with samples such as concentrated sap, concentrated sap + 1% CSL, concentrated sap + 3% CSL, concentrated sap + 5% CSL (v/v), concentrated sap + 1% YE, concentrated sap + 3% YE, concentrated sap + 5% YE (v/w), concentrated sap + 25% SW, concentrated sap + 50% SW,

concentrated sap+ 75% SW (v/v) were prepared. The sugar content in these samples was assessed by the DNS method as described in Miller (1959). Two strains of *S. cerevisiae* (MTCC 170 and MTCC 180) were used to inoculate the samples. As reported by Caputi et al. (1968), the chromic acid technique was used to estimate the ethanol concentration in the fermentation medium. Briefly, 25 mL of potassium dichromate was combined with 10 mL of fermented broth and heated at 80ºC for 15 min in a hot water bath. About 5 mL of the distillate was then recovered. After those samples were cooled, a UV-Vis spectrophotometer was used to determine their absorbance at 600 nm. A solution of ethanol and water was used to prepare ethanol standards.

3.3.6 Statistical analysis

All the experiments were carried out in the triplicates. The data were examined by Analysis of Variance (ANOVA) using Graph pad prism software version 5.

3.4 *In vitro* evaluation of bioactive properties of banana sap

3.4.1 Screening of antimicrobial activity

3.4.1.1 Sample collection

Fresh banana pseudo-stem (Grand Naine cultivar) was obtained from the Thapar Institute of Engineering and Technology Campus. The sap from the banana pseudo-stem was collected using a commercial squeezer from the local market. The squeezer was carefully cleaned and disinfected before extracting the sap. After the juice extraction, banana sap was concentrated ten times (IKA RV 10) with the help of a rota evaporator to study the antimicrobial activity.

3.4.1.2 Preparation of un-oxidized banana sap

In an amber colored bottle, 80% ethanol containing 100 mM NaCl, 40 mM citric acid, 0.2 mM ascorbic acid, 0.1 mM $Na_2S_2O_5$, 0.2 mM EDTA and 0.25% Triton X-100 was prepared (Pothavorn et al. 2010). Sap was produced by processing stems. Subsequently, a solution of 80% ethanol solution was mixed with freshly extracted sap in a ratio of 1:1 to prevent oxidation. After 45 min of 80°C heating, the sap was centrifuged at 11250 g for 15 min. at 25°C using a centrifuge (Thermo Fischer Scientific, Sorvall Legend XFR Centrifuge, 75004538, Germany). The rota evaporator was used to collect and concentrate the supernatant. Afterward, it was

lyophilized followed by dissolving in 10% DMSO (dimethyl sulfoxide) and stored at 4°C till further use.

3.4.1.3 Test microorganisms

The microbial test panel employed for the antimicrobial action *investigation comprised Bacillus megaterium* FH 1127, *Escherichia coli ESS 2231, Pseudomonas aeruginosa* M35, *Staphylococcus aureus* ATCC 33591, and *Candida albicans* ATCC10231. Individual bacterial cultures were streaked on Muller Hinton (MH) agar plate (Appendix) and incubated overnight. Afterwards, a single colony was transferred aseptically to 100 mL of pre-sterilized MH broth (Appendix) and incubated at 37°C overnight. In the case of anti-candidal activity, *Candida albicans* was streaked on the Sabouraud Dextrose Agar plate (Appendix) and incubated overnight. A single colony was picked and transferred from this plate to the Sabouraud Dextrose broth (Appendix) and incubated at 37°C before the test.

3.4.1.4 *In vitro* antibacterial assay

The antibacterial activity of the banana sap extract was assessed with the help of *in vitro* micro broth dilution assay (Jorgensen et al. 1999; EUCAST 2018). The antibacterial activity was assessed in a 96-well microtiter plate, and the visual minimum inhibitory concentration was determined (MIC). 10% dimethyl sulfoxide (DMSO) was used to dissolve the banana sap extract and evaluated in a concentration range from 500 mg/mL to 1.95 mg/mL, i.e., 2-fold serial dilutions. A final bacterial cell concentration (10^5 cells) in the wells was achieved by adding 50 µL of the 0.5 McFarland (Appendix) adjusted bacterial suspension in saline to 125 µL of MH broth. Amoxicillin (0.1mg/mL) was used as a positive control. The titer plate was incubated for 24 hrs at 37°C. After 24 hrs, 20 µl of 0.02% of TTC (2, 3, 5-triphenyl tetrazolium chloride) was used to visualize the MIC. The assay was performed in the triplicates.

3.4.1.5 *In vitro* anti-candidal activity

The anti-candidal activity of the banana sap extract was determined by *in vitro* micro broth dilution assay (Jorgensen et al. 1999; EUCAST 2018). Briefly, a 96-well microtiter plate was used to evaluate the anti-candidal activity and arrive at a visual minimal inhibitory concentration (MIC). Further, banana sap extract was dissolved in 10% dimethyl sulfoxide (DMSO) and evaluated in a concentration ranging from 500 mg/mL to 1.95 mg/mL, i.e., 2-fold serial dilutions. 50 µL of the 0.5 McFarland

adjusted suspension of *Candida albicans* in saline was added to 125 µL of SD broth to achieve a final concentration of 10^5 cells in the well. Fluconazole (0.1mg/mL) was used as a positive control. The titer plate was incubated for 24 hrs at 37°C. After 24 hrs, 0.01% of Resazurin was used to visualize the MIC. The assay was performed in triplicates.

3.4.2 Screening of antioxidant and cytotoxic activity

3.4.2.1 Preparation of extracts

Fresh banana stem (Grand Naine cultivar) was collected from the Thapar Institute of Engineering and Technology Campus. With the help of the local market, banana stem juice was extracted from the stems. After juice extraction, banana sap was concentrated ten times with the help of a rota evaporator (IKA RV 10). Subsequently, the concentrated sap was extracted with dichloromethane (DCM) and ethyl acetate (EA) solvents. 100 mL of concentrated banana sap was mixed with three times 70 mL of solvents (DCM/EA) to obtain the DCM/EA solvent extract with the help of solvent extraction process at 35^{o}C (Vasundhara et al. 2017). Further, the solvent was removed by rota evaporator, and subsequently, the extract was dried with the help of a lyophilizer. The obtained dried powder was dissolved in dimethyl sulfoxide (DMSO).

3.4.2.2 Chemical and reagents

3.4.2.2.1 Cell lines procurement and maintenance of MCF-7

Human breast cancer (MCF-7) cell lines were procured from the National Centre for Cell Sciences (NCCS), Pune, India. The cell line was maintained in a complete DMEM medium in the humidified incubator with 5% CO_2 at 37^{o}C in T25 flasks. Complete medium (Appendix and Annexure) means DMEM supplemented with 100 µg/mL streptomycin, 10% (v/v) FBS, 100 IU/mL penicillin, and 2.5 µg/mL amphotericin. An inverted microscope and vertical laminar were used to examine the cells (Figure 3.1).

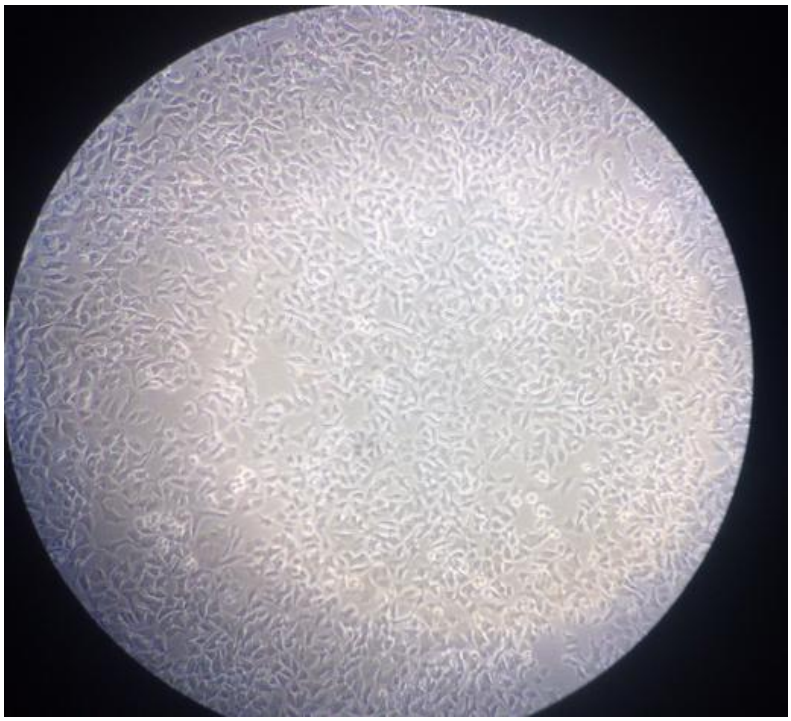


Figure 3.1: MCF-7 cell lines under 40X magnification (Nikon Eclipse T5100)

3.4.2.2.2 Revival of MCF-7 cells

The vial containing cells was removed from the storage and thawed quickly in a 37°C water bath. Afterward, a complete medium was added to the vial containing frozen cells followed by gently mixing. The cells were then centrifuged at room temperature for 10 min at 1000 rpm. The supernatant was discarded. Subsequently in complete medium cells were re-suspended. The cells were plated in T25, incubated at 37°C and 5% CO_2 (New Brunswick Galaxy; Eppendorf, Haupage, NY, USA).

3.4.2.2.3 Subculturing of cells and media change

MCF-7 cells were grown in a complete medium in a T25 flask and maintained at 37°C and 5% CO_2. As MCF-7 cells reached approximately 80% confluence in the flask, the media was removed from the flask and rinsed with 1X Phosphate Buffered Saline (PBS) (Appendix). After that, 2-3 mL of Trypsin EDTA solution (Appendix) was incorporated to MCF-7 cells to detach the cell layer from T25. For a detachment of the cell layer from T25, the T25 was placed in a 37°C incubation chamber and observed under an inverted microscope. Once the MCF-7 cell layer is dispersed, the same volume of complete medium is added in T25 and then transferred to eppendorf tubes. The MCF-7 cells in complete medium were centrifuged for 10 min at 1000

rpm. The MCF-7 cells were re-suspended from the complete medium. The MCF-7 cells were counted using a hemocytometer. The cells were again seeded in T25 and placed in a CO_2 incubator. The confluence of the MCF-7 cells was checked regularly under an inverted microscope.

3.4.2.2.4 Cell counting

A hemocytometer was used to count the cells, which were stained with Trypan blue. Trypan blue is a stain that penetrates the cell wall of dead cells and stains them blue while leaving live cells uncolored. The cell was diluted ten times (10 µL of cell suspension, 80 µL of media, and 10 µL of Trypan blue solution). The Trypan blue-diluted suspension was placed into a hemocytometer. Hemocytometer was focused on using the 10X objective of the microscope, and cells were counted in all four sets of squares of the hemocytometer using the 40X objective of the microscope.

Cell count was calculated using the formula:

$$\text{Cell count} = \frac{\text{Total number of cells counted x Dilution Factor x } 10^4 \text{ cells /mL}}{\text{Number of chambers counted}}$$

3.4.2.2.5 MTT assay

Cell proliferation of MCF-7 cells was examined by using a 3-(4, 5- dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide (MTT) assay. The cytosolic compartment of active cells in viable cells contains NADPH-dependent oxidoreductases that reduce MTT to an insoluble formazan (Mosmann 1983).

Principle: MTT is a calorimetric assay that measures the reduction of MTT (yellow color) by mitochondrial succinate dehydrogenase to insoluble formazan crystals (dark purple color), which were further solubilized in DMSO. The level of activity is a measure of the viability of the cells as the MTT reduction can occur in metabolically active cells.

3.4.2.2.6 Cell growth inhibition assay

2 x 10^4 MCF-7 cells were seeded in 96-well microtiter plates and kept overnight in the incubator. The extract was added to the wells at an increasing concentration (5-100 µg/mL), followed by incubation. After 72 hrs incubation of plates, 20 µL MTT (5 mg/mL) was added to each well and incubated for 4 hrs. Subsequently, 100 µL DMSO was added to each well to dissolve the formazan crystals. On an ELISA plate reader (Tecan Infinite, Groedig, Austria, Pro ELISA reader), the absorbance was

recorded at 570 nm, taking the reference wavelength at 620 nm. Paclitaxel was employed as the positive control at 20 µg/mL concentration. Proliferative index was expressed as:

$$\text{Proliferative index} = (A_{treated} / A_{untreated})$$

3.4.2.2.7 Antioxidant assay

The antioxidant potential of the concentrated banana sap extract was assayed with the help of the free radical scavenging effect (Sharma et al. 2017). In a 96 well microtiter plate, 50 µL of banana sap extracts in increasing concentrations (100, 250, 500, and 1000 µg/mL) were incorporated with 150 µL of DPPH (100µM) in methanol. Ascorbic acid (100 µg/mL) was used as the positive control. The plate was incubated for 45 min in the dark, and after that, absorbance was recorded at 517 nm using a microplate reader (Tecan infinite, Austria).

The scavenging activity was expressed as:

$$\text{Scavenging activity (\%)} = \{(A_{control} - A_{sample}) / A_{control}\} * 100$$

3.4.2.2.8 Statistical analysis

All the experiments were performed in triplicate. The results were represented as mean ± standard deviation. The data were analyzed by analysis of variance, and the means were compared by Tukey's test at $p < 0.05$.

3.4.3 Nutritional analysis of banana sap

Standard nutritional components such as vitamins, minerals, proteins, and amino acids in banana sap were analyzed by PBTI, Mohali.

3.4.4 LCMS analysis

3.4.4.1 Sample preparation

Fresh banana stem (Grand Naine cultivar) was collected from Thapar Institute of Engineering and Technology Campus, Patiala, Punjab, India. The juice was extracted from the stems using three roller cane crushers from the local market. After extraction, the banana sap was concentrated ten times using a rota evaporator (IKA RV 10). The concentrated sap was further extracted with solvents such as dichloromethane (DCM) and ethyl acetate (EA).

3.4.4.2 Extraction procedure

Concentrated banana sap (100 mL) was extracted with DCM (70 mL x 3 times) and EA (70 mL x 3 times) to obtain the respective solvent extract. The organic layer of DCM and EA were subsequently rota evaporated and stored in vials at 4℃ until further use.

3.4.4.3 LCMS analysis

It was done at IIT Bombay using Orbitrap LCMS (OHRLCMS) with Hypersil gold column (3micron 100 x 2.1 MM) with a positive polarity, and each run for 30 min. The instrument used was VANQUISH with a range of 80 to 1200 m/z and a loop count of 5.

CHAPTER -4
RESULTS

4.1 Preliminary test of banana sap

4.1.1 Chemical characterization of banana sap

The chemical characterization of banana sap reveals a pH of 5.7, high organic matter COD, BOD (Table 4.1) and low levels of reducing sugar (Table 4.2, Figure 4.1) and proteins (Table 4.3, Figure 4.2)

Table 4.1 Chemical characterization of banana sap

Parameters	**Results**
pH	5.73±0.12
COD (mg/L)	28966.67±57.74
BOD (mg/L)	12555.33±419.74
BOD : COD ratio	0.43±0.01
Organic Carbon %	14.83±0.76
Reducing Sugars (mg/mL)	4.61±0.35
Protein (mg/mL)	0.96±0.01

All values are mean of the triplicate test

Table 4.2: Standard table of Glucose concentration (DNS method)

Stock concentration (µL)	**Stock volume (µL)**	**Distilled water (µL)**	**DNS (mL)**	**Absorbance 540nm**
0	Blank	1000	3	0
0.2	200	800	3	0.233
0.4	400	600	3	0.435
0.6	600	400	3	0.659
0.8	800	200	3	0.91
1	1000	0	3	1.2

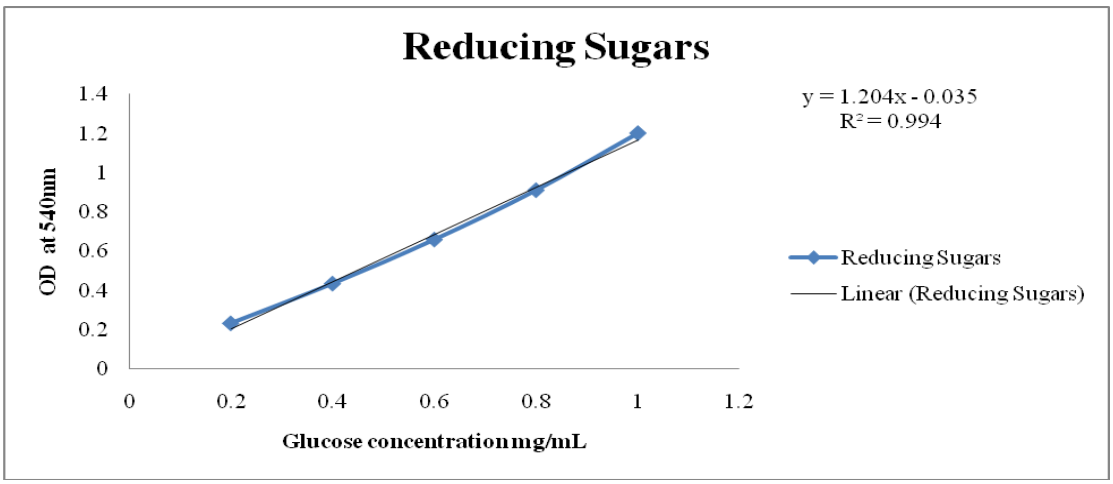


Figure 4.1: Standard curve of Glucose concentration (DNS method)

Table 4.3: Standard table of protein concentration (Folin and Lowry method)

Stock concentration (mg/ml)	Stock volume (µl)	Distilled Water (µl)	Solution C(ml)	Folin Reagent (ml)	Absorbance 660nm
0	Blank	1000	5	0.5	0
0.05	50	950	5	0.5	0.118
0.1	100	900	5	0.5	0.186
0.15	150	850	5	0.5	0.262
0.2	200	800	5	0.5	0.314
0.25	250	750	5	0.5	0.346
0.3	300	700	5	0.5	0.43
0.35	350	650	5	0.5	0.52
0.4	400	600	5	0.5	0.547

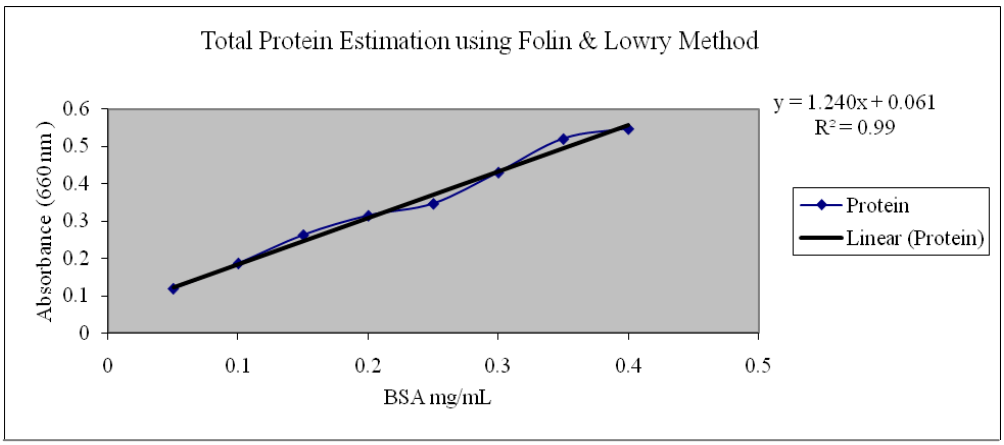
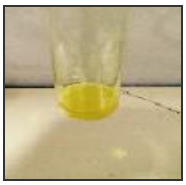


Figure 4.2: Standard curve of protein concentration (Folin and Lowry method)

4.2 Phytochemical screening of banana sap

Phytochemical screening of banana sap reveals the presence of amino acids, carbohydrates, fats, triterpenoides, alkaloids and absence of glycosides (Table 4.4).

Table 4.4: Phytochemical screening of banana sap

S.NO	Test	Inference	
1	Carbohydrates (Molisch's test)	+ve	
2	Amino acids (Ninhydrin Test)	+ve	
3	Proteins estimation (Biuret test)	+ve	
4	Triterpenoides (Salkowoski's test)	+ve	
5	Fats and Fixed oils (Saponification test)	+ve	
6 (a)	Alkaloids (Wagner's reagent test)	+ve	
(b)	Hager's reagent	+ve	
7	Cellulose	+ve	
8	Flavonoids	-ve	
9	Tannins and phenolic compounds (Ferric chloride test)	-ve	
10	Glycosides (Keller-Killiani test for deoxy sugars)	-ve	

4.3 Conversion of banana sap into bioethanol

4.3.1 Analysis of samples

The chemical characterization of concentrated banana sap, spent wash, and corn steep liquor was performed and represented in Table 4.5. It was observed that the industrial waste spent wash had maximum BOD whereas banana sap showed the lowest BOD, indicating that in the spent wash, aerobic microorganisms require more dissolved oxygen for the breakdown of the organic material present in it. Examination of the COD shows that spent wash contains high organic matter, which suggests that it consumes more oxygen than banana sap, which has low COD. Total solid, total dissolved solids, and total suspended solids were maximum in CSL and lower in banana sap when compared with the content of concentrated banana sap and spent wash. Total solids are the amount of both suspended solids and dissolved solids. Phosphorus levels were also estimated, which are low in banana sap as compared to the CSL.

Table 4.5: Chemical composition of concentrated banana sap, corn steep liquor, and spent wash

Parameter	**Concentrated banana sap**	**Corn steep liquor**	**Spent wash**
pH	5.9 ± 0.3	4.0 ± 0.2	7.5 ± 0.2
Chemical oxygen demand [gL^{-1}]	6.7 ± 2.6	29.6 ± 2.5	70.0 ± 10.0
Biological oxygen demand[gL^{-1}]	24.4 ± 1.8	15.5 ± 2.3	45.6 ± 1.1
Total solids [gL^{-1}]	10.17 ± 0.8	12.56 ± 0.6	11.96 ± 0.3
Total dissolved solids [gL^{-1}]	9.30 ± 0.1	11.97 ± 0.4	11.38 ± 0.2
Total suspended solids [gL^{-1}]	0.39 ± 0.1	0.59 ± 0.2	0.58 ± 03
Total phosphorus [gL^{-1}]	0.002 ± 0.0	0.060 ± 0.05	0.002 ± 0.03
Total nitrogen [%]	0.4 ± 0.1	0.5 ± 0.0	0.06 ± 0.5
Sugar content [gL^{-1}]	7.1 ± 0.3	2.2 ± 0.2	3.2 ± 0.2

All values are mean ±SD of the triplicate test.

4.3.2 Optimization conditions

The production of ethanol with concentrated sap supplemented with 0.3% yeast extract inoculated with *Saccharomyces cerevisiae* (MTCC 170 and MTCC 180) was optimized for a number of days for fermentation (Figure 4.3). After every 24 hrs, the sample was withdrawn from the inoculated flasks, and the ethanol content was estimated (Table 4.7 and Figure 4.4). During optimization, it was observed that till 96

hrs, the ethanol content increased, and at 120 hrs, the ethanol content decreased. Hence further experiments were performed by incubating the samples for four days.

4.3.3 Fermentation conditions

No detectable ethanol was produced when banana sap, corn steep liquor, or spent wash fermented alone with *Saccharomyces cerevisiae* strains. Reducing sugars are observed higher in acid hydrolyzed banana sap compared to alkaline hydrolyzed samples (Table 4.6). Concentrated banana sap alone, when fermented, did not yield significant levels of ethanol with both *Saccharomyces strains*. On the other hand, the supplementation of sap with either corn steep liquor or yeast extract or spent wash and fermentation with *Saccharomyces cerevisiae strains* (MTCC 170 and MTCC180) after acid hydrolysis showed much better ethanol production. Of the two strains, MTCC 180 gave slightly more ethanol yield than strain MTCC 170. When compared to banana sap alone, the highest ethanol production was observed with concentrated banana sap supplemented with 25% SW (v/v) with MTCC170 (Figure 4.5), where the ethanol concentration was 2.5 g L^{-1} (sixteen-fold higher), followed by MTCC 180 with 1.85 g L^{-1} (eight-fold higher). Compared to concentrated banana sap alone, banana sap treated with 5% CSL yielded three and two-fold higher ethanol concentrations for MTCC170 and MTCC180, respectively. Significant variation was observed between the yeast strains and the sap treatments concerning ethanol production, as revealed by the two-way analysis of variance (ANOVA).

In alkali hydrolysis (Figure 4.6), concentrated sap alone had shown little ethanol production. The ethanol content improved when the sap was supplemented with other industrial by-products such as CSL, SW, and YE. When banana sap was supplemented with 5% CSL, the greatest ethanol level was recorded, around one- and 1.5-fold greater than banana sap alone. The maximum ethanol production of 1.25 and 1.22 g L^{-1} (six and nine times greater, respectively) in MTCC 170 and MTCC 180 strains were observed when the sap was supplemented with 25% SW (v/v) compared to all other treatments.

Supplementation of yeast extract also increased ethanol production with maximum yield recorded at sap with 5% yeast extract among the yeast treatments. Compared to control, CSL also showed the increase in the ethanol production (Figures 4.5 and 4.6). Two-way ANOVA revealed a significant variation among the treatments and between the yeast strains in ethanol production. When compared to acid and alkali hydrolyzed

banana samples, acid hydrolyzed samples supplemented with CSL, SW, and YE yielded more ethanol than alkali hydrolyzed samples.

Table 4.6 Sugar content in banana sap (concentrated) and banana sap (concentrated) supplemented with corn steep liquor, yeast extract, spent wash after hydrolysis (Acid and Alkali)

Sample Name	**Sugar content (mg/mL)**	
	Acid hydrolysis	**Alkali hydrolysis**
Sap	7.93±0.15	7.5±0.30
Sap + 1 % CSL	7.95±0.15	7.52±0.30
Sap + 3% CSL	7.98±0.16	7.55±0.31
Sap + 5% CSL	8.02±0.15	7.59±0.30
Sap + 1 % YE	7.93±0.15	7.5±0.30
Sap + 3% YE	7.93±0.15	7.5±0.30
Sap + 5% YE	7.93±0.15	7.5±0.30
Sap + SW1:3	4.06±0.23	3.95±0.23
Sap + SW3:1	6.64±0.18	6.32±0.18
Sap + SW1:1	5.35±0.20	5.14±0.20

All values are mean ±SD of triplicate test

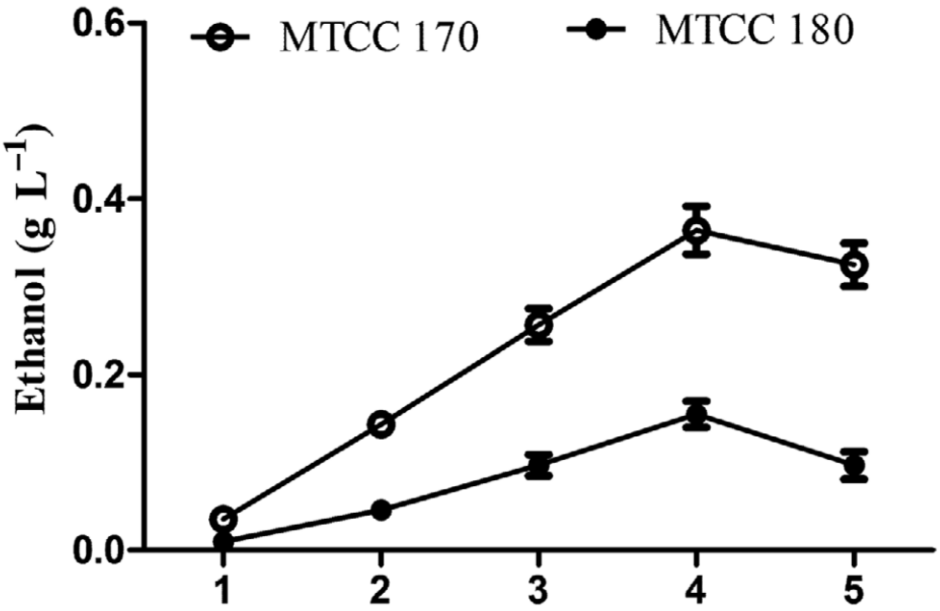


Figure 4.3 Optimization of number of days for fermentation using concentrated sap supplemented with 0.3% yeast extract inoculated with *Saccharomyces cerevisiae* (MTCC 170 and MTCC 180).

Table 4.7 Standard table of ethanol

Ethanol (µL)	Distilled water (mL)	Ethanol (%)	DBP (ml)	Potassium Dichromate (mL)	O.D
0	2	0	2	1.5	0
4	1.996	0.2	2	1.5	0.146
8	1.992	0.4	2	1.5	0.299
12	1.988	0.6	2	1.5	0.452
16	1.984	0.8	2	1.5	0.601
20	1.98	1	2	1.5	0.717

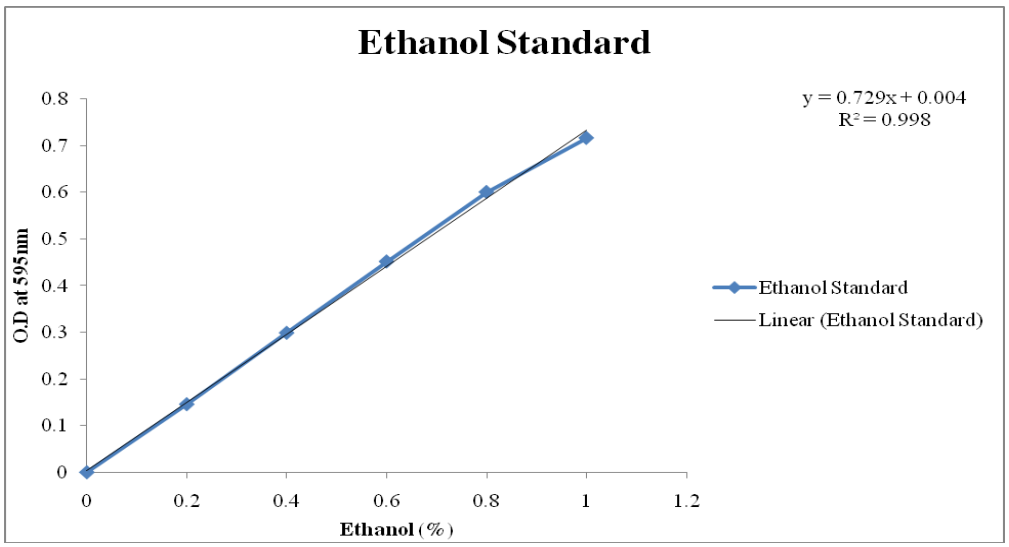


Figure 4.4: Standard curve of ethanol

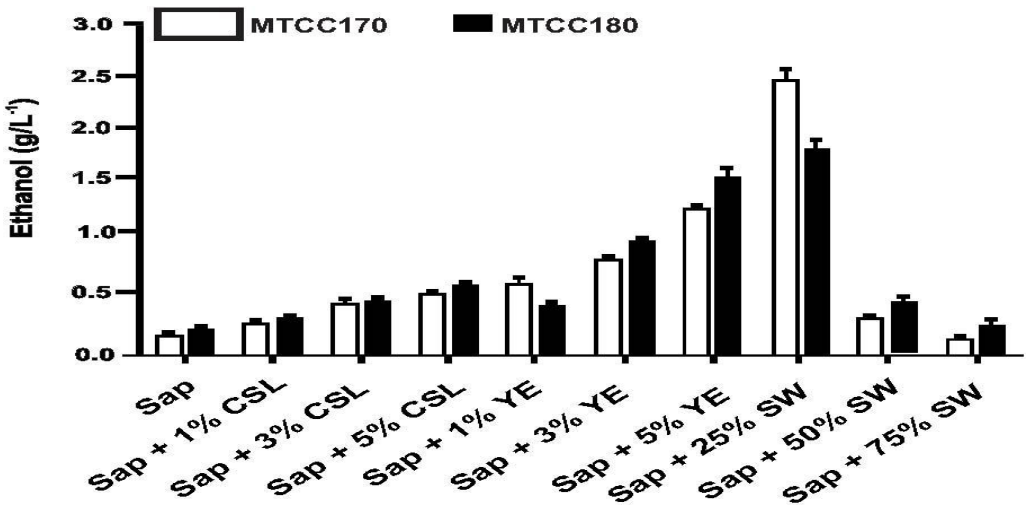


Figure 4.5: Ethanol production after acid hydrolysis with different banana sap samples (sap: concentrated banana sap, CSL: corn steep liquor, YE: yeast extract, SW: spent wash) with strains MTCC 170 and MTCC180.

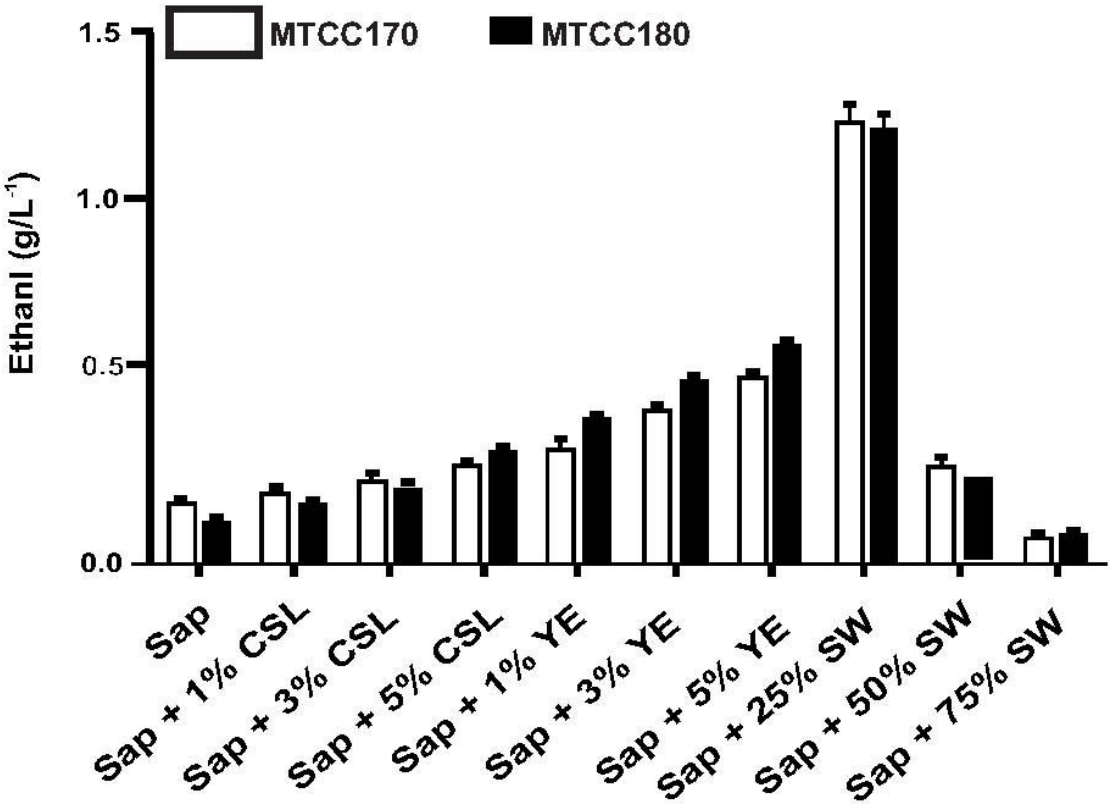


Figure 4.6: Ethanol production after alkali hydrolysis with different banana sap samples (sap: concentrated banana sap, CSL: corn steep liquor, YE: yeast extract, SW: spent wash) with strains MTCC 170 and MTCC180.

4.4 *In vitro* evaluation of bioactive properties of banana sap

4.4.1 Screening of antimicrobial activity

Because of tannins, banana sap becomes light brown to black when exposed to air. However, two methodologies were used throughout the study of antimicrobial potential evaluation: (i) assessment of banana sap exposed to air (light/dark brown) (Figure 4.7 a) (ii) Banana sap remained green because tannins were chelated as it was not directly exposed to air (Figure 4.7 b). The colored sap had a higher Minimal inhibitory concentration (MIC) against gram-positive and gram-negative bacteria, ranging from 125 mg/mL to 500 mg/mL, but the green sap had a significantly lower MIC, ranging from 15.625 mg/mL to 62.5 mg/mL (Table 4.8). This significant decline in the MIC in the tannin-chelated (green sap) is most probably attributable to tannin non-interference. However, both extracts did not exhibit any anti-candidal activity.

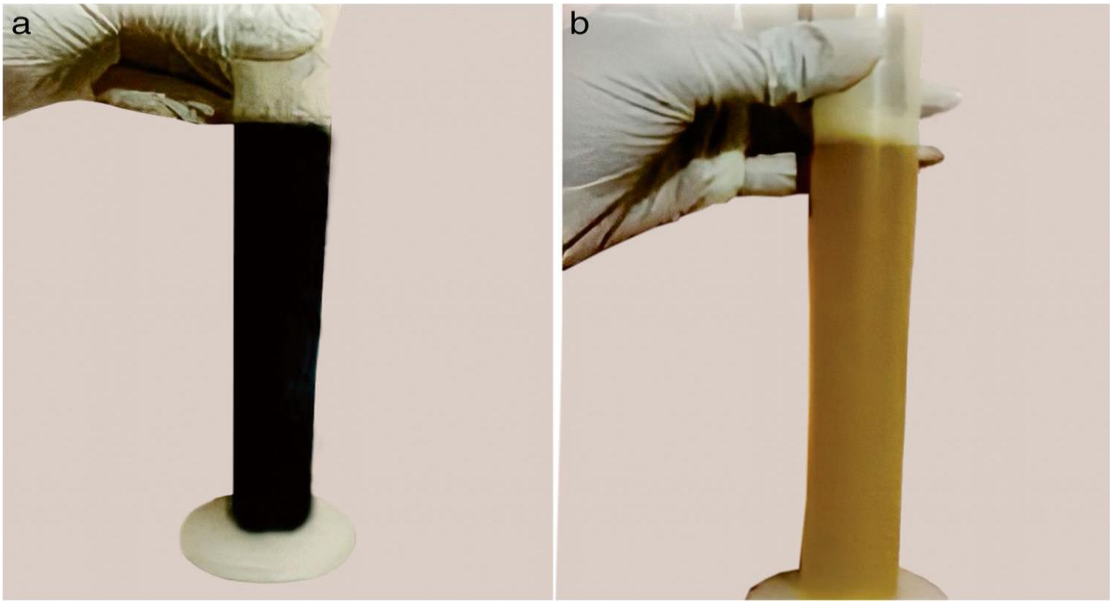


Figure 4.7 a) Banana sap exposed to air **b**) Banana sap when not exposed to air

Table 4.8: Comparative *in vitro* antimicrobial activity as MIC of oxidized and unoxidized banana sap

Test Sample	***MIC values against the test microorganisms (mg/mL)****				
	E. coli	*B. megaterium*	*P. aeruginosa*	*S. aureus*	*Candida albicans*
Oxidized banana sap	125	250	500	125	No inhibition
Unoxidized banana sap	15.625	62.5	62.5	15.625	No inhibition
Positive control (antibacterial) @ 0.1 mg/mL	No growth	No Growth	No growth	No growth	No growth
Positive control (anti-fungal) @ 0.1 mg/mL	No growth	No Growth	No growth	No growth	No growth

**All values are means of triplicate test*

4.4.2 Cytotoxic/Anticancer activity

Banana extracts (DCM/EA) were screened for their effect on MCF-7 breast cancer cell lines based on MTT assay. Initial experiments were carried out with EA and DCM solvents extract (50 to 1500 µg/mL). The result of EA was inconsistent, and there the proliferative index was found to be > 1, indicating no cell growth inhibition (Figures 4.8 and 4.9). DCM extract has shown growth inhibition (proliferative index < 1) at all different concentrations (Figures 4.8 and 4.9). Hence, DCM extract was selected for further study. DCM extract exhibited a concentration-dependent decrease

in a proliferative index, indicating a cytotoxic effect. The IC_{50} values were calculated and found to be 34.15±8.75 µg/mL (Figure 4.10). Paclitaxel, an anticancer drug, was used as a positive control where the proliferative index was observed to be 0.11± 0.03.

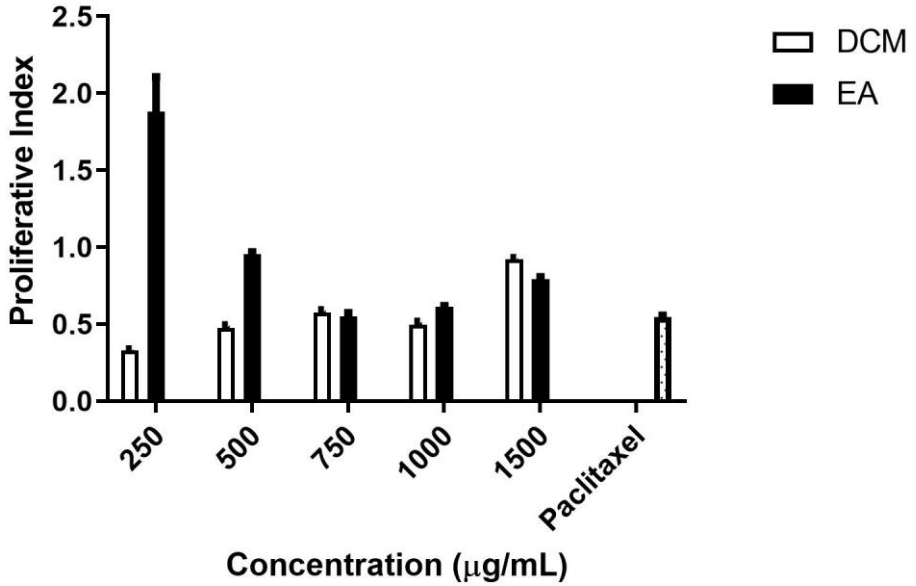


Figure 4.8: Proliferative index of banana sap dichloromethane and ethyl acetate extracts against human breast cancer (MCF-7) cell lines.

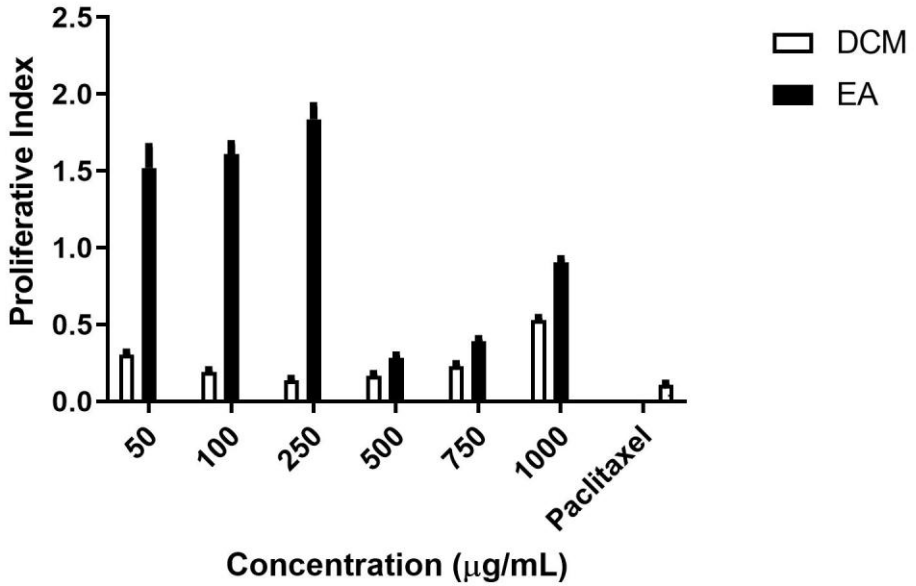


Figure 4.9: Proliferative index of banana sap dichloromethane and ethyl acetate extracts against human breast cancer (MCF-7) cell lines.

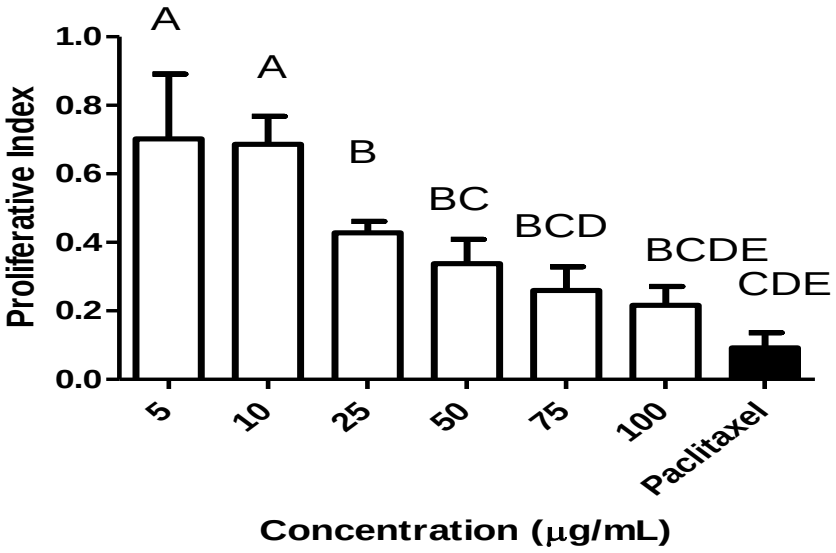


Figure 4.10: Proliferative Index of banana sap dichloromethane extract against human breast cancer (MCF-7) cell lines. Means followed by the same letter are not significantly different.

4.4.3 Antioxidant assay

The free radical scavenging activity was done to evaluate the antioxidant capacity of banana sap extract. Scavenging activity increased significantly with the banana sap extract's concentration (Figures 4.11 and 4.12). The highest antioxidant activity was observed to be 54.62±1.09% (DCM) and 79±1.05% (EA) at 1000 µg/mL concentration. Ascorbic acid, an antioxidant used as a positive control, showed 89.67 ±1.53% scavenging activity.

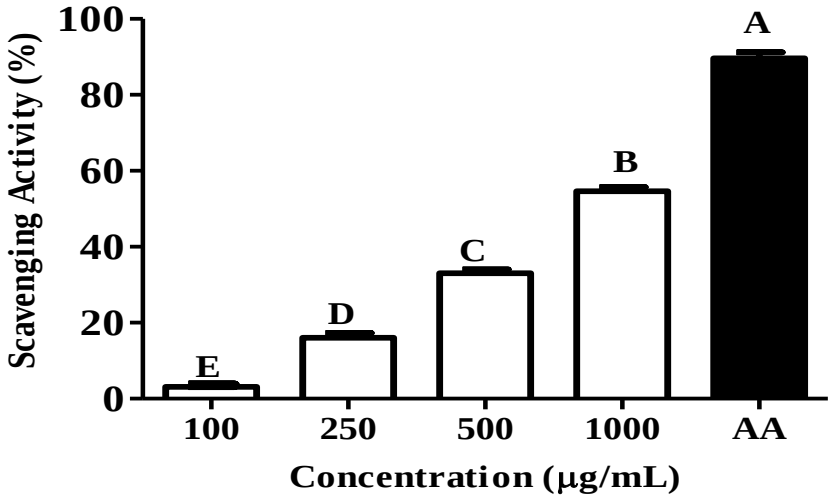


Figure 4.11: Antioxidant potential of banana sap dichloromethane extract based on free radical scavenging activity. Means followed by the same letter are not significantly different.

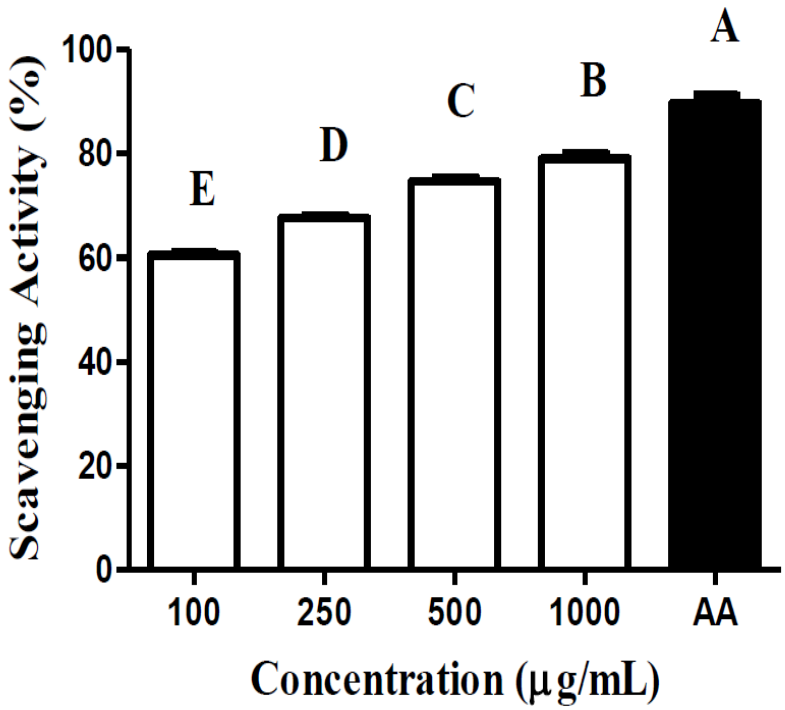


Figure 4.12: Antioxidant potential of banana sap ethyl acetate extract based on free radical scavenging activity. Means followed by same letter are not significantly different.

4.4.4 Nutritional analysis of banana sap

Banana sap contained vitamins B12, E, and C (Table 4.9), while other vitamins like Vitamin A, B1, B2, B3, and D were below the detection limit. Amino acids analysis has shown that banana sap is rich in different amino acids. Further, banana sap was tested for minerals such as lead, copper, cobalt, nickel, arsenic, tin, zinc, cadmium, mercury, and methyl mercury, out of which sap contains zinc (Table 4.9), and others were found below the detection limit. It was found that the sap is devoid of saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, and trans fatty acids. The sap also contains energy, a low amount of carbohydrates, and ash, whereas sugars and proteins were found below limit.

Table 4.9 Nutritional analysis of banana sap

Parameter	Results
Energy	10.55 Kcal/100ml
Carbohydrates	1.86%
Ash	0.35%
Vitamins	
Vitamin B12	0.07 µg/100g
Vitamin C	2.2 mg/100g
Vitamin E	0.157 mg/100g
Amino Acid Profiling	mg/100mL
Histidine	0.83
Serine	1.93
Arginine	5.80
Glycine	5.76
Aspartic Acid	18.45
Glutamic Acid	17.64
Threonine	2.71
Alanine	8.04
Proline	2.75
Cysteine	0.22
Lysine	6.99
Tyrosine	1.26
Methionine	0.60
Valine	1.69
Isoleucine	1.14
Leucine	4.62
Phenylalanine	1.72
Metals	mg/kg
Zinc	1.73

4.4.5 LCMS analysis of banana sap

LCMS chromatograph of the DCM extract of banana sap having major peaks appeared at 12.28 min, 12.83 min, and 15.49 min of compounds A, B, and C (Figure 4.13). LC chromatograph of the EA extract of banana sap showing the, compounds A, B, and C appeared at 12.28 min, 12.83 min, and 15.49 min, respectively (Figure 4.14). Compounds A, B and C were predicted as rescinnmaine, dihydrorescinnamine and epimedin A respectively based on the m/z cloud results of the LC-MS Library (© Reported with Compound Discoverer 2.1) represented in Figures 4.15, 4.16 and 4.17. Both Figures 4.13 and 4.14 point to significant compounds A and B.

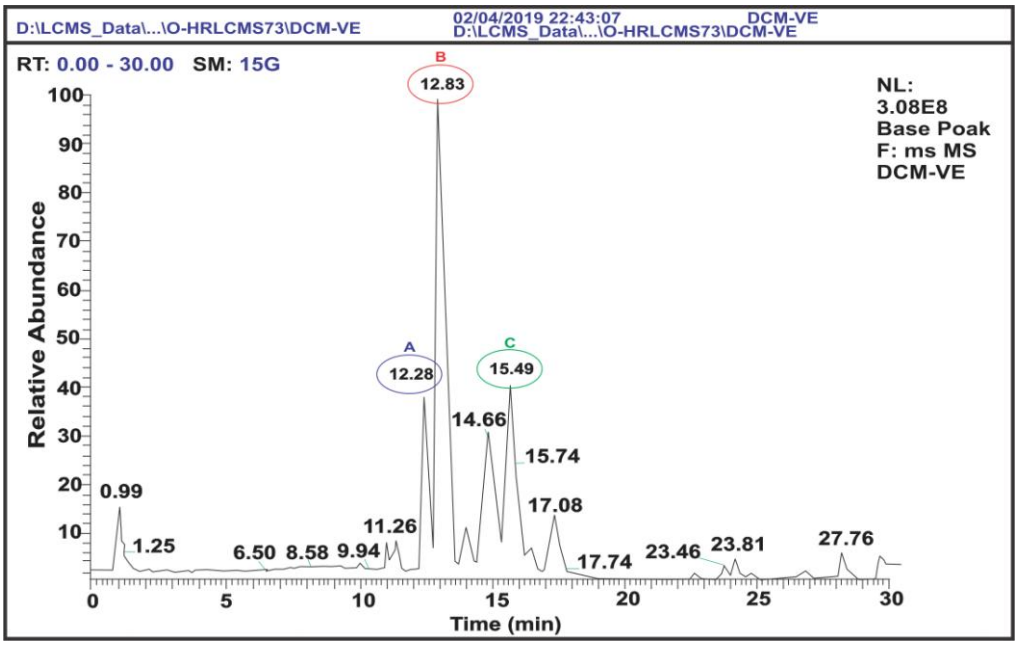


Figure 4.13: LC chromatograph of banana sap DCM extract

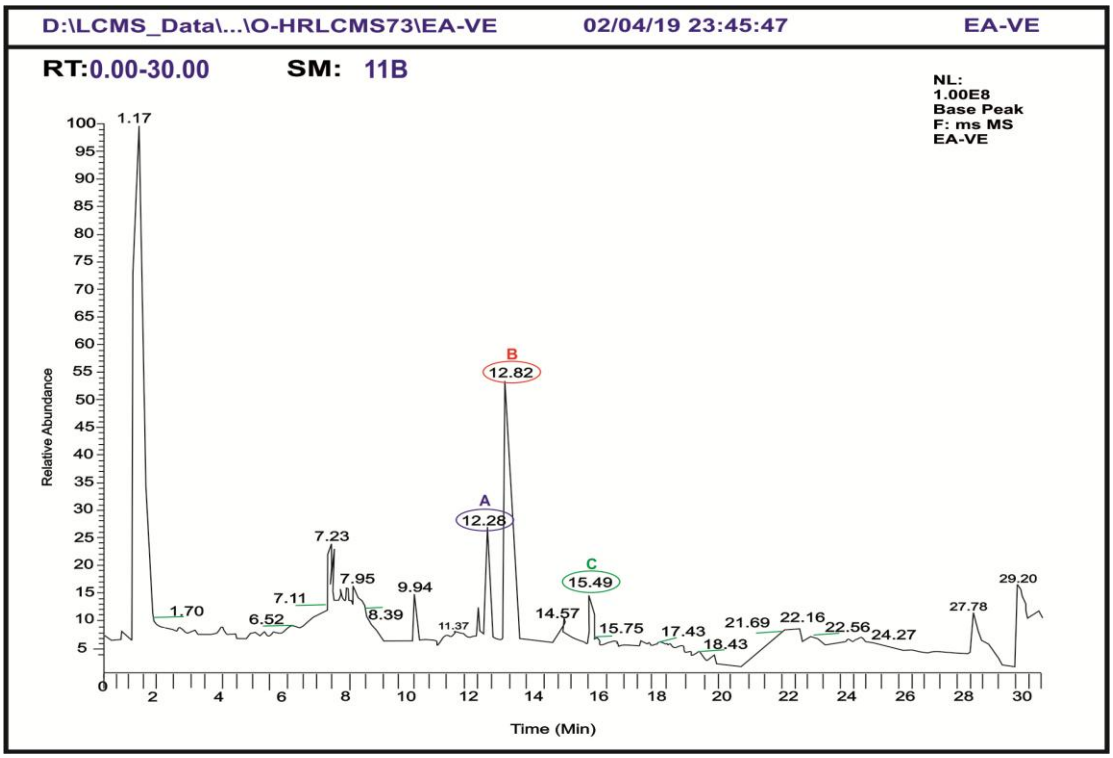


Figure 4.14: LC chromatograph of banana sap EA extract

4.4.5.1 Identification of compounds

Use of fragmentation pattern of MS mzCloud Results on LCMS Library and correlation with the molecular mass suggested structures of compounds A, B, and C as shown in Figures 4.18, 4.19, and 4.22. In the MS spectra, no peak corresponding to

rescinnamine was observed due to its unstable nature under electron impact ionization. The most stable fragment was observed at [M+1] value of 327.22 m/z ratio corresponding to intermediate a-2 appearing as a base peak (100% intensity) having m/z ratio equal to 362.12. The intermediate a-2 was derived from fragment a-1 having a molecular mass of 362.13, appearing at [M+1] value at 363.19 m/z value. Fragmentation of 2,3,4-trimethoxycinnamyl moiety from Rescinnamine (compound A) zone fragment a-1. The dimerization of the main skelton zone's peak at m/z value of 655.44 correspond to [M+1] molecular mass of compound a-3. Figure 4.18 above/below MS and the structure of discussed fragments.

Compound B has a molecular mass of 636.74 and MS pattern similarity to rescinnamine. It was identified as dihydrorescinnamine having structure, as shown in Figure 4.19. There was no molecular ion peak identified in this case also. The fragments after electron impact gave ions similar to that of rescinnamine, having a m/z mass equivalent to that of intermediates b-1, b-2, and b-3, as shown in Figure 4.19. The MS spectra of both rescinnamine and dihydrorescinnamine were similar for both DCM, and EA extracts, as shown in Figures 4.20 and 4.21.

The quantity of compound C that appeared in the chromatograph at 15.49 min was relatively higher in DCM extract than in the EA extract. Using mzcloud on LCMS library, this compound's fragmentation pattern matched that of a known compound Epimedin A. Compound C; a flavanone had one monosaccharide and a disaccharide attached at 7 and 3 positions, respectively. No molecular ion peak for the Epimedin A, compound C was observed in the MS spectra. A peak at [M] m/z value of 802.55 was observed for compound c-1, corresponding to the elimination and the dehydration product of compound C. The peak at 627.48 [M+1] m/z value represented the breaking away of the monosaccharide unit at the C-7 position of the flavanone, as shown in the c-2 fragment (Figures 4.22 and 4.23). Further, the C-3 positioned disaccharide fragmented from the c-2 fragment to give a c-3 compound that appeared at [M+1] m/z value of 349.2. Finally, the fragmentation of isoprene unit attached at C-8 carbon zone fragment c-4 at m/z value of [M+1] having a value of 313.24. This was, in fact, the most stable fragment with the base peak. The MS spectra of epimedin A were similar for both DCM, and EA extracts, as shown in Figures 4.22 and 4.24.

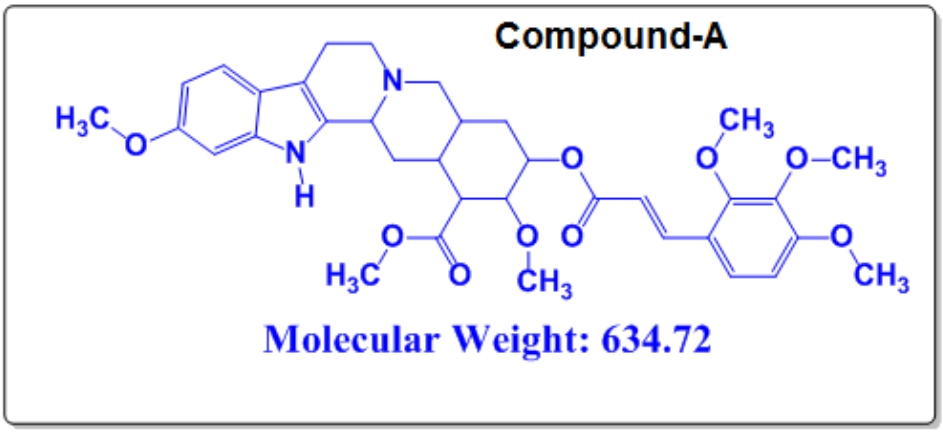


Figure 4.15: Rescinnamine RT 12.28 min in both DCM and EA extracts

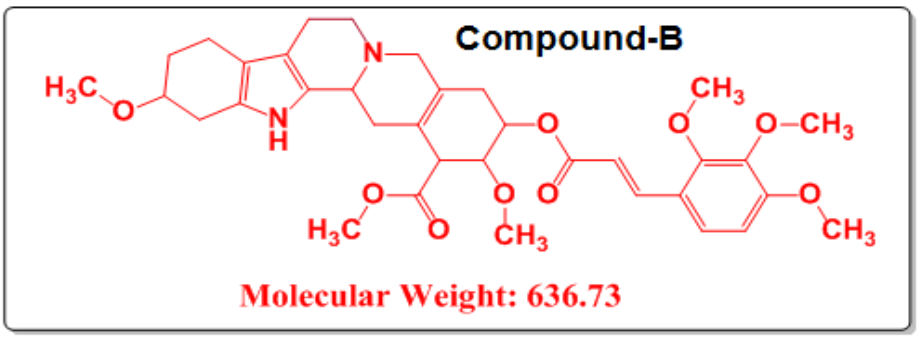


Figure 4.16: Dihydrorescinnamine RT 12.82 min in both DCM and EA extracts

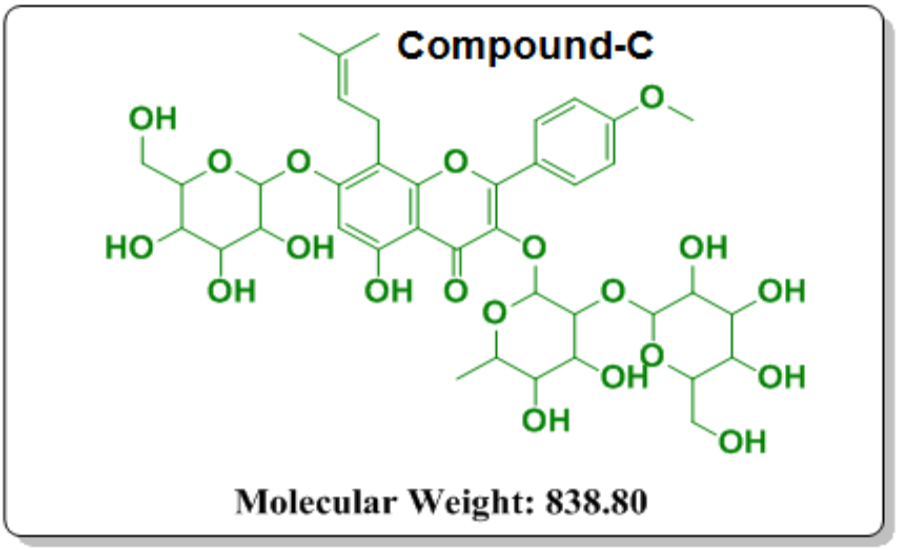


Figure 4.17: Epimedin A RT 15.49 min in both DCM and EA extracts

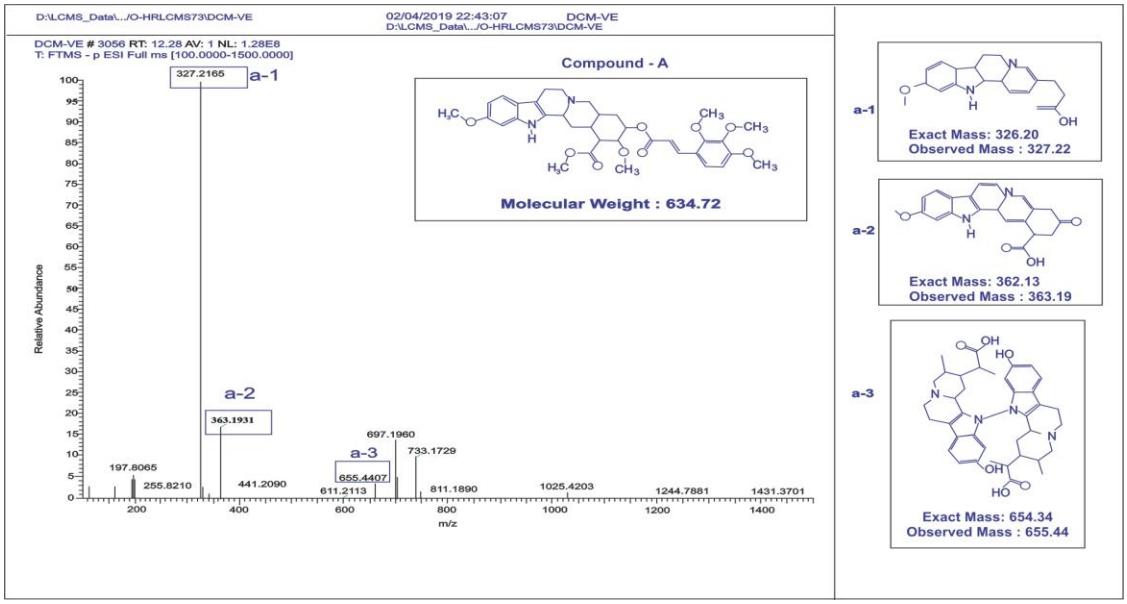


Figure 4.18: Spectra of the DCM extract of the banana sap at RT 12.28, highlighted masses are common in both DCM and EA extract of the banana sap

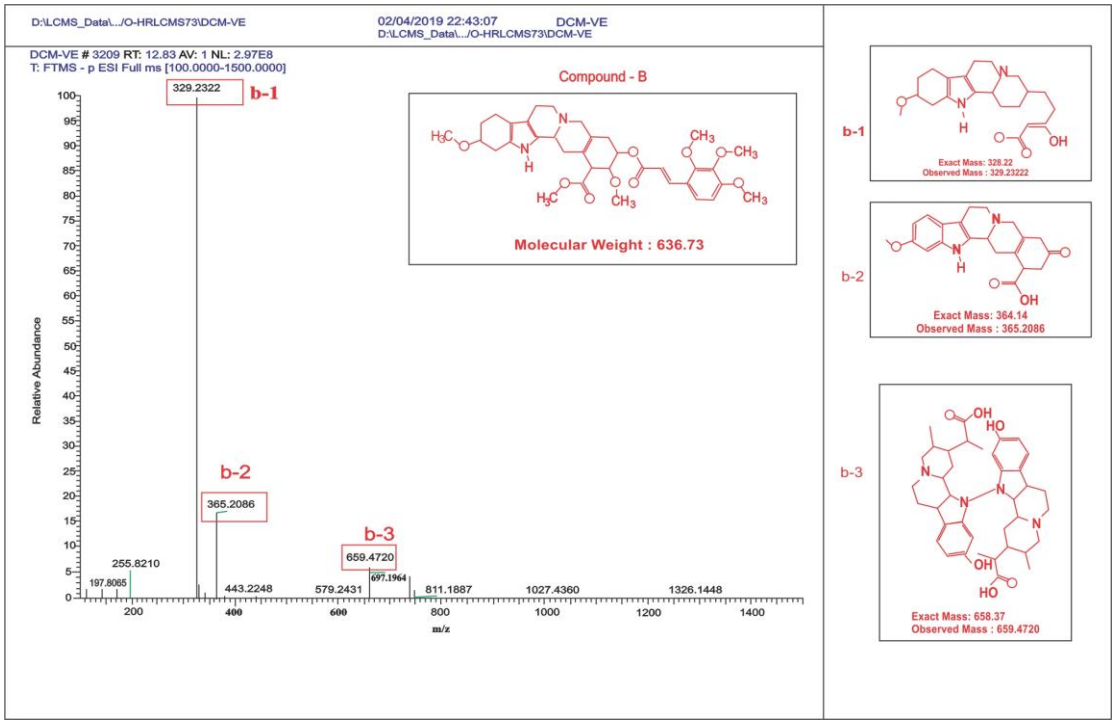


Figure 4.19: Spectra of the DCM extract of the banana sap at RT 12.83, highlighted masses are common in both DCM and EA extract of the banana sap

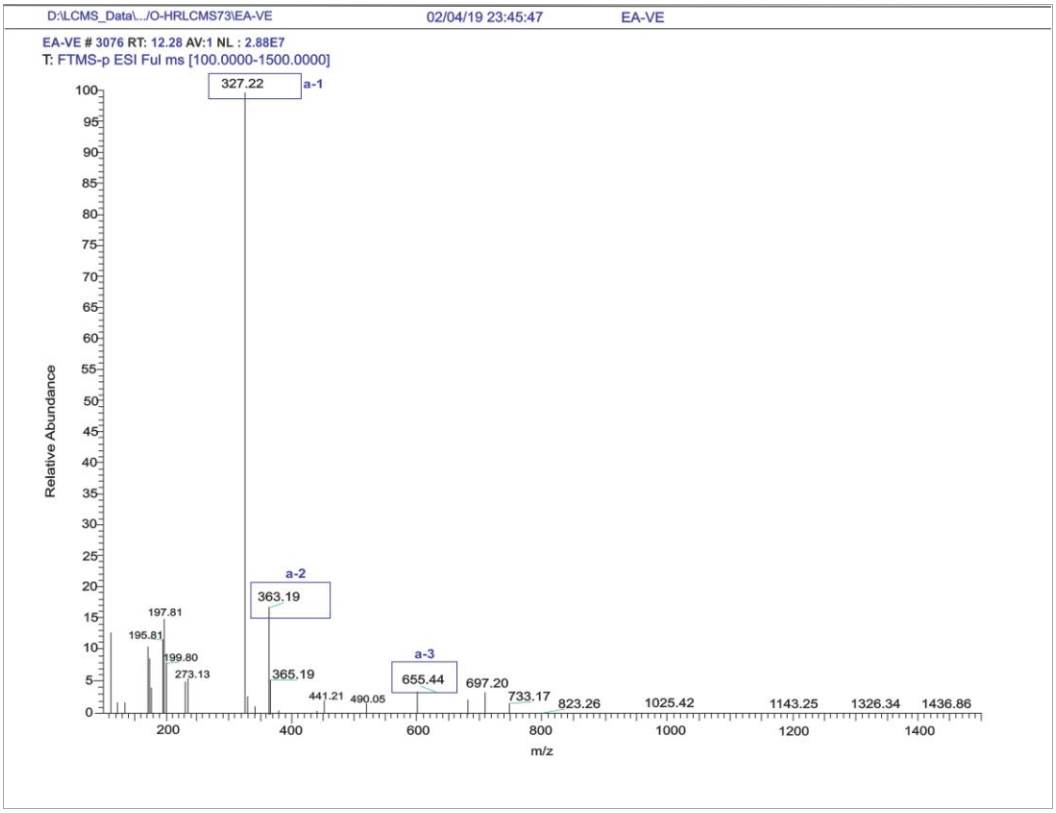


Figure 4.20: Spectra of the EA extract of the banana sap at RT 12.28

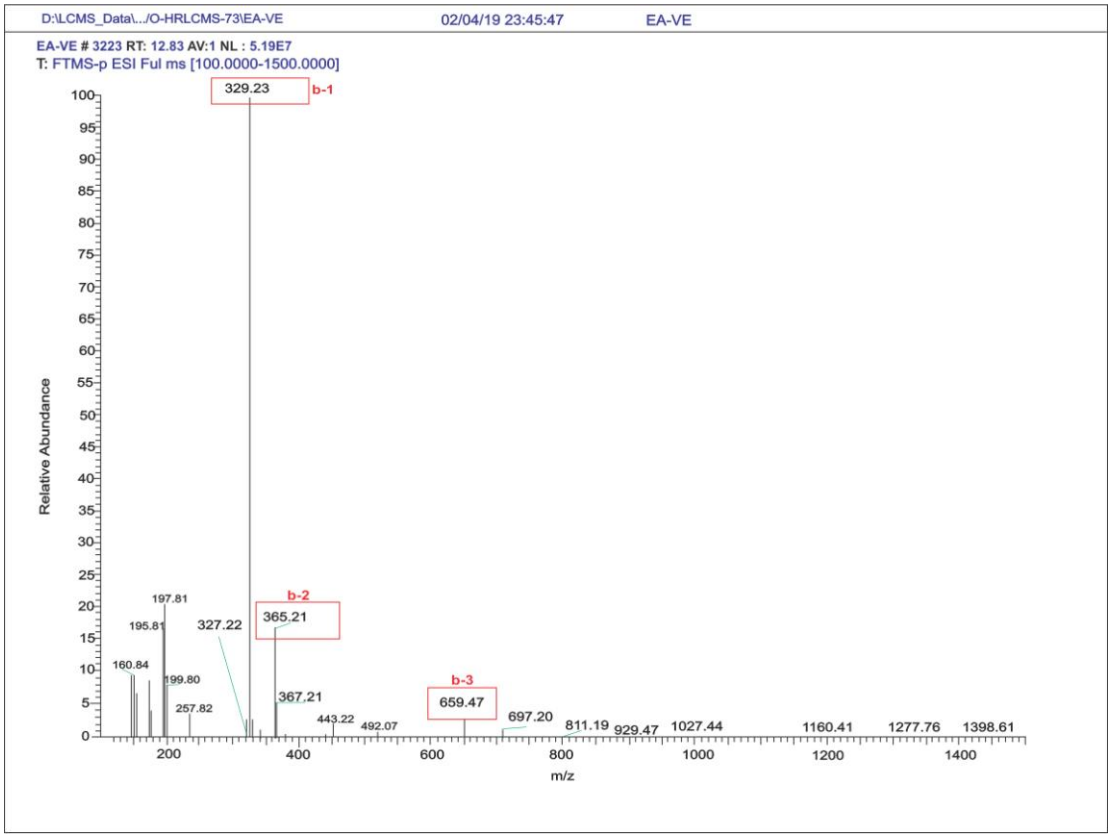


Figure 4.21: Spectra of the EA extract of the banana sap at RT 12.83

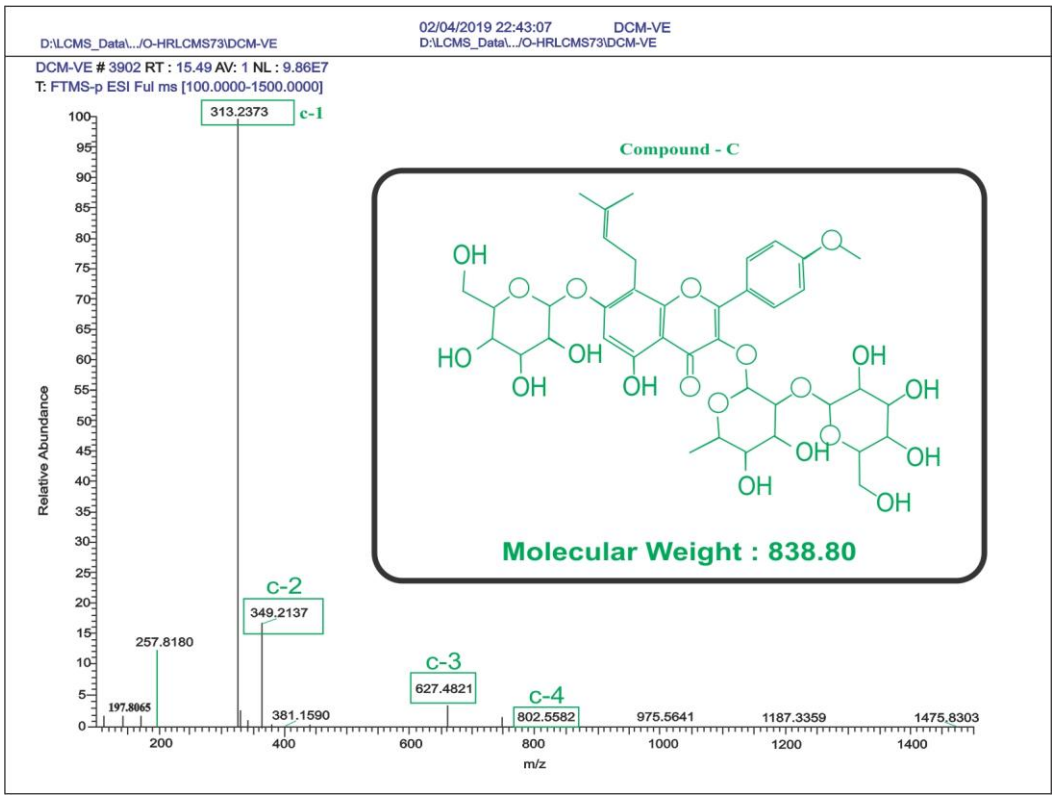


Figure 4.22: Spectra of the DCM extract of the banana sap at RT 15.49, highlighted masses are common in both DCM and EA extract of the banana sap

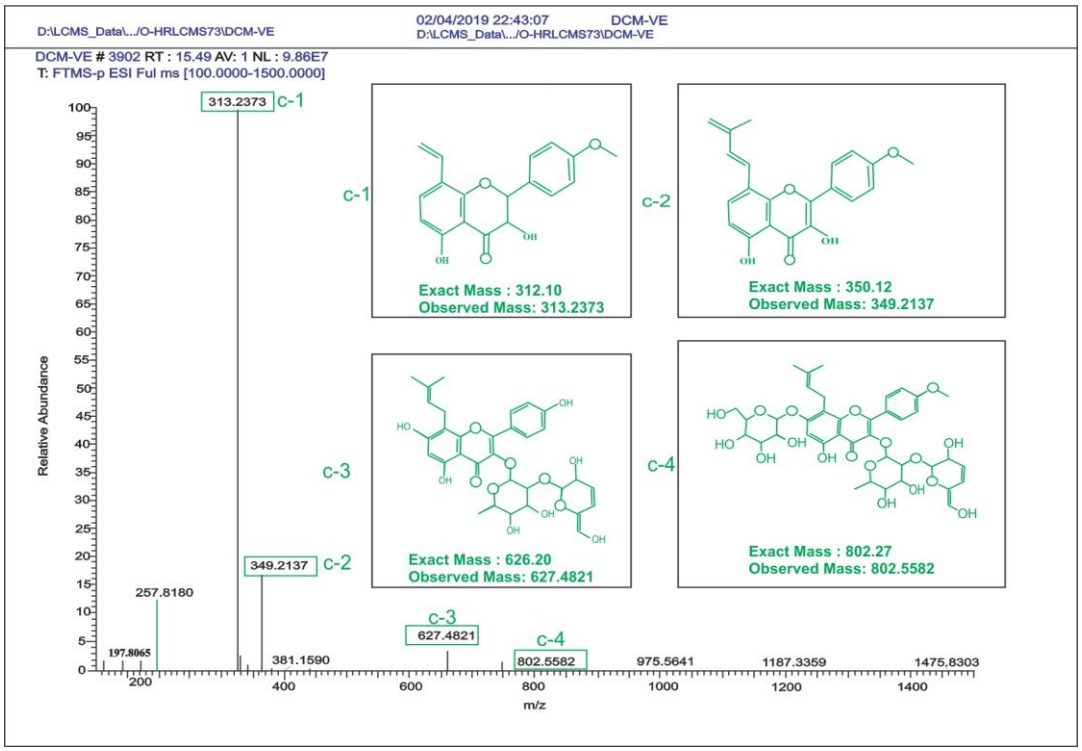


Figure 4.23: Spectra of the DCM extract of banana sap at RT 15.49, displaying structures of the common compounds in both DCM and EA extract of the banana sap

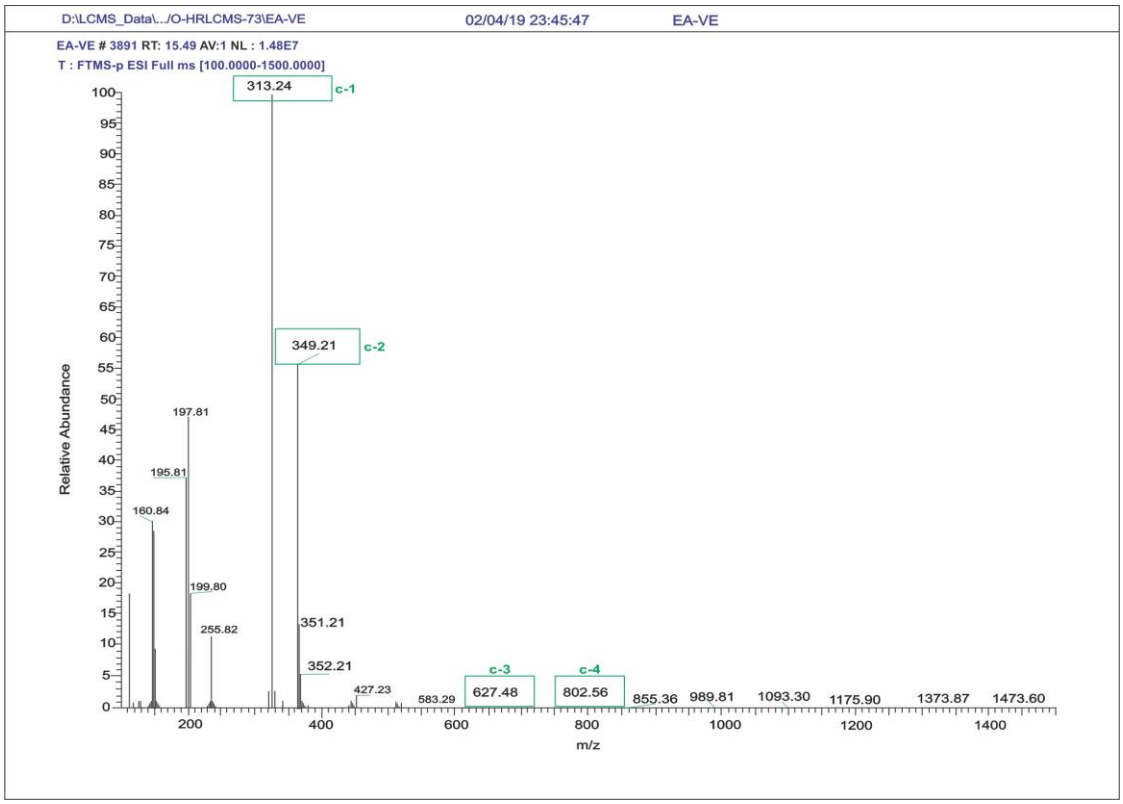


Figure 4.24: Spectra of the EA extract of banana sap at RT 15.49

CHAPTER -5
DISCUSSIONS

5.1 Conversion of banana sap into bioethanol

The high production rates make bananas a suitable candidate for bioethanol production. Literature abounds ethanol production from banana biomass such as banana pulp, peels, and crushed pseudo-stem produced 1.02g $L^{-1}h^{-1}$ (Uchoa et al. 2021), dry pseudo-stem bran 0.82 g $L^{-1}h^{-1}$ (De Souza et al. 2017), 0.56 g $L^{-1}h^{-1}$(Guerrero et al. 2018), pulp 3.75 g $L^{-1}h^{-1}$(Souza et al. 2012) by using *S. cerevisiae* yeast strain. Moreover, usually discarded banana peels possess rich reserves of growth supplements that act as a medium for yeast strain growth. Their high carbohydrate content and other essential nutrients promote the growth of microorganism that facilitates ethanol production (Brooks 2008).

Further, Pandey et al. (2000) reported in their study that in order to fulfill the increasing need for fuel globally, the fermentation technology must be made affordable and simple to use. Further, research strategies had subdivided into two divisions: ethanol production from cheaper raw materials and the identification of novel microbe or yeast strains effective in ethanol production. Factors such as the appropriate microbial strain, the fermentation substrate, and the optimal process technology all have an impact on the ethanol yield produced by microbial fermentation (Akin et al. 2008). A suitable microorganism for producing ethanol must have a strong fermentative potential, higher flocculating ability, acceptable osmo-tolerance, improved ethanol tolerance, and good thermo-tolerance. In the majority of the research, *S. cerevisiae* has been identified as the appropriate microbe for industrial ethanol production. Additionally, yeast has the ability to produce ethanol that is free from contamination by other substrate products (El-Diwany et al. 1992).

Limited studies have focused on using banana pseudo-stem and other parts such as the peel and rotten bananas for bioethanol production. Banana peel is suitable for fermenting alcohol (Tewari et al. 1986). According to Hammond et al. (1996), ripe bananas produce more ethanol per dry weight than the other agro-waste. Further, Arredondo et al. (2009) examined the enzymatic and acid hydrolysis of banana peel, fruit, and flower stalks. The results demonstrated that banana agro-waste could be utilized to produce ethanol by fermenting, distilling, and hydrolyzing it.

The findings of a case study conducted in Costa Rica using banana shade trees and organic and conventional banana producers from Ecuador are presented in a paper by Graefe et al. (2011). The study demonstrated that high yield of ethanol could be produced from banana bunches and low agroforestry systems that do not exceed quality requirements and are abandoned to rot in the fields. Oberoi et al. (2011) have proved that banana peel is a suitable substrate for ethanol production by simultaneously saccharification and fermentation (SSF).

Ahmed et al. (2011) analyzed bioethanol production from rotten bananas. They found that this could produce low emissions in motor vehicle engines. Consequently, it can be used as a waste management procedure that recycles the environment. Arumugam and Manikandan (2011) studied the feasibility of using banana pulp and peel agro-waste in bioethanol production by dilute acid pretreatment and enzymatic hydrolysis.

Characterization of banana sap and other industrial byproducts revealed that these products contain high-strength organic waste (BOD, COD), solids (TS, TDS, and TSS), and nutrients (phosphorus and nitrogen), which must be treated before waste disposal (Galbe et al. 2007). After hydrolysis of the banana sap, the BOD and COD levels are significantly more significant than the observed sugar levels (Table 4.1 and 4.5). This could be due to high total organic carbon (14.83%) in unhydrolyzed sap and volatile fatty acids, small quantities of proteins, and organic acids, all comprising its higher BOD and COD. Different pretreatment methods are used, including treatment with concentrated acids, solvents, wet oxidation and metal complexes. These approaches are more expensive than the standard glucose-based ethanol production. Depending on the economic sustainability, steam and alkali-based pretreatments are suitable for pretreatment (Ingale et al. 2014). Alkaline pretreatment, in particular, has been found to successfully remove lignin without affecting the carbohydrate component of the material (Galbe and Zacchi 2007). Hence, acid and alkali treatments were carried out in our study, followed by fermentation to analyze the amount of ethanol produced. The use of pretreatments like alkali, acids, steam, and enzymatic degradation of lignocellulosic by-products, as well as its fermentation by microbes, are highlighted in several studies listed in Table 5.1.

Table 5.1 Comparative analysis of ethanol production from different biomass

Biomass	Pretreatment	Microorganism	Ethanol yield	References
Hardy sugar cane	0.5% NaOH	*Saccharomyces cerevisiae*	30g/L	Muthuvelu et al. 2019
Olive Tree	1% (v/v) H_2SO_4	*Saccharomyces cerevisiae*	130g/L	Fernandes-Klajn et al. 2018
	1% of $Ca(OH)_2$	*Trichoderma reesei*	30g/L	Mamaní et al. 2022
Rice Straw	Ball milling pretreatment	*Saccharomyces cerevisiae*	11.6.65g/L	Zhang et al. 2017
Sugarcane baggase pith	1% (v/v) H_2SO_4	*Pichia stipitis*	250g/L	Sritrakul et al. 2017
Banana pseudostem	Alkali and microbial treatment	*Aspergillus ellipticus*	17.1g/L	Ingale et al. 2014
Barley straw	NaOH (0.58 M)	*Saccharomyces cerevisiae*	50g/L	Han et al. 2013
Banana sap	Acid and alkali treatment followed by supplementation of Corn steep liquor, spent wash and yeast extract	*Saccharomyces cerevisiae*	2.5g/L;1.25g/L	This study

Sugars formed during biomass preparation and enzymatic digestion of pretreated biomass can be transformed into compounds, including 1,3-propanediol, acetone, n-butanol, itaconic acid, and xylitol. As a result, a part of the hydrolysate stream produced during the second generation bioethanol process can be used to produce biochemicals. The development of microorganisms or catalysts that are inhibitor-resistant, however, prevents the rapid incorporation of the sugar-to-biochemical technologies into the bioethanol process. The existence of by-products produced during the pretreatment step has a negative impact on the microorganisms. Further, there is a need for commercial technologies to be developed to convert starch/molasses derivate sugar streams, high in glucose concentration, to chemicals. Most commercialized technologies, such as cellulosic hydrolysate, are not designed to

manage sugar mixtures containing pentose and hexose sugars. As a result, the design of microorganisms and catalysts capable of converting multiple sugars is needed to maximize production yields. Technologies for converting ethanol to chemicals are considered mature and can be immediately applied. While techno-economic analyses are needed to determine the viability of producing chemicals from cellulosic ethanol, market analyses are crucial to assessing the market value and size of the selected biochemicals (Rosales-Calderon and Arantes 2019).

It is generally known that the presence of sugar alone has no substantial effect on the amount of ethanol produced and fermentation might stress the organism and that ethanol might not be produced significantly. A nitrogen supply is added into the culture to increase an organism's efficiency in producing ethanol (Jones and Ingledew 1994; Laopaiboon et al. 2009). Researchers have used peptone as a nitrogen source to enhance ethanol production in sorghum juice (Laopaiboon et al. 2009). Corn steep liquor is an inexpensive substitute of nitrogen source than expensive materials like yeast extract and peptone (Ligget and Koffler 1948). We also observed that there was an increase in ethanol production when corn steep was added to banana sap at different concentration (1%, 3%, and 5%) and evaluated for ethanol fermentation. Using corn steep liquor improved ethanol yield in acid and alkali hydrolysis with *Saccharomyces cerevisiae strains* (MTCC 170 and MTCC180).

Spent wash is the residual liquid waste produced during alcohol production. Pollution caused by spent wash poses a significant risk to the environment (Mohana et al. 2009; Kumar et al. 1997). The spent wash is a cheap, readily available source (Jain and Srivatsava 2012). Spent wash is a good source of carbon and sugar with less nitrogen and would provide ancillary factors that could be required for effective fermentation (Kumar et al. 1997). So, in the present study, banana sap and spent wash was mixed with 25%, 50% and 75% SW (v/v), and it is observed that sap and 25% SW (v/v) gave the highest ethanol yield in acid and alkali hydrolysis with *Saccharomyces cerevisiae strains* (MTCC 170 and MTCC180).

Yeast extract has been shown to be particularly useful for enhancing fermentation since it contains amino acids, nucleotides, peptides, and other soluble components of yeast cells (Jorgensen 2009). We observed that when we supplemented yeast extract in concentrations of 1, 3, and 5%, respectively, as nitrogen source, ethanol production

increased significantly in acid and alkali hydrolysis with *Saccharomyces cerevisiae strains* (MTCC 170 and MTCC180).

In our study, concentrated banana sap supplemented with other industrial byproducts was investigated for bioethanol production. In comparison to sap alone, the ethanol concentration increased with the addition of byproducts such as CSL, SW, and YE. When sap was combined with 25% SW (v/v) with both *S. cerevisiae* strains in acid hydrolyzed samples, the ethanol concentration was highest compared to sap alone. Adding 5% YE to banana sap improved ethanol output by eight and six fold in acid hydrolyzed samples treated with MTCC170 and MTCC180, respectively. The findings indicate that banana sap discarded into the environment could be used as a possible supply for bioethanol production. However, research is needed to optimize the hydrolysis of the banana sap and further concentrate the sap to 20 or even 50 times, which could be commercially feasible and also choose an appropriate yeast strain for its optimum fermentation.

5.2 *In vitro* evaluation of bioactive properties of banana sap

5.2.1 Antimicrobial activity

Antimicrobial susceptibility varies greatly amongst microorganisms (Ismail et al. 2018). The potential for antimicrobial activity is influenced by several factors, including polarity, solubility, extract stability, and solvent extraction (Jouneghani et al. 2020). Additionally, the gram type and species of the tested microbial strain may influence the extent of the inhibition zone (Norfaradhiah and Rapeah 2017). The agar disc diffusion technique is employed for the quantitative analysis. Although this approach cannot reliably test all fastidious bacteria, it has been standardized to screen certain bacterial diseases utilizing a specific culture medium, different incubation settings, and interpretative criteria for inhibitory zones. Dilution procedures are the most appropriate for determining MIC values for quantitative analysis since they determine the level of the tested antimicrobial agent in the agar or broth medium. Several authorized guidelines exist for dilution antimicrobial susceptibility testing of fastidious or non-fastidious bacteria, filamentous fungus, and yeast. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) offer relevant and significant standards (Balouiri et al. 2016). According to Coico (2006), Gram staining may distinguish between different bacterial species based on their cell walls' structural

and chemical characteristics. Gram-negative bacteria contain an extra lipopolysaccharide structure that makes the cell wall impermeable to lipophilic solutes, in contrast to gram-positive bacteria, which have a layer of peptidoglycan that acts as a weak permeability barrier. Several fungi species such as *Aspergillus*, *Candida*, *Cryptococcus*, *Penicillium*, and *Trichophyton* have been examined for their antifungal activity (Kumar et al. 2014; Jouneghani et al. 2020). Most scientists have investigated the antimicrobial properties of banana leaves (Naikwade et al. 2014; Asuquo and Udobi, 2016; Sivasamugham et al. 2021). In addition to leaves, research has been conducted on the inflorescence (Padam et al. 2012), peel (Ehiowemwenguan et al. 2014), and sap (Kumar et al. 2014). The antimicrobial spectrum of banana agro-waste extracts has been examined in the organic (Kumar et al. 2014) and aqueous fractions (Ehiowemwenguan et al. 2014). Researchers have demonstrated that in bananas, the aqueous fraction was less efficient against bacteria than organic extracts. Among organic solvents, the most potent extracting solvent was ethyl acetate, followed by ethanol. Except for peel extract, none of the plant part aqueous extracts indicated an inhibitory zone or antibacterial action (Norfaradhiah and Rapeah 2017). However, peel extracts inhibits the gram-negative bacterium *P. aeruginosa* growth (Evbuomwan et al. 2018). Furthermore, it was established that water can extract and dissolve non-bioactive polysaccharides but is ineffective at extracting substances that bind to the lipophilic component (Al- Mqbali and Hossain 2019). Banana agro-waste that demonstrated the most inhibitory effect against several gram-positive and gram-negative bacteria were the peel (ripe or green) extracts (Kavitha et al. 2019; Mokbel and Hashinaga 2005), then the root (Biswas et al. 2011). This may be attributed to the prevalence of several phenolic chemicals, which contribute to the antibacterial action against diseases and bacteria that produce spores (Bisht et al. 2016). Banana corm and blossom extracts exhibited a low effect, particularly against *S. aureus* and *S. paratyphi* (Jouneghani et al. 2020). Methanolic extracts of banana peel, pulp, and stem exhibit distinct antimicrobial activity (Sirajudin et al. 2014). When extracted with either aqueous or organic solvent, a puree made of banana fruit exhibits action against a broad spectrum of microorganisms (Richter and Vore 1989). Aqueous and ethanolic sap extracts demonstrated significant antibacterial activity against a broad spectrum of bacteria (Kumar et al. 2014). The minimum inhibitory concentration (MIC) is the lowest concentration that prevents bacterial growth during incubation. As

a result, substances having a lower MIC function as antimicrobial agents more effectively (Karuppiah and Mustaffa 2013). All banana plant extracts exhibit varying MIC and degrees of inhibition depending on the solvent. Both ethyl acetate and methanol extracts can suppress bacteria. According to Ismail et al. (2018), the banana leaf stalk acetone extract exhibited low MIC value because it can extract several active metabolites, including phenolics, terpenoids, fatty acids, hydrocarbon, and tocopherol. Banana peel and leaf extracts had the minimum MIC values (0.14 and 0.0312 mg/mL) compared to the other plant components (Mostafa 2021).

Our antimicrobial panel included gram-positive and gram-negative microorganisms. Our approach was unique in that we investigated the antibacterial action of oxidized and unoxidized banana sap and found that the unoxidized banana sap, which was green in color, had more strong antimicrobial potential in terms of MIC than the oxidised sap.

In the unoxidized sap, there was an eight-fold reduction. Tannins oxidize, turning black and rendering them ineffective for antibacterial purposes. Chelating prevents oxidation, keeping it active and therefore enhancing its antimicrobial action. To our best knowledge, this research of exploration of unoxidized sap has been carried out for the first time. The banana sap presumably remains unoxidized in plants under in vivo conditions, thereby having antimicrobial action. According to Kumar et al. (2014), banana sap shows modest antibacterial activity but no antifungal activity as only oxidized sap was tested. However, our anti-candidal activity results coincide with Kumar et al. (2014).

5.2.2 Anticancer/Cytotoxic effect

Floral extracts of bananas have inhibited the growth of cancer cells *in vitro* (Nadumane and Timsina 2014). Banana fruits have been extracted with multiple solvents. The extracts exhibited cytotoxic effect in different cancerous cell lines such as A-549 (human lung cancer cells), HepG-2 (human hepatoma cells), MCF-7 (human breast cancer cells), and HT-29 (human colon cancer cells). Both banana peel and banana pulp have exhibited considerable anticancer potential against cell lines (Li et al. 2013). Peels of both raw and ripe bananas have been demonstrated to exhibit good anticancer activity. The banana extracts have been shown to demonstrate an anti-leukemic effect (Chadarat et al. 2010).

The MCF-7 cell line susceptibility for apoptosis has emerged as a popular model system for breast cancer research. The efficiency of a drug in blocking a particular biological or biochemical activity is measured by the half maximum inhibitory concentration (IC). This quantitative measurement shows the amount of an inhibitor (sample) needed to stop inhibiting a specific biological process (Durgadevi et al. 2019). With known IC_{50} values, the extracts demonstrated different actions. For instance, banana peel demonstrated up to 33% antiproliferative potential for MCF-7 cell lines (El-Zawawy 2015), while banana blossom demonstrated activity with IC_{50} less than 10 µg/mL for CHO and HeLa cell lines (Nadumane and Timsina 2014). Hexane extracts have been shown to exhibit anticancer activity against HCT- 116 and MCF-7 cell lines (Dahham et al. 2015).

Several methods have been worked out to harvest the bioactive compounds from the banana flower (Sumathy and Sumathy 2011). The extraction of compounds from flower using supercritical CO_2 and propane has resulted in obtaining extracts that showed high bioactivity (Correa et al. 2017).

Ethanolic extracts of banana peels have been studied for effective anti-tumor activity. Alkaloids were present in most of the peels. However, polyphenols, tannins, and proteins were also reported in most tested peels, such as lemon, carrot, orange, kiwi, goldenberry, tangerine, watermelon, and banana (El-Zawawy 2015).

Studies done in giant water prawns for effects of banana extracts had shown positive effects of using the extracts. Hot water extracts of banana peel helped improve the overall haemocyte count and phagocyte activity in giant water prawns. Banana peel extract has been shown to be an excellent immunostimulant for prawns. The pulp, seed and peel methanolic extracts exhibited specific cytotoxic activity (Rattanavichai and Cheng 2014). Although the anticancer effects of banana sap have not been firmly established in animal models or cell lines, the presence of flavones and flavanol suggests that the sap has anticancer properties (Pothavorn et al. 2010). However, we have shown that the MCF-7 breast cancer cell line is inhibited by the dichloromethane extract of concentrated banana sap.

5.2.3 Antioxidant studies

Research studies have been conducted on the antioxidant potential of the banana agro-waste, including the pulp, and peel (Sulaiman et al. 2011; Mokbel and Hashinaga 2005; Nagarjaiah and Prakash 2011), fruit (Alothman et al. 2009), pseudo-stem and rhizome (Sararvanan and Aradhya 2011a; Kumar et al. 2014) inflorescence (Loganayaki et al. 2010) and leaf (Karuppiah and Mustaffa 2013). The most popular technique for determining the antioxidant action is the 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) method, followed by ferric reducing antioxidant power (FRAP) and total antioxidant activity (TAA). FRAP tests assess the reducing power, whereas antioxidant assays like DPPH evaluate antioxidant capacity to scavenge free radicals. DPPH is a strong free radical that, when exposed to antioxidants, is converted to colorless DPPH (Mishra et al. 2012). The discrepancies in antioxidant potential observed by various groups might be attributed to changes in cultivars, extraction processes, geographical location, and prevailing variables such as soil, temperature, sunlight, and horticulture techniques (Deng et al. 2010; Durgadevi et al. 2019). For the assessment of their antioxidant potential, various extracts were made in water (Mokbel and Hashinaga 2005; Alothman et al. 2009; Nagarjaiah and Prakash 2011), acetone (Loganayaki et al. 2010), ethanol (Nagarjaiah and Prakash 2011), methanol (Kumar et al. 2014), and hexane (Sulaiman et al. 2011). Researchers showed that different banana parts had various antioxidant properties, and they found that solvent-based extracts had stronger antioxidant potential than aqueous extracts. The concentration of antioxidant enzymes such as APOX, CAT, POX, and SOD were more significant in the pulp than in the peel. These enzymes are significant antioxidants that scavenge active oxygen species from plant cells. CAT is activated during heating and persists throughout cold storage, suggesting that it may be a significant antioxidant enzyme implicated in defense mechanisms. The synthesis of lignin and ethylene, pathogen defense, wound healing, and stress responses are only a few of the physiological processes involved in POX, APOX, and SOD. Moreover, POXs are one of the plant enzymes that are most heat stable, and it is found more in the peel followed by the pulp (Durgadevi et al. 2019).

Moreover, the banana pulp and peel contain beta carotene, vitamins, and phenolic compounds such as catechin, lignin, anthocyanins, and tannin contributing to its potent antioxidant property (Sulaiman et al. 2011). Extraction of the fruit pulp with

various solvents results in differential extraction of polyphenols from the material. Based on the extent of extraction, differential antioxidant abilities have been observed. Extracts from banana peels have demonstrated higher antioxidant capability than banana pulp (Nagarajaiah and Prakash 2001). Organic solvent extracts of banana stem and flower show high phenolic compounds. These extracts have been shown to exhibit potent free radical scavenging activity. The flowers of bananas are identified to contain a higher concentration of phenolics than any other part of the plant. This high concentration of phenolics gives a higher antioxidant property to the floral parts of the banana. Higher mineral content helps in the good metal chelating activity of the extracts (Apriasari et al. 2014). The bioactive molecules caffeoylquinic acid or chlorogenic acid, responsible for the antioxidant action, are found in the banana sap (Pothavorn et al. 2010). In our study, for the first time, the antioxidant activity of the dichloromethane and ethyl acetate (DCM/EA) extract of concentrated banana sap was assessed using the DPPH assay. The non-polar components in the extract (DCM/EA) may be responsible for the antioxidant potential.

5.2.4 Nutritional analysis

Phytochemical analysis of banana sap revealed that sap is abundant in flavonols and flavanes. According to HPLC analysis, sap contains significant levels of apigenin glycosides and naringenin glycosides, which inhibit a number of cancer cell lines by inducing cell cycle arrest and death. Flavanols like kaempferol, quercetin glycosides, and myrecetin glycoside are also found in high amounts in sap. Besides flavone and flavanols components, N acetyl serotonin and dopamine are also detected in sap. Flavanonols and flavones are known anticancer compounds (Pothavorn et al. 2010). Banana sap found to contain vitamin E has an antioxidant function, helps in scavenging the free radicals, and minimizes the risk of tissue injury. Vitamin E also protects against cancer (Kaur et al. 2013; Logan 2004). Banana sap contains a good amount of vitamin C, essential for the biosynthesis of collagen, carnitine, and neurotransmitters (Dhanasekaran and Ren 2005). It is associated with health benefits as it has antioxidant, anti-athergenic, anti-carcinogenic, and immunomodulatory activity (Kaur et al. 2013). Trace amounts of vitamin B12 are also present in banana sap which protects against hydrogen peroxide-induced oxidative stress (Birch et al. 2009). Out of minerals, banana sap contains a fair amount of zinc which serves as an

antioxidant by regulating the expression of glutamate-cysteine ligase (Eide 2011). Katayama and Mine (2007) revealed that amino acids having the potential for antioxidant activity could be classified into four groups; sulfhydryl-containing amino acids (cysteine, methionine), branched-chain amino acids (leucine, isoleucine, and valine), heterocyclic amino acids (tryptophan, histidine) and other (lysine and alanine). Our study indicated that a trace amount of sulfhydryl amino acids (methionine 0.60 mg/100mL, cysteine 0.22 mg/100mL) and heterocyclic amino acids (histidine 0.83 mg/100mL), a good amount of branched amino acids (isoleucine 1.14 mg/100mL, leucine 4.62 mg/100mL, valine 1.69 mg/100mL) and other amino acids (serine 1.93 mg/100mL, arginine 5.80 mg/100mL, glycine 5.76 mg/100mL, aspartic acid 18.45 mg/100mL, glutamic acid 17.64 mg/100mL, threonine 2.71 mg/100mL, alanine 8.04 mg/100mL, proline 2.75 mg/100mL, cysteine 0.22 mg/100mL, lysine 6.99 mg/100mL, tyrosine 1.26 mg/100mL, and phenylalanine 1.72 mg/100mL) were present in banana sap. No fatty acids were found in banana sap.

5.2.5 LCMS analysis

Plants are the primary sources of natural phenolics, such as different kinds of phenolic acids, including a group of hydroxybenzoic acids (Tomas-Barberan and Clifford 2000) and hydroxycinnamic acids (Umar and Xia 2005), flavonoids (Shukla and Gupta 2010), flavanone naringenin (Patel et al. 2018) anthocyanins, isofavones, chalcones, and nonflavonoids, including tannins, stilbenes, and lignans (González-Sarrías et al. 2020). Major phytochemicals compounds reported from the banana sap can be grouped as phenolics, alkaloids, lignins, saponins, coumarins and cardiac glycosides (Onyema et al. 2016; Pothavorn et al. 2010). Furthermore, phytochemical analysis of banana stem juice (sap) reported some antidiabetic compounds such as ferulic acid, lupeol, vanillic acid, trans-cinnamic acid, p-hydroxybenzoic acid, p-coumaric acid, catechin/ epicatechin, rutin, chlorogenic acid, gallic acid, cafeic acid and nicotiforin (Nguyen et al. 2017) . In our study LC–MS data detected the presence of alkaloid and flavonoids as major compounds such as rescinnamine derivative, dihydrorescinnamine and epimedin A were predicted to be significant compounds in both solvents, i.e., dichloromethane (DCM) and ethyl acetate (EA). LCMS data revealed that these compounds are more soluble in the DCM extract and less soluble in the EA extract.

Rescinnamine is an alkaloid produced by *Rauwolfia serpentina*. It belongs to the Apocynaceae family. All parts of the plant include indole alkaloids such as reserpine, rescinnamine, serpentine, and many others (Lobay 2015). A systematic analysis done for the presence of rescinnamine in various plant parts of *Rauvolfia* has shown it is expressed to very high levels in flower and floral stem, respectively (Verma 2017). Rescinnamine is abundant in members of *the Rauvolfia* genus. However, there are no reports of its presence in other plants outside this genus. Banana stem, flower, pseudo-stem, fruit, and peel are rich in alkaloids. Ogbonna et al. (2016) studied the levels of alkaloids at different stages of banana fruit development. They found that the highest alkaloid content is seen in ripe fruits. Similarly, the banana peel is rich in alkaloids (Romelle et al. 2016). Though total alkaloid content is high in these, the nature of alkaloids has not been investigated in great detail. The presence of Rescinnamine is not established in any of the banana plant parts. Rescinnamine, an antihypertensive drug, prevents coronavirus from attaching to a receptor on the surface of human cells (Wu et al. 2020). On this basis, we may conclude that our study's antibacterial action in banana sap is attributable to alkaloid chemicals such as rescinnamine and dihydrorescinnamine.

Epimedia herba is a weed utilized in traditional Chinese medicine. The herb has exhibited multiple biological activities, including anti-tumor, anti-hepatotoxic, and anti-inflammatory activity. The principal constituents in epimedium responsible for such broad-spectrum activity include icariin and its metabolites. They all belong to the glycosylated flavonoid family. Icariin derivatives include epimedin A, B, and C, respectively. Epimedin C has been studied extensively for its bioactivity. Various epimedins are important in controlling many inflammatory conditions. Epimedin A, B and C have been expressed in Epimedia (Kim et al. 2017). Other plants have not been shown to express these molecules. The banana plant produces flavonoids in different plant parts. Studies done using unripe banana peel have shown that it protects gastric mucosa against aspirin effects. Leucocyanidin was identified from dried unripe banana powder as the main component of the protective effect (Lewis et al. 1999). In bananas, epimedins have not been documented till date. There are no reports indicating that bananas contain glycosylated flavonoids. Additionally, flavonoids are potent antioxidants with hepatoprotective, anti-inflammatory, anti-cancer, anti-

allergic, and anti-viral effects (Lewis et al. 1999; Xi et al. 2014; Xie et al. 2015; Ogbonna et al. 2016).

Studies conducted *in vivo* and *in vitro* have revealed that epimedin compounds have potent anticancer effects on a variety of cancer cells through a number of pathways, including cell cycle regulation, immunological modulation, anti-metastasis, anti-angiogenesis, and apoptosis. (Tan et al. 2016; Lone et al. 2018).All four epimedium species markers—epimedin A, epimedin B, epimedin C, and icariin—showed potent anticancer potential in a different study on *E. koreanum* (Yasukawa et al. 2016). Epimedium herbs may therefore help prevent cancer. Our findings revealed the existence of epimedin A in banana sap, as well as antioxidant, anticancer, and antibacterial activities. Thus, the results of our investigation coincide with those of Yasukawa et al. (2016).

CONCLUSIONS

Chemical characterization of the banana sap showed that it would be used as a resource material for the production of different value added products.

Concentrated banana sap supplemented with other industrial wastes such as corn steep liquor, and spent wash significantly increased the ethanol content when fermented with *S. cerevisiae* strains (MTCC170 and MTCC180).

Our antimicrobial study indicated that the concentrated banana sap has the good potential against antibacterial activity. This opens up avenues on harnessing different phytochemicals from banana plant sap and evaluating them individually or in combinations for their antimicrobial action and possible use in herbal cosmeceutical formulations.

Further antioxidant, anticancer revealed that concentrated banana sap has a good potential of cell growth inhibition and antioxidant activity in correlation with the common nutrition present in banana sap which indicates it contains bioactive compounds which can act as a good natural resource for the preparation of antioxidant formulation and development of drugs with least side effects. Moreover, LCMS studies indicated the presence of an alkaloid and flavonoids as major bioactive compounds in the concentrated banana sap revealing that in this way banana sap will come into utilization of value added product as of now it is discarded in environment which causes environmental pollution.

REFERENCES

1. Agarwal PK, Singh A, Gaurav K, Goel S, Khanna HD, Goel RK (2009) Evaluation of wound healing activity of extracts of plantain banana (*Musa sapientum* var. paradisiaca) in rats. Indian Journal of Experimental Biology 47:32-40.
2. Ahmed MA, Faris MA, Hadeel M, Norah H (2011) Bioethanol fuel production from rotten banana as an environmental waste management and sustainable energy. African journal of microbiology research 5:586-598.
3. Akaninwor JO, Sodje M (2005) The effect of storage on the nutrient composition of some Nigerian foodstuffs. Journal of Applied Sciences and Environmental Management 9:9-11.
4. Akin-Osanaiye BC, Nzelibe HC, Agbaji AS (2008) Ethanol production from Carica papaya (pawpaw) fruit waste. Asian journal of Biochemistry 3:188-193.
5. Al-Mqbali LR, Hossain MA (2019) Cytotoxic and antimicrobial potential of different varieties of ripe banana used traditionally to treat ulcers. Toxicology Reports 6:1086-1090.
6. Alothman M, Bhat R, Karim AA (2009) Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chemistry 115:785-788.
7. Al-Sahlany ST, Al-musafer AM (2020) Effect of substitution percentage of banana peels flour in chemical composition, rheological characteristics of wheat flour and the viability of yeast during dough time. Journal of the Saudi Society of Agricultural Sciences 19:87-91.
8. Ammayappan (2004) Natural Dye from Pseudostem of Banana for Silk. Man-made Textile India 47:218-220.
9. Angulo-Mosquera LS, Alvarado-Alvarado AA, Rivas-Arrieta MJ, Cattaneo CR, Rene ER, García-Depraect O (2021) Production of solid biofuels from organic waste in developing countries: A review from sustainability and economic feasibility perspectives. Science of The Total Environment 795:1-7.
10. Anhwange B A (2008) Chemical Composition of Musa sapientum (Banana) Peels. Journal of Food Technology 6:263-266.
11. Apeda (2010) Product Profiles of banana. http://apeda.in/agriexchange /Market%20Profile/one/BANANA.aspx (accessed Aug. 10, 2022).
12. Apeda (2015) India production of banana. https://agriexchange.apeda.gov.in/ India%20Production/India_Productions.aspx?cat=fruit&hscode=1042 (accessed Aug. 10, 2022).
13. APHA (2012) Standard methods for the examination of water and wastewater, 22nd edition edited by E. W. Rice, R. B. Baird, A. D. Eaton and L. S. Clesceri. American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF), Washington, D.C., USA.

14. Apriasari ML, Suhartono E (2014) Bioactive compound and antioxidant activity of methanol extract mauli bananas (Musa sp) stem. International Journal of Bioscience, Biochemistry and Bioinformatics 4:110-115.
15. Arredondo HV, Colorado AR, Júnior SD (2009) Ethanol production from banana fruit and its lignocellulosic residues: Energy and renewability analysis. International Journal of Thermodynamics 12:155-162.
16. Arumugam R, Manikandan M (2011) Fermentation of pretreated hydrolyzates of banana and mango fruit wastes for ethanol production. Asian Journal of Experimental Biological Sciences 2: 246-256.
17. Asif M, Kaur A (2018) Biologically active phytochemical contents and biological activities of whole Musa Acuminata (banana) plant. International Journal of Recent Advances in Medical and Pharmaceutical Research 1:5-33.
18. Asuquo EG, Udobi CE (2016) Antibacterial and toxicity studies of the ethanol extract of Musa paradisiaca leaf. Cogent Biology 2:1-10.
19. Aynehchi Y, Salehi Sormaghi MH, Amin GH, Ghahreman A (1981) Survey of Iranian Plants for Saponins, Alkaloids, Flavonoids and Tannins. Quarterly Journal of Crude Drug Research 19:53-63.
20. Ayoola GA, Coker HA, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, Atangbayila TO (2008) Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Tropical Journal of Pharmaceutical Research 7:1019-1024.
21. Azad AK, Rasul MG, Khan MM, Sharma SC, Hazrat MA (2015) Prospect of biofuels as an alternative transport fuel in Australia. Renewable and Sustainable Energy Reviews 43:331-351.
22. Bagavan A, Rahuman AA, Kaushik NK, Sahal D (2011) In vitro antimalarial activity of medicinal plant extracts against Plasmodium falciparum. Parasitology research 108:15-22.
23. Balda S, Sharma A, Capalash N, Sharma P (2021) Banana fibre: a natural and sustainable bioresource for eco-friendly applications. Clean Technologies and Environmental Policy 23:1389-401.
24. Balouiri M, Sadiki M, Ibnsouda SK (2016) Methods for in vitro evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis 6:71-79.

25. Banana Market review (2017) released by Food agricultural organization (FAO). Available on internet
26. Barhanpurkar S, Kumar A, Purwar R (2015) Charcterisation of banana pseudostem sap used as a mordant for dying. SSRG International Journal of Polymer and Textile Engineering 2:1-7.
27. Binod P, Gnansounou E, Sindhu R, Pandey A (2019) Enzymes for second generation biofuels: recent developments and future perspectives. Bioresource Technology Reports 5:317-325.
28. Birch CS, Brasch NE, McCaddon A, Williams JH (2009) A novel role for vitamin B12: cobalamins are intracellular antioxidants in vitro. Free Radical Biology and Medicine 47:184-188.
29. Bisht R, Chanyal S, Agrawal PK (2016) Antimicrobial and phytochemical analysis of leaf extract of medicinal fruit plants. Asian Journal of Pharmaceutical and Clinical Research 9:131-136.
30. Biswas AK, Kumar V, Bhosle S, Sahoo J, Chatli MK (2011) Dietary fibers as functional ingredients in meat products and their role in human health. International Journal of Livestock Production 2:45-54.
31. Borborah K, Borthakur SK, Tanti B (2016) Musa balbisiana Colla-Taxonomy, Traditional knowledge and economic potentialities of the plant in Assam, India. Indian Journal of Traditional Knowledge 15:116-120.
32. Borges MH, Alves DLF, Raslan DS, Piló-Veloso D, Rodrigues VM, Homsi-Brandeburgo MI, De Lima ME (2005) Neutralizing properties of Musa paradisiaca L.(Musaceae) juice on phospholipase A2, myotoxic, hemorrhagic and lethal activities of crotalidae venoms. Journal of ethnopharmacology 98:21-29.
33. BP Statistical Review of World Energy (2021) 70th ed. Available online: https://www.bp.com/en/global/corporate/energyeconomics/statistical-review-of-world-energy.html (accessed on 12 October 2021).
34. Brooks AA (2008) Ethanol production potential of local yeast strains isolated from ripe banana peels. African Journal of Biotechnology 7:3749-3752.
35. Budhisatria R, Rosaria R, Jap L, Jan TT (2017) In vitro and in vivo prebiotic activities of purified oligosaccharides derived from various local bananas (Musa sp.): Tanduk, Uli, Raja Sereh, and Cavendish. Microbiology Indonesia 11:55-61.

36. Buranawit K, Laenoi W (2015) Effects of supplementing banana (Musa spp.) as prebiotic, probiotic (Toyocerin®) and their combination on growth performance, Carcass and meat quality in broilers. Asia-Pacific Journal of Science and Technology 20:419-427.

37. Bušić A, Marđetko N, Kundas S, Morzak G, Belskaya H, Ivančić Šantek M, Komes D, Novak S, Šantek B (2018) Bioethanol production from renewable raw materials and its separation and purification: a review. Food technology and biotechnology 56:289-311.

38. Business standard report (2021) Available online: https://www.business-standard.com/article/economy-policy /india-targeting-20-ethanol-blending-with-petrol-by-2023-24-piyush-goyal-121071 601169_1.html, 2021 (accessed on July 17, 2021).

39. Caputi A, Ueda M, Brown T (1968) Spectrophotometric determination of ethanol in wine. American Journal of Enology and Viticulture 19:160-165.

40. Castelo-Branco VN, Guimarães JN, Souza L, Guedes MR, Silva PM, Ferrão LL, Miyahira RF, Guimarães RR, Freitas SM, Reis MC, Zago L (2017) The use of green banana (Musa balbisiana) pulp and peel flour as an ingredient for tagliatelle pasta. Brazilian Journal of Food Technology 20:1-8.

41. Chabuck ZA, Al-Charrakh AH, Hindi NK, Hindi SK (2013) Antimicrobial effect of aqueous banana peel extract, Iraq. Research Gate. Pharmaceutical Sciences 1:73-75.

42. Chadarat A, Siriporn O, Songyot A (2010) Cytotoxicity of extracts from fruit plants against leukemic cell lines. African Journal of Pharmacy and Pharmacology 4:013-021.

43. Chaudhary N, Chand S, Kaur N (2014) Bioethanol production from fruit peels using simultaneous saccharification and fermentation. GSTF Journal of BioSciences 3:48-53.

44. China R, Dutta S, Sen S, Chakrabarti R, Bhowmik D, Ghosh S, Dhar P (2011) In vitro antioxidant activity of different cultivars of banana flower (Musa paradicicus L.) extracts available in India. Journal of Food Science 76:C1292-C1299.

45. Chitra P (2015) Development of banana-based weaning food mixes for infants and its nutritional quality evaluation. Reviews on Environmental Health 30:125-130.

46. Coico R (2006) Gram staining. Current protocols in microbiology A:3C.

47. Cordoba LD, da Silva RG, Gomes DD, Schnitzler E, Waszczynskyj N (2018) Brazilian green banana. Journal of Thermal Analysis and Calorimetry 134:2065-2073.
48. Correa M, Bombardelli MC, Fontana PD, Bovo F, Messias-Reason IJ, Maurer JB, Corazza ML (2017) Bioactivity of extracts of Musa paradisiaca L. obtained with compressed propane and supercritical CO2. The Journal of Supercritical Fluids 122:63-69.
49. Dahham SS, Mohamad TA, Tabana YM, Majid AM (2015) Antioxidant activities and anticancer screening of extracts from banana fruit (Musa sapientum). Academic Journal of Cancer Research 8:28-34.
50. Dang H, Fawcett D, Poinern GE (2017) Biogenic synthesis of silver nanoparticles from waste banana plant stems and their antibacterial activity against Escherichia coli and Staphylococcus Epidermis. International Journal of Research in Medical Sciences 5:3769-3775.
51. Datta A, Hossain A, Roy S (2019) An overview on biofuels and their advantages and disadvantages. Asian Journal of Chemistry 8:1851-1858.
52. De Souza EL, Sellin N, Marangoni C, Souza O (2017) The influence of different strategies for the saccharification of the banana plant pseudostem and the detoxification of concentrated broth on bioethanol production. Applied biochemistry and biotechnology 183:943-965.
53. Deng S, West BJ, Jensen CJ (2010) A quantitative comparison of phytochemical components in global noni fruits and their commercial products. Food Chemistry 122:267-270.
54. Dhanasekaran M, Ren J (2005) The emerging role of coenzyme Q-10 in aging, neurodegeneration, cardiovascular disease, cancer and diabetes mellitus. Current Neurovascular Research 2:447-459.
55. Divya RS, Venkatalakshmi P, Vadivel V, Brindha P (2016) In vitro studies on the biological activities of flowers of banana (Musa Paradisiaca L.). Der Pharmacia Lettre 8:238-246.
56. Divyabharathi R, Angeeswaran R, Jagadeeshkumar K, Pugalendhi S (2017) Characterization and batch anaerobic digestion study of banana wastes. International Journal of Current Microbiology and Applied Sciences 6:2307-2315.

57. Durgadevi PK, Saravanan A, Uma S (2019) Antioxidant potential and antitumour activities of Nendran banana peels in breast cancer cell line. Indian Journal of Pharmaceutical Sciences 81:464-473.
58. Ehiowemwenguan G, Emoghene AO, Inetianbor JE (2014) Antibacterial and phytochemical analysis of Banana fruit peel. IOSR Journal of Pharmacy 4:18-25.
59. Eide DJ (2011) The oxidative stress of zinc deficiency. Metallomics 3:1124-1129.
60. El Zawawy NA (2015) Antioxidant, antitumor, antimicrobial studies and quantitative phytochemical estimation of ethanolic extracts of selected fruit peels. International Journal of Current Microbiology and Applied Sciences 4:298-309.
61. El-Diwany AI, El-Abyad MS, El-Refai AH, Sallam LA, Allam RF (1992) Effect of some fermentation parameters on ethanol production from beet molasses by Saccharomyces cerevisiae Y-7. Bioresource technology 42:191-195.
62. Evbuomwan LU, Onodje GO, Jacob I, Patric CE (2018) Evaluating the antibacterial activity of Musa acuminata (banana) fruit peels against multidrug resistant bacterial isolates. International Journal of Novel Research in Life Sciences 5:26-31.
63. FAO (2019) Production Year book. Food and Agricultural Organization, Rome, Italy.
64. FAOSTAT (2012) FAO statistical database (FAOSTAT). Retrieved 2019, from http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor
65. FAOSTAT (2013) FAO statistical database (FAOSTAT). Retrieved 2019, from http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor
66. FAOSTAT (2018) http://www.fao.org/faost at/en/#data/QC
67. FAOSTAT (2019) Crop production, statistics division. Food and Agriculture OrganizationoftheUnitedNations. http://fao.org/faostat/en/#data/QC
68. Feriotti DG, Iguti AM (2012) Proposal for use of pseudostem from banana tree (Musa cavendish). In International Congress of Engineering and Food.
69. Fernandes ER, Marangoni C, Souza O, Sellin N (2013) Thermochemical characterization of banana leaves as a potential energy source. Energy conversion and management 75:603-608.
70. Fernandes-Klajn F, Romero-García JM, Díaz MJ, Castro E (2018) Comparison of fermentation strategies for ethanol production from olive tree pruning biomass. Industrial Crops and Products 122:98-106.

71. Food and Agriculture Organisation of the United Nations (FAO) (2020) Banana production, 1961 to 2018. http://www.fao.org/faostat/en/?#data/, online: 18 February 2021.
72. Forster M, Rodríguez Rodríguez E, Darias Martín J, Díaz Romero C (2003) Distribution of nutrients in edible banana pulp. Food Technology and Biotechnology 41:167-171.
73. Galbe M, Zacchi G (2007) Pretreatment of lignocellulosic materials for efficient bioethanol production. Biofuels, Springer, Berlin, Heidelberg, pp 41-65.
74. Gebregergs A, Gebresemati M, Sahu O (2016) Industrial ethanol from banana peels for developing countries: Response surface methodology. Pacific Science Review A: Natural Science and Engineering 18:22-29.
75. González-Montelongo R, Lobo MG, González M (2010) Antioxidant activity in banana peel extracts: Testing extraction conditions and related bioactive compounds. Food Chemistry 119:1030-1039.
76. González-Sarrías A, Tomás-Barberán FA, García-Villalba R (2020) Structural diversity of polyphenols and distribution in foods.In Dietary Polyphenols: Their Metabolism and Health Effects 1-29.
77. Gopalan G, Prabha B, Joe A, Reshmitha TR, Sherin DR, Abraham B, Sabu M, Manojkumar TK, Radhakrishnan KV, Nisha P (2019) Screening of Musa balbisiana Colla. seeds for antidiabetic properties and isolation of apiforol, a potential lead, with antidiabetic activity. Journal of the Science of Food and Agriculture 99:2521-2529.
78. Graefe S, Dufour D, Giraldo A, Muñoz LA, Mora P, Solís H, Garcés H, Gonzalez A (2011) Energy and carbon footprints of ethanol production using banana and cooking banana discard: A case study from Costa Rica and Ecuador. Biomass and Bioenergy 35:2640-2649.
79. Guerrero AB, Ballesteros I, Ballesteros M (2018) The potential of agricultural banana waste for bioethanol production. Fuel 213:176-185.
80. Gupta G, Baranwal M, Saxena S, Reddy MS (2019) Utilization of banana stem juice as a feedstock material for bioethanol production. CLEAN–Soil, Air, Water 47:1-5.
81. Gupta G, Baranwal M, Saxena S, Reddy MS (2022) Utilization of banana waste as a resource material for biofuels and other value-added products. Biomass Conversion and Biorefinery 17:1-20.

82. Häkkinen M (2009) Musa chunii Häkkinen, a new species (Musaceae) from Yunnan, China and taxonomic identity of Musa rubra. Journal of systematics and evolution 47:87-91.

83. Hameed RS, Fayyad RJ, Nuaman RS, Hamdan NT, Maliki SA (2019) Synthesis and characterization of a novel titanium nanoparticals using banana peel extract and investigate its antibacterial and insecticidal activity. Journal of Pure and Applied Microbiology 13:2241-2249.

84. Hammond JB, Egg R, Diggins D, Coble CG (1996) Alcohol from bananas. Bioresource Technlogy 56:125-130

85. Han M, Kang KE, Kim Y, Choi GW (2013) High efficiency bioethanol production from barley straw using a continuous pretreatment reactor. Process Biochemistry 48:488-495.

86. Harini K, Sukumar M (2019) Development of cellulose-based migratory and nonmigratory active packaging films. Carbohydrate polymers 204:202-213.

87. Hariprasad T, Dharmalingam G, Praveen Raj P (2013) Study of mechanical properties of banana-coir hybrid composite using experimental and fem techniques. Journal of Mechanical Engineering and Sciences 4:518-531.

88. Hernandez-Salas JM, Villa-Ramírez MS, Veloz-Rendón JS, Rivera-Hernández KN, González-César RA, Plascencia-Espinosa MA, Trejo-Estrada SR (2009) Comparative hydrolysis and fermentation of sugarcane and agave bagasse. Bioresource technology 100:1238-1245.

89. Hikal WM, Ahl SA, Hussein AH, Bratovcic A, Tkachenko KG, Sharifi-Rad J, Kačániová M, Elhourri M, Atanassova M (2022) Banana Peels: A Waste Treasure for Human Being. Evidence-Based Complementary and Alternative Medicine 2022:1-9.

90. Hirani AH, Javed N, Asif M, Basu SK, Kumar A (2018) A review on first-and second-generation biofuel productions. Biofuels: greenhouse gas mitigation and global warming, Springer, New Delhi, pp 141-154.

91. Hoekman SK (2009) Biofuels in the US–challenges and opportunities. Renewable energy 34:14-22.

92. Horigome T, Sakaguchi E, Kishimoto C (2002) Hypocholesterolaemic effect of banana (*Musa sapientum* L. var. Cavendishii) pulp in the rat fed on a cholesterol-containing diet. British Journal of Nutrition 68:231-244.

93. Hossain MS, Alam MB, Asadujjaman M, Zahan R, Islam MM, Mazumder ME, Haque ME (2011) Antidiarrheal, antioxidant and antimicrobial activities of the Musa sapientum seed Avicenna Journal of Medical Biotechnology 3:95-105.

94. Ibrahim HM (2015) Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. Journal of radiation research and applied sciences 8:265-275.

95. IEA World Energy Outlook (2021). Available online: https://www.iea.org/reports/world-energy-outlook-2021 (accessed on 28 October 2021).

96. Ighodaro OM (2012) Evaluation study on Nigerian species of Musa paradisiaca peels. Researcher 4:17-20.

97. Imam MZ, Akter S (2011) Musa paradisiaca L. and Musa sapientum L.: A phytochemical and pharmacological review. Journal of Applied Pharmaceutical Science 01:14-20.

98. Ingale S, Joshi SJ, Gupte A (2014) Production of bioethanol using agricultural waste: banana pseudo stem. Brazilian Journal of Microbiology 45:885-892.

99. Ismail TN, Awang RA, Mohamad S, Shahidan WN (2018) Chemical compounds and antimicrobial activity of acetone Musa acuminate AA/AAA leaf stalk extracts on selective gram-negative bacteria. Malaysian Journal of Analytical Sciences 22:957-964.

100. Itelima J, Onwuliri F, Onwuliri E, Onyimba I, Oforji S (2013) Bio-ethanol production from banana, plantain and pineapple peels by simultaneous saccharification and fermentation process. International Journal of Environmental Science Development 4:213-216.

101. Jagessar RC, Fraser C (2016) The fermentation of banana (*Musa acuminata*), Mango (*Mangifera indica* L.) and pineapple (*Ananas comosus*) mash in the absence and presence of additives. American Journal of Research Communication 4:131-140.

102. Jahan M, Warsi MK, Khatoon F (2010) Concentration influence on antimicrobial activity of banana blossom extract-incorporated chitosan-polyethylene glycol (CS-PEG) blended film. Journal of Chemical and Pharmaceutical Research 2:373-378.

103. Jain P, Bhuiyan MH, Hossain KR, Bachar SC (2011) Antibacterial and antioxidant activities of local seeded banana fruits. African Journal of Pharmacy Pharmacology 5:1398-1403.

104. Jain R, Srivastava S (2012) Nutrient composition of spent wash and its impact on sugarcane growth and biochemical attributes. Physiology and Molecular Biology of Plants 18:95-99.
105. Ji L, Srzednicki G (2013) Extraction of aromatic compounds from banana peels. InII Southeast Asia Symposium on Quality Management in Postharvest Systems 1088:541-546.
106. Jones AM, Ingledew WM (1994) Fuel alcohol production: appraisal of nitrogenous yeast foods for very high gravity wheat mash fermentation. Process Biochemistry 29:483-488.
107. Jørgensen H (2009) Effect of nutrients on fermentation of pretreated wheat straw at very high dry matter content by Saccharomyces cerevisiae. Applied biochemistry and Biotechnology153:44-57.
108. Jorgensen JH, Turnidge JD, Washington JA, Murray EJ, Pfaller MA, Tenover FC, Yolken RH (1999) Manual of clinical microbiology 7thed, American Society for Microbiology, Washington DC: ASM Press 1526-1543.
109. Jouneghani RS, Castro AH, Panda SK, Swennen R, Luyten W (2020) Antimicrobial activity of selected banana cultivars against important human pathogens, including candida biofilm. Foods 9:1-9.
110. Jull C, Redondo PC, Vapnek J, Mosoti V (2007) Recent trends in the law and policy of bioenergy production, promotion and use. Food and Agriculture Organisation 95.
111. Kanazawa K, Sakakibara H (2000). High content of dopamine, a strong antioxidant, in cavendish banana. Journal of agricultural and food chemistry 48:844-848.
112. Kandasamy S, Aradhya SM (2014) Polyphenolic profile and antioxidant properties of rhizome of commercial banana cultivars grown in India. Food Bioscience 8:22-32.
113. Kandasamy S, Baggu C, Javagal MR, Lingamallu JR, Yenamandra V, Aradhya SM (2014) Antioxidant properties of isolated compounds from banana rhizome. Journal of food science 79:988-1001.
114. Karuppiah P, Mustaffa M (2013) Antibacterial and antioxidant activities of Musa sp. Leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection. Asian Pacific Journal of Tropical Biomedicine 3:737-742.
115. Katayama S, Mine Y (2007) Antioxidative activity of amino acids on tissue oxidative stress in human intestinal epithelial cell model. Journal of Agricultural and Food Chemistry 55:8458-8464.

116. Kaur H, Gupta Shalini, Verma Minni, Singh Kamaljit, Singh Jagmohan (2013) Free radicals and antioxidants in human heakth and disease- An overview. Biosciences 7:187-191.
117. Kavitha V, Manonmani G, Devakumar J (2019) Antimicrobial efficacy of fresh and dried banana peel extracts: An in vitro study. Drug Invention Today 11:1237-1240.
118. Khandelwal K (2000) Practical pharmacognosy Pragati Books Pvt. Ltd.
119. Kim DH, Jung HA, Sohn HS, Kim JW, Choi JS (2017) Potential of icariin metabolites from Epimedium koreanum Nakai as antidiabetic therapeutic agents. Molecules 22:1-14.
120. Kirti N, Tekade SP, Tagade A, Sawarkar AN (2022) Pyrolysis of pigeon pea (Cajanus cajan) stalk: Kinetics and thermodynamic analysis of degradation stages via isoconversional and master plot methods. Bioresource Technology 347:126440.
121. Kitdamrongsont K, Pothavorn P, Swangpol S, Wongniam S, Atawongsa K, Svasti J, Somana J (2008) Anthocyanin composition of wild bananas in Thailand. Journal of Agricultural Food Chemistry 56:10853-10857.
122. Klotoé JR, Dougnon TV, Sacramento TI, Dandjesso C, Edorh AP, Koudokpon H, Fanou VB, Fah L, Ategbo JM, Loko F, Dramane K (2012) Hemostatic potential of the sap of Musa sapientum L.(Musaceae). Journal of Applied Pharmaceutical Science 2:65-69.
123. Kraithong S, Issara U (2021) A strategic review on plant by-product from banana harvesting: A potentially bio-based ingredient for approaching novel food and agro-industry sustainability. Journal of the Saudi Society of Agricultural Sciences 20:530-543.
124. Krishna C (1995) Microbial enzyme production utilizing banana wastes (Doctoral dissertation, Cochin University of Science and Technology).
125. Krishna C, Chandrasekaran M (1996) Banana waste as substrate for α-amylase production by Bacillus subtilis (CBTK 106) under solid-state fermentation. Applied Microbiology and Biotechnology 46:106-111.
126. Kumar KS, Bhowmik D, Duraivel S, Umadevi M (2012) Traditional and medicinal uses of banana. Journal of Pharmacognosy Phytochemistry 1:51-63.
127. Kumar PR, Srivastava S, Singh KK, Mathad C, Thin PS (2014) Study of antioxidant and antimicrobial properties, phytochemical screening and analysis of sap extracted

from banana (Musa acuminata) pseudostem. International Journal of Advanced Biotechnology and Research 5:649-658.

128. Kumar V, Wati L, FitzGibbon F, Nigam P, Banat IM, Singh D, Marchant R (1997) Bioremediation and decolorization of anaerobically digested distillery spent wash. Biotechnology Letters 19:311-314.

129. Kumari S, Katare PB, Elancheran R, Nizami HL, Paramesha B, Arava S, Sarma PP, Kumar R, Mahajan D, Kumar Y, Devi R (2020) Musa balbisiana fruit rich in polyphenols attenuates isoproterenol-induced cardiac hypertrophy in rats via inhibition of inflammation and oxidative stress. Oxidative medicine and cellular longevity 2020:1-14.

130. Kurhade A, Patil S, Sonawane SK, Waghmare JS, Arya SS (2016) Effect of banana peel powder on bioactive constituents and microstructural quality of chapatti: unleavened Indian flat bread. Journal of Food Measurement and Characterization 10:32-41.

131. Kusmiyati, Mustofa A, Jumarmi (2018) Bioethanol production from banana stem by using Simultaneous Saccharification and Fermentation (SSF). IOP Conference Series: Materials Science and Engineering 358: 3rd international Conference on Global Sustainability and Chemical Engineering (ICGSCE) Putrajaya, Malaysia.

132. Laopaiboon L, Nuanpeng S, Srinophakun P, Klanrit P, Laopaiboon P (2009) Ethanol production from sweet sorghum juice using very high gravity technology: effects of carbon and nitrogen supplementations. Bioresource technology 100:4176-4182.

133. Lee, Uisung, Hoyoung Kwon, May Wu, Michael Wang (2021) Retrospective analysis of the US corn ethanol industry for 2005–2019: implications for greenhouse gas emission reductions. Biofuels, Bioproducts and Biorefining 15:1318-1331.

134. Lewis DA, Fields WN, Shaw GP (1999) A natural flavonoid present in unripe plantain banana pulp (Musa sapientum L. var. paradisiaca) protects the gastric mucosa from aspirin-induced erosions. Journal of ethnopharmacology 65:283-288.

135. Li F, Li S, Li HB, Deng GF, Ling WH, Wu S, Xu XR, Chen F (2013) Antiproliferative activity of peels, pulps and seeds of 61 fruits. Journal of Functional Foods 5:1298-1309.

136. Liggett RW, Koffler H (1948) Corn steep liquor in microbiology. Bacteriological reviews 12:297-311.

137. Liu Y, Song X, Cao F, Li F, Wang M, Yang Y, Liu M, Liu A, Xin H, Wang X (2020) Banana peel-derived dendrite-shaped Au nanomaterials with dual inhibition toward tumor growth and migration. International Journal of Nanomedicine 15:2315-2322.
138. Lobay D (2015) Rauwolfia in the treatment of hypertension. Integrative Medicine: A Clinician's Journal 14:40-46.
139. Logan AC (2004) Omega-3 fatty acids and major depression: a primer for the mental health professional. Lipids in health and disease 3:1-8.
140. Loganayaki N, Rajendrakumaran D, Manian S (2010) Antioxidant capacity and phenolic content of different solvent extracts from banana (Musa paradisiaca) and mustai (Rivea hypocrateriformis). Food Science and Biotechnology 19:1251-1258.
141. Lone SA, Gupta AP, Manzoor MM, Goyal P, Hassan QP, Gupta S (2018) Epimedium elatum (morr & decne): a therapeutic medicinal plant from northwestern himalayas of India. In Plant and Human Health, Springer, Cham, pp 619-656.
142. Lowry OH (1951) Protein measurement with the Folin phenol reagent. Journal of biological Chemistry 193:265-275.
143. Lustikaiswi DK, Yuliani S, Annura R, Rahmadani E (2021) Tryptophan in banana peel (Musa paradisiaca) as an anti-dementia alternative treatment: A narrative review. JKKI: Jurnal Kedokteran dan Kesehatan Indonesia 12:175-181.
144. Maduwanthi SD, Marapana RA (2019) Comparative study on aroma volatiles, organic acids, and sugars of Ambul banana (Musa acuminata, AAB) treated with induced ripening agents. Journal of Food Quality 1-9.
145. Mamaní A, Maturano Y, Herrero L, Montoro L, Sardella F (2022) Increase in fermentable sugars of olive tree pruning biomass for bioethanol production: application of an experimental design for optimization of alkaline pretreatment. Periodica Polytechnica Chemical Engineering 66:269-278.
146. Manthey, John A, Jaitrong S (2016) An HPLC-MS Analysis of Phenolic Antioxidants in Banana Peel. In Proceedings of the Florida State Horticultural Society 129:181-184.
147. Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical chemistry 31:426-428.
148. Mishra K, Ojha H, Chaudhury NK (2012) Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. Food chemistry 130:1036-1043.

149. Mohana S, Acharya BK, Madamwar D (2009) Distillery spent wash: treatment technologies and potential applications. Journal of Hazardous Materials 163:12-25.
150. Mohapatra D, Mishra S, Sutar N (2010) Banana and its by-product utilisation: an overview. Journal of Scientific and Industrial Research 69:323-329.
151. Mohiuddin AKM, Saha MK, Hossian MS, Ferdoushi A (2014) Usefulness of banana (*Musa paradisiaca*) wastes in manufacturing of bio-products: a review. The Agriculturists 12:148-158.
152. Mokbel MS, Hashinaga F (2005) Antibacterial and antioxidant activities of banana (Musa, AAA cv. Cavendish) fruits peel. American journal of Biochemistry and Biotechnology 1:125-131.
153. Morales M, Arvesen A, Cherubini F (2021) Integrated process simulation for bioethanol production: Effects of varying lignocellulosic feedstocks on technical performance. Bioresource Technology 328:1-15.
154. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods 65:55-63.
155. Mostafa HS (2021) Banana plant as a source of valuable antimicrobial compounds and its current applications in the food sector. Journal of Food Science 86:3778-3797.
156. Muthuvelu KS, Rajarathinam R, Kanagaraj LP, Ranganathan RV, Dhanasekaran K, Manickam NK (2019) Evaluation and characterization of novel sources of sustainable lignocellulosic residues for bioethanol production using ultrasound-assisted alkaline pre-treatment.Waste Management 87:368-374.
157. Nadumane VK, Timsina B (2014) Anticancer potential of banana flower extract: An in vitro study. Bangladesh Journal of Pharmacology 9:628-635.
158. Nagarajaiah SB, Prakash J (2011) Chemical composition and antioxidant potential of peels from three varieties of banana. Asian Journal of Food Agro-Industry 4:31-46.
159. Naikwade PV, Gaurav S, Sharayu D, Kailas J (2014) Evaluation of antibacterial properties of Musa paradisiaca L. Leaves. In Proceeding of the national conference on conservation of natural resources & biodiversity for sustainable development.
160. Narasimhan R, Bennick A, Palmer B, Murray RK (1982) Studies on the glycolipids of human saliva and gastric juice. Journal of Biological Chemistry 257:15122-15128.
161. Nguyen D, Novakova A, Spurna K (2017) Antidiabetic compounds in stem juice from banana. Czech Journal of Food Sciences 35:407–413.

162. Norfaradhiah R, Rapeah S (2017) Antibacterial activity of water and methanol extracts of banana pulps against Vibrio cholera. Health and Environment Journal 8:86–103.
163. Novidiyanto N, Enardi OP, Devriany A, Pratiwi AP, Airuni M (2020) Acceptability and antioxidant activity level of shredded banana flower-chicken meat. Amerta Nutrition 4:299-306.
164. Nyandiga GO, Siagi ZO, Makokha AB (2014) Optimization of anaerobic fermentation conditions for bioethanol production from banana (Ngombe) peels using yeast in a still reactor. In Proceedings of Sustainable Research and Innovation Conference, 5:120-123.
165. Oberoi HS, Vadlani PV, Saida L, Bansal S, Hughes JD (2011) Ethanol production from banana peels using statistically optimized simultaneous saccharification and fermentation process. Waste management 31:1576-1584.
166. OECD, FAO. OECD-FAO Agricultural Outlook 2021–2030.
167. Ogbonna OA, Izundu AI, Okoye NH, Ikeyi AP (2016) Phytochemical compositions of fruits of three Musa species at three stages of development. IOSR Journal of Pharmacy and Biological Sciences 11:48-59.
168. Okolie JA, Mukherjee A, Nanda S, Dalai AK, Kozinski JA (2021) Next‐generation biofuels and platform biochemicals from lignocellulosic biomass. International Journal of Energy Research 45:14145-14169.
169. Oloyede OI (2005) Chemical profile of unripe pulp of Carica papaya. Pakistan journal of nutrition 4:379-381.
170. Onyema CT, Ofor CE, Okudo VC, Ogbuagu AS (2016) Phytochemical and antimicrobial analysis of banana pseudo stem (Musa acuminata). British Journal of Pharmaceutical Research 10:1-9.
171. Padam BS, Tin HS, Chye FY, Abdullah MI (2012) Antibacterial and Antioxidative Activities of the Various Solvent Extracts of Banana (Musa paradisiaca cv. Mysore) Inflorescences. Journal of Biological Sciences 12:62-73.
172. Padam BS, Tin HS, Chye FY, Abdullah MI (2014) Banana by-products: an under-utilized renewable food biomass with great potential. Journal of food science and technology 51:3527-3545.
173. Palacios S, Ruiz HA, Ramos-Gonzalez R, Martínez J, Segura E, Aguilar M, Aguilera A, Cristóbal GM, Ilyina A (2017) Comparison of physicochemical pretreatments of

banana peels for bioethanol production. Food Science and Biotechnology 26:993-1001.

174. Pandey A, Soccol CR, Nigam P, Soccol VT, Vandenberghe LP, Mohan R (2000) Biotechnological potential of agro-industrial residues. II: cassava bagasse. Bioresource technology 74:81-87.

175. Pari L, Maheswari JU (1999) Hypoglycaemic effect of Musa sapientum L. in alloxan-induced diabetic rats. Journal of ethnopharmacology 68:321-325.

176. Patel H, Patel A, Surati T, Shah G (2012) Potential use of banana peels for the production of fermented products. International Journal of Ecology & Development 9:1-7.

177. Patel K, Singh GK, Patel DK (2018) A review on pharmacological and analytical aspects of naringenin. Chinese journal of integrative medicine 24:551-60.

178. Patle DS, Pandey A, Srivastava S, Sawarkar AN, Kumar S (2021) Ultrasound-intensified biodiesel production from algal biomass: a review. Environmental Chemistry Letters19:209-229.

179. Pei P, Zhang C, Li J, Chang S, Li S, Wang J, Zhao M, Li J, Yu M, Chen X (2014) Optimization of NaOH pretreatment for enhancement of biogas production of banana pseudo-stem fiber using response surface methodology. BioResources 9:5073-5087.

180. Pereira A, Maraschin M (2015) Banana (Musa spp) from peel to pulp: ethnopharmacology, source of bioactive compounds and its relevance for human health. Journal of ethnopharmacology 160:149-163.

181. Pisutpaisal N, Boonyawanich S, Saowaluck H (2014) Feasibility of biomethane production from banana peel. Energy Procedia 50:782-788.

182. Pothan LA, Potschke P, Habler R, Thomas S (2005) The static and dynamic mechanical properties of banana and glass fiber woven fabric-reinforced polyester composite. Journal of composite materials 39:1007-1025.

183. Pothavorn P, Kitdamrongsont K, Swangpol S, Wongniam S, Atawongsa K, Svasti J, Somana J (2010) Sap phytochemical compositions of some bananas in Thailand. Journal of Agricultural and Food Chemistry 58:8782-8787.

184. Powthong P, Jantrapanukorn B, Suntornthiticharoen P, Laohaphatanalert K (2020) Study of prebiotic properties of selected banana species in Thailand. Journal of food science and technology 57:2490-2500.

185. Prasad S, Singh A, Joshi HC (2007) Ethanol as an alternative fuel from agricultural, industrial and urban residues. Resources, Conservation and Recycling 50:1-39.
186. Qamar S, Shaikh A (2018) Therapeutic potentials and compositional changes of valuable compounds from banana-A review. Trends in Food Science & Technology 79:1-9.
187. Rabbani GH, Teka T, Zaman B, Majid N, Khatun M, Fuchs GJ (2001) Clinical studies in persistent diarrhea: dietary management with green banana or pectin in Bangladeshi children. Gastroenterology 121:554-60.
188. Raksakulpiwat Y, Kluengsamrong J, Somnuk U, Sutapun W, Supakarn N (2005) 31st Congress on Science and Technology of Thailand. Thailand: Suranaree University of Technology.
189. Rattanavichai W, Cheng W (2014) Effects of hot-water extract of banana (Musa acuminata) fruit's peel on the antibacterial activity, and anti-hypothermal stress, immune responses and disease resistance of the giant freshwater prawn, Macrobrachium rosenbegii. Fish & shellfish immunology 39:326-35.
190. Ravi U, Menon L, Gomathy G (2011) Development and quality assessment of value added plantain stem juice incorporated with grape juice. Indian Journal of Natural Products and Resources 2:204-210.
191. Reddy CA, Prashanthi N, Babu PH, Mahale JS (2015) Banana peel as a biosorbent in removal of nitrate from water. International advanced research journal in science, engineering and technology 2:94-98.
192. Renewable energy market update Outlook for 2022-2023. Available online: https://www.iea.org/reports/renewable-energy-market-update-may-2022
193. Revadigar V, Al-Mansoub MA, Asif M, Hamdan MR, Majid AM, Asmawi MZ, Murugaiyah V (2017) Anti-oxidative and cytotoxic attributes of phenolic rich ethanol extract of Musa balbisiana Colla inflorescence. Journal of Applied Pharmaceutical Science 7:103-110.
194. Richter ER, Vore LA (1989) Antimicrobial activity of banana puree. Food microbiology 6:179-87.
195. Romelle FD, Rani A, Manohar RS (2016) Chemical composition of some selected fruit peels. European Journal of Food Science and Technology 4:12-21.

196. Rosales-Calderon O, Arantes V (2019) A review on commercial-scale high-value products that can be produced alongside cellulosic ethanol. Biotechnology for biofuels 12:1-58.

197. Saha S, Sharma A, Purkayastha S, Pandey K, Dhingra S (2019) Bio-plastics and biofuel: is it the way in future development for end users? In Plastics to Energy, William Andrew Publishing, pp 365-376.

198. Saravanan K, Aradhya SM (2011a) Polyphenols of pseudostem of different banana cultivars and their antioxidant activities. Journal of Agriculture and Food Chemistry 59: 3613-3623.

199. Saravanan K, Aradhya SM (2011b) Potential nutraceutical food beverage with antioxidant properties from banana plant bio-waste (pseudostem and rhizome). Food and Function 2:603-610.

200. Sarawong C, Schoenlechner R, Sekiguchi K, Berghofer E, Ng PK (2014) Effect of extrusion cooking on the physicochemical properties, resistant starch, phenolic content and antioxidant capacities of green banana flour. Food chemistry 143:33-39.

201. Sarma PP, Gurumayum N, Verma AK, Devi R (2021) A pharmacological perspective of banana: Implications relating to therapeutic benefits and molecular docking. Food & Function 12:4749-4767.

202. Sawhney S, Mir M, Amin S (2011) Phytochemical screening and antioxidant properties of Bauhinia variegate. International Journal of Research in Phytochemistry & Pharmacology 2:21-24.

203. Schieber A, Stintzing FC, Carle R (2001) By-products of plant food processing as a source of functional compounds—recent developments. Trends in Food Science & Technology 12:401-413.

204. Shanmuga SC, Subramanian S (2011) Biochemical evaluation of hypoglycemic activity of Musa paradisiaca (plantain) flowers in STZ-induced experimental diabetes in rats. Asian Journal of Research in Chemistry 4:827-833.

205. Sharma V, Lohia N, Handa V, Baranwal M (2017) Amomum subulatum seed extract exhibit antioxidant, cytotoxic and immune-suppressive effect. Indian Journal of Biochemistry and Biophysics 54:135-139.

206. Sharmila YD (2015) Development and evaluation of banana blossom incorporated dark chocolate. International Journal of Science and Research 4:1409-1411.

207. Shukla S, Gupta S (2010) Apigenin: a promising molecule for cancer prevention. Pharmaceutical research 27:962-978.
208. Sidhu JS, Zafar TA (2018) Bioactive compounds in banana fruits and their health benefits. Food Quality and Safety 2:183-188.
209. Singh AK, Rath S, Kumar Y, Masih H, Jyotsna K, Peter, Benjamin JC, Singh PK, Singh DP (2014) Bio-ethanol production from banana peel by simultaneous saccharification and fermentation process using cocultures *Aspergillus niger* and *Saccharomyces cerevisiae*. International Journal of Current Microbiology Applied Sciences 3:84-96.
210. Singh JP, Kaur A, Shevkani K, Singh N (2016) Composition, bioactive compounds and antioxidant activity of common Indian fruits and vegetables. Journal of food science and technology 53:4056-4066.
211. Singh R, Kaushik R, Gosewade S (2018) Bananas as underutilized fruit having huge potential as raw materials for food and non-food processing industries: A brief review. The Pharma Innovation Journal 7:574-580.
212. Singh RK, Patil T, Sawarkar AN (2020) Pyrolysis of garlic husk biomass: physico-chemical characterization, thermodynamic and kinetic analyses. Bioresource Technology Reports12:1-10.
213. Singh S, Sawarkar AN (2020) Thermal decomposition aspects and kinetics of pyrolysis of garlic stalk. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects1-11.
214. Singh SK, Kesari AN, Rai PK, Watal G (2007) Assessment of glycemic potential of *Musa paradisiaca* stem juice. Indian Journal of Clinical Biochemistry 22: 48-52.
215. Singh YN (1986) Traditional medicine in Fiji: some herbal folk cures used by Fiji Indians. Journal of ethnopharmacology 15:57-88.
216. Sirajudin ZN, Ahmed QU, Chowdhury AJ, Kamarudin EZ, Khan AV, Uddin AB (2014) Antimicrobial activity of banana (Musa paradisiaca L.) peels against food borne pathogenic microbes. Journal of Pure and Applied Microbiology 8:3627-3639.
217. Sivaram V, Saha S (2018) The geopolitical implications of a clean energy future from the perspective of the United States. In The geopolitics of renewables 125-162.
218. Sivasamugham LA, Nimalan V, Subramaniam G (2021) Antibacterial effects of Musa sp. ethanolic leaf extracts against methicillin-resistant and susceptible Staphylococcus aureus. South African Journal of Chemical Engineering 35:107-110.

219. Soccol, Carlos R., Luciana PS Vandenberghe, Bill Costa, Adenise Lorenci Woiciechowski, Julio Cesar de Carvalho, Adriane BP Medeiros, Antonio Maria Francisco, Luiz José Bonomi (2005) Brazilian biofuel program: an overview. Journal of Scientific and Industrial Research 64:897-904.

220. Souza O, Schulz MA, Fischer GA, Wagner TM, Sellin N (2012) Energia alternativa de biomassa: bioetanol a partir da casca e da polpa de banana. Revista Brasileira de Engenharia Agrícola e Ambiental 16:915-921.

221. Sritrakul N, Nitisinprasert S, Keawsompong S (2017) Evaluation of dilute acid pretreatment for bioethanol fermentation from sugarcane bagasse pith. Agriculture and Natural Resources 51:512-519.

222. Subagyo A, Chafidz A (2018) Banana pseudo-stem fiber: Preparation, characteristics, and applications. Banana nutrition-function and processing kinetics 28:1-19.

223. Sulaiman SF, Yusoff NAM, Eldeen IM, Seow EM, Sajak AAB, Ooi KL (2011) Correlation between total phenolic and mineral contents with antioxidant activity of eight Malaysian bananas (Musa sp.) Journal of Food Composition and Analysis 24:1-10.

224. Sumathy N, Sumathy J (2011) Antibacterial and antifungal activity of musa fruit peels against skin and gastrointestinal tract diseases. Herbal Tech Industry 2:9-11.

225. Tan HL, Chan KG, Pusparajah P, Saokaew S, Duangjai A, Lee LH, Goh BH (2016) Anti-cancer properties of the naturally occurring aphrodisiacs: icariin and its derivatives. Frontiers in pharmacology 7:1-18.

226. Tewari HK, Marwaha SS, Rupal K (1986) Ethanol from banana peels. Agricultural wastes 16:35-146.

227. Tewtrakul T, Subhadhirasakul S (2007) Anti-allergic activity of some selected plants in the Zingiberaceae family. Journal of Ethnopharmacology 109:535-538.

228. Thakur S, Shrivastava B, Ingale S, Kuhad RC, Gupte A (2013) Degradation and selective ligninolysis of wheat straw and banana stem for an efficient bioethanol production using fungal and chemical pretreatment. 3 Biotech 3:365-372.

229. Thancharoen T (2015) Rotten banana waste management for bioethanol producing ethanologenic yeasts. In International Conference on Biological, Civil and Environmental Engineering (BCEE-20153-4).

230. The European Committee on Antimicrobial Susceptibility Testing (2018) Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 8.0. http;//www.eucast.org.
231. Tibolla H, Pelissari FM, Martins JT, Vicente AA, Menegalli FC (2018) Cellulose nanofibers produced from banana peel by chemical and mechanical treatments: characterization and cytotoxicity assessment. Food Hydrocolloids 75:192-201.
232. Toh PY, Leong FS, Chang SK, Khoo HE, Yim HS (2016) Optimization of extraction parameters on the antioxidant properties of banana waste. Acta Scientiarum Polonorum Technologia Alimentaria 15:65-78.
233. Tomás‐Barberán FA, Clifford MN (2000) Dietary hydroxybenzoic acid derivatives–nature, occurrence and dietary burden. Journal of the Science Food and Agriculture 80:1024-1032.
234. Trache D, Tarchoun AF, Derradji M, Hamidon TS, Masruchin N, Brosse N, Hussin MH (2020) Nanocellulose: from fundamentals to advanced applications. Frontiers in Chemistry 8:1-33.
235. Tripathi SK, Kaur D, Alam I, Bhardwaj NK, Pathak P, Kumar S (2020) Utilization of different microbes to enhance the biogas production from banana stem juice. Journal of Environmental Engineering 146.
236. Tsamo CV, Herent MF, Tomekpe K, Emaga TH, Quetin-Leclercq J, Rogez H, Larondelle Y, Andre C (2015) Phenolic profiling in the pulp and peel of nine plantain cultivars (Musa sp.). Food chemistry 167:197-204.
237. Uchoa PZ, Porto RC, Battisti R, Marangoni C, Sellin N, Souza O (2021) Ethanol from residual biomass of banana harvest and commercialization: a three-waste simultaneous fermentation approach and a logistic-economic assessment of the process scaling-up towards a sustainable biorefinery in Brazil. Industrial Crops and Products 174:114170.
238. Umar Lule S, Xia W (2005) Food phenolics, pros and cons: A review. Food Reviews International 21:367-388.
239. Vasundhara M, Baranwal M, Sivaramaiah N , Kumar A (2017) Isolation and characterization of trichalasin-producing endophytic fungus from Taxus baccata. Annals of Microbiology 67:255-261.
240. Verma OP (2017) Comparative study of alkaloids from different parts of Ravolfia tetraphylla. Journal of pharmacognosy and phytochemistry 1: 770-772.

241. Vigneswaran C, Pavithra V, Gayathri V, Mythili K (2015) Banana fiber: scope and value added product development Journal of textile and apparel, technology and management 9:1-7.
242. Vilela C, Santos SA, Villaverde JJ, Oliveira L, Nunes A, Cordeiro N, Freire CS, Silvestre AJ (2014) Lipophilic phytochemicals from banana fruits of several Musa species. Food chemistry 162:247-252.
243. Villaverde JJ, Oliveira L, Vilela C, Domingues RM, Freitas N, Cordeiro N, Freire CS, Silvestre AJ (2013) High valuable compounds from the unripe peel of several Musa species cultivated in Madeira Island (Portugal). Industrial crops and products 42:507-512.
244. Voca N, Ribic B (2020) Biofuel production and utilization through smart and sustainable biowaste management. Journal of cleaner production 259:120742.
245. Vu HT, Scarlett CJ, Vuong QV (2017) Optimization of ultrasound‐assisted extraction conditions for recovery of phenolic compounds and antioxidant capacity from banana (Musa cavendish) peel. Journal of Food Processing and Preservation 41:1-18.
246. Waghmare AG, Arya SS (2016) Utilization of unripe banana peel waste as feedstock for ethanol production. Bioethanol 2:146-156.
247. Waghmare JS, Kurhade AH (2014) GC-MS analysis of bioactive components from banana peel (Musa sapientum peel). European journal of experimental biology 4:10-15.
248. Wahab NB, Ismail SN, Abidin MH (2020) Physicochemical and Sensory Characteristics of Banana Blossom Nuggets. International Journal of Research and Innovation Management 6:56-66.
249. Waldron KW, Nichols E (2009) Composting of food-chain waste for agricultural and horticultural use. In Handbook of waste management and co-product recovery in food processing, Woodhead Publishing, pp 583-627.
250. Walkley A, Black IA (1934) An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil science 37:29-38.
251. Weremfo A, Adinortey MB, Pappoe ANM (2011) Haemostatic effect of the stem juice of Musa paradisiaca L.(Musaceae) in Guinea pigs. Advances in Biological Research 5:190-192.

252. Winkler LW (1888) The determination of dissolved oxygen in water. Berlin DeutChem Gas 21:2843-55.
253. Wu C, Chen X, Cai Y, Zhou X, Xu S, Huang H, Zhang L, Zhou X, Du C, Zhang Y, Song J (2020) Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA internal medicine 180:934-943.
254. Xi W, Fang B, Zhao Q, Jiao B , Zhou Z (2014) Flavonoid composition and antioxidant activities of Chinese local pummelo (Citrusgrandis Osbeck.) varieties. Food Chemistry 161: 230–238.
255. Xie Y, Zheng Y, Dai X, Wang Q, Cao J , Xiao J (2015) Seasonal dynamics of total flavonoids contents and antioxidant activity of Dryopteriserythrosora. Food Chemistry 186:113–118.
256. Yasukawa K, Ko SK, Whang WK (2016) Inhibitory effects of the aerial parts of Epimedium koreanumon TPA-induced inflammation and tumour promotion in two-stage carcinogenesis in mouse skin. Journal of Pharmacy and Nutrition Sci 6:38–42.
257. Yin X, Quan J, Kanazawa T (2008) Banana prevents plasma oxidative stress in healthy individuals. Plant Foods for Human Nutrition 63:71-76.
258. Zafar IM, Saleha A, Hoque MME, Sohel RM (2011) Antimicrobial and cytotoxic properties of different extracts of Musa sapientum L. subsp. sylvestris. International Research Journal of Pharmacy 2:62-65.
259. Zhang H, Zhang P, Ye J, Wu Y, Liu J, Fang W, Xu D, Wang B, Yan L, Zeng G (2017) Comparison of various pretreatments for ethanol production enhancement from solid residue after rumen fluid digestion of rice straw. Bioresource technology 247:147-156.
260. Zhang L, Xu CC, Champagne P (2010) Overview of recent advances in thermo-chemical conversion of biomass. Energy conversion and management 51:969-982.

APPENDIX

Media Composition

Composition of Media used YEPD Agar

Peptone	10.0g
Yeast extract	3.0g
Dextrose	20.0g
Agar	15.0g
Distilled water	1000 ml

Mueller Hinton Broth

Ingredient	**Quantity (Gms/Lit)**
Infusion from Beef	300 g
Casein acid hydrolysate	17 g
Starch	1 g
Final pH (at 25^0C)	7.3

Volume made upto 1000 ml with distilled water

Sterilized by autoclaving at 15 Ibs pressure (121℃) for 15 minutes

Mueller Hinton Agar	Mueller Hinton Broth containing 20 g/L Agar

Sabouraud Dextrose Broth

Ingredient	10 g
Mixture of peptic digest of animal tissue & pancreatic digest of casein (1:1)	20 g
Dextose	5.6
Final pH (at 25^0C)	

Volume made upto 1000 ml with distilled water

Sterilized by autoclaving at 15 Ibs pressure (121^0C) for 15 minutes

Sabouraud Dextrose Agar	Sabouraud Dextrose Broth containing 20 g/L Agar

<u>05 Mc Farland</u>

$BaCl_2$ (0.048 M)	0.5 ml
H_2SO_4 (0.18 M)	99.5 ml

<u>Saline</u>

NaCl	0.85 g
DDW	100.0 ml

Complete DMEM Medium

Ingredients	mg/L
INORGANIC SALTS	
Calcium chloride dehydrate	265.000
Ferric nitrate nonahydrate	0.100
Magnesium sulphate anhydrous	97.720
Potassium chloride	400.000
Sodium bicarbonate	1500.000
Sodium chloride	6400.000
Sodium phosphate monobasic anhydrous	109.000
AMINO ACIDS	
Glycine	30.000
L-Arginine hydrochloride	84.000
L-Cystine dihydrochloride	62.570
L-Glutamine	584.000
L-Histidine hydrochloride monohydrate	42.000
L-Isoleucine	105.000
L-Leucine	105.000
L-Lysine hydrochloride	146.000
L-Methionine	30.000
L-Phenylalanine	66.000

L-Serine	42.000
L-Threonine	95.000
L-Tryptophan	16.000
L-Tyrosine disodium salt	103.790
L-Valine	94.000
VITAMINS	
Choline chloride	4.000
D-Ca-Pantothenate	4.000
Folic acid	4.000
Nicotinamide	4.000
Pyridoxal 5 phosphate	4.000
Riboflavin	0.400
Thiamine hydrochloride	4.000
i-Inositol	7.200
OTHERS	
D-Glucose	4500.000
Phenol red sodium salt	15.900
Sodium pyruvate	110.000

Fetal Bovine Serum
10% of DMEM
Antibiotic Solution 100X
500µL
Amphotericin B
250 µL

Antibiotic Solution (100X Liquid)

Ingredients	Quantity
Penicillin	10,000 units
Streptomycin	10 mg/mL
Normal Saline	0.9%

Buffer Solutions

Trypsin EDTA solution (1X)

Ingredients	Quantity
Trypsin	0.25%
EDTA	0.002%

Dulbecco ‘s Phosphate Buffered saline without Phenol Red

PBS (1X)

Ingredients	mg/L
Disodium hydrogen phosphate anhydrous	726
Potassium dihydrogen phosphate	210
Sodium chloride	9000

ANNEXURE

List of Chemicals	Make
DMEM medium	HiMedia
Fetal bovine serum (FBS)	HiMedia
Antibiotic solution (penicillin and streptomycin),	HiMedia
Amphotericin B	HiMedia
Trypsin EDTA	HiMedia
MTT	HiMedia
DPPH	HiMedia
Ethyl acetate solvent	MERCK
Dichloromethane solvent	MERCK
Plasticware	Tarsons
Glass ware	Schott Duran

LIST OF PUBLICATIONS

Published

1. Gupta, G., Baranwal, M., Saxena, S., & Reddy, M. S. (2019). Utilization of banana stem juice as a feedstock material for bioethanol production. CLEAN–Soil, Air, Water, 47(9), 1900047. https://doi.org/10.1002/clen.201900047 IF 2.404

2. Gupta, G., Baranwal, M., Saxena, S., & Reddy, M. S. (2022). Utilization of banana waste as a resource material for biofuels and other value-added products. Biomass Conversion and Biorefinery, 1- 20. https://doi.org/10.1007/s13399-022-02306-6 IF 4.050

3. Gupta, G., Saxena, S., Baranwal, M., & Reddy, M. S. (2022). *In vitro* evaluation of bioactive properties of banana sap. Biologia, 1-12. https://doi.org/10.1007/s11756-022-01159-8 IF 1.653

PARTICIPATION IN CONFERENCES

National

1. Geetika Gupta and M. S Reddy (2016): Importance of banana sap as a resource material in the national conference on Fungal Biotechnology (NCFB-2016) organized at Birla institute of Scientific Research, Jaipur, Rajasthan from 16th -18th November 2016.

International

2. Geetika Gupta, Manoj Baranwal,Sanjai Saxena and M. S. Reddy (2018): Utilization of banana stem juice as a nutritional resource at the International conference on Food Security Challenges and Opportunities held on December 07-08, 2018 at Department of Biotechnology, TIET, Patiala, India

Thesis

ORIGINALITY REPORT

11%	7%	7%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	tudr.thapar.edu:8080 Internet Source	2%
2	Heba Sayed Mostafa. "Banana plant as a source of valuable antimicrobial compounds and its current applications in the food sector", Journal of Food Science, 2021 Publication	1%
3	link.springer.com Internet Source	1%
4	www.science.gov Internet Source	<1%
5	Roger Hoel Bello, Poliana Linzmeyer, Cláudia Maria Bueno Franco, Ozair Souza et al. "Pervaporation of ethanol produced from banana waste", Waste Management, 2014 Publication	<1%
6	Supaluck Kraithong, Utthapon Issara. "A strategic review on plant by-product from banana harvesting: A potentially bio-based ingredient for approaching novel food and	<1%

CLEAN Soil Air Water
www.clean-journal.com

Utilization of Banana Stem Juice as a Feedstock Material for Bioethanol Production

*Geetika Gupta, Manoj Baranwal, Sanjai Saxena, and Mondem Sudhakara Reddy**

Bananas are widely cultivated in tropical and subtropical countries and about 220 tons of biomass waste is produced per hectare of banana plantation. Banana pseudostem contains nearly 90% of moisture and about 4–5 m^3 sap is generated from one ton of dried stem with high chemical oxygen demand(COD) and biological oxygen demand (BOD). The feasibility of using banana sap as a feedstock to produce ethanol is evaluated in this study. Banana sap is obtained by crushing the pseudostems and concentrated ten times and supplementing with other industrial byproducts such as corn steep liquor(CSL), spent wash (SW), and yeast extract (YE) for ethanol production. Acid and alkali hydrolyzes are performed to enhance the sugar levels of the sap before fermentation. Two different strains of *Saccharomyces cerevisiae* (MTCC170 and MTCC180) are used for fermentation. In general, supplementation of banana sap with industrial byproducts significantly enhanced the ethanol production. The maximum ethanol production (2.5 g^{-1}) is observed with concentrated banana sap supplemented with 25% SW (v/v) with MTCC170, which is 16-fold higher than banana sap alone. Theethanol content is also higher in alkali-hydrolyzed banana sap supplemented with 25% SW compared to control. These results suggest that banana sap can be used as a renewable source to produce ethanol by supplementing with other industrial byproducts.

1. Introduction

Bananas are widely grown in large plantations in tropical and subtropical countries and approximately 106.54 million tons of banana fruits are produced every year, which contributes to 16% of total food production in the world.[1] Cultivation of banana generates a large amount of byproducts, which includes pseudostems, leaves, peduncle, and those ungraded bananas that do not meet the high-quality standards. The pseudostems and leaves are left in the fields. The byproducts left in the open produce copious amount of gases such as carbon dioxide, methane and hydrogen sulfide. It can also lead to an outbreak of notorious banana fungus, *Fusraium oxysporum*.[2] The banana pseudostem gives a single bunch of fruit before drying and replaced by new pseudostem. The pseudostems supply nutrients from the soil to the fruits and become waste after the harvest of banana fruits. It is estimated that about 220 tons of biomass waste is produced in each hectare of banana plantation.[3]

Climate change and depletion of natural reserves arising from fossil fuel use are leading to enhanced investment in renewable energy. It has been reported that presently 14% of total energy comes from renewable sources,[4] of which 11% comes from biomass.[5] It has been expected that by 2040, nearly 50% of energy generated will come from sun, wind, and biomass.[6] As per the International Energy Agency report, equal percentage of land is required to grow sufficient feedstock for production of biofuels that will be displaced by the biofuel.[7] Hence, this option may not be sustainable as it demands more land to grow energy crops. Therefore, it is essential to evaluate other biomass resources like agricultural and municipal residuals and wastes. The biomass residues, such as straw, sugarcane bagasse, corn stover, sugar beet pulp and tailings and fruit-processing wastes, were explored for ethanol and biogas production.[8–10]

In the present study, utilization of banana pseudostem juice (sap) as a feedstock material for ethanol production is presented. As the sugar content in the fruits and peels of banana is high and so is the cellulosic fiber content of stems and leaves.[11–13] The fruits of banana, as well as residual materials are rich in amylose and lignocellulose and need to be hydrolyzed and then fermented for ethanol production. The enzymatic hydrolysis of ligninolytic material and acid hydrolysis of amylaceous material are frequently used methods for ethanol production from banana.[9] Many studies have been reported on the utility of these wastes for the ethanol production. Bhatia et al. and Joshi et al. reported ethanol production from wasted banana peels,[14,15] while Harish et al. used stems and leaves by using *Clostridium* sp.[16] Pazmiño-Hernandez et al. used wasted banana peduncle as feedstock for biofuel production.[17] Currently not much work has been done on the production of ethanol using banana sap as a feedstock.

Banana pseudostem is rich in fibers and when processed, yields pulp that can be used for paper production.[18] The major

G. Gupta, Dr. M. Baranwal, Prof. S. Saxena, Prof. M. S. Reddy
Department of Biotechnology
Thapar Institute of Engineering and Technology
Patiala 147004, Punjab, India
E-mail: msreddy@thapar.edu

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/clen.201900047

DOI: 10.1002/clen.201900047

component of banana stem is the sap, which constitutes up to 90% of the stem. In most cases, the sap is discarded as it has high water content. It is also rich in proteins, carbohydrates, and fibers and serves as a potential feedstock for biofuel production.[19] About 4–5 m^3 sap is generated from one ton of dried stems. In India, there is potential of generation of about 40–50 million tons of banana sap per annum.[20] Apart from this, the sap also has very high chemical oxygen demand (COD) and biological oxygen demand (BOD).[21] The draining of sap as wastewater will create a problem to environment, whereas its utilization as biomass for production of biofuel will serve as an extra source for renewable energy. In the present study, banana sap has been explored for ethanol production with other industrial byproducts to use banana sap effectively.

2. Experimental Section

2.1. Biological Materials

Saccharomyces cerevisiae strains (MTCC170 and MTCC180) used in this study were procured from the Institute of Microbial Technology, Chandigarh, India. These cultures were maintained on YEPD medium (3 g yeast extract (YE), 10 g peptone, 20 g dextrose, 1 L distilled water, and 15 g agar). Banana stems were collected from Thapar Institute of Engineering & Technology Campus, Patiala. The stems were crushed through sugarcane presser and filtered through a cheesecloth to remove solid particles. The clarified liquid was collected and concentrated ten times using a rotary evaporator and was further stored at 4 °C. The corn steep liquor (CSL) was procured from Sukhjit Starch and Chemicals, Phagwara, Punjab, India, and spent wash (SW) from Patiala Distillers and Manufacturers, Mann village, Patiala, India. The YE was procured from Himedia (Mumbai, India).

2.2. Analysis of Samples

Initially, the banana sap was analyzed for the different physicochemical properties such as pH, total organic carbon, fatty acids, COD, and BOD. Banana sap (ten times concentrated), SW, and CSL were then analyzed for COD, BOD, total suspended solids (TSS), total dissolved solids (TDS), total solids (TS), sugar content, phosphorus content, and nitrogen levels according to the standard methods.[22]

2.3. Hydrolysis of Banana Sap

The ten times concentrated banana sap was subjected for acid and alkali hydrolyses to enhance the sugar level. Banana sap was hydrolyzed with concentrated hydrochloric acid (HCl) as described by Hernández-Salas et al.[23] Briefly, HCl was added to banana sap samples to reach a final concentration of 0.5, 1.0, and 1.5 N (v/v). Each suspension was then autoclaved at 121 °C for 30 min. After hydrolysis, the pH of the sample was adjusted to 5.6 with 0.1 N NaOH. Alkali hydrolysis was performed by adding 0.5, 1.0, and 1.5 N NaOH to banana sap (v/v) and autoclaved at 121 °C at 30 min, cooled, and pH was adjusted to 5.6. The sugar content was estimated in hydrolyzed samples.

2.4. Ethanol Fermentation

The fermentation of banana sap seems to be a new approach and not much information is available about the optimal conditions and the microorganisms used for ethanol production. Therefore, in the present study, ethanol fermentation of the ten times concentrated hydrolyzed banana sap was evaluated by mixing other industrial byproducts. Two strains *S. cerevisiae* MTCC170 and MTCC180 were selected as biocatalysts for fermentation as these strains were well established for fermentation and known to ferment glucose and fructose.[24,25] Batch fermentations were carried out in triplicate in sterilized 250-mL Erlenmeyer flasks with capped with screw cap. The working volume of each flask was 50 mL.

To optimize the time period for maximum fermentation, 50 mL of the banana sap supplemented with 0.3% YE (v/w) was taken in 250-mL Erlenmeyer flasks and the pH was adjusted to 5.6. Then the two yeast strains *S. cerevisiae* MTCC170 and MTCC180 were inoculated separately and the flasks were incubated at 30 °C in a shaker at 150 rpm. The samples were analyzed for ethanol production at different time intervals, i.e., at days 1, 2, 3, 4, and 5.

The acid and alkali hydrolyzed samples mixed along with CSL, SW, and YE resulted in samples such as sap, sap + 1% CSL, sap + 3% CSL, sap + 5% CSL (v/v), sap + 1% YE, sap + 3% YE, sap + 5% YE (v/w), sap + 25% SW, sap + 50% SW, and sap+ 75% SW (v/v). The sugar content in these samples was determined by dinitrosalicylic acid method as described by Miller.[26] Banana sap supplemented with the above-mentioned byproducts (50 mL) was taken in 250-mL Erlenmeyer flasks and the pH was adjusted to 5.6. Then the two yeast strains *S. cerevisiae* MTCC170 and MTCC180 were inoculated separately and the flasks were incubated at 30 °C in a shaker at 150 rpm. The samples were harvested after 4 days and analyzed for ethanol production. The ethanol content in the fermentation medium was estimated using the chromic acid method as described by Caputi et al.[27] Briefly, 10 mL of fermented broth was distilled, approximately 5 mL of distillate was collected and mixed with 25 mL of potassium dichromate and kept in water bath at 80 °C for 15 min. The samples were cooled and their optical density (600 nm) was recorded by UV-vis spectrophotometry. Ethanol standards were made by using ethanol/water (v/v) solution.

All the experiments were carried out in triplicate. The data were analyzed by ANOVA by using GraphPad Prism software version 5.

3. Results

3.1. Analysis of Samples

The physicochemical characteristics of the banana sap is as follows: pH: 5.6, 6.5 g L^{-1} total organic carbon, 0.9 g L^{-1} fatty acids, 6000 mg L^{-1} BOD, and 24 000 mg L^{-1} COD. The chemical characterization of concentrated banana sap, SW, and CSL is presented in **Table 1** The highest BOD was observed in SW followed by concentrated banana sap and CSL, which indicates that in SW aerobic biological organisms need more amount of dissolved oxygen to break down the organic material. The COD values were

Table 1. The chemical characteristics of the banana sap, corn steep liquor, and spent wash.

Parameter	Concentrated banana sap	Corn steep liquor	Spent wash
pH	5.9 ± 0.3	4.0 ± 0.2	7.5 ± 0.2
Chemical oxygen demand [g L^{-1}]	6.7 ± 2.6	29.6 ± 2.5	70.0 ± 10.0
Biological oxygen demand [g L^{-1}]	24.4 ± 1.8	15.5 ± 2.3	45.6 ± 1.1
Total solids [g L^{-1}]	10.17 ± 0.8	12.56 ± 0.6	11.96 ± 0.3
Total dissolved solids [g L^{-1}]	9.30 ± 0.1	11.97 ± 0.4	11.38 ± 0.2
Total suspended solids [g L^{-1}]	0.39 ± 0.1	0.59 ± 0.2	0.58 ± 0.3
Total phosphorus [g L^{-1}]	0.002 ± 0.0	0.060 ± 0.05	0.002 ± 0.03
Total nitrogen [%]	0.4 ± 0.1	0.5 ± 0.0	0.06 ± 0.5
Sugar content [g L^{-1}]	7.1 ± 0.3	2.2 ± 0.2	3.2 ± 0.2

Values are mean ±SD.

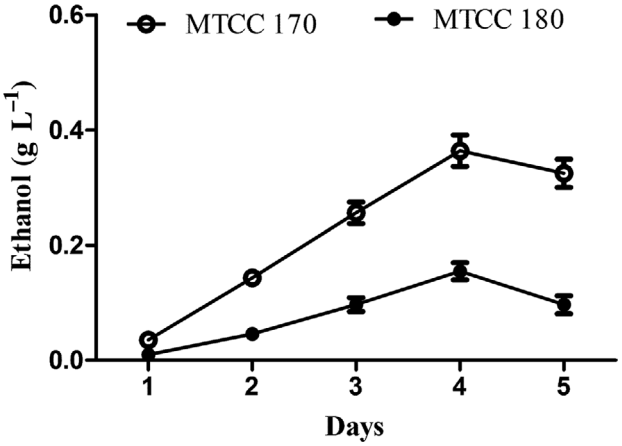


Figure 1. Optimization of number of days for fermentation using concentrated sap supplemented with 0.3% yeast extract inoculated with *Saccharomyces cerevisiae* (MTCC 170 and MTCC 180).

also higher in SW followed by concentrated banana sap and least was observed in CSL. TS, TDS, and TSS were high in CSL and lower in concentrated banana sap. Phosphorus levels were also estimated, which are less in banana sap as compared to the CSL and SW. The nitrogen content was higher in CSL compared to other samples. The sugar content was higher in concentrated banana sap followed by SW.

3.2. Optimization Conditions

To optimize the time period for maximum ethanol production, concentrated banana sap supplemented with 0.3% YE was inoculated with *S. cerevisiae* strains MTCC 170 and MTCC 180. The ethanol production was increased with increase in time period up to 4 days and decreased thereafter. Maximum ethanol production was observed at day 4 of fermentation in both yeast strains. High titer value of ethanol was observed when MTCC 170 was used as inoculant compared to MTCC 180 yeast strain (**Figure 1**). Based on these results, the fermentation time was optimized as 4 days for further experiments.

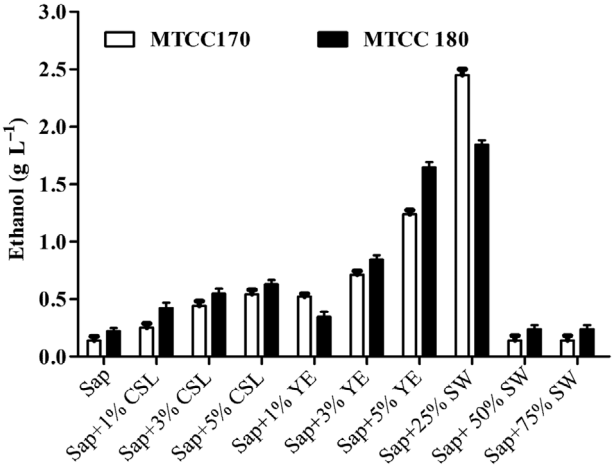


Figure 2. Ethanol production after acid hydrolysis with different banana sap samples (sap: concentrated banana sap, CSL: corn steep liquor, YE: yeast extract, SW: spent wash) inoculated with *S. cerevisiae* strains MTCC170 and MTCC180.

3.3. Ethanol Fermentation

Acid and alkali hydrolyses were performed to enhance the sugar levels in concentrated banana sap. The maximum sugar levels were obtained when the concentrated banana sap was hydrolyzed with 1 N HCl or 1 N NaOH compared to other treatments and, hence, these conditions were selected for further studies. The reducing sugar levels increased from 7.1 to 7.93 g L^{-1} (11% increase) in acid hydrolyzed sample, while it was from 7.1 to 7.5 g L^{-1} (5% increase) in alkali hydrolyzed sample. Reducing sugars are higher in acid hydrolyzed banana sap compared to alkaline hydrolyzed sample. Concentrated banana sap alone when fermented did not produce significant levels of ethanol with both *S. cerevisiae* strains.

In acid hydrolyzed samples, the supplementation of banana sap with industrial byproducts, such as CSL, SW, or YE, and fermented with *S. cerevisiae* strains MTCC170 and MTCC180 enhanced the ethanol production. The MTCC180 strain gave slightly better yield of ethanol than strain MTCC 170 when the sap was supplemented with different ratios of CSL, YE, and SW except the banana sap supplemented with 25% SW (v/v). The maximum ethanol production was observed with concentrated banana sap supplemented with 25% SW (v/v) with MTCC170 where the ethanol content was 2.5 g L^{-1} (16-fold higher) followed by MTCC 180 with 1.85 g L^{-1} (eightfold higher) compared with banana sap alone (**Figure 2**). The ethanol content also significantly increased when the sap is supplemented with 5% YE (v/v) where the two strains showed about sevenfold higher ethanol production than banana sap alone. Banana sap supplemented with 5% CSL yielded three- and twofold higher ethanol content for MTCC170 and MTCC180, respectively, compared to banana sap alone (Figure 2). Analysis of variance showed a significant variation between the yeast strains and the different sap treatments in respect to ethanol production (Table S1, Supporting Information).

In alkali hydrolysis, concentrated sap alone showed lower amount of ethanol production with *S. cerevisiae* strains MTCC170

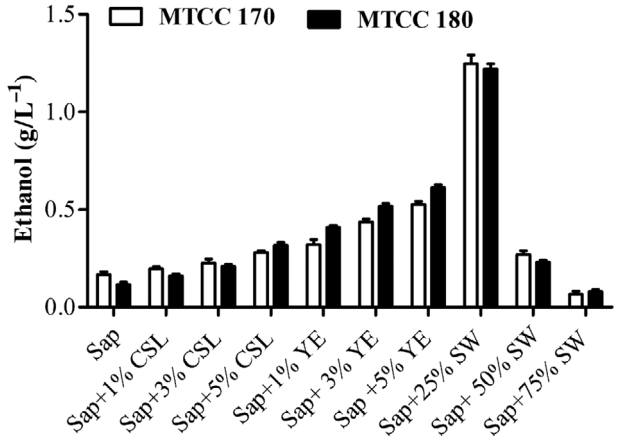


Figure 3. Ethanol production after alkali hydrolysis with different banana sap samples (sap: concentrated banana sap, CSL: corn steep liquor, YE: yeast extract, SW: spent wash) inoculated with *S. cerevisiae* strains MTCC170 and MTCC180.

Table 2. Sugar content in banana sap and banana sap supplemented with corn steep liquor (CSL), yeast extract (YE) and spent wash (SW).

Sample name	Sugar content [g/L]	
	Acid hydrolysis	Alkali hydrolysis
Sap	7.93 ± 0.15	7.5 ± 0.30
Sap + 1% CSL	7.95 ± 0.15	7.52 ± 0.30
Sap + 3% CSL	7.98 ± 0.16	7.55 ± 0.31
Sap + 5% CSL	8.02 ± 0.15	7.59 ± 0.30
Sap + 1% YE	7.93 ± 0.15	7.5 ± 0.30
Sap + 3% YE	7.93 ± 0.15	7.5 ± 0.30
Sap + 5% YE	7.93 ± 0.15	7.5 ± 0.30
Sap + 25% SW	6.64 ± 0.18	6.32 ± 0.18
Sap + 50% SW	5.35 ± 0.20	5.14 ± 0.20
Sap + 75% SW	4.06 ± 0.23	3.95 ± 0.23

Values are mean ± SD.

and MTCC180. The ethanol content increased when the sap was supplemented with other industrial byproducts such as CSL, SW, and YE. The maximum ethanol production was observed when the sap was supplemented with 25% SW (v/v) compared to all other treatments (**Figure 3**). The ethanol content was 1.25 and 1.22 g L^{-1} (six- and ninefold higher) in MTCC170 and MTCC180 strains, respectively, in this treatment compared to banana sap alone (Figure 3). Supplementation of YE also increased the production of ethanol with maximum yield recorded with banana sap supplemented with 5% YE where the content of ethanol increased two- and fourfold with MTCC170 and MTCC180 strains, respectively (Figure 3). Compared to control, CSL also increased the ethanol production and the maximum ethanol content was observed when sap was supplemented with 5% CSL where it increased about one- and 1.5-fold higher compared to banana sap alone. Two-way ANOVA revealed a significant variation among the treatments as well as between the yeast strains in relation to ethanol production (Table S1, Supporting Information). When compared to acid and alkali hydrolyzed banana samples, acid hydrolyzed samples supplemented with CSL, SW, and YE yielded more ethanol than alkali hydrolyzed samples.

4. Discussion

Characterization of banana sap along with other industrial byproducts showed that these products contain high-strength organic waste (COD, BOD), solids (TS, TDS, and TSS), nutrients (phosphorus and nitrogen), and before disposal, those require proper treatment.[28] The BOD and COD levels of the banana sap are much higher compared to the measured sugar levels (**Table 2**) after hydrolysis of the banana sap. This might be due to the presence of higher total organic carbon (6.5 g L^{-1}) in unhydrolyzed sap, presence of volatile fatty acids, small amount of proteins and organic acids, which contribute to its higher BOD and COD. Various pretreatment methods, such as treating the substrates with concentrated acids, solvents, and metal complexes or wet oxidation, are widely practiced for ethanol production.[29] These methods are expensive when compared to the traditional glucose-based production of ethanol. Steam and alkali-based pretreatments are the best methods suited for pretreatment based on the economic feasibility.[30] Alkaline pretreatment has shown promising results to effectively remove lignin without altering the carbohydrate portion of the material.[17] In the present study, the banana sap was subjected for acid and alkali hydrolysis to increase sugar levels, which could be used for ethanol produced.

The presence of high amounts of sugar alone has no increase in amount of ethanol production. It is well established that presence of sugar alone for fermentation might stress the organism and that ethanol might not be produced effectively. So, when nitrogen source is supplemented into the medium it might enhance the efficiency of organism to produce ethanol.[31] To enhance the ethanol production in sorghum juice, peptone was used as nitrogen source.[17] CSL is an inexpensive alternative nitrogen source compared to more expensive materials such as YE and peptone.[32] We also observed that when CSL was included in banana sap at different proportions (1%, 3%, and 5%), it enhanced the ethanol fermentation. The use of CSL gave better ethanol yield in both acid and alkali hydrolyzed samples with *S. cerevisiae* strains MTCC170 and MTCC80 used in this study.

SW is the residual liquid waste generated during alcohol production. Pollution caused by SW poses a significant threat to the environment.[33–35] The SW is an inexpensive source, which is easily available.[18] SW is good source of carbon and sugar with less nitrogen and also would supply ancillary factors that could be required for effective fermentation.[33] Hence, in the present study, banana sap was mixed with 25%, 50%, and 75% SW (v/v) and it was observed that banana sap and 25% SW (v/v) yielded the highest titer of ethanol with both *S. cerevisiae* strains MTCC170 and MTCC180.

YE is proven to be very efficient for increasing fermentation rate because it mainly consists of amino acids, peptides, nucleotides, and other soluble components of yeast cells.[36] We observed that when YE was added at different concentrations (1%, 3%, and 5%) as nitrogen source, the ethanol production was enhanced in both acid and alkali hydrolyzed samples inoculated with *S. cerevisiae* strains MTCC170 and MTCC180.

5. Conclusions

In the present study, banana sap supplemented with other industrial byproducts was explored for bioethanol production. The ethanol content increased with supplementation of byproducts, such as CSL, SW, and YE, compared to the sap alone. The maximum ethanol content was recorded when the sap is mixed with 25% SW (v/v) with both *S. cerevisiae* strains in acid hydrolyzed samples compared to sap alone. Supplementation of banana sap with 5% YE also increased the ethanol production by eight- and sixfold in acid hydrolyzed sample treated with MTCC170 and MTCC180, respectively. The results suggest that the banana sap disposed into the environment can be used as a potential source for bioethanol production. However, studies are required to optimize the hydrolysis of the banana sap, as well as further upconcentration of the sap to 20 or even 50 times, which could be economically viable and also select a suitable yeast strain for its maximum fermentation.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

Authors are thankful to Institute of Microbial Technology (IMTECH), Chandigarh for providing the *Saccharomyces cerevisiae* strains (MTCC170 and MTCC180), and Sukhjit Starch and Chemical Limited, Phagwara and Patiala Distillers & Manufacturers Limited, Patiala, for providing the CSL (CSL) and SW respectively. We are also thankful to Dr. Anita Rajor, School of Energy and environment, TIET for helping in the chemical characterization and Mr. Sandeep Tripathi, ACIRD, Yamunanagar, Haryana, India for helping to analyze the total sugar and fatty acid content of the sap. MSR is thankful to Department of Science and Technology, Government of India, New Delhi for financial support under DST/SSTP/Haryana/345.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

acid hydrolysis, banana sap, ethanol, fermentation, *Saccharomyces cerevisiae*

Received: February 3, 2019
Revised: May 16, 2019
Published online: September 17, 2019

[1] D. Mohapatra, S. Mishra, N. Sutar, *J. Sci. Ind. Res.* **2010**, *69*, 323.
[2] P. Pei, C. Zhang, J. Li, S. Chang, S. Li, J. Wang, M. Zhao, J. Li, M. Yu, X. Chen, *BioResources* **2014**, *9*, 5073.
[3] M. P. Shah, G. Reddy, R. Banerjee, P. R. Babu, I. Kothari, *Process Biochem.* **2005**, *40*, 445.
[4] N. Panwar, S. Kaushik, S. Kothari, *Renew. Sust. Energ. Rev.* **2011**, *15*, 1513.
[5] J. P. Dorian, H. T. Franssen, D. R. Simbeck, *Energy Policy* **2006**, *34*, 1984.
[6] I. Kralova, J. Sjöblom, *J. Dispersion Sci. Technol.* **2010**, *31*, 409.
[7] A. Demirbas, *Energy Convers. Manage.* **2008**, *49*, 2106.
[8] J. Sheehan, A. Aden, K. Paustian, K. Killian, J. Brenner, M. Walsh, R. Nelson, *J. Ind. Ecol.* **2003**, *7*, 117.
[9] J. Nigam, *J. Biotechnol.* **2001**, *87*, 17.
[10] A. Koppar, P. Pullammanappallil, *Energy* **2013**, *60*, 62.
[11] H. Velásquez-Arredondo, A. Ruiz-Colorado, *Energy* **2010**, *35*, 3081.
[12] N. Cordeiro, M. Belgacem, I. Torres, J. Moura, *Ind. Crops Prod.* **2004**, *19*, 147.
[13] J. B. Hammond, R. Egg, D. Diggins, C. G. Coble, *Bioresour. Technol.* **1996**, *56*, 125.
[14] L. Bhatia, S. Paliwal, *Int. J. Biotechnol. Bioeng. Res.* **2010**, *1*, 213.
[15] S. Joshi, R. Dhopeshwarkar, U. Jadhav, R. Jadhav, L. D'souza, J. Dixit, *Indian J. Chem. Technol.* **2001**, *8*, 153.
[16] K. R. Y. Harish, M. Srijana, R. D. Madhusudhan, R. Gopal, *Afr. J. Biotechnol.* **2010**, *9*, 1926.
[17] M. Pazmiño-Hernandez, C. M. Moreira, P. Pullammanappallil, *Biofuels* **2019**, *10*, 473.
[18] N. Reddy, Y. Yang, *Trends Biotechnol.* **2005**, *23*, 22.
[19] P. Pothavorn, K. Kitdamrongsont, S. Swangpol, S. Wongniam, K. Atawongsa, J. Svasti, J. Somana, *J. Agric. Food Chem.* **2010**, *58*, 8782.
[20] *Indian Horticulture Database* (Ed: B. Kumar), Ministry of Agriculture, Govt. of India, New Delhi, India **2012**.
[21] H. Alwi, J. Idris, M. Musa, K. H. K. Hamid, *J. Chem.* **2013**, *2013*, 1.
[22] American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF), *Standard Methods for the Examination of Water and Wastewater* (Eds: E. W. Rice, R. B. Baird, A. D. Eaton, L. S. Clesceri), Apha, Washington, DC, USA **2012**.
[23] J. Hernández-Salas, M. Villa-Ramírez, J. Veloz-Rendón, K. Rivera-Hernández, R. González-César, M. Plascencia-Espinosa, S. Trejo-Estrada, *Bioresour. Technol.* **2009**, *100*, 1238.
[24] K. Suresh, L. V. Rao, *Bioresour. Technol.* **1999**, *68*, 301.
[25] K. Jeyaram, J. P. Tamang, A. Capece, P. Romano, *Antonie Van Leeuwenhoek* **2011**, *100*, 569.
[26] G. L. Miller, *Anal. Chem.* **1959**, *31*, 426.
[27] A. Caputi, M. Ueda, T. Brown, *Am. J. Enol. Vitic.* **1968**, *19*, 160.
[28] M. Galbe, G. Zacchi, in *Biofuels* (Eds: L. Olsson), Springer, New York **2007**, Ch.12.
[29] K. Robak, M. Balcerek, *Food Technol. Biotechnol.* **2018**, *56*, 174.
[30] S. Ingale, S.J. Joshi, A. Gupte, *Braz. J. Microbiol.* **2014**, *45*, 885.
[31] L. Laopaiboon, S. Nuanpeng, P. Srinophakun, P. Klanrit, P. Laopaiboon, *Bioresour. Technol.* **2009**, *100*, 4176.
[32] R. W. Liggett, H. Koffler, *Bacteriol. Rev.* **1948**, *12*, 297.
[33] R. Jain, S. Srivastava, *Physiol. Mol. Biol. Plants* **2012**, *18*, 95.
[34] S. Mohana, B. K. Acharya, D. Madamwar, *J. Hazard. Mater.* **2009**, *163*, 12.
[35] V. Kumar, L. Wati, F. FitzGibbon, P. Nigam, I. Banat, D. Singh, R. Marchant, *Biotechnol. Lett.* **1997**, *19*, 311.
[36] S. Klotz, A. Kuenz, U. Prüße, *Green Chem.* **2017**, *19*, 4633.

Biomass Conversion and Biorefinery
https://doi.org/10.1007/s13399-022-02306-6

ORIGINAL ARTICLE

Utilization of banana waste as a resource material for biofuels and other value-added products

Geetika Gupta[1] · Manoj Baranwal[1] · Sanjai Saxena[1] · M. Sudhakara Reddy[1]

Received: 9 August 2021 / Revised: 24 December 2021 / Accepted: 3 January 2022

Abstract
Banana is one of the most important food crops which is generally planted in tropical countries and has beneficial applications in the food industry. A large amount of by-products such as leaves, inflorescence, pseudostem, and rhizomes serves as a source for different industries. Most of these by-products may serve as an undervalued commodity with a limited commercial value, application and in some cases, it is considered as an agricultural waste. This also paves the way to utilize a huge amount of untapped biomass and resolve some of the environmental issues. Most of the edible bananas are cultivated mainly for their fruits, thus, banana farms could generate several tons of underused by-products and wastes. The present review mainly discusses the utilization of banana by-products such as peels, leaves, pseudostem, pseudostem juice, stalk, and inflorescence in various industries as a thickening agent, alternative source for renewable energy, nutraceuticals, livestock feed, natural fibers, coloring agents, bioactive compounds, and bio-fertilizers. Banana waste serves as a potential source for the production of valuable products and preserves renewable resources and provides additional income to the farming industries.

Keywords Banana waste · Banana sap · Bioethanol · Renewable resources · Waste utilization · Bioconversion

Abbreviations

ITO	Indium tin oxide
PEDOT	Poly(3,4-ethylenedioxythiophene)
PSS	Polystyrene sulfonate
PEG	Polyethylene glycol
Al	Aluminum
CMC	Carboxymethyl cellulose
SR	Schopper-Riegler

1 Introduction

Banana (*Musa paradisiaca*, family Musaceae) is a popular fruit crop grown worldwide. It is one of the tallest herbaceous plants with a pseudostem and probably the world's oldest cultured crop [1]. The tough tree-like flexible stem consists of sheathing twisting leaf bases consisting of fibers that provide adequate strength to maintain the tree upright. About 300 varieties of bananas are grown across the world, and the majority of them are cultivated in tropical Asia [2]. Around 1200 seedless fleshy fruit varieties and cultivars of banana and plantain are grown around the world, mostly for food. Although there are significant challenges involved with the use of whole-plant or floral morphology, particularly dealing with somaclonal variation and recognizing clones, morphological identification is nevertheless commonly employed to determine the variety of cultivated banana. The genus Musa contains the majority of edible bananas; approximately, 70 species have been recognized. The majority of edible and cultivated bananas are derived from hybridization between two species: *Musa acuminata* and *Musa balbisiana*. Edible bananas are diploid, triploid, or tetraploid hybrids, containing genetic information from subspecies *M. acuminata* (the "A" genome) and *M. balbisiana* (the "B" genome). An investigation of triploid varieties of banana fruit pulp and peel revealed that it had high nutritional value. Banana inflorescences from *Musa acuminata* and *Musa balbisiana* are rich in anthocyanins. One of the catecholamines, dopamine, was identified in significant concentrations in the pulp of yellow bananas (*Musa acuminata*), red bananas (*Musa sapientum* var. *baracoa*), and Cavendish banana peels. Bracts and male flowers of *Musa paradisiaca* have antioxidant, anti-cancer, and antibacterial activity. Peels of *Musa acuminata* cv. Cavendish have anti-inflammatory, anti-cholesterol, antioxidants, and antibacterial activity. Banana flower (*Musa Paradisaca*), banana fruit

✉ Geetika Gupta
geetika_12_gupta@yahoo.com

[1] Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala 147004, India

Published online: 17 January 2022

(*Musa sapientum*), and banana peel (*Musa acuminata*) have anti-cancer activity. There are significant variances in the quality of banana by-product fibers derived from different varieties and cultivars. Banana is cultivated in India, Sri Lanka, Bangladesh, and African countries for the fruits, leaves, and cooked vegetables [3–5]. Banana is considered as one of the most essential fruit crops worldwide. This means that the banana fruit crops are widely cultivated in tropical countries for its valuable applications in the food industry. Banana is the second-largest producer, which contributes about 16% of the total fruit production. Apart from being highly nutritious, this has various other economic benefits as well. Banana leaves serve as food wrappers, weaving mats, and baskets. Furthermore, banana fiber serves as raw material for paper production and making [5–8]. The components of the banana plant include a fleshy rhizome, pseudostem, and long, oblong-shaped leaves. Its oval-shaped inflorescence protrudes from the pseudostem which surrounds the male and female flowers. Its flowers transform or mature into berry fruits which then will develop flesh [9, 10]. Currently, India is the highest producer of bananas, where all year-round production of the banana is possible along with a suitable climate and soil conditions [11]. Deep, rich loamy soil with a pH between 6.5 and 7.5 is most preferred for banana cultivation. Soil for bananas should have good drainage, adequate fertility and moisture. Saline solid, calcareous soils are not suitable for banana cultivation.

Despite different uses of banana plant, it has been observed that large parts of the banana plant are barely discarded as waste, which not only causes environmental hazards but also affects the ecosystem. The various by-products of bananas are a significant source of extremely valuable and required raw materials not just for farming but for various other industries also, through agricultural waste recycling. Most of the edible bananas are cultivated mainly for their fruits; thus, banana farms could generate several tons of underused by-products and wastes. Therefore, without proper agricultural waste management practice, huge amounts of valuable untapped commodities will be lost, thus, causing serious ecological damages. Millions of tonnes of banana pseudostems are disposed of as waste and the farmers are facing problems in disposing of the accumulated banana pseudostems. Hence, there is a need to convert this waste biomass into useful products. The present review mainly describes the use of banana waste (pseudostem) for the production of some valuable products.

2 Banana waste

Cultivation of bananas gives rise to a large number of by-products, which include pseudostem, leaves, inflorescence, rhizomes, pith, sap, and fibers. Often considered environmental waste, these by-products have a lot of unused biomass, which can be tapped from various processes and put to good use. These by-products can be obtained from crushed components to replenish the lost nutrients or simply leave these components in an empty area for degradation. Due to copious amounts of gases produced from the by-products left, it is harmful for the current environment. It can also lead to an outbreak of notorious banana fungus, *Fusraium oxysporum* [12]. Reports have shown that banana peel can be efficiently used for the production of alpha-amylase [13]. Various banana by-products can be used for wide applications in different industries as mentioned in Figs. 1 and 2. These by-products can be utilized in the production of enzymes, certain polysaccharides, or even methane for fulfilling the energy requirement [14–18].

Alkaline pretreatment of banana pseudostem fiber has shown the production of good amounts of bio methanol [12]. The fiber obtained from the banana pseudostem has been utilized for making value-added products such as ropes, baskets, and threads [19]. Banana pseudostem sap is used as

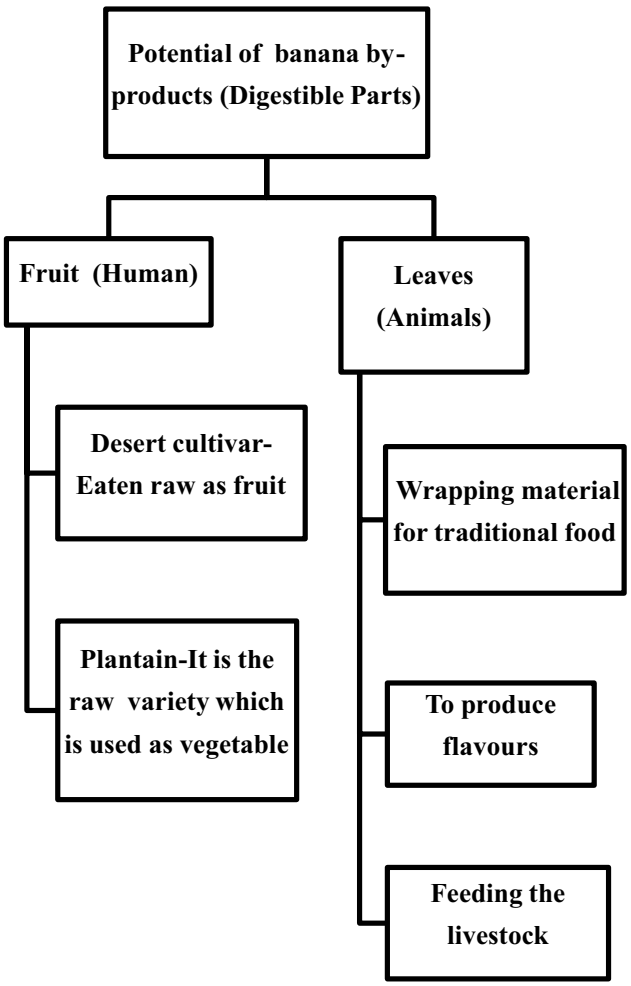


Fig. 1 Banana by-products (digestible parts) as a potential raw material in different industries

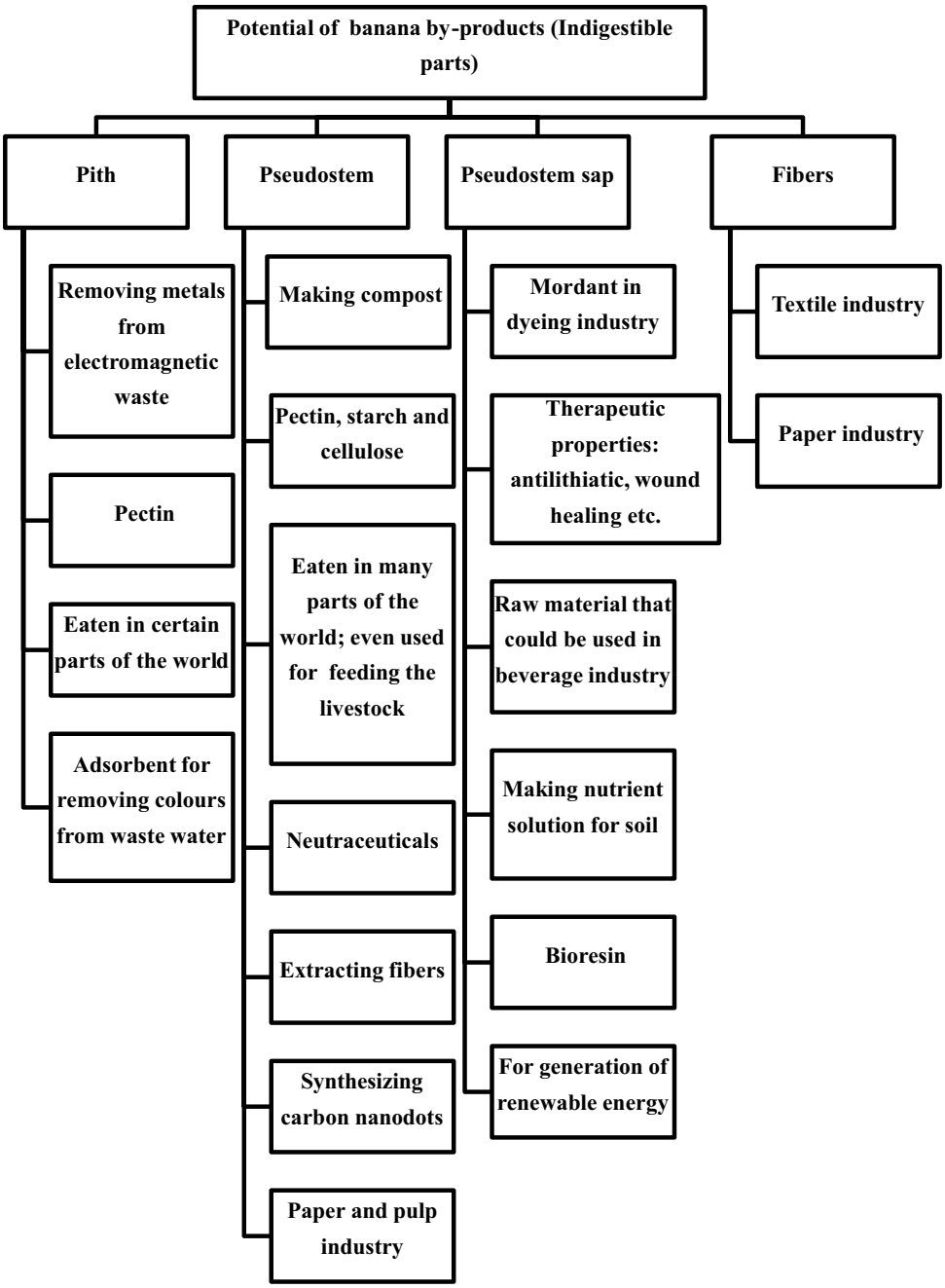


Fig. 2 Banana by-products (indigestible parts) as a potential raw material in different industries

mordant in the dye industry and as a fertilizer. The banana pseudostem has been used to make pickles, candy, and soft drinks [20]. Ripe banana peel increases ash content, crude fiber, total dietary fiber, and vitamin C to increase the nutritional properties of the cake product. The use of banana peel improves free radical scavenging activity, phytochemical content, and antioxidant activity in the bread to develop functional chapatti. In bread and bakery productions, both ripe and unripe banana peels have been used to substitute (1–30%) other key ingredients like wheat flour, wholemeal flour, maize, starch, rice flour, and brown rice flour. The mixed banana peel and pulp increase dietary fiber content and it could be useful for controlling starch hydrolysis of yellow noodles. Banana peel improved the hardness, cooking yield, water holding capacity, and the dietary fiber content of fish patty. Banana peel has been successfully used in the production of enzymes such as alpha-amylase and laccase [21].

2.1 Bioremediation

Banana waste directly or by processing is converted into material that is useful for metal ion removal. Banana peel,

stem, and carbon foam made from banana peel have been shown to adsorb heavy metals such as copper, lead, cadmium, and chromium. Fiber derived from the banana adsorbs cadmium, copper, iron, and zinc. Cellulose obtained from banana peel binds a range of heavy metals such as copper, lead, zinc, copper, and cadmium respectively. Carbon isolated from banana pith is an effective binder of mercury, and nickel respectively [22]. Acid or base-activated banana stalks bind to mercury and lead respectively [23]. Treatment of industrial effluents with such banana waste components has shown removal of these toxic metals up to 95%. Banana waste-derived products have been used to remove pesticides from contaminated waters. Activated carbon derived from banana stalk has been used for the removal of carbofuran, 2,4 diphenoxy acetic acid and benzaton from contaminated waters [24]. Charred banana peel treated with phosphoric acid has been used to remove atrazine from contaminated waters [22]. Banana peel and other components have been successfully used in removing dyes from contaminated material. Activated banana pith, banana stalk waste, and a banana bunch have been used successfully for removing methyl orange [25]. Banana leaf and banana empty fruit bunch treated with acid/base have been useful in removing the methylene blue. Banana pith is useful for removing rhodamine B and acid brilliant blue. Different parts of banana plant have been represented in order to improve the absorption of water-soluble radioactive nuclides, cyanides, fluorides, and other toxic elements [26, 27].

2.2 Characteristics of the banana waste

Banana fruit is one of the most commonly consumed products in the world. Asian and Latin American countries contribute to the maximum production of bananas. The banana tree generates a tremendous amount of waste which includes peel, pulp, pseudostem, pith, flowers, and leaves. Potential technologies for the utilization of banana waste are mentioned in Fig. 3. Furthermore, a well-labeled diagram of banana parts such as rhizome, pseudostem, inflorescence, pith, and fiber are represented in Fig. 4. Banana leaves are richer in lignin content compared to their pseudo stems. Peels of the banana contains lignin (6–12%), cellulose (7.6–9.6%), pectin (10–21%), hemicelluloses (6.4–9.4%), and starch (3%) [32]. The moisture content in banana peel is about 83% and in pulp 70%. The overall sugar content is quite high in pulp that accounts for up to 40% of the sugar. In both banana pulp and peel, sucrose and cellulose are the major sugar components followed by glucose and fructose [5]. Pseudostem of the banana contains 90% of water and minerals such as sodium, potassium, calcium, magnesium, and chloride [33]. Banana pseudostem, petioles/midrib, rachis, leaf sheath, and floral stalk are good sources of nutrients such as glucose, xylose, galactose, arabinose, and minerals like potassium, calcium, magnesium, silicon, and phosphorus [5]. This has resulted in the development of methods for the utilization of waste in both chemical and biotechnological industries. Banana development creates a huge measure of buildups. The spoiled banana natural product, banana strip, banana pseudostem (BPS), leaves, stalks, rhizome, and organic product bundle stem are regularly delegated banana biomass. Roughly, four tons of pseudo-stems are abandoned in the field for every huge load of banana organic product collected. The assessed banana buildup rate in India is around 1.2×107 tones/year. Generally, this colossal measure of biomass is simply unloaded off at removal locales. As a result, the regular decomposition of waste from banana biomass for an open climate discharges harmful gases, like CH_4, H_2S. There is a requirement for innovative improvement for powerful use of banana squander.

2.3 Cellulose, pectin, and starch source

By-products such as pseudostem pith and green culled bananas (which are rejected at the time of selection of the edible fruits) can be used as a primary element of pectin, cellulose, and starch. Banana starch has relatively low amylase

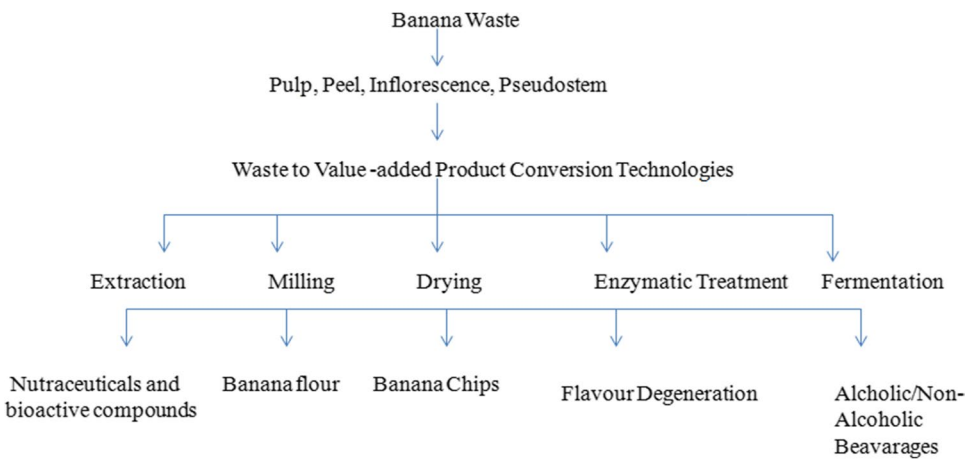


Fig. 3 Potential technologies for utilization of banana waste

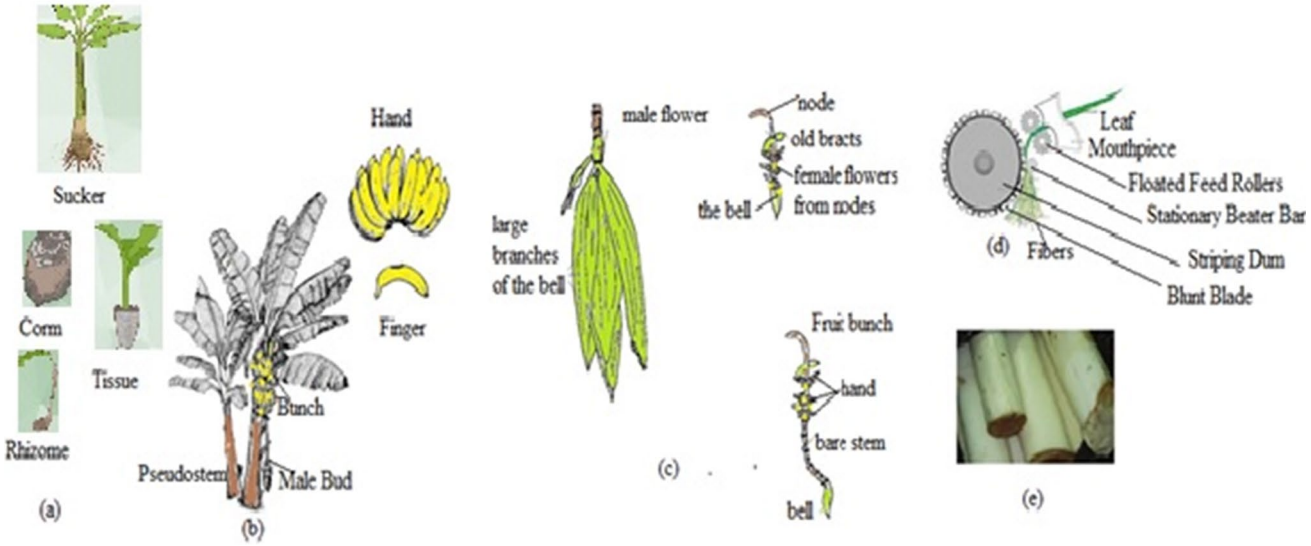


Fig. 4 A well-labeled diagram of different banana by-products (**a**) rhizome of banana [28], (**b**) pseudostem of banana [29], (**c**) inflorescence/blossom/heart of banana [30], (**d**) fibers of banana (82), (**e**) pith of banana [31]

content and resistance to heat and amylase attack, low swelling and solubility properties, and is superior to corn starch [34]. The pectin content in a banana is less than the pectin isolated from citrus peels like lime and pomelo [35]. Elanthikkal et al. obtained the microcrystalline cellulose from the waste of the banana fibers with the help of acid hydrolysis [36]. These three substances, including cellulose, pectin, and starch, have a huge need in the food sector as a thickening agent, gelling agent, etc. Pectin can be extracted from banana peels through various extraction methods. Similarly, cellulose can also be isolated from the banana waste fiber using different extraction processes [34, 37, 38]. Cellulose, pectin, and starch isolated from banana by-products have many applications. In the human diet, starch, cellulose, and pectin are of great nutritional importance as they are major carbohydrates. Emaga et al. [39] reported that the pectins isolated from banana peels contain various monosaccharides such as glucose, galactose, arabinose, rhamnose, and xylose. From an industrial perspective, starch and cellulose serves as a major feedstock for bioethanol due to the relative ease with which it can be converted into fermentable sugars [40].

Jadhav et al. [40] examined the production of amylase from the potato and banana peel by *Aspergillus niger* and *Bacillus subtilis*. Also, Oshoma et al. [41] reported that banana peel is primarily a substrate for the production of amylase and single-cell proteins assuring the possibility of banana peel waste into proteinaceous food along with enzymes. Studies also revealed that banana peel can be used as a substrate for the production of lactic acid [42]. Lactic acid is an organic acid with a wide range of applications in the food (as preservatives, pH regulators, flavoring agents, etc.), chemical (neutralizers, cleaning agents, chiral intermediates, etc.), cosmetic (moisturizers, skin lightening, anti-acne agents, etc.), and pharmaceutical industries (mineral preparations, prostheses, controlled drugs, etc.) [43]. Banana peel has been successfully used in the production of enzymes such as alpha-amylase and laccase. Distinct groups of fungi such as *Aspergillus*, *Penicillium*, and *Saccharomyces* are known to grow on a banana peel. Several of the bacterial species are also known to grow on the peel. The growth of such a broad range of organisms helps in fermenting the peel and production of several important biomolecules at the industrial level [20].

2.4 Source for Anthocyanins

Anthocyanins are naturally found in fruits and vegetables [44]. Other than that, current research has found that anthocyanins have an inhibiting effect on digestive enzymes. Due to its anti-inflammatory, antioxidant, and chemoprotective qualities, anthocyanin has the potential to prevent chronic and degenerative diseases [45]. Few research studies were conducted to develop anthocyanin-fortified foods [46]. Banana bracts have a lot of promise as a banana waste product, especially because of the attractive color of the pigments. UV–visible spectroscopy, physicochemical reactions, HPLC, and electrospray mass spectrometry were used to interpret anthocyanins extracted with acidified methanol, purified using C-18 resin, and characterized by UV–visible spectroscopy, physicochemical reactions, HPLC, and electrospray mass spectrometry. Furthermore, it is a useful tool in anthocyanin identification since it contains the six most prevalent anthocyanidins: cyaniding, delphinidin, pelargonidin, petunidin, peonidin, and malvidin [47]. Sutikno et al.

[48] effectively built organic solar cells with a layer structure of ITO/PEDOT: PSS/PEG/PEG + Anthocyanin/Anthocyanin/Al/ITO, demonstrating the potential of banana flowers as electron acceptors. Based on parameters such as pH and solvent concentration, Ove et al. [49] extracted and evaluated the anthocyanins derived from the banana bracts as a natural colorant that can be used as a natural colorant in different food sectors instead of synthetic colorants which have a carcinogenic effect. Six different anthocyanin pigments, petunidin-3-rutinoside, delphinidin-3-rutinoside, malvidin-3-rutinoside, cyanidin-3-rutinoside, peonidin-3-rutinoside, and pelargonidin-3-rutinoside, were identified from wild bananas of Thailand by Kitdamrongsont et al. [50].

2.5 Flavor creator

Banana leaves are rich in lipoxygenase, which is a membrane-bound enzyme having many applications in the food industry. This enzyme can be used to produce different flavors like oolong tea, melon, and cucumber when treated with oils (soyabean oil, linoleic oil). Thus, using banana leaves for the generation of flavors in the food industry can pave new ways for handling the huge amount of banana leaves that are usually thrown as waste [17]. Bananas have a particular aroma and flavor. They contain different nutrients, which will aid in the development of banana-based drinks. Chen et al. [51] investigated the physicochemical flavor and sensory characteristics of banana juice and wines at different stages of the fermentation process. Post-fermentation banana wine had a lower pH and higher brix, as well as more reducing sugar, alcohol, and total acid than primary fermentation banana wine. Hasbullah et al. [52] examined the sensory properties of banana peel analog coffee (Modern American coffee shop located in the Capitol Hill neighborhood of Seattle. Serving a rotating cast of exceptional local roasters and Fresh Breeze Organic dairy) and the impact of banana peel maturity along with oven time. The study concluded that banana peels had the potential to be utilized as an alternative to coffee. Consumer acceptability and production potential will be determined by the sensory characteristics of analog coffee.

2.6 Source of several nutrients

Banana pith from the pseudostem as well as inflorescence have long been eaten which is an important part of the culinary culture of the people of southeast Asia. It has been shown that banana pith and inflorescence have a substantial amount of dietary fiber, protein, and amino acids. Banana peels are also quite high in potassium [53, 54]. Potassium is abundant in bananas, which offer 23% of our daily potassium requirements. Potassium aids in the reduction of blood pressure and risk of stroke. The peel of a banana is high in carbs and fiber. Bananas include a variety of vitamins, including A, B_6, C, and D, which help to keep the body healthy in many different ways. Peels relieve constipation and aid in the treatment of diarrhea and dysentery due to their high fiber content. Bananas are intended to help children with worm issues. At a concentration of 158 mg/100 g dry weight (DW), gallocatechin is found in banana peel [55]. Bananas contain around 20% sugar and are a good source of vitamin B and calcium. It has around 116 kcal energy/100 g of ripe banana. Leucocyanidin is a flavonoid that thickens the mucus membrane in the stomach and protects against ulcers, heartburn, and hangovers [1]. Major nutritional components such as lipids, proteins, and carbs are abundant in banana peels, accounting for 91.50% of the dry weight. It also contains high content of indigestible fiber [56].

2.7 Source of phenolic compounds

Several natural phenolic compounds are present in banana by-products exhibiting anti-bacterial, anti-oxidants, anti-cancer, and anti-inflammatory activities which are presented in Table 1. Banana peel is rich in many antibacterial compounds (12-hydroxy stearic acid, ß-sitosterol, and malic acid). Studies have also shown that bananas are rich in antioxidants, which furthermore enhances its food preservation capacity [57, 58].

Epigallocatechin and its derivatives have been found in the male banana flowers. Studies have also detected (+)-catechin, gentisic acid, protocatechuic acid, ferulic acid, caffeic acid, and cinnamic acid in the banana pseudostem [59, 60]. Studies have shown that compounds such as caffeic acid, gallocatechin, catechin possess unique medicinal properties such as antimicrobial activity, antioxidative properties, neuroprotective features, chemo-preventive properties, and anti-cancerous properties [60–63]. Other components such as anthocyanins also have many notable properties such as antioxidant anti-inflammatory and anti-cancerous properties which can be explored to their full potential. Usually found in the pulp, dopamine is a catecholamine that also has anti-inflammatory properties [10, 64, 65]. Bananas can furthermore contribute to the food industry as the bioactive components present in them can be used for food preservation. Different parts of the banana plant such as banana fruit, pulp, peel, stem, flower, sap, leaf, and pseudostem have shown antioxidant activities (Table 2). There is a strong link between phenolics content and the ability to scavenge oxygen radicals and reduce free radicals [66]. Based on the presence of the individual phenols, they can be grouped into phenolic acid and hydroxycinnamic acids. Moreover, banana by-products also exhibit extracts that show selective anti-bacterial activity in some of the studies against a wide range of bacteria such as *B. subtilis*, *E. coli*, *S. aureus*, *V.cholerae*, *Micrococcus*, *Klebsiella*, *Salmonella*,

Table 1 Natural phenolic compounds present in banana by-product

Phenolic acids	Source	Banana by-products	References
Hydroxybenzoic	Vanillic acid	Banana sap	[123]
	Gallic acid	Banana sap	[123]
	p-Hydroxybenzoic acid	Banana sap	[123]
Hdroxycinnamic	Ferulic acid	Banana pulp, peel, sap, and leaves	[123, 133, 134]
	Caffeic acid	Banana sap	[123]
	p-Coumaric acid	Banana sap	[123]
	Caffeoylquinic acid	Banana sap	[123]
	Chlorogenic acid	Banana sap	[123]
	Synapic acid	Banana peel and pulp	[133]
Flavonoids			
Anthocyanins	Petunidin-3-rutinoside	Banana bract	[50]
	Delphinidin-3-rutinoside		
	Malvidin-3-rutinoside		
	Cyanidin-3-rutinoside		
	Peonidin-3-rutinoside		
	Pelargonidin-3-rutinoside		
Flavonols	Quercetin	Banana flower, sap	[82, 135]
	Kaempferol	Banana peel, pulp, and sap	[82, 133]
	Myricetin	Banana peel, pulp, and sap	[82, 133]
Flavones	Apigenin	Banana sap	[82]
	Rutin	Banana flower	[135]
Flavanones	Naringenin	Banana sap	[82]
Flavanols	Catechin	Banana pulp	[136, 137]

Table 2 Antioxidant activities of banana by-products

Type of banana by-product	Activity	Applications	References
Banana fruit extracted with aqueous, methanol, ethanol, and acetone extracts	To scavenge free radicals either by lipid peroxidation or chelating metal ions	Fruits contains antioxidant	[138]
Banana pseudostem and rhizome juices	Electron transfer or hydrogen donors	Functional beverage	[139]
Banana pseudo stem and flower extracted with chloroform, acetone, and methanol solvents	Prevent lipid oxidation via chain breaking reaction	Nutraceutical for the replacement of synthetic antioxidants	[140]
Banana sap extracted with methanol and ethanol solvents	Strong antioxidant	Preparation of formulations in pharmaceutical industries	[141]
Banana leaf extracted with hexane, ethyl acetate, and methanol solvents	Inactivate lipid free radicals	Helps to understand the use of *Musa* species in traditional medicine as an antioxidant agent	[142]
Banana pulp, seed, and peel extracted with hexane, ethyl acetate, and ethanol solvents	Free radical terminators	Potential source of bioactive compounds	[143]

and *P. aeruginosa* (Table 3). A large number of studies have been conducted that have effectively shown the antibacterial activity of various plant parts of banana extracts by organic solvents. Furthermore, various parts of the banana have been tested for anti-proliferative/anti-cancer activity on various cancerous cell lines presented in Table 4. The extracts show distinct activity with defined IC_{50} values. Other than cell lines, studies have been done by various groups to demonstrate the enhancement of immunomodulatory activity of the immune cells using various banana extracts [67, 68]. All the natural phenolic compounds present in banana by-products are bioactive compounds mentioned in Table 5. Due to the presence of these bioactive compounds, banana by-products such as pulp, fruit, peel, and sap showed various therapeutic

Table 3 Applications of banana by-products based on antimicrobial activities

Type of banana by-product	Activity	Applications	References
Banana leaf extracted with petroleum ether, chloroform, and ethanol solvents	Chloroform and ethanol showed inhibition against all the tested bacteria (*E. coli*, *B. subtilis*, *P. aeruginosa*, and *S. aureus*) whereas petroleum ether did not show any activity	Antiseptic and disinfectant formulations	[144]
Banana peels extracted with ethanolic extract and aqueous extract	Ethanolic extract showed inhibition against all the tested bacteria (*B. subtilis*, *E. coli*, *P. aeruginosa*, and *S. aureus* Micrococcus, *Klebsiella*, and *Salmonella*) whereas aqueous extract does not show activity against *Salmonella*, *S. aureus*, and *Micrococcus*	Medicinal plant with antimicrobial activity	[145]
Banana sap extracted with aqueous, methanol and ethanol solvents	Only *Streptococcus* showed activity against all the solvents out of the tested bacteria	Preparation of antibacterial formulations in pharmaceutical industries	[141]
Banana inflorescence extracted with aqueous, chloroform, methanolic, and ethanolic solvents	*V. parahemolyticus* showed no inhibition in case of aqueous and chloroform extract, chloroform extract showed activity against *B. cereus* and *S. aureus* whereas methanol and ethanol extracts showed activity against all the tested bacteria *B. cereus*, *S. aureu*, *V. parahemolyticus*, and *L. monocytogenes*	Bacteriostatic as banana inflorescence is a source of natural antibacterials	[146]
Banana pulp, seed, peel extracted with methanolic solvent	Pulp and peel showed activity against all the bacteria *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*, *Shigella*, *Vibrio*, and *Salmonella* whereas seed showed no activity	Treatment of dysentery and diarrhea	[147]

applications (hypocholesterolemic, wound healing, antiallergic, and antioxidant, etc.) presented in Table 6. Lupeol is a dietary triterpene reported to have a therapeutic role as an anti-inflammatory and anti-cancer agent [69]. Hydroxybenzoic and hydroxycinnamic acids are the derivatives of phenolic acids. Hydroxycinnamic acids are present in the bound form [70]. Hydroxybenzoic acid such as gallic acid is the component of hydrolyzable tannins whereas p-hydroxybenzoic acid and vanillic acid are the components of lignins [71]. Due to the presence of polyphenolics, gallic acid has high free radical scavenging activity [72]. Caffeic acid is the most important hydroxycinnamic acid which is found in foods primarily as chlorogenic acid. Hydroxycinnamic acids due to their antioxidant activity play a role in the prevention of diseases associated with the risk of oxidative stress such as cardiovascular diseases and cancer [73]. Gulcin [74] reported that caffeic acid is the superior antioxidant when compared with p-coumaric and ferulic acids in inhibiting the LDL oxidation and quenching of singlet oxygen and radicals. Apigenin is a bioactive flavonoid that helps to protect against cancer and monitor cardiovascular conditions [75]. Rutin, also known as vitamin P, and Quercetin-3-o-rutinoside has been reported to protect organs against the free radical agents and can be used for the treatment of gastrointestinal diseases and hepatotoxicity [76]. Quercetin, kaempferol, rutin, and myricetin act as antioxidants and have therapeutic effects in the treatment of inflammation, allergy, viral infection, and cancer [77]. Naringenin is a flavanone and has anti-cancer, antioxidant, antiproliferative, anti-inflammatory, antiatherogenic, and antimutagenic activities and is found in fruits such as grapefruit and oranges [78]. Dopamine plays a role as a neurotransmitter in the brain, precursors for epinephrine and norepinephrine, an accumulation of the oxidized products of dopamine is associated with Parkinson's disease [79, 80]. Kanzawa et al. [81] reported that bananas contain dopamine which acts as a strong antioxidant due to the o-dihydroxy structure and its amino residue which is responsible for the hydrophilic character. N-acetyl serotonin and furthermore metabolite serotonin have a role in the ripening period due to the melanin production [82]. Serotonin is responsible for feelings of well-being and happiness. In bananas, serotonin benefits to overcome or prevent depression by altering mood and calming the body [83].

2.8 Source for animal feed

The banana by-products will be an inexpensive source of animal feed along with the high nutritive value. It possesses a significant amount of nutrition in the form of proteins, lipids, carbohydrates, and fibrous content as well as other essential minerals such as calcium, potassium, iron, and manganese, which makes it a suitable choice as an animal feed [20, 84]. Akinyele and Agbro reported the increase in

Table 4 Applications of banana by-products based on anti-cancer activities

Type of banana by-product	Effects	Applications	References
Banana peel, pulp extracted with hexane, water, and ethanol	Hexane extracts of peel and pulp showed high cytotoxicity against HCT-116 and MCF-7 whereas water and ethanol extracts showed least activity with HCT-116 and MCF-7 cell lines	Pulp and peel has biological activities lead to therapeutic applications	[148]
Banana peel, pulp extracted with mixture of ethanol–water	Pulp and peel showed anti-cancer activity against A549, MCF-7, HepG2, and HT-29 cell lines	Prevention and treatment of different cancers in different ways	[149]
Banana flower extracted with ethanol solvent	Flowers exhibited good cytotoxic effect with both HeLa and CHO cell lines	Natural source for the development of an anti-cancer lead molecule	[150]
Banana peel extracted with ethanol solvent	Peel showed cytotoxic activity against MCF-7 cell line	Banana peel as a source of antitumor and anti-cancer agent	[151]

Table 5 Bioactive compounds obtained from banana by-products

Banana by-product	Bioactive compound	Applications	References
Banana peel	Myricetin, quercetin, kaempferol, rhamnetin	Strong antioxidant and metal chelating properties	[152]
Banana sap	Lupeol, ferulic acid, vanillic acid, trans-cinnamic acid, p-hydroxybenzoic acid, p-coumaric acid, rutin, catechin/epicatechin, chlorogenic acid, gallic acid, caffeic acid, and nicotiflorin	Antidiabetic activity	[123]
Banana sap	Apigenin glycosides, myricetin glycoside, myricetin-3-O-rutinoside, naringenin glycosides, kaempferol-3-O-rutinoside, quericitin-3-O-rutinoside, dopamine, and N-acetyl serotonin	Inhibition of cancer cell lines, neurodegenerative disorders, anti-estrogen, anti-inflammation, antivenom, capillaries strengthening	[82]
Banana flowers	Rutin, quericetin	Treatment of gastrointestinal diseases	[135]
Banana pulp	Ferulic acid, synapic acid	Inhibiting LDL oxidation	[133]
Banana peel	Isorhamnetin, kaempferol, quericitein, myricetin, and methylmyricetin	Treatment of inflammation, allergy, viral, and cancer	[133]
Banana bract	Anthocyanin	Antioxidant activity	[50]

Table 6 Therapeutic applications of banana by-products

Banana by-products	Therapeutic applications	References
Green banana	Antidiarrhoeal activity	[153]
Banana pulp	Hypocholesterolaemic activity	[154]
Banana meal	Antioxidant activity	[155]
Green plantain banana fruit	Wound healing activity	[156]
Banana (peel, flesh) and ripe banana (peel, flesh)	Anti-allergic activity	[157]
Banana sap	Antioxidant and antimicrobial	[1]
Banana sap	Wound healing activity	[121]
Banana sap	Hyperglycemic effect	[124]
Banana sap	Antilithiatic	[157]

protein and sugar content by solid-state fermentation by incubating the banana peels with *Aspergillus niger*, *A. flavus*, and *Pennicilium* sp. These fungi can break down the banana peels non-starchy polysaccharide content, converting it to simple sugars with a large increase in protein. According to the findings, fungal biotechnology is a useful method for improving the nutritional content of agricultural by-products. This gives rise to the notion of incorporating these biodegradable by-products into the diets of chickens, pigs, and goats to reduce the amount of maize consumed by these species [84]. This enhancement through microbial degradation will furthermore increase the nutritional value of the animal feed. In a feeding experiment, where the livestock was given leaves and pseudostems of banana, it was found that banana leaves possess many superior qualities as compared to the conventional fodder. Features such as low partition factor, high ATP, and last but not the least, high microbial biomass benefitted the organism. The study indicated that bananas are a potent source of ruminant feed [85]. Banana peel included low amounts of anti-nutritive chemicals, such as hydrogen cyanide, a toxic toxin, which was determined to be less than the acceptable limit (0.5–3.5 mg/g). Other anti-nutrient chemicals, such as oxalate and phytate, are reduced in comparison to maize and sorghum, which are

typical animal feed. As a result, the banana peel has been utilized in the production of animal feed [56].

2.9 Source of natural fibers

Banana pseudostems produce fibers that offer a wide range of value-added products such as ropes and baskets. Pickles, sweets, and soft beverages can also be made from the stem's inner core [86]. Studies have shown that banana by-products also have an exponential capacity to be used as the substituent of natural fibers. Fibers from the fruit stalk, pseudostem, and the leaves have been extensively studied for their potential [87–89]. Zuluga et al. [88] examined the potential of cellulose microfibrils extracted from banana rachis vascular bundles. The impact of the treatment on the morphology and structure of the resultant products was studied using SEM, ion chromatography, ATR-FTIR, TEM, electron, and X-ray diffraction. The partial elimination of hemicelluloses was verified using ATR-FTIR. The strong link of xyloglucans and cellulose appears to be the reason for non-cellulosic components. Furthermore, the appearance in all ATR-FTIR spectra is due to the presence of xylans related to hemicelluloses. Diffraction, 13C NMR, and ATR-FTIR results indicate that the cellulose microfibrils isolated from banana rachis belong to cellulose IV1 and are very close to cellulose Ib. Cherian et al. [89] proposed a unique technique for synthesizing natural fiber nanofibrils from banana fibers. Different contemporary methods were used to analyze the produced nanofibrils. The primary components of these fibers were discovered to be cellulose during the chemical analysis. The IR analyses show that the fibers disintegrated and transformed chemically during steam explosion, as well as when the fibers were furthermore treated for steam explosion in an acidic media. The modified fiber size and crystallinity were investigated using XRD. The XRD experiments furthermore indicated that during steam explosion in an alkaline medium, fiber size is reduced to the nanometer range, and with repeated steam explosion in an acidic medium, fiber size is reduced to the nanoscale range. The scanning force microscopy (SFM) study also reveals that the size of banana fibers has decreased to the nanometer range (below 40 nm). The evidence for the development of nanofibrils in banana fibers by repeated steam explosion in acidic circumstances is furthermore supported by transmission electron microscopy (TEM) examination. Oliveira et al. [87] extracted dioxane lignin (DL) from banana plant leaf sheaths. The HGS type (having a 12:25:63 molar proportion of p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units) lignin isolated from the leaf sheaths has a specific lignin fraction rich in H and S units that is strongly structurally linked with aliphatic chemicals. It is suggested that lignin from the banana plant's leaf sheath is chemically linked to suberin-like components of cell tissues via ester bonds involving hydroxycinnamic acid residues. The composite structure of banana fibers has been discovered through research. The use of cellulosic fibers, such as banana fiber, as a filler in the plastic industry helps to reduce costs. Furthermore, by combining banana with glass fiber in the fabric form, the requisite high tensile strength can be achieved. The impact of the composites on strength improves as the number of layers and fiber volume fraction increase [90]. Furthermore, researchers have also shown that fibers extracted from the pseudostem enhance the epoxy composites, i.e., increase/raise the tensile potency of epoxy by almost 40% [91]. These fibers obtained from pseudostem have been in use since a long time in making traditional handicraft and clothes. Banana fibers have a huge potential to be used as textile fibers after degumming through microorganisms. *Streptomyces lydicus* was used for the production of poly-galacturonase for converting the raw banana fibers into processed and modified fibers that could be used in the textile industry [92].

2.10 Pulp and paper production

Banana by-products are widely used as raw materials in the paper industry. Pseudostems are utilized in the processing of pulp and paper. Preparing the pulp at a lower temperature and utilizing formic acid yields better quality of the pulp [93]. Papers manufactured from these by-products were found to be water-resistant and stronger as compared to the traditional paper made from wood pulp. It was their low water absorption capacity that made them resistant to water. In a comparative study conducted between three agricultural wastes, it was found that banana leaf and the peduncle are excellent sources of short fiber pulp. Its fiber characteristics are at par with those that are obtained from other woody and non-woody sources. The features of the fibers from the agricultural wastes performed well in terms of burst factor, tear index, stretch, and tensile properties and have a huge potential in various industries [94]. Nassar et al. [95] optimized banana stem pulping parameters to replace softwood pulp in writing and printing paper. A furnish mix ratio of 20 to 80%, banana stem pulp, and commercial bagasse pulp was used to produce high-quality writing and printing paper.

The increased pentosan content of particular banana plant sheaths, along with gums and mucilage, makes it a suitable source for making grease resistant paper. With the help of kraft and soda processes, pulp from banana pseudostem waste was used to manufacture grease resistant paper. On the basis of yield, it was concluded that the kraft process is safer and more efficient than the soda process [96]. For the manufacturing of greaseproof paper, banana pseudostem fiber pulp can successfully substitute wood pulp. In comparison to base paper produced with a higher degree of pulp refining, base paper produced with a lower degree of pulp refining will require a greater quantity of coating per unit

area. Only the carboxymethyl cellulose (CMC)-coated paper surpassed the other two coating materials in terms of grease resistance. The handsheets generated with 70° SR pulp freeness in conjunction with a 5% CMC coating yielded the best results. When the amount of coating absorbed per unit area of the sheet is considered, handsheets generated with 70° SR pulp freeness and 3% CMC coating appear to be more efficient [97].

2.11 Carbon nanodots

Carbon nanodots are nanoparticles that have the property of fluorescence which are used to track biological processes occurring inside the living cell [98]. Banana pseudostem has been used for the manufacture of these carbon dots. These carbon dots exhibited water solubility, high photostability, high cell permeability, low toxicity, and above all, high biocompatibility which has made them an attractive choice. These nanoparticles were prepared using one-pot hydrothermal treatment. Furthermore, studies conducted depict that these carbon dots can be used for cellular imaging by using it as a fluorescent probe for $Fe3^{+}$ [99]. Nanoparticles were made using starch extracted from a green banana. Citric acid was used to cross-link these nanoparticles. Thus, demonstrating that it is a powerful chemical alteration for encapsulating extremely hydrophobic compounds like β-carotene. This study confirmed that these cross-linked nanoparticles are suitable as vehicles for intestinal-specific targeting [100]. As banana pith extract has a high bacteriostatic effect, gold nanoparticles made from it can be utilized in biomedical applications. Moreover, nanoparticles were shown to be effective in degrading Malachite green dye, suggesting that they might be utilized for bioremediation of Malachite green dye-containing textile industry effluent [101].

3 Production of renewable fuels

Renewable resources, also known as biomass, are a naturally plentiful resource that can comprise any biologically derived elements such as plants and animals, agricultural products, and biological leftovers or wastes [102]. These resources can be converted into raw materials or products that are recyclable and quickly biodegradable, with good environmental acceptability or "green label" qualities as well as commercial feasibility [103]. Excessive consumption of the non-renewable resources has put a big question mark over the duration for which they can be used. Hence, alternative forms of energy are required to meet societal needs. Not only has demand been fast depleting non-renewable resources, but it has also resulted in the abundant generation of harmful gases which have led to the deterioration of the earth's natural resources. Utilization/transformation of biomass such as agricultural waste into renewable fuel is one of the environmentally friendly approaches. The general methods adapted in this technology are dependent on enzymatic hydrolysis and microbial fermentation of solid matters [104]. Renewable resources have opened the way for industry and have been utilized to replace non-renewable resources, such as petroleum and gas products, precious metals, and minerals, for decades. In order to ensure a sustainable development of technology, it is critical that the use of low-cost agricultural by-products and biological wastes be spread to all relevant sectors. This may provide an extra source of income for farmers and processing companies while also reducing non-renewable resource depletion [105]. Additional useful outputs from present farming activities may be able to prevent our important forest from being destroyed to generate similar commodities. Green technology refers to an approach that is environmentally friendly and focuses on conserving natural resources while providing the least amount of risk to existing species on the planet, including humans. There are a number of food processing methods available for food processing. Few of those methods are canning, fermentation, air-drying food, blanching, and preserving in salt or sugar. Because using agro-food based items to drive green technology would eventually result in food instability, ethical concerns, and unsustainable energy returns, the technology should be independent of the present agro-food stock market [106]. Banana by-products are widely available as a source of raw materials for the green technology industry due to its abundance of biomass. The fact that bananas have been consumed by humans for a long time without any major negative effects provides some reassurance that these by-products are free of harmful phytochemicals. However, compared to other plants with potent and harmful chemical ingredients, by-product harvesting, handling, and storage may require less vigilance. Therefore, it is not dependent on the present agro-food-based market, the utilization of banana by-products for industrial purposes could promote "green technology," which may not pose any food security or ethical difficulties. Furthermore, except for the accessible banana plantation for fruit, it does not need supplementary planting space [10].

3.1 Production of bioethanol

Ethanol is extensively utilized in the industry as a renewable fuel as well as a solvent. Bioethanol production has been one of the major applications of banana waste. Bioethanol is produced through a fermentation process using either bacteria or yeast by converting cellulosic materials into ethanol [107]. It has been found that banana peels are an extremely good substrate for the production of ethanol by using microorganisms. The yield is affected by different parameters such as the substrate concentration,

Table 7 Ethanol production with different parts of banana by-products

Source of fermentation	Amount of ethanol	References
Whole banana	0.009%	[110]
	0.009%	[110]
	7.45%	[158]
Banana peel	6.54%	[159]
	45%	[160]
	3.56%	[161]
	1.309%	[162]
	0.16%	[163]
	3.6–5.8%	[111]
	2.1%	[164]
Plantain peel	20%	[158]
Chiku and banana peels	2.66–78.9%	[165]
Banana pulp	28.45%	[166]
	0.008%	[110]
Banana stem hydrolysate	0.2%	[167]
Rotten banana	0.113%	[168]
Banana fruit mash	7%	[169]
Banana pseudostem	84%	[170]
Banana sap	0.014–0.3%	[118]

fermentation conditions, and the organism performing the process of fermentation [108]. The yield of ethanol obtained from different parts of banana in several studies is mentioned in Table 7. Harish et al. reported the production of ethanol from banana pseudostem and leaves by using thermophilic fungi *Clostridium thermocellum* CT2 co-cultured with *Clostridium thermosaccharolyticum* HG8 [109]. Hammond et al. [110] reported ethanol yields of 0.091, 0.082, and 0.006 L/kg from whole fruit waste bananas. Green, normal ripe, and overripe green whole bananas yielded 0.090, 0.082, and 0.069 L/kg of ethanol by enzymatic hydrolysis, respectively. Brooks [111] investigated the abilities of five yeast strains obtained from ripe banana peels. Saccharomyces cerevisiae R-8 had the finest ethanol-producing characteristics because it was extremely flocculent. On maltose, S. cerevisiae T-7 and S. cerevisiae R-2 had rapid fermentative activity. *Debaryomyces hansenii* B-2 and *Saccharomyces kluvveri* K-6 fermented 40% (v/v) glucose at 30 °C to produce 3.6 and 5.8% ethanol, respectively. The pseudostem of banana has been utilized in producing up to 17.1 g/L of ethanol. In many of the cases, the pseudostem can be pre-treated along with a mix of enzymes or chemicals that can result in substrate hydrolysis. Pretrated banana leaves and fruits have also been proved to produce ethanol upon fermentation.

3.2 Production of biomethane

Methane is a very useful fuel that is currently used in industries and households. However, with a growing population, more of this fuel is required. As of now, sewage is used to generate methane gas, but banana waste has tremendous potential to be used in the production of methane gas. First, the plant material needs to be digested by anaerobic bacteria in an air-tight container. It is a completely natural process that does not require the external supplementation of any sludge or sewage [14, 112]. Except banana pseudostem, banana peel, stalk, and waste have been successfully shown to produce biogas. Even the briquettes prepared from the banana peel had a less burning rate, and equivalent briquette strength to the existing ones. Banana by-products can also be used for making compost by solid-state fertilization. Utilizing this as soil manure greatly enriched the nutrition content and resulted in an increased yield [10]. Biomethanation of banana peel and pineapple wastes was achieved with 0.76 v/v and 0.93 v/v gas output per day and 36% and 58% substrate utilization, respectively [113]. Kalia et al. [114] investigated the high organic content (83%) as well as the lignin and cellulose content of 15–20% (w/w), all of which contribute to the sheath-like structure of banana peels. In batch culture, banana stem slurries (BSS) containing 2–16% total solids (TS) were anaerobically digested under mesophilic (37–40 °C) and thermophilic (50–55 °C) conditions. Under mesophilic conditions, ultimate biogas yields of 267–271 L/kg TS fed were reported with 2–4% TS slurries. Biogas yields of 212–229 L/kg TS fed were obtained with 2–8% TS slurries in the thermophilic range. Thermophilic digestion rates, on the other hand, were 2.4 times quicker than those obtained from the mesophilic rates. In both temperature ranges, methane production was highest at 2% TS BSS. The method resulted in organic solid reductions of 45–50% and COD reductions of 40–55%. Biogas generation from banana and plantain peels was reported by Ilori et al. [115], with gas produced from feedstocks of 8800 cm^3 and 2409 cm^3, respectively. The digester produced a total volume gas of 13,356 cm^3 and used equal amounts of banana and plantain peels in combination as feedstock. The physical chemistry of the digested feedstocks indicated an initial decrease in pH to the acidic region, followed by a gradual rise up to 7.4 and the temperature remained consistent in the mesophilic range of 32–35 °C.

4 Banana sap

Banana stem juice or banana sap is an exudate of the banana plant that oozes out when the stem is cut. Initially, it is transparent, changes to a pink color when exposed to air, and later changes into brown. Also, it is the sap which constitutes

about 90% of the weight of the pseudostem [116]. The watery content of the banana pseudostem is referred to as banana sap. Banana sap has many unique properties. Its constituents include carbohydrates, lignins, tannins, and alpha-cellulose [117]. Banana sap can be put to numerous uses as well. The sap can be utilized to develop liquid fertilizers and nutrient spray solution, mordant for natural dyes [4]. Banana sap/banana stem juice discarded into the environment has the potential to produce ethanol. By combining the banana sap with other industrial wastes such as corn steep liquor, spent wash, and yeast extract production of ethanol can be significantly increased [118]. Furthermore, studies reported the enhanced methane/biogas production via co-digestion of banana sap/banana stem juice (BSJ) with agricultural residue washings such as bagasse washing (BW) and wheat straw washing (WSW). Biomethanation potential was demonstrated by BSJ alone, as well as in combination with BW and WSW [119]. Among the unique medicinal properties it possesses, the notable ones are the following: it can be used for the treatment of epilepsy, fever, leprosy, insect bites, digestive ailments, and hemorrhoids along with astringent properties [1]. Various studies conducted to analyze the therapeutic properties of the pseudostem sap have shown antimicrobial properties. Also, it showed substantial antioxidant activity [1]. In a study, it was found that the sap has hemostatic properties with non-specific action on the coagulation cascade. Tannins present in the sap might be an important factor for mediating the interaction between the sap and proteins in serum which leads to the formation of a network thus effectively reducing blood loss. This network not only traps the red blood cells but the leukocytes also and inhibits their movement. Another important factor is the astringent properties of the sap in determining its hemostatic properties. The sap also showed vasoconstriction properties. It was a combination of the two that helped in reducing the time of clotting [120]. The banana sap is rich in phenols and certain aromatic amino acids; dopamine was found in the sap, which is a potential vasoconstrictor. The presence of dopamine can explain the medicinal properties of the sap where it was often used to stop the bleeding from an injury. The sap was also found to contain N-acetylserotonin, whose function remains unclear. Apart from these, it is also responsible for antioxidant activity. Naringenin was found to have beneficial effects such as lowering the cholesterol level in blood. Other compounds such as kaempferol and quercetin also possess unique therapeutic values such as anti-inflammation and anti-venom. Myricetin, also found in the banana sap, is said to reduce the risk of neurodegenerative disorders in critical diseases such as Alzheimer's disease. Another compound extracted from the sap, apigenin, is also said to have anti-cancerous properties as it promotes cell division and apoptosis [83]. Research has shown that the banana pseudostem juice is quite effective in the stimulation of wound healing whose action was quite comparable to that of silver sulphadoxine [121]. Banana stem sap (BSS) has a higher potential for improving wound healing in diabetic mice than virgin coconut oil (VCO) [122]. Banana stem sap was found to have hypoglycemic effects [123] and hyperglycemic effects as depicted in the in vivo studies conducted by Singh et al. [124]. Mordant is any chemical substance that is crucial for the establishment of dye (color) in a particular material. Using banana pseudostem sap as mordant not only gave efficient results, it was also cost-effective. Tannins present in the sap are the main chemical compound responsible for the dyeing action of the sap [117]. Scientists have also found out that banana sap can also be effectively used as an anti-corrosive agent. Mixing the concrete with banana sap can amplify the steel reinforcement thus enabling it to withstand harsh environmental conditions [116]. To enhance the nutritional value of sap, researchers tried to find out whether the plantain stem juice could be blended with fruit juice. Results showed that banana juice can be added to grape fruit juice that will increase the nutritional content and antioxidant activity. This will open up the novel view point for the utilization of the plantain stem juice. Furthermore, this will also produce a low-cost blended average required by the consumers [125]. It was found out that banana sap had all the requirements of raw material for a sports drink. However, other ingredients including water, sodium, and sugar need to be added. It shows that this sap has the potential to be exploited as a sports drink, although more research needs to be done to assess its safety, i.e., whether it is safe for human consumption or not [23]. In another study, the banana sap was mixed with non-digestible gluco-oligosaccharides and D-allulose. Dextransucrase enzyme transfers D-glucose moiety of sucrose into α-(1–6) linked oligosaccharides in the presence of an acceptor. These oligosaccharides are beneficial as they promote the growth of good bacteria in the mouth. Furthermore, calcium alginate has been studied as one of the most efficient carriers for glucan sucrase immobilization. It was seen that dextransucrase-based biocatalytic reactions could change the sucrose into probiotic oligosaccharides in the banana pseudostem juice. These could function as probiotics because non-digestible gluco-oligosaccharides act as an apt substrate for the growth of beneficial bacteria. D-allulose has many beneficial effects. It has almost zero calorific value and has positive health impacts, some of which are anti-diabetic, anti-dyslipidemic, anti-hyper glycemic, and neuroprotective [126]. The stem juice has an antilithiatic activity which was confirmed while conducting a study to assess whether the juice can dissolve kidney stones. Results indicated that the juice could dissolve calcium, phosphate, and oxalate ions from the stones and exerted a beneficial effect in cases of kidney stones [127]. In the industrial field also, the banana sap has been tried as a unique resin that was environmentally friendly. The hybrid

bio-based resin that was prepared had many unique features such as improved strength of the resin, completely biodegradable, and has a significant potential to be used in the automobile industry. Results showed that resins with 50% banana sap by weight had the best strength and preferable qualities [128].

5 Challenges and future prospects

Waste management and by-product handling in the agriculture sector generate environmental and sustainability issues [129]. It may be difficult to dispose of this garbage due to the following factors: pathogen development and biological stability. Many types of garbage already include a large number of bacteria or will be rapidly transformed by microbial action. The present techniques for furthermore utilizing product-specific waste have been established along conventional lines and are inextricably linked to the agricultural origins of the raw materials themselves.

In modern agriculture, bananas are classified as fruit crops or cash crop commodities. These commodities generate a large quantity of cellulosic waste, often known as agricultural waste or biomass. It is a constant challenge to invent new approaches to overcome such a large volume of agricultural waste or biomass. Recent trends emphasize the use of this biomass for value-added applications to meet needs in renewable energy, fiber composites and textiles, food alternatives, and animal feed [130].

Several researches have been conducted to enhance the utilization of banana by-products in order to fulfill the rising demand for raw materials in various sectors [14–17]. These studies paved the door for new and innovative approaches to develop consumer items and applications using a value-added strategy while recycling banana agricultural wastes. As a tool to promote a productive community, there is an ongoing need to generate and invent new technologies with value-added capabilities from alternative bio-resources. Because of the rising need for food, energy, and other essentials, a gradual shift in present technical progress toward the use of alternative resources in many sectors is required to meet the demands of the world's growing population.

To establish economic impacts and fulfill the demands of the market, it is highly preferable to convert the waste biomass and by-products into highly beneficial products [131]. There is a long way to go before banana waste can be efficiently used to meet the needs and requirements of diverse fields. Although the products from banana wastes such as papers, bio-resins, biofuels, and fibers will be of extremely good quality, there is a need to take initiative and spread awareness so that these banana by-products can be made widely and easily available. Furthermore, the quality of the product generated from the banana by-product should be comparable or better as compared to the competition existing in the market. Before releasing the product into the market, it has to be assessed for its purity and ensured that it fulfills all the criteria and regulations set by the regulatory bodies. Coming from the customer's point of view, the product should be suitable for the consumer in every way. Food products developed from banana by-products need to be fortified on this aspect as well.

Considering the fact that the immediate issue will always be research innovation in order to provide high-value, high-quality products with low costs. Bananas, which cover a wide range of well-known varieties and cultivars, have been explored, and by-products such as pseudostems, rhizomes, leaves, fruit stalks, and peels from common varieties have been identified as potential raw materials in the food and non-food industries, with each application providing a unique solution. Banana by-products that have been evaluated and found to have potential applications for food additives, nutraceuticals, food supplements, feeds, renewable fuel, fibers, bioactive and other organic chemicals, fertilizers, and contaminant absorbers should be furthermore addressed for their safety aspects to meet market demand. In order to make these unprocessed raw materials available for industrial scale processing, a standardized collecting system and processing of banana by-products had to be resolved. The trend of using more sustainable and economical by-products is increasing along with the current situation of worldwide population expansion. It gives a suitable and significant reason for the development of sustainable goods from banana by-products and waste, making it a long-term income-generating commodity. Income source from waste, such as banana by-products, should be considered as one of the most significant methods to ensure an environmentally sustainable future for future generations.

There exists a huge variety of cultivars of banana that need to be studied to identify which species gives the best raw material that can be furthermore processed. Moreover, more detailed research is required to develop molecular markers or any other technology that can easily identify and differentiate between the different varieties of banana species. This can be followed by the use of genetic engineering techniques as well as marker assisted breeding to generate products that have better features and are tuned to the needs of the consumer. Efforts are needed to be taken for educating the farmers as well as the managers who supervise the collection of by-products as well as their processing. Recently, there has been a shift toward adopting agricultural waste as a renewable source of energy which lowers the cost and has beneficial effects on the environment. Land degradation is prevented as well as the waste is utilized into something productive. In this way, the stress on the environment is also reduced. This will also help the banana farmers by generating extra income. There has been very limited research

conducted to explain the relationship between the quality of the banana by-product and the geographic location as well as the climate of that particular location [132]. More research and assessment is required to calculate the cost of modern equipment that will be in use to convert the by-products into the feasible processed compound. More regulation is required concerning the collection of the by-products and sorting them so that the product does not start to decay. For example, lignocellulosic materials will start degrading if a certain period of storage interval is exceeded. The storage and handling procedures need to be standardized so that the quality of the by-product remains the same without fluctuating. Efforts are needed to be taken for educating the farmers as well as the managers who supervise the collection of by-products as well as their processing. With the non-renewable resources declining, it is time to look for alternate sources of energy in nature only. The agricultural wastes provide a lucrative option. It is also essential to exploit the various fields in which banana waste products can be used, i.e., as food additives, nutraceuticals, in the paper-making industry, making sports juice, renewable energy, compost, resource of bioactive, and other organic chemicals. This approach will help us in leaving a greener and healthier sustainable future to the generations.

6 Conclusions

The banana by-products such as pith, leaves, pseudostem, and fibers have great potential to fulfill the need of the raw material in different industries such as pulp, paper, beverage, and textile industries. The banana by-products such as pseudostem, peel, pulp, sap and flowers are reported to possess bioactive compounds having anti-bacterial, anti-oxidants, anti-cancer, and anti-inflammatory activity. It can also be used for the generation of biofuel (bioethanol and methane). Banana waste by-products can be investigated for use in foods as food additives or pharmaceuticals as a bacteriolytic or fungicidal agent, or separated into various phenolic-rich fractions or specific phenolic compounds as these are potent sources of anti-angiogenic and anti-cancer agents. It may have a key function in lowering the risk of neurodegenerative and cardiovascular diseases. Individual compounds and enhanced fractions can be utilized as food fortifiers or as functional agents in pharmaceutical products. As a result, the banana industry can acquire better significance. Even though there is a long way to go in terms of optimizing the use of biomass waste, biomass-derived adsorbents have a bright future in terms of societal and environmental sustainability. The best part of the products developed from the banana is that it will reduce the amount of biomass that is wasted, and will result in the utilization of the by-product into something good for the environment. Furthermore, studies should be conducted to assess the feasibility of utilizing the banana sap in various industries.

Acknowledgements Authors thankfully acknowledge the financial assistance provided by the Department of Science and Technology, Ministry of Science and Technology, Government of India under the project "Utilization of Banana Stem Juice for renewable energy and value added products." The authors are grateful to the Director, Thapar Institute of Engineering and Technology, Patiala, India, for providing the facilities to complete the research work.

Author contributions MSR involved in conceptualization and funding acquisition; GG participated in writing—original draft preparation; MBW and SS participated in review and editing. All authors participated in the final improvement of the manuscript.

Funding The financial support for carrying out the study was received under project entitled "Utilization of banana stem juice for renewable energy and value added products" from Department of Science and Technology, Govt. of India (DST/SSTP/Haryana/345).

Data availability All relevant data are included in the paper.

Declarations

Conflict of interest The authors declare no conflict of interests.

References

1. Kumar KS, Bhowmik D, Duraivel S, Umadevi M (2012) Traditional and medicinal uses of banana. J Pharmacogn Phytochem 1:51–63
2. Simmonds NW, The evolution of the bananas. The Evolution of the Bananas (1962). https://www.cabdirect.org/cabdirect/abstract/19630303919
3. Khanum F, Swamy MS, Krishna KS, Santhanam K, Viswanathan KR (2000) Dietary fiber content of commonly fresh and cooked vegetables consumed in India. Plant Foods Hum Nutr 55:207–218
4. Mohiuddin AKM, Saha MK, Hossian MS, Ferdoushi A (2014) Usefulness of banana (*Musa paradisiaca*) wastes in manufacturing of bio-products: a review. The Agriculturists 12:148–158
5. Mohapatra D, Mishra S, Sutar N (2010) Banana and its by-product utilisation: an overview. Food Bioprocess Technol 69:323–329
6. Sharrock S and Lusty C, Nutritive value of banana, INIBAP Annual Report (2000)
7. Muraleedharan H, Perumal K (2010) Ecofriendly handmade paper making. Booklet published from Shri AMM Murugappa Chettiar Research Centre, Taramani, Chennai
8. Cordeiro N, Belgacem MN, Torres IC, Moura JCVP (2004) Chemical composition and pulping of banana pseudo-stems. Indus Crops Prod 19:147–154
9. Arvanitoyannis IS, Mavromatis A (2009) Banana cultivars, cultivation practices, and physicochemical properties. Crit Rev Food Sci Nutr 49:113–135
10. Padam BS, Tin HS, Chye FY, Abdullah MI (2014) Banana by-products: an under-utilized renewable food biomass with great potential. J Food Sci Technol 51:3527–3545
11. Seth K, Top Banana Producing Countries In The World (2014). Retrieved from http://www.worldatlas.com/articles/top-banana-producing-countries-in-the-world.html

12. Pei P, Zhang C, Li J, Chang S, Li S, Wang J, Zhao M (2014) Li, Yu M, and Chen X, Optimization of NaOH pretreatment for enhancement of biogas production of banana pseudo-stem fiber using response surface methodology. BioResources 9:5073–5087
13. Kokab S, Asghar M, Rehman K, Asad MJ, Adedyo O (2003) Bioprocessing of banana peel for α-amylase production by Bacillus subtilis. Int J Agri Biol 5:36–39
14. Clarke WP, Radnidge P, Lai TE, Jensen PD, Hardin MT (2008) Digestion of waste bananas to generate energy in Australia. Waste Manage 28:527–533
15. Doran I, Sen B, Kaya Z (2005) The effects of compost prepared from waste material of banana on the growth, yield and quality properties of banana plants. J Environ Biol 26:7–12
16. Emaga TH, Ronkart SN, Robert C, Wathelet B, Paquot M (2008) Characterisation of pectins extracted from banana peels (Musa AAA) under different conditions using an experimental design. Food Chem 108:463–471
17. Kuo JM, Hwang A, Yeh DB, Pan MH, Tsai ML, Pan BS (2006) Lipoxygenase from banana leaf: purification and characterization of an enzyme that catalyzes linoleic acid oxygenation at the 9-position. J Agric Food Chem 54:3151–3156
18. Rosli WW, Law KN, Zainuddin Z, Asro R (2004) Effect of pulping variables on the characteristics of oil-palm frond-fiber. Bioresour Technol 93:233–240
19. Vigneswaran C, Pavithra V, Gayathri V, Mythili K (2015) Banana fiber: scope and value-added product development. J Text Appar Technol Manag 9:1–7
20. Jamal P, Saheed OK, Alam Z (2012) Bio-valorization potential of banana peels (Musa sapientum): an overview. Asian Journal of Biotechnology 4:1–14
21. Naik SN, Goud VV, Rout PK, Dalai AK (2010) Production of first and second generation biofuels: a comprehensive review. Renew Sustain Energy Rev 14:578–597
22. Kadirvelu K, Kavipriya M, Karthika C, Radhika M, Vennilamani N, Pattabhi S (2003) Utilization of various agricultural wastes for activated carbon preparation and application for the removal of dyes and metal ions from aqueous solutions. Bioresour Technol 87:129–132
23. Ogunleye OO, Ajala MA, Agarry SE (2014) Evaluation of biosorptive capacity of banana (Musa paradisiaca) stalk for lead (II) removal from aqueous solution. J Environ Prot Sci 5:1451
24. Ahmed T, Danish M (2018) Prospects of banana waste utilization in waste water treatment: a review. J Environ Manage 206:330–348
25. Prachpreecha O, Pipatpanyanugoon K, Sawangwong P (2016) A study of characterizations and efficiency of activated carbon prepared from peel and bunch of banana for methyl orange dye adsorption. IOSR J Environ Sci Toxicol Food Technol 10:17–26
26. Abbas MN, Abbas FS, Ibrahim SA (2014) Cyanide removal from wastewater by using banana peel. J Asian Sci Res 4:239–247
27. Bhaumik R, Mondal NK (2016) Optimizing adsorption of fluoride from water by modified banana peel dust using response surface modelling approach. Appl Water Sci 6:115–135
28. Moran M, How to grow banana plants (2020) Retrieved from https://www.wikihow.com/Grow-Banana-Plants
29. Macrae R, Robinson RK, Sadler MJ (1993) Bananas and plantains. Food Technology and Nutrition Academic Press, Encyclopaedia of Food Science
30. School Science Lessons, Banana Project (2021) Retrieved from https://www.uq.edu.au/_School_Science_Lessons/BaProj.html
31. Alwi H, Idris J, Musa M and Ku Hamid KH, A preliminary study of banana stem juice as a plant-based coagulant for treatment of spent coolant wastewater. J. Chem. (2013)
32. Asif M, Kaur A (2018) Biologically active phytochemical contents and biological activities of whole Musa Acuminata (banana) plant. International Journal of Recent Advances in Medical and Pharma Research 1:5–33
33. Feriotti DG and Iguti AM (2012)Proposal for use of pseudostem from banana tree (Musa cavendish). In: International Congress of Engineering and Food
34. Zhang P, Whistler RL, BeMiller JN, Hamaker BR (2005) Banana starch: production, physicochemical properties, and digestibility-a review. Carbohydr Polym 59:443–458
35. Apsara M, Pushpalatha PB (2002) Quality upgradation of jellies prepared using pectin extracted from fruit wastes. J Trop Agric 40:31–34
36. Elanthikkal S, Gopalakrishnapanicker U, Varghese S, Guthrie JT (2010) Cellulose microfibres produced from banana plant wastes: isolation and characterization. Carbohydr Polym 80:852–859
37. Aziz NAA, Ho LH, Azahari B, Bhat R, Cheng LH, Ibrahim MNM (2011) Chemical and functional properties of the native banana (Musa acuminata× balbisiana colla cv Awak) pseudo-stem and pseudo-stem tender core flours. Food Chem 128:748–753
38. da Mota RV, Lajolo FM, Cordenunsi BR, Ciacco C (2000) Composition and functional properties of banana flour from different varieties. Starch-Stärke 52:63–68
39. Emaga TH, Robert C, Ronkart SN, Wathelet B, Paquot M (2008) Dietary fibre components and pectin chemical features of peels during ripening in banana and plantain varieties. Bioresour Technol 99:4346–4354
40. Jadhav SA, Kataria PK, Bhise KK, Chougule SA (2013) Amylase production from potato and banana peel waste. Int J Curr Microbiol Appl Sci 2:410–414
41. Oshoma CE, Eguakun-Owie SO, Obuekwe IS (2019) Utilization of banana peel as a substrate for single cell protein and amylase production by Aspergillus niger. African Scientist 18:143–150
42. Liang S, Gliniewicz K, Gerritsen AT, McDonald AG (2016) Analysis of microbial community variation during the mixed culture fermentation of agricultural peel wastes to produce lactic acid. Bioresour Technol 208:7–12
43. Alsaheb RAA, Aladdin A, Othman NZ, Malek RA, Leng OM, Aziz R, Enshasy HAE (2015) Lactic acid applications in pharmaceutical and cosmeceutical industries. J Chem Pharm Res 7:729–735
44. Manach C, Scalbert A, Morand C, Remesy C, Jimenez L (2004) Polyphenols: food sources and bioavailability. Am J Clin Nutr 79:727–747
45. from plant to health (2008) de Pascual-Teresa S. and Sanchez-Ballesta MT, Anthocyanins. Phytochem Rev 7:281–299
46. Sui X, Zhang Y, Zhou W (2016) Bread fortified with anthocyanin-rich extract from black rice as nutraceutical sources: its quality attributes and in vitro digestibility. Food Chem 196:910–916
47. Pazmiño-Durán EA, Giusti MM, Wrolstad RE, Glória MBA (2001) Anthocyanins from banana bracts (Musa X paradisiaca) as potential food colorants. Food Chem 73:327–332
48. Sutikno NMD, Rahayu S (2014) Fabrication and characterization of banana flower extract anthocyanin-based organic solar cell. J Adv Agric Technol 1:89–93
49. Ove TA, Kamal MM, Nasim SMNI, Momin MMI, Mondal SC (2019) Extraction and quantification of anthocyanin from banana bracts using different pH and solvent concentration. Int J 4:060–064
50. Kitdamrongsont K, Pothavorn P, Swangpol S, Wongniam S, Atawongsa K, Svasti J, Somana J (2008) Anthocyanin composition of wild bananas in Thailand. J Agric Food Chem 56:10853–10857
51. Chen L, Li D, Hao D, Ma X, Song S, Rong Y (2020) Study on chemical compositions, sensory properties, and volatile compounds of banana wine. J Food Process Pres 44:14924

52. Hasbullah UHAA, Mentari AB, Kholisoh SN and Hidayat TN (2021) Sensory properties of analog coffee from banana peels. *AGROINTEK 15*
53. Kennedy J (2009) Bananas and people in the homeland of genus Musa: not just pretty fruit. Ethnobot Res Appl 7:179–197
54. Emaga TH, Andrianaivo RH, Wathelet B, Tchango JT, Paquot M (2007) Effects of the stage of maturation and varieties on the chemical composition of banana and plantain peels. Food Chem 103:590–600
55. Someya S, Yoshiki Y, Okubo K (2002) Antioxidant compounds from bananas (Musa Cavendish). Food Chem 79:351–354
56. Anhwange BA (2008) Chemical composition of *Musa sapientum* (banana) peels. J Food Technol 6:263–266
57. Mokbel MS, Hashinaga F (2005) Antibacterial and antioxidant activities of banana (Musa, AAA cv. Cavendish) fruits peel. Am J Biochem Biotechnol 1:125–131
58. Devatkal SK, Kumboj R, Paul D (2014) Comparative antioxidant effect of BHT and water extracts of banana and sapodilla peels in raw poultry meat. J Food Sci Technol 51:387–391
59. Saravanan K, Aradhya SM (2011) Polyphenols of pseudostem of different banana cultivars and their antioxidant activities. J Agric Food Chem 59:3613–3623
60. Chye FY, Sim KY (2009) Antioxidative and antibacterial activities of *Pangium edule* seed extracts. Int J Pharmacol 5:285–297
61. Chanwitheesuk A, Teerawutgulrag A, Kilburn JD, Rakariyatham N (2007) Antimicrobial gallic acid from *Caesalpinia mimosoides* Lamk. Food Chem 100:1044–1048
62. Lu Z, Nie G, Belton PS, Tang H, Zhao B (2006) Structure–activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives. Neurochem Int 48:263–274
63. Artali R, Beretta G, Morazzoni P, Bombardelli E, Meneghetti F (2009) Green tea catechins in chemoprevention of cancer: a molecular docking investigation into their interaction with glutathione S-transferase (GST P1–1). J Enzyme Inhib Med Chem 24:287–295
64. Zheng J, Ding C, Wang L, Li G, Shi J, Li H, Wang H, Suo Y (2011) Anthocyanins composition and antioxidant activity of wild Lycium ruthenicum Murr. from Qinghai-Tibet Plateau. Food Chem 126:859–865
65. Kulma A, Szopa J (2007) Catecholamines are active compounds in plants. Plant Sci 172:433–440
66. Vu HT, Scarlett CJ, Vuong QV (2018) Phenolic compounds within banana peel and their potential uses: a review. J Funct Foods 1:238–248
67. Correa M, Bombardelli MC, Fontana PD, Bovo F, Messias-Reason IJ, Maurer JBB, Corazza ML (2017) Bioactivity of extracts of Musa paradisiaca L. obtained with compressed propane and supercritical CO2. J Supercrit Fluids 122:63–69
68. Rattanavichai W, Cheng W (2014) Effects of hot-water extract of banana (Musa acuminata) fruit's peel on the antibacterial activity, and anti-hypothermal stress, immune responses and disease resistance of the giant freshwater prawn. Macrobrachium rosenbegii Fish Shellfish Immunol 39:326–335
69. Saleem M (2009) Lupeol, a novel anti-inflammatory and anticancer dietary triterpene. Cancer Lett 285:109–115
70. Umar Lule S, Xia W (2005) Food phenolics, pros and cons: a review. Food Rev Int 21:367–388
71. Tomás-Barberán FA, Clifford MN (2000) Dietary hydroxybenzoic acid derivatives–nature, occurrence and dietary burden. J Sci Food Agric 80:1024–1032
72. Locatelli C, Filippin-Monteiro FB, Creczynski-Pasa TB (2013) Alkyl esters of gallic acid as anticancer agents: a review. Eur J Med Chem 60:233–239
73. Rocha LD, Monteiro MC, Teodoro AJ (2012) Anticancer properties of hydroxycinnamic acids-a review. Cancer Clin Oncol 1:109–121
74. Gülçin İ (2006) Antioxidant activity of caffeic acid (3, 4-dihydroxycinnamic acid). Toxicology 217:213–220
75. Shukla S, Gupta S (2010) Apigenin: a promising molecule for cancer prevention. Pharm Res 27:962–978
76. Hosseinzadeh H, Nassiri-Asl M (2014) Review of the protective effects of rutin on the metabolic function as an important dietary flavonoid. J Endocrinol Invest 37:783–788
77. Tapas AR, Sakarkar DM, Kakde RB (2008) Flavonoids as nutraceuticals: a review. Trop J Pharm Res 7:1089–1099
78. Patel K, Singh GK, Patel DK (2018) A review on pharmacological and analytical aspects of naringenin. Chin J Integr Med 24:551–560
79. Luo Y, Umegaki H, Wang X, Abe R, Roth GS (1998) Dopamine induces apoptosis through an oxidation-involved SAPK/JNK activation pathway. J Biol Chem 273:3756–3764
80. Cadet JL, Brannock C (1998) Invited review free radicals and the pathobiology of brain dopamine systems. Neurochem Int 32:117–131
81. Kanazawa K, Sakakibara H (2000) High content of dopamine, a strong antioxidant, in cavendish banana. J Agric Food Chem 20:844–848
82. Pothavorn P, Kitdamrongsont K, Swangpol S, Wongniam S, Atawongsa K, Svasti J, Somana J (2010) Sap phytochemical compositions of some bananas in Thailand. J Agric Food Chem 58:8782–8787
83. Singh B, Singh JP, Kaur A, Singh N (2016) Bioactive compounds in banana and their associated health benefits–a review. Food Chem 206:1–1
84. Akinyele BJ, Agbro O (2007) Increasing the nutritional value of plantain wastes by the activities of fungi using the solid state fermentation technique. Res J Microbiol 2:117–124
85. Amarnath R, Balakrishnan V (2007) Evaluation of the banana (*Musa paradisiaca*) plant by-product's fermentation characteristics to assess their fodder potential. Int J Dairy Sci 2:217–225
86. Subagyo A and Chafidz A (2018) Book chapter published by Intechopen. Available on internet
87. Oliveira L, Evtuguin DV, Cordeiro N, Silvestre AJ, Silva AM, Torres IC (2006) Structural characterization of lignin from leaf sheaths of "Dwarf Cavendish" banana plant. J Agric Food Chem 54:2598–2605
88. Zuluaga R, Putaux JL, Cruz J, Vélez J, Mondragon I, Gañán P (2009) Cellulose microfibrils from banana rachis: effect of alkaline treatments on structural and morphological features. Carbohydr Polym 76:51–59
89. Cherian BM, Pothan LA, Nguyen-Chung T, Mennig G, Kottaisamy M, Thomas S (2008) A novel method for the synthesis of cellulose nanofibril whiskers from banana fibers and characterization. J Agric Food Chem 56:5617–5627
90. Pothan LA, Potschke P, Habler R, Thomas S (2005) The static and dynamic mechanical properties of banana and glass fiber woven fabric-reinforced polyester composite. J Compos Mater 39:1007–1025
91. Maleque M, Belal FY, Sapuan SM (2007) Mechanical properties study of pseudo-stem banana fiber reinforced epoxy composite. Arab J Sci Eng 32:359–364
92. Jacob N, Prema P (2008) Novel process for the simultaneous extraction and degumming of banana fibers under solid-state cultivation. Br J Microbiol 39:115–121
93. Mire MA, Benjelloun-Mlayah B, Delmas M, Bravo R (2005) Formic acid/acetic acid pulping of banana stem (Musa Cavendish). Appita Journal: Journal of the Technical Association of the Australian and New Zealand Pulp and Paper Industry 58:393
94. Fagbemigun TK, Fagbemi OD, Buhari F, Mgbachiuzo M, Igwe CC (2016) Fibre characteristics and strength properties of Nigerian pineapple leaf (*Ananas cosmosus*), banana peduncle

and banana leaf (*Musa sapientum*) - potential green resources for pulp and paper production. J Sci Res Reports 12:1–13
95. Nassar M, Ebrahim EE, Sherif HM, Ali MA (2021) Optimization of banana stem pulp to substitute softwood pulp for high quality paper. Egypt J Chem 64:1461–1469
96. Marella JBR, Madireddy S, Maripi AN (2014) Production of pulp from banana pseudo stem for grease proof paper. Table of Content Topics 61(2):61–99
97. Sakare P, Bharimalla AK, Dhakane-Lad J, Patil PG (2020) Development of greaseproof paper from banana pseudostem fiber for packaging of butter. J Nat Fibers 18:1974–1982
98. Puvvada N, Kumar BP, Konar S, Kalita H, Mandal M, Pathak A (2012) Synthesis of biocompatible multicolor luminescent carbon dots for bioimaging applications. Sci Technol Adv Mater 13:045008
99. Vandarkuzhali SAA, Jeyalakshmi V, Sivaraman G, Singaravadivel S, Krishnamurthy KR, Viswanathan B (2017) Highly fluorescent carbon dots from pseudo-stem of banana plant: applications as nanosensor and bio-imaging agents. Sens Actuators, B Chem 252:894–900
100. Santoyo-Aleman D, Sanchez LT, Villa CC (2019) Citric-acid modified banana starch nanoparticles as a novel vehicle for β-carotene delivery. J Sci Food Agric 99:6392–6399
101. Nayak S, Sajankila SP, Rao CV (2021) Green synthesis of gold nanoparticles from banana pith extract and its evaluation of antibacterial activity and catalytic reduction of malachite green dye. J Microbiol, Biotechnol Food Sci 7:641–645
102. Xu YX, Hanna MA, Isom L (2008) "Green" Chemicals from renewable agricultural biomass—a mini review. Open Agr J 2:54–61
103. Mohanty AK, Misra M and Drzal LT (2018) Sustainable biocomposites from renewable resources: opportunities and challenges in the green materials world. Renew Energy 396–409
104. Saxena RC, Adhikari DK, Goyal HB (2009) Biomass-based energy fuel through biochemical routes: a review. Renewable Sustainable Energy Rev 13:167–178
105. Reddy N, Yang YQ (2005) Biofibers from agricultural by-products for industrial applications. Trends Biotechnol 23:22–27
106. Pimentel D, Patzek TW (2005) Ethanol production using corn, switchgrass and wood; biodiesel production using soybean and sunflower. Nat Resour Res 14:65–76
107. Raposo S, Pardão JM, Diaz I, Lima-Costa ME (2009) Kinetic modelling of bioethanol production using agro-industrial by-products. Int J of Energy Env 3:8
108. Manikandan K, Saravanan V, Viruthagiri T (2008) Kinetics studies on ethanol production from banana peel waste using mutant strain of Saccharomyces cerevisiae. Indian J Biotechnol 7:83–88
109. Harish KRY, Srijana M, Madhusudhan RD, Gopal R (2010) Coculture fermentation of banana agro-waste to ethanol by cellulolytic thermophilic *Clostridium thermocellum* CT2. Afr J Biotechnol 9:1926–1934
110. Hammond JB, Egg R, Diggins D, Coble CG (1996) Alcohol from bananas. Bioresour Technol 56:125–130
111. Brooks AA (2008) Ethanol production potential of local yeast strains isolated from ripe banana peels. Afr J Biotechnol 7:3749–3752
112. Chanakya HN, Sharma I, Ramachandra TV (2009) Micro-scale anaerobic digestion of point source components of organic fraction of municipal solid waste. Waste Manage 29:1306–1312
113. Bardiya N, Somayaji D, Khanna S (1996) Biomethanation of banana peel and pineapple waste. Bioresour Technol 58:73–76
114. Kalia VC, Sonakya V, Raizada N (2000) Anaerobic digestion of banana stem waste. Bioresour Technol 73:191–193
115. Ilori MO, Adebusoye SA, Lawal AK and Awotiwon OA, Production of biogas from banana and plantain peels Adv Environ. Biol 33–39 (2007)
116. El-Sayed M, Mansour OY, Selim IZ, Ibrahim MM (2001) Identification and utilization of banana plant juice and its pulping liquor as anti-corrosive materials. J Sci Ind Res 60:738–747
117. Barhanpurkar S, Kumar A, Purwar R (2015) Charcterisation of banana pseudostem sap used as a mordant for dying. SSRG Int J Polym Text Eng 2:1–7
118. Gupta G, Baranwal M, Saxena S, Reddy MS (2019) Utilization of banana stem juice as a feedstock material for bioethanol production. CLEAN–Soil. Air, Water 47:1–5
119. Tripathi SK, Kaur D, Alam I, Bhardwaj NK, Pathak P and Kumar S, Utilization of different microbes to enhance the biogas production from banana stem juice. J Environ Eng 7: (2020)
120. Klotoé JR, Dougnon TV, Sacramento TI, Dandjesso C, Edorh AP, Fanou VBA, Fah L, Atego JM, Loto F, Dramane K (2012) Hemostatic potential of the sap of Musa sapientum L. (Musaceae). J Appl Pharm Sci 2:65–69
121. Weremfo A, Adinortey MB, Pappoe ANM (2011) Haemostatic effect of the stem juice of Musa paradisiaca L.(Musaceae) in guinea pigs. Adv Biol Res 5:190–192
122. Sari Y, Isworo A, Upoyo AS, Sumeru A, Kurniawan DW and Sutrisna E, A comparison of the effectiveness banana stem sap and virgin coconut oil on diabetic wound healing. In IOP Conference Series: Earth and Environmental Science, 746: (2021)
123. Nguyen D, Novakova A, Spurna K (2017) Antidiabetic compounds in stem juice from banana. Czech J Food Sci 35:407–413
124. Singh SK, Kesari AN, Rai PK, Watal G (2007) Assessment of glycemic potential of *Musa paradisiaca* stem juice. Indian J Clin Biochem 22:48–52
125. Ravi U, Menon L, Gomathy G (2011) Development and quality assessment of value added plantain stem juice incorporated with grape juice. Indian J Nat Prod Resour 2:204–210
126. Sharma M, Patel SN, Sangwan RS, Singh SP (2017) Biotransformation of banana pseudostem extract into a functional juice containing value added biomolecules of potential health benefits. Indian J Exp Biol 55:453–462
127. Prasad KV, Bharathi K, Srinivasan KK (1993) Evaluation of Musa (Paradisiaca Linn. cultivar)—"Puttubale" stem juice for antilithiatic activity in albino rats. Indian J Physiol Phannacol 37:337–341
128. Paul V, Kanny K, Redhi GG (2013) Formulation of a novel bioresin from banana sap. Ind Crops Prod 43:496–505
129. Russ W, Pittroff RM (2004) Utilizing waste products from the food production and processing industries. Crit Rev Food Sci Nutr 44:57–62
130. Rosentrater K, Todey D, Persyn R (2009) Quantifying total and sustainable agricultural biomass resources in South Dakota—a preliminary assessment. Agricultural Engineering International: the CIGR Journal of Scientific Research and Development Manuscript 1059(1058):1
131. Jayathilakan K, Sultana K, Radhakrishna K, Bawa AS (2012) Utilization of byproducts and waste materials from meat, poultry and fish processing industries: a review. J Food Sci Technol 49:278–293
132. Weathers PJ, Arsenault PR, Covello PS, McMickle A, Teoh KH, Reed DW (2011) Artemisinin production in *Artemisia annua*: studies in planta and results of a novel delivery method for treating malaria and other neglected diseases. Phytochem Rev 10:173–183
133. Tsamo CV, Herent MF, Tomekpe K, Emaga TH, Quetin-Leclercq J, Rogez H, Larondelle Y, Andre C (2015) Phenolic profiling in the pulp and peel of nine plantain cultivars (Musa sp.). Food Chem 167:197–204

134. Mahouachi Mahouachi J, López-Climent MF, Gómez-Cadenas A (2014) Hormonal and Hydroxycinnamic acids profiles in banana leaves in response to various periods of water stress. Sci World J 145:496–504
135. China R, Dutta S, Sen S, Chakrabarti R, Bhowmik D, Ghosh S, Dhar P (2011) In vitro antioxidant activity of different cultivars of banana flower (Musa paradicicus L.) extracts available in India. J Food Sci 76:C1292–C1299
136. Borges CV, de Oliveira Amorim VB, Ramlov F, da Silva Ledo CA, Donato M, Maraschin M, Amorim EP (2014) Characterisation of metabolic profile of banana genotypes, aiming at biofortified Musa spp. cultivars. Food Chem 145:496–504
137. Bennett RN, Shiga TM, Hassimotto NMA, Rosa EAS, Lajolo FM, Cordenunsi BR (2010) Phenolics and antioxidant properties of fruit pulp and cell wall fractions of postharvest banana (Musa acuminata Juss.) Cultivars. J Agr 58:7991–8003
138. Alothman M, Bhat R, Karim AA (2009) Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chem 115:785–788
139. Saravanan K, Aradhya SM (2011) Potential nutraceutical food beverage with antioxidant properties from banana plant biowaste (pseudostem and rhizome). Food Funct 2:603–610
140. Loganayaki N, Rajendrakumaran D, Manian S (2010) Antioxidant capacity and phenolic content of different solvent extracts from banana (Musa paradisiaca) and mustai (Rivea hypocrateriformis). Food Sci Biotechnol 5:1251–1258
141. Kumar PR, Srivastava S, Singh KK, Mathad C, Thin PS (2014) Study of antioxidant and antimicrobial properties, phytochemical screening and analysis of sap extracted from banana (Musa acuminata) pseudostem. Int J Adv Biotechnol Res 5:649–658
142. Karuppiah P, Mustaffa M (2013) Antibacterial and antioxidant activities of Musa sp. leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection. Asian Pac J Trop Biomed 3:737–42
143. Jain P, Bhuiyan MH, Hossain KR, Bachar SC (2011) Antibacterial and antioxidant activities of local seeded banana fruits. Afr J Pharmacy Pharmacol 5:1398–1403
144. Naikwade PV, Gaurav S, Sharayu, D. and Kailas J, Evaluation of antibacterial properties of Musa paradisiaca L. leaves. In Proceeding of the national conference on conservation of natural resources & biodiversity for sustainable development (2014)
145. Ehiowemwenguan G, Emoghene AO, Inetianbor JE (2014) Antibacterial and phytochemical analysis of banana fruit peel. Iosr J Pharm 4:18–25
146. Padam BS, Tin HS, Chye FY, Abdullah MI (2012) Antibacterial and antioxidative activities of the various solvent extracts of banana (Musa paradisiaca cv. Mysore) inflorescences. Journal Biol Sci 12:62–73
147. Zafar IM, Saleha A, Hoque MME, Sohel RM (2011) Antimicrobial and cytotoxic properties of different extracts of Musa sapientum L. subsp. sylvestris. International Res. J. Pharm 2:62–65
148. Dahham SS, Mohamad TA, Tabana YM, Majid AM (2015) Antioxidant activities and anticancer screening of extracts from banana fruit (Musa sapientum). Academic J Cancer Res 8:28–34
149. Li F, Li S, Li HB, Deng GF, Ling WH, Wu S, Xu XR, Chen F (2013) Antiproliferative activity of peels, pulps and seeds of 61 fruits. J Funct Foods 5:1298–1309
150. Nadumane VK, Timsina B (2014) Anticancer potential of banana flower extract: an in vitro study. Bangladesh J Pharmacol 9:628–635
151. El Zawawy NA (2015) Antioxidant, antitumor, antimicrobial studies and quantitative phytochemical estimation of ethanolic extracts of selected fruit peels. Int J Curr Microbiol Appl Sci 4:298–309
152. Manthey, John A and Jaitrong S. An HPLC-MS analysis of phenolic antioxidants in banana peel (2016)
153. Rabbani GH, Teka T, Zaman B, Majid N, Khatun M, Fuchs GJ (2001) Clinical studies in persistent diarrhea: dietary management with green banana or pectin in Bangladeshi children. Gastroenterology 121:554–560
154. Horigome T, Sakaguchi E, Kishimoto C (2002) Hypocholesterolaemic effect of banana (Musa sapientum L. var. Cavendishii) pulp in the rat fed on a cholesterol-containing. Diet.x Br J Nutr 68:231–244
155. Yin X, Quan J, Kanazawa T (2008) Banana prevents plasma oxidative stress in healthy individuals. Plant Foods Hum Nutr 63:71–76
156. Agarwal PK, Singh A, Gaurav K, Goel S, Khanna HD, Goel RK (2009) Evaluation of wound healing activity of extracts of plantain banana (Musa sapientum var. paradisiaca) in rats. Indian J Exp Biol 47:32–40
157. Tewtrakul T, Subhadhirasakul S (2007) Anti-allergic activity of some selected plants in the Zingiberaceae family. J Ethnopharmacol 109:535–538
158. Itelima J, Onwuliri F, Onwuliri E, Onyimba I, Oforji S (2013) Bio-ethanol production from banana, plantain and pineapple peels by simultaneous saccharification and fermentation process. Int J Env Sci Dev 4:213–216
159. Singh AK, Rath S, Kumar Y, Masih H, Jyotsna K (2014) Peter, Benjamin JC, Singh PK and Singh DP, Bio-ethanol production from banana peel by simultaneous saccharification and fermentation process using cocultures *Aspergillus niger* and *Saccharomyces cerevisiae*. Int J Curr Microbiol App Sci 3:84–96
160. Gebregergs A, Gebresemati M, Sahu O (2016) Industrial ethanol from banana peels for developing countries: response surface methodology. Pac Sci Rev A: Nat Sci Eng 18:22–29
161. Waghmare AG, Arya SS (2016) Utilization of unripe banana peel waste as feedstock for ethanol production. Bioethanol 2:146–156
162. Nyandiga GO, Siagi ZO, Makokha AB (2014) Optimization of anaerobic fermentation conditions for bioethanol production from banana (Ngombe) peels using yeast in a still reactor. In Proceedings of Sustainable Research and Innovation Conference 5:120–123
163. Patel H, Patel A, Surati T, Shah G (2012) Potential use of banana peels for the production of fermented products. International Journal of Ecology & Development (IJED) 9:1–7
164. Palacios S, Ruiz HA, Ramos-Gonzalez R, Martínez J, Segura E, Aguilar M, Aguilera A, Cristóbal GM, Ilyina A (2017) Comparison of physicochemical pretreatments of banana peels for bioethanol production. Food Sci Biotechnol 26:993–1001
165. Chaudhary N, Chand S and Kaur N, Bioethanol production from fruit peels using simultaneous saccharification and fermentation. GSTF J Bio Sci 3: (2014)
166. Arumugam R, Manikandan M (2011) Fermentation of pretreated hydrolyzates of banana and mango fruit wastes for ethanol production. Asian J Exp Biol Sci 2:246–256
167. Thakur S, Shrivastava B, Ingale S, Kuhad RC, Gupte A (2013) Degradation and selective ligninolysis of wheat straw and banana stem for an efficient bioethanol production using fungal and chemical pretreatment. 3 Biotech 3:365–372
168. Thancharoen T (2014) Rotten banana waste management for bioethanol producing ethanologenic yeasts. In International Conference on Biological, Civil and Environmental Engineering (BCEE-20153–4) (3–4)
169. Jagessar RC, Fraser C (2016) The fermentation of banana (Musa acuminata), mango (Mangifera indica L.) and pineapple

(Ananas comosus) mash in the absence and presence of additives. Am J Res Commun 4:131–140
170. Ingale S, Joshi SJ, Gupte A (2014) Production of bioethanol using agricultural waste: banana pseudo stem. Br J Microbiol 45:885–892

Biologia
https://doi.org/10.1007/s11756-022-01159-8

ORIGINAL ARTICLE

In vitro evaluation of bioactive properties of banana sap

Geetika Gupta[1] · Sanjai Saxena[1] · Manoj Baranwal[1] · M. Sudhakara Reddy[1]

Received: 12 August 2021 / Accepted: 14 June 2022

Abstract

Banana sap is currently designated as a waste subsequent to utilization of pseudo stem in pulp and paper industry as well as other applications which is contributing to the environmental pollution. In the present study, banana sap and its crude extracts were evaluated for antimicrobial, antioxidant and anticancer properties. The role of oxidized and un-oxidized banana sap for its antimicrobial potential against a microbial test panel comprising gram positive as well as gram negative bacteria and *Candida albicans* using *in vitro* micro broth dilution assay. The un-oxidized banana sap exhibited a significantly higher antibacterial potential as evident by a lower minimal inhibitory concentration (MIC) ranging between 15.625 to 62.5 mg/mL. *In vitro* radical scavenging activity of dichloromethane (DCM) extract of banana sap by DPPH method exhibited 54.62 ± 1.09 (µg/mL) IC_{50} value at the concentration of 1 mg/mL. Dichloromethane extract of banana sap showed maximum cytotoxic effect with human breast cancer (MCF-7) cell proliferation at the concentration of 100 µg/mL which was $78.37 \pm 0.05\%$ and the cytotoxic effect significantly increased with increasing concentration of banana sap extract. Furthermore, LCMS studies revealed the presence of bioactive compounds in dichloromethane extract of banana sap, such as rescinnamine derivative, dihydrorescinnamine and epimedin A. The present study suggested that banana sap is a promising source of bioactive compounds with relevant antimicrobial, antioxidant and anticancer properties.

Keywords Banana sap · Scavenging ability · Antimicrobial activity · Cytotoxic · LCMS · Phytochemical constituents

Abbreviations

LCMS	Liquid chromatography mass spectrometry
DCM	Dichloromethane
MIC	Minimum Inhibitory Concentration
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing antioxidant power
TAA	Total antioxidant activity
NaCl	Sodium chloride
$Na_2S_2O_5$	Sodium Pyrosulfite
EDTA	Ethylenediamine tetraacetic acid
DMSO	Dimethyl Sulfoxide
MH	Muller Hinton
SDA	Sabouraud Dextrose Agar
EUCAST	European Committee on Antimicrobial Susceptibility Testing
TTC	2, 3, 5-Triphenyl tetrazolium chloride
NCCS	National Centre for Cell Sciences
DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal Bovine Serum
MTT	3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
ELISA	Enzyme Linked Immuno Sorbent Assay
OHRLCMS	Orbitrap Liquid Chromatography Mass Spectroscopy
IC_{50}	Inhibitory Concentration $_{50}$
Minutes	Min
Hours	H

Introduction

Plant-derived natural products have played a key role in the process of drug discovery and development ever since the advent of modern medicine. The diverse chemical scaffolds present in plants exhibit a spectrum of biological activities and thus have been the mainstay for the development of drugs that have been classified as semi-synthetic or natural product derived. They have proven to be used for the development of effective therapeutic interventions in the treatment of cancer,

✉ Geetika Gupta
geetika_12_gupta@yahoo.com

[1] Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, Punjab 147004, India

Published online: 04 July 2022

diseases caused by multi-drug resistant bacteria and viruses apart from curing immunity-associated disorders (Majhi and Das 2021). Banana is a flowering plant that belongs to *Musa* spp. It is grown in the tropical regions of the world primarily for its fruits and contributes an important food source after rice, wheat, and maize. Traditionally and scientifically, banana has been found to contain medicinal properties (Nadumane and Timsina 2014). Different parts of the banana such as pseudo-stem, leaves, sap, and flowers have been documented to possess medicinal or curative properties such as anti-snake venom (Borges et al. 2005) anti-gastric ulcer (Khamboonruang et al. 2015), antimicrobial (Budi et al. 2020), antidepressant (Kar et al. 2019), antihypercholesterolemic (Dikshit et al. 2016), antioxidant effect (Dikshit et al. 2016), antidiarrheal (Yakubu et al. 2015) and antidiabetic activity (Sheng et al. 2017). After harvesting the bananas, the plant dies. The dead plants comprising of leaves, pseudo-stems contribute to huge agro-waste from the banana farms into the environment (Gupta et al. 2019). Moreover, the pseudo-stem of banana is finding application in the paper manufacturing industry along with a wide range of value-added products such as ropes, mats etc. However, the immediate concern is the huge amount of sap present in the pseudo-stem of the plant (Gupta et al. 2022). Previous studies on the phytochemical composition of banana sap have indicated the presence of compounds comprising of hydrocinnamic acids, flavones, and flavonoids (broadly phenolics) (Pothavorn et al. 2010). More specifically, stem juice of *Musa paradisiaca* reported the presence of antidiabetic compounds such as lupeol, ferulic acid, vanillic acid, trans-cinnamic acid, p-hydroxybenzoic acid, p-coumaric acid, rutin, catechin/ epicatechin, chlorogenic acid, gallic acid, caffeic acid, and nicotiflorin (Nguyen et al. 2017). The banana stem juice is contributing as liquid waste and is an environmental concern as it is being disposed of in the environment after the utilization of the banana stem for different applications (Gupta et al. 2022). Phytochemical studies on banana stem juice/ sap have been initially carried out by Pothavorn et al. (2010) and Nguyen et al. (2017) for possible exploitation of the banana stem juice. Based on this premise, the present investigation was undertaken to explore the bioactive potential of the banana stem sap by fractionating with solvents and analysing their antioxidant, anticancer and antimicrobial activities. Further, the composition of these solvent extracts were analysed by LC–MS to find out the phytochemicals which possibly would be providing the medicinal potential to the banana sap.

Materials and methods

Preparation of extracts

Fresh banana pseudo-stem of Grand naine cultivar was collected from Thapar Institute of Engineering and Technology Campus, Patiala, India. Banana stem juice was extracted from the stems with the help of local market. The sap from the banana pseudo-stem was extracted with the help of a commercial squeezer in the local market. Prior to extracting the sap, the squeezer was thoroughly washed and disinfected. Subsequent to the extraction of juice, banana sap was concentrated to ten times with the help of rotary evaporator. The concentrated sap was further extracted with dichloromethane (DCM). One hundred (100) mL of concentrated banana sap was mixed three times with 70 mL of DCM to obtain the DCM residue with the help of solvent extraction process at 35 ºC (Vasundhara et al. 2017). Further, the solvent was removed by rotary evaporator (IKA RV 10) and extract was dried by using lyophilizer. The obtained dried residue was dissolved in dimethyl sulfoxide (DMSO). DCM extract was used for the evaluation of anticancer, antioxidant activities and LCMS analysis.

Antioxidant assay

The antioxidant capacity of the dichloromethane extracts of banana sap was carried out via free radical scavenging effect (Sharma et al. 2017). In 96 well micro titer plate, 50 µL of banana sap extracts in increasing concentrations (100, 250, 500 and 1000 µg/mL) were mixed with 150 µL of DPPH (100 µM) in methanol. Ascorbic acid (Stock solution: 100 µg/mL; Working volume: 4µL) was used as the positive control. The microtitre plate was incubated in the dark for 45 min and subsequently absorbance was recorded at 517 nm using microplate reader (Tecan infinite, Austria).

The scavenging activity was expressed as:

$$\text{Scavenging activity } (\%) = \{(A_{control} - A_{sample})/A_{control}\} \times 100$$

Preparation of un-oxidized banana sap

80% ethanol containing 100 mM NaCl, 0.2 mM ascorbic acid, 40 mM citric acid, 0.1 mM $Na_2S_2O_5$, 0.25% Triton X-100 and 0.2 mM EDTA was prepared in amber coloured bottle (Pothavorn et al. 2010). Stems were processed to make sap. To prevent oxidation, freshly extracted sap was mixed with 80% ethanol solution in the ratio of 1:1. The sap was then heated at 80ºC for 45 min and subsequently centrifuged (Thermo Fischer Scientific, Sorvall Legend XFR Centrifuge, 75004538, Germany) at 11250 × g for 15 min at 25˚C. The supernatant was collected and concentrated in rota evaporator. Subsequently it was lyophilized and dissolved in 10% DMSO (dimethyl sulfoxide) and stored at 4 °C until further use.

Test microorganisms

The microbial test panel comprised of *Escherichia coli* ESS 2231, *Bacillus megaterium* FH 1127, *Pseudomonas aeruginosa* M35, *Staphylococcus aureus* ATCC 33591 and *Candida albicans* ATCC10231 used for the study of the anti-candidal activity. The bacterial cultures were individually streaked on Muller Hinton (MH) agar plate and incubated overnight and subsequently transferred a single colony aseptically to 100 mL of pre-sterilized MH broth and incubated at 37 °C overnight. In case of *Candida albicans*, it was streaked on Sabouraud Dextrose Agar (SDA) plate and incubated overnight. A single colony was picked from this plate and transferred to Sabouraud Dextrose broth and incubated at 37 °C prior to test.

In vitro antibacterial assay

Antibacterial activity of the oxidized and unoxidized extract of the banana sap was determined through *in vitro* microbroth dilution assay (Jorgensen et al. 1999; EUCAST 2018). Briefly, 96-well microtiter plate was used to evaluate the antibacterial activity and determine visual minimal inhibitory concentration (MIC) determination. The oxidized and unoxidized extract of the banana sap was dissolved in 10% dimethyl sulfoxide (DMSO) and tested in a concentration from 500 mg/mL to 1.95 mg/mL i.e., 2-fold serial dilutions. Fifty (50) µL of the 0.5 McFarland adjusted bacterial suspension in saline was added to 125 µl of MH broth to achieve a final bacterial cell concentration of 10^5 cells in the well. Amoxicillin (0.1 mg/mL) was used as a positive control. The titer plate was incubated at 37 °C for 24 h. After 24 h, 20 µL of 0.02% of TTC (2, 3, 5-triphenyl tetrazolium chloride) was used for the visualization of the MIC.

In vitro anti-candidal activity

The anti-candidal activity of the oxidized and unoxidized of the banana sap extract was evaluated by *in vitro* microbroth dilution assay (Jorgensen et al. 1999; EUCAST 2018). Briefly, 96-well microtiter plate was used to evaluate the anti-candidal activity as well as to visualize minimal inhibitory concentration (MIC). The banana sap extract was dissolved in 10% dimethyl sulfoxide (DMSO) and tested in a concentration from 500 mg/mL to 1.95 mg/mL i.e., 2-fold serial dilutions. Fifty (50) µl of the 0.5 McFarland adjusted suspension of *Candida albicans* in saline was added to 125 µl of MH broth to achieve a final concentration of 10^5 cells in the well. Fluconazole (0.1 mg/mL) was used as a positive control. The titer plate was incubated at 37 °C for 24 h. After 24 h, 0.01% of Resazurin was used for the visualization of the MIC.

Maintenance of cell lines

Human breast cancer (MCF-7) cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. The cell line was maintained in complete Dulbecco's Modified Eagle Medium (DMEM) in the humidified incubator with 5% CO_2 in 37 °C in T25 flasks. Complete medium means DMEM supplemented with 10% (v/v) FBS, 100 IU/mL penicillin, 100 µg/mL streptomycin and 2.5 µg/mL amphotericin.

Cell growth inhibition assay

The growth effect of dichloromethane extract of banana sap on MCF-7 cells was measured by 3-(4, 5 dimethylthiazol-2-yl)-2–5 diphenyl tetrazolium bromide (MTT assay) (Lohia and Baranwal 2017). 2×10^4 cells MCF-7 cells were seeded in 96-well microtiter plates and kept overnight in the incubator. The plate was incubated at 37 °C in a humidified incubator maintained at 5% CO_2 (New Brunswick Galaxy; Eppendorf, Hauppauge, NY, USA). After incubation, the extract was added in increasing concentration (5, 10, 25, 50, 75 and 100 µg/mL) to the wells. After 72 h incubation of plates, 20 µL MTT (5 mg/mL) was added in each well and again incubated for 4 h. 100 µL DMSO was added to each well to dissolve the formazan crystals. The absorbance was recorded at 570 nm taking reference wavelength at 620 nm on ELISA plate reader (Tecan Infinite, Groedig, Austria, Pro ELISA reader). Paclitaxel was used as the positive control at a concentration of 20 µg/mL (Working volume: 4 µL). Cell growth inhibition was expressed as:

$$\text{Cell growth inhibition } (\%) = \{(A_{\text{untreated}}\text{cell} - A_{\text{treated}})/A_{\text{untreated}}\} \times 100$$

LC–MS analysis

The organic layer of DCM was further rota-evaporated and stored in vials at 4°C until further use and sent to SAIF, IIT Bombay, for Orbitrap LCMS (OHRLCMS) analysis. The column used for orbitrap LC–MS was Hypersil gold 3 micron 100 × 2.1 MM with run time 30 min on instrument (VANQUISH on IITB_QE-PC). 5 µl of DCM sample gradient was injected for the analysis.

Statistical analysis

All the experiments were performed in triplicate. The data were analyzed by one way ANOVA (analysis of variance) and

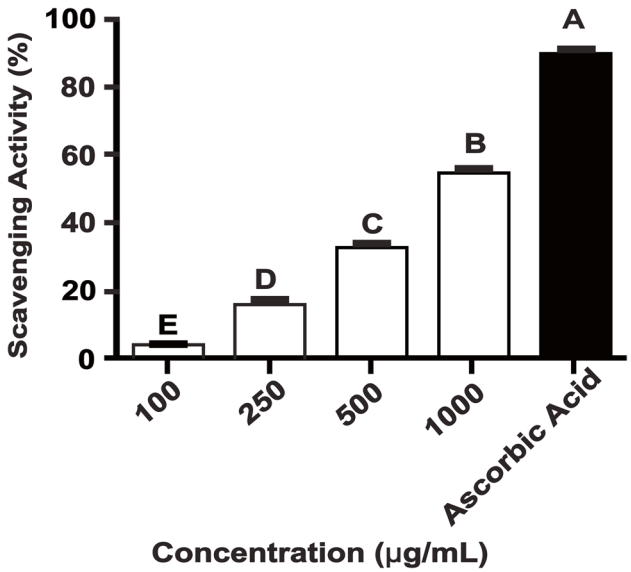


Fig. 1 Antioxidant potential of dichloromethane extract of banana sap. Mean followed by same letter are not significantly different

the means were compared by Tukey's test at $p < 0.05$ using GraphPad Prism (GraphPad Software, Inc., San Diego, CA).

Results

Antioxidant effect

The free radical scavenging activity was done to assess the antioxidant capacity of banana sap extract. With the increase in concentration of the banana sap extract, scavenging activity increased significantly. Figure 1 represented antioxidant potential of dichloromethane extract of banana sap. Mean followed by same letter are not significantly different. The highest antioxidant activity was observed to be $54.62 \pm 1.09\%$ at the concentration of 1000 µg/mL. Ascorbic acid (Stock solution: 100 µg/mL; Working volume: 4µL) which was used as a positive control, had a scavenging activity of $89.67 \pm 1.53\%$.

Antimicrobial potential of the banana sap

Banana sap when exposed to air becomes light brown to dark brown due to the presence of tannins. However, during the course of antimicrobial potential evaluation two strategies were adopted: (a) evaluation of banana sap exposed to air (black/dark brown) [(Fig. 2a)] (ii) banana sap remained green as tannins were chelated and was not directly exposed to air [Fig. 2b]. It was observed that the colored sap exhibited a higher minimal inhibitory concentration (MIC) against gram-positive and gram-negative bacteria which was in range of 125 mg/mL to 500 mg/mL while the green sap exhibited a much lower MIC in range of 15.625 mg/mL to 62.5 mg/mL (Table 1). This drastic reduction in the MIC in the tannin chelated (green sap) is probably due to non-interference of tannins. However, both extracts did not exhibit any anti-candidal activity. Amoxicillin is semi-synthetic penicillin that has both gram-positive and gram-negative activity and therefore has been used as a positive control.

Cytotoxic effect in cancer cell lines

DCM extract of banana sap was tested for their effect on MCF-7 (breast cancer cell lines) cell growth based on MTT

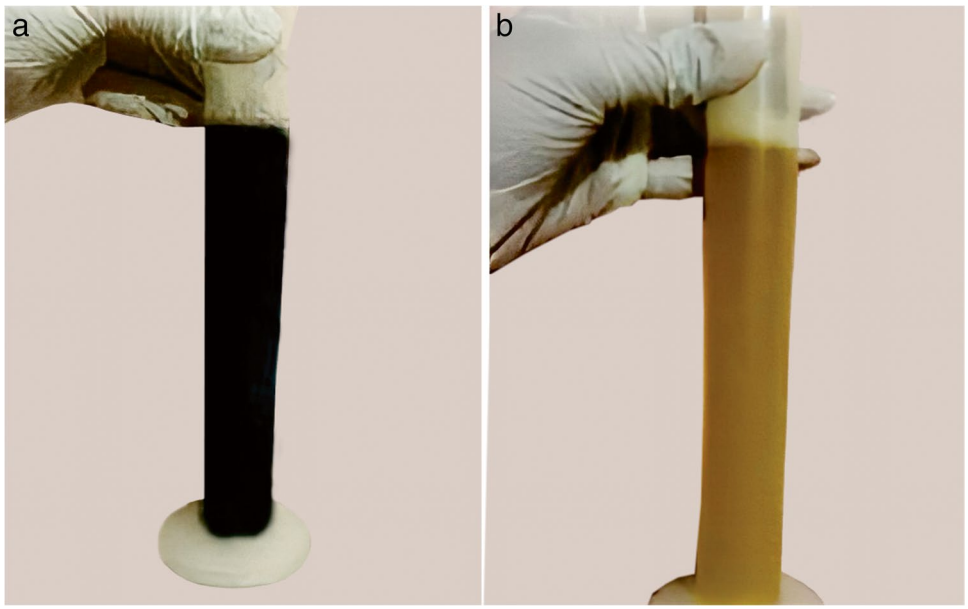


Fig. 2 **a**) Banana sap exposed to air. **b**) Banana sap when not exposed to air

Table 1 Comparative *in vitro* antimicrobial activity as MIC of oxidized and unoxidized Banana sap

Test sample	*MIC values against the test microorganisms (mg/ml)**				
	E. coli	*B. megaterium*	*P. aeruginosa*	*S. aureus*	*C. albicans*
Oxidized banana sap	125	250	500	125	-
Unoxidized banana sap	15.625	62.5	62.5	15.625	-
Positive control (antibacterial) @ 0.1 mg/ml	-	-	-	-	
Positive control (anti-fungal) @ 0.1 mg/ml	-	-	-	-	-

*All values are means of triplicate group

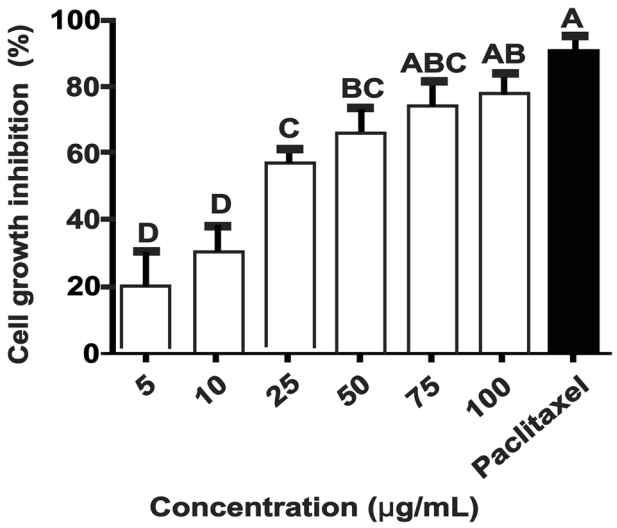


Fig. 3 Cytotoxic effect of dichloromethane extract of banana sap against human breast cancer (MCF-7) cell lines. Mean followed by same letter are not significantly different

assay. It was observed that extracts inhibit the growth of MCF-7 cells representing cytotoxic effect which is found to be significantly increased with concentration. Figure 3 presented cytotoxic effect of dichloromethane extract of banana sap against human breast cancer (MCF-7) cell lines. Mean followed by same letter are not significantly different. The IC_{50} value was calculated and found to be 34.15 ± 8.75 µg/mL. Paclitaxel, an anticancer drug was used as a positive control where the growth inhibition was observed to be $90.81 \pm 4.42\%$.

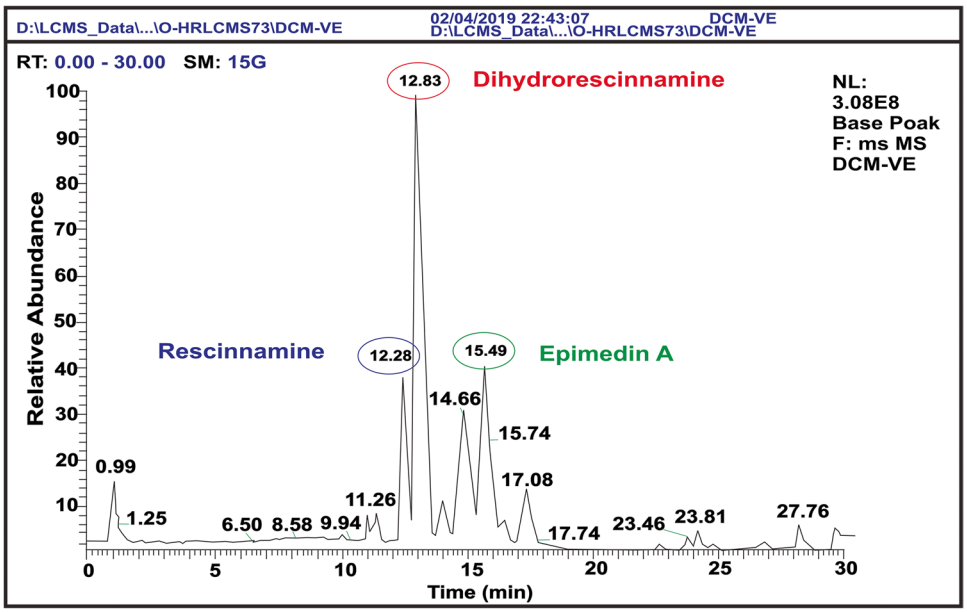
LC–MS analysis

The proposed identifications are based on Orbitrap Liquid Chromatography Mass Spectroscopy chromatograms, mass spectra and a comparison with previous literature and reference data from the m/z library compound database.

Compounds such as rescinnamine, dihydrorescinnamine and epimedin A were predicted based on the mzCloud results of the LC–MS Library (© Reported with Compound Discoverer 2.1). Compounds having RT = 12.28, 12.83 and 15.49 appeared in DCM extracts in major quantities and have been resolved and detected under the column condition. Figure 4 represented spectra of the DCM extract of the banana sap. Figure 5 explained spectra of the RT 12.28 of the DCM extract of the banana sap, displaying fragmentation pattern of compounds in DCM extract of the banana sap. Further, fragmentation pattern of the rescinanmine can be explained by the loss of the $C_{15}H_{16}O_7$ Fig. 5a. Figure 5b explains the fragmentation pattern and products by the loss of $C_{14}H_{24}O_5$ whereas Fig. 5c exhibits the fragmentation pattern by the gain of $C_3H_4N_2$ because of the dimerization of the molecules and loss of the O_3 from the parent compound. Figure 6 presented spectra of the RT 12.83 of the DCM extract of the banana sap, displaying fragmentation pattern of compounds in DCM extract of the banana sap. Moreover, fragmentation pattern of the dihydrorescinnamine Fig. 6a can be explained by the loss of $C_{15}H_{16}O_{7,}$ (b) can be explained by the loss of the $C_{14}H_{22}O_{5,}$ (c) can be explained by the gain of $C_3H_6N_2$ because of the dimerization of the molecules and loss of the O_3 from the parent compound. Figure 7a represented spectra of the RT 15.49 of the DCM extract of the banana sap whereas Fig. 7b explained the fragmentation pattern of compounds at spectra of the RT 15.49 of the DCM extract of the banana sap. Moreover, fragmentation pattern of the Fig. 7b of Epimedin A (a) can be explained by the loss of $C_{21}H_{34}O_{15,}$ (b) can be explained by the loss of $C_{18}H_{32}O_{15,}$ (c) can be explained by the loss of $C_7H_{16}O_{7,}$ (d) can be explained by the loss of the H_4O_2, from the parent compound. There was a difference of the ± 1 in the exact mass and the observed mass which is due to the loss and gain of the H atom.

Discussions

Herbal extracts or plant extracts have been a part of traditional and folklore medicines. Medicinal systems like Ayurveda, Unani, Traditional Chinese medicine rely on the use of plant extracts that have been time tested for their medical potential (Pandey 2021). The interdisciplinary applications of different plant components such as the flower, pulp, stem, and leaves enlist the banana plant among the most beneficial plants (Lopes et al. 2020). The sap from bananas has high



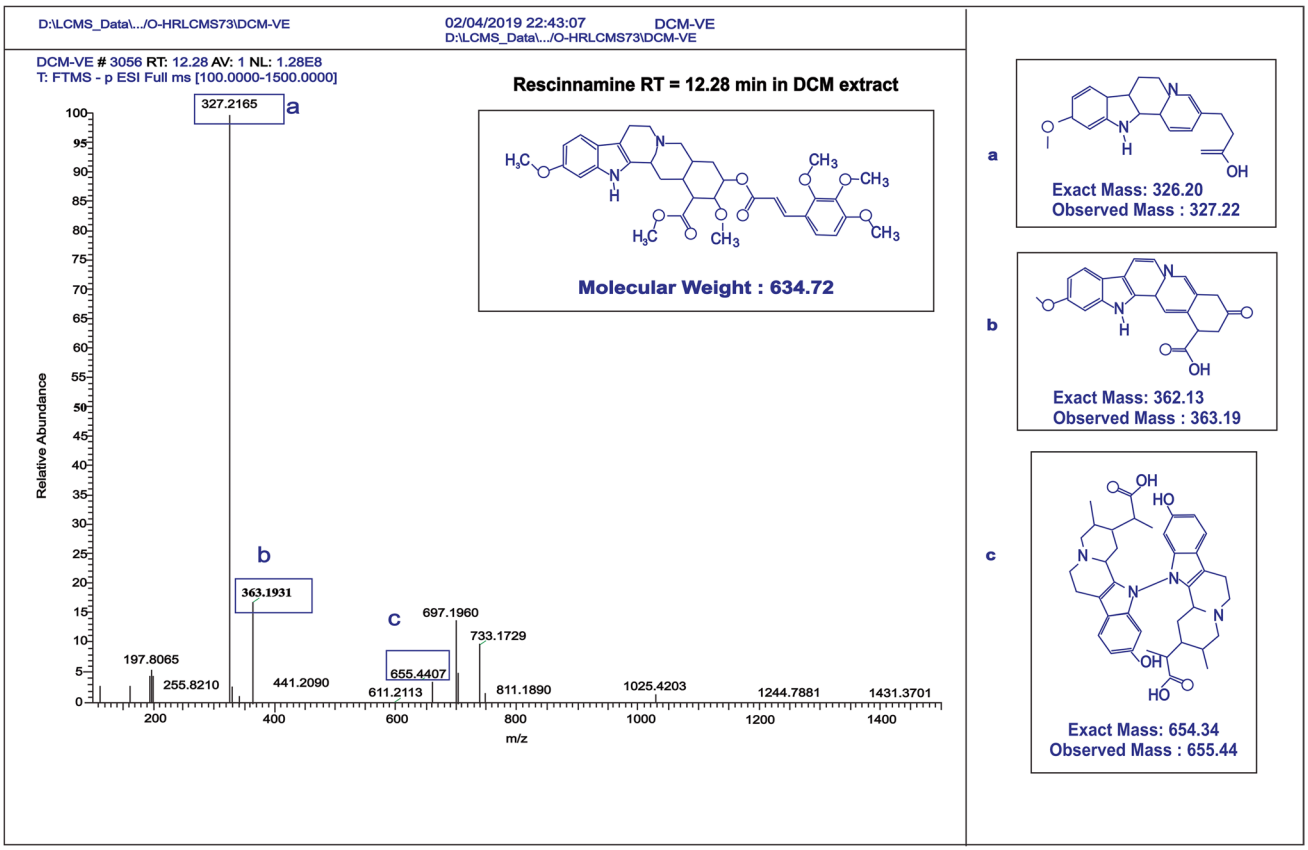


Fig. 4 Spectra of the DCM extract of the banana sap

Fig. 5 Spectra of the RT 12.28 of the DCM extract of the banana sap, displaying fragmentation pattern of compounds in DCM extract of the banana sap

medicinal value and is being used to cure a range of ailments, including bleeding, hysteria, fever, leprosy, digestive problems, epilepsy, hemorrhoids, and insect bites (Kumar et al. 2012 and Gupta et al. 2022). Furthermore, banana sap is an excellent source of the bioactive and antioxidants phytochemicals, such as phenolic and flavonoid compounds

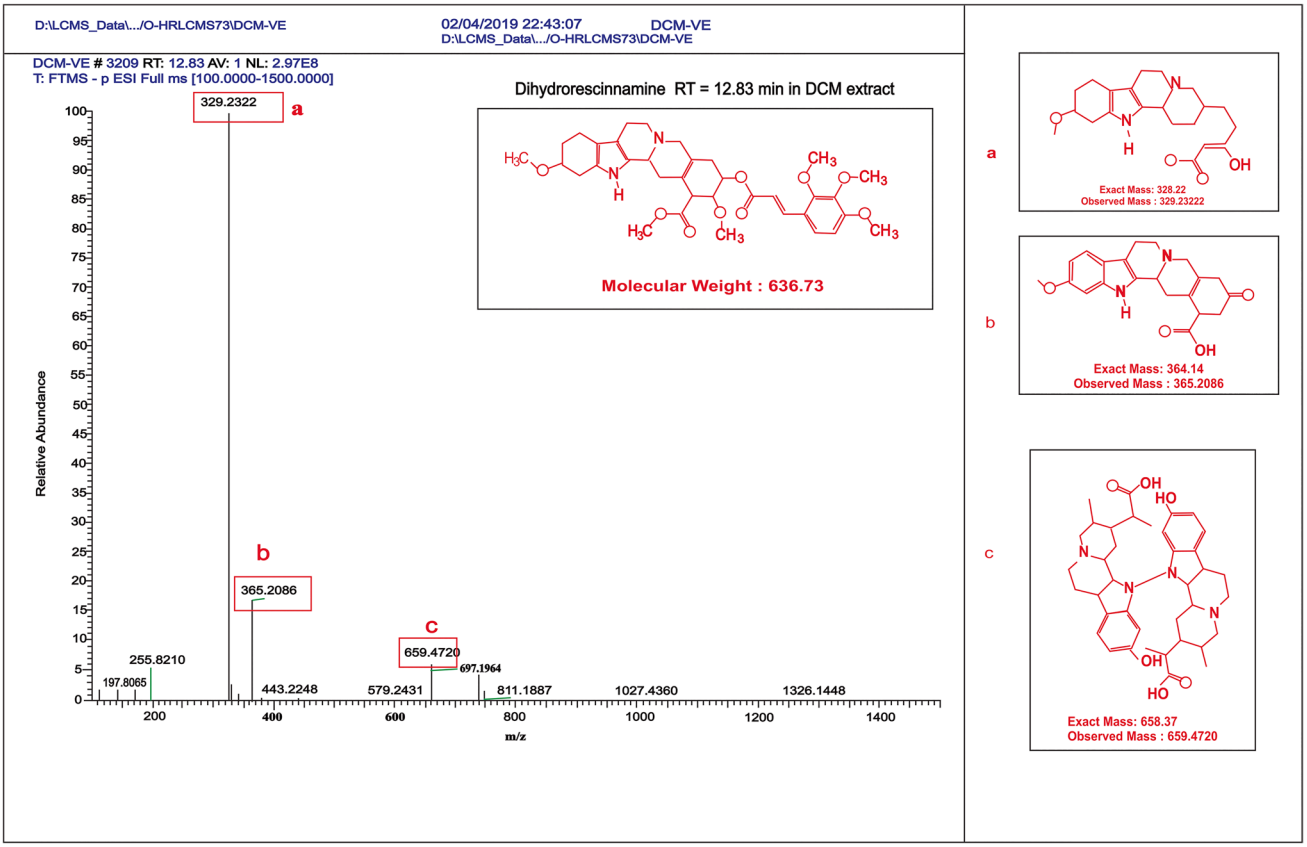


Fig. 6 Spectra of the RT 12.83 of the DCM extract of the banana sap, displaying fragmentation pattern of compounds in DCM extract of the banana sap

such as apigenin glycosides, myricetin glycoside, myricetin-3-o-rutinoside, naringenin glycosides, kaempferol-3-o-rutinoside, quericitin-3-o-rutinoside, dopamine and N-acetyl serotonin (Pothavorn et al. 2010). Hence in this study, we explored the antioxidant, anticancer and antimicrobial potential of banana sap from the pseudo stem. Literature abounds on the antioxidant activity in different parts of the banana plant such as fruit (Alothman et al. 2009), pulp and peel (Sulaiman et al. 2011; Mokbel and Hashinaga 2005; Nagarajaiah and Prakash 2011), leaf (Karuppiah and Mustaffa 2013), pseudo-stem and rhizome (Saravanan and Aradhya 2011; Kumar et al. 2014), and flower (Loganayaki et al. 2010). 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) method is the most commonly used followed by ferric reducing antioxidant power (FRAP), total antioxidant activity (TAA) to assess the anti-oxidant potential. Moreover, for the evaluation of antioxidant potential, various extracts were prepared by researchers in water (Alothman et al. 2009; Mokbel and Hashinaga 2005; Nagarajaiah and Prakash 2011), acetone (Loganayaki et al. 2010), hexane (Sulaiman et al. 2011), ethanol (Nagarajaiah and Prakash 2011), and methanol (Kumar et al. 2014). Researchers have demonstrated distinct antioxidant activity of various parts of banana and revealed that solvent-based extracts exhibited higher antioxidant activity compared to aqueous extracts. Pothavorn et al. (2010) reported that the banana sap contains bioactive compounds caffeoylquinic acid or chlorogenic acid which is responsible for its antioxidant activity. In the present study, the antioxidant potential of dichloromethane extract of concentrated banana sap has been evaluated using DPPH assay, however, the anti-oxidant activity was moderate as compared to the ascorbic acid. The antioxidant potential could possibly be attributed to non-polar compounds present in the dichloromethane extract.

Several studies have reported antimicrobial activity from different plant parts of *Musa* spp. The most common microorganisms used for the studies included *B. subtilis, E. coli, S. aureus* and *P. aeruginosa* (Naikwade et al. 2014; Asuquo and Udobi 2016). Although some researchers have also studied *Micrococcus, Klebsiella* and *Salmonella* (Ehiowemwenguan et al. 2014; Kumar et al. 2014), in the test panel of microorganisms. The various fungal species tested include *Candida, Aspergillus, Penicillium, Cryptococcus* and *Trichophyton* (Kumar et al. 2014; Jouneghani et al. 2020). The majority of researchers have studied the antimicrobial activities of banana leaves (Naikwade et al. 2014; Asuquo and Udobi,

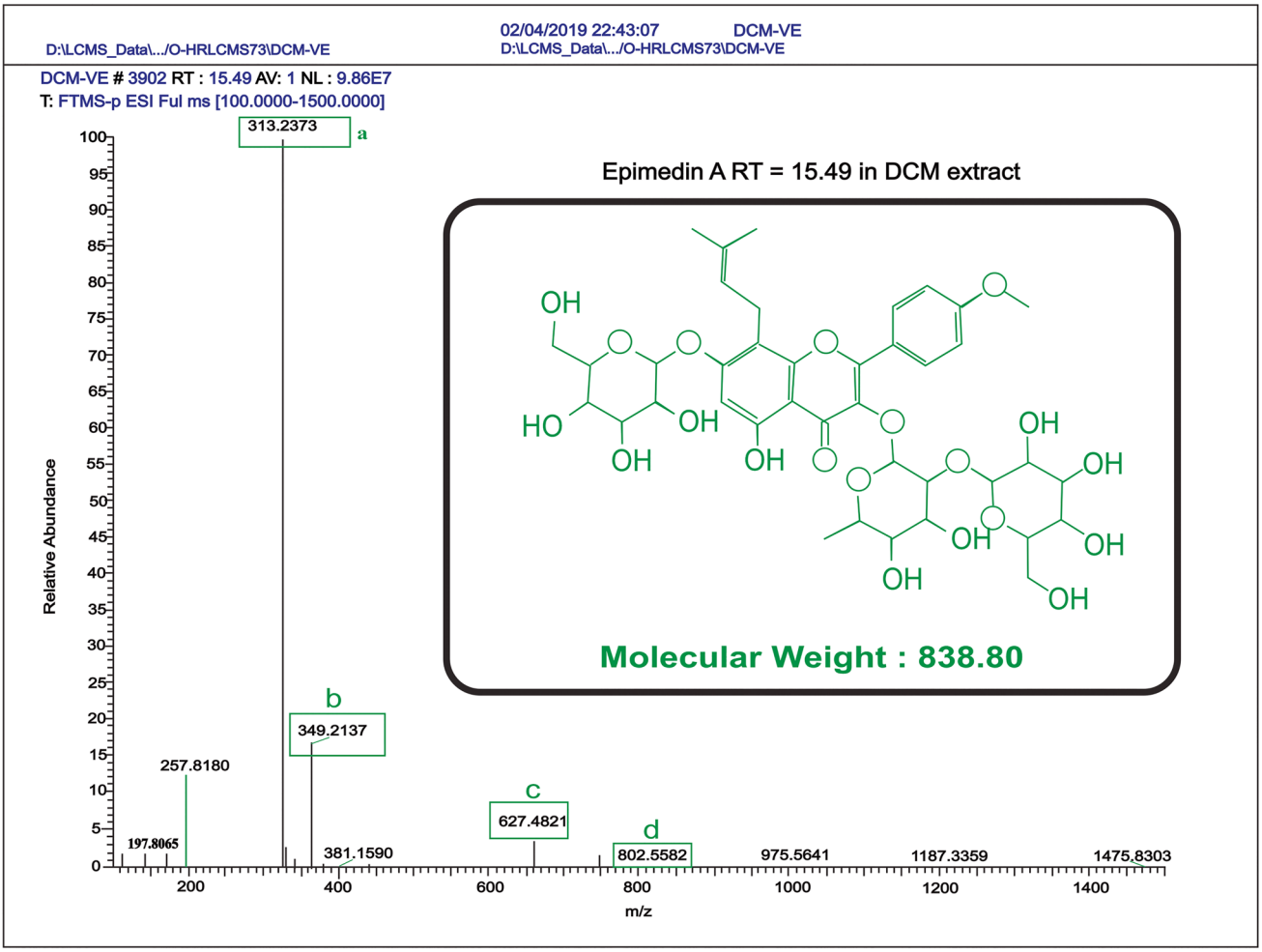


Fig. 7 **a** Spectra of the RT 15.49 of the DCM extract of the banana sap, **b** Spectra of the RT 15.49 of the DCM extract of the banana sap, displaying fragmentation pattern of compounds in DCM extract of the banana sap

2016; Sivasamugham et al. 2021). Apart from leaves, peel (Ehiowemwenguan et al. 2014), inflorescence (Padam et al. 2012) and sap (Kumar et al. 2014) have also been studied for the presence of antimicrobial activities. Both aqueous (Ehiowemwenguan et al. 2014) and organic fractions (Padam et al. 2012; Kumar et al. 2014) of extracts from different parts of banana plants have been tested for their antimicrobial spectrum. It has been observed by researchers in the case of bananas that the aqueous fraction has poor antimicrobial potency as compared to organic extracts. The antimicrobial panel in our studies comprised of both Gram-positive and Gram-negative bacteria. However, our approach was unique in the sense that we evaluated the antimicrobial potential of the oxidized and unoxidized banana sap and proved that the unoxidized banana sap, which was green in colour had potent antimicrobial activity as compared to the oxidized sap in terms of MIC. There was an 8-fold reduction which happened in the unoxidized sap. The oxidation of tannins leads to a black color and becomes unavailable for antibacterial actions. By chelating, its oxidation is stopped and it remains active and induces antibacterial action. To the best of our knowledge the same has never been investigated. Kumar et al. (2014) reported that the banana sap has little antimicrobial activity but no anti-fungal activity was reported since only oxidized sap was evaluated. However, our results of anti-candida activity are in agreement with the results of Kumar et al. (2014).

Moreover, researchers have reported that different parts of banana such as peel, pulp and seed (Li et al. 2013; Zawawy 2015), fruit (Ampasavate et al. 2010; Dahham et al. 2015), flower (Nadumane and Timsina 2014), and banana leaf (Asuquo and Udobi 2016) to possess anti-proliferative activity when tested on various cancerous cell lines such as A549, MCF-7, Hep G2, HT-29, U937, K562, HL60, Molt4, CHO, HUVEC, HCT-116 and swiss albino mice selected from the different cultivars of the Musa. Mostly MCF-7 cell lines have been studied by the researchers. The extracts showed distinct activity with defined IC_{50} values. For instance,

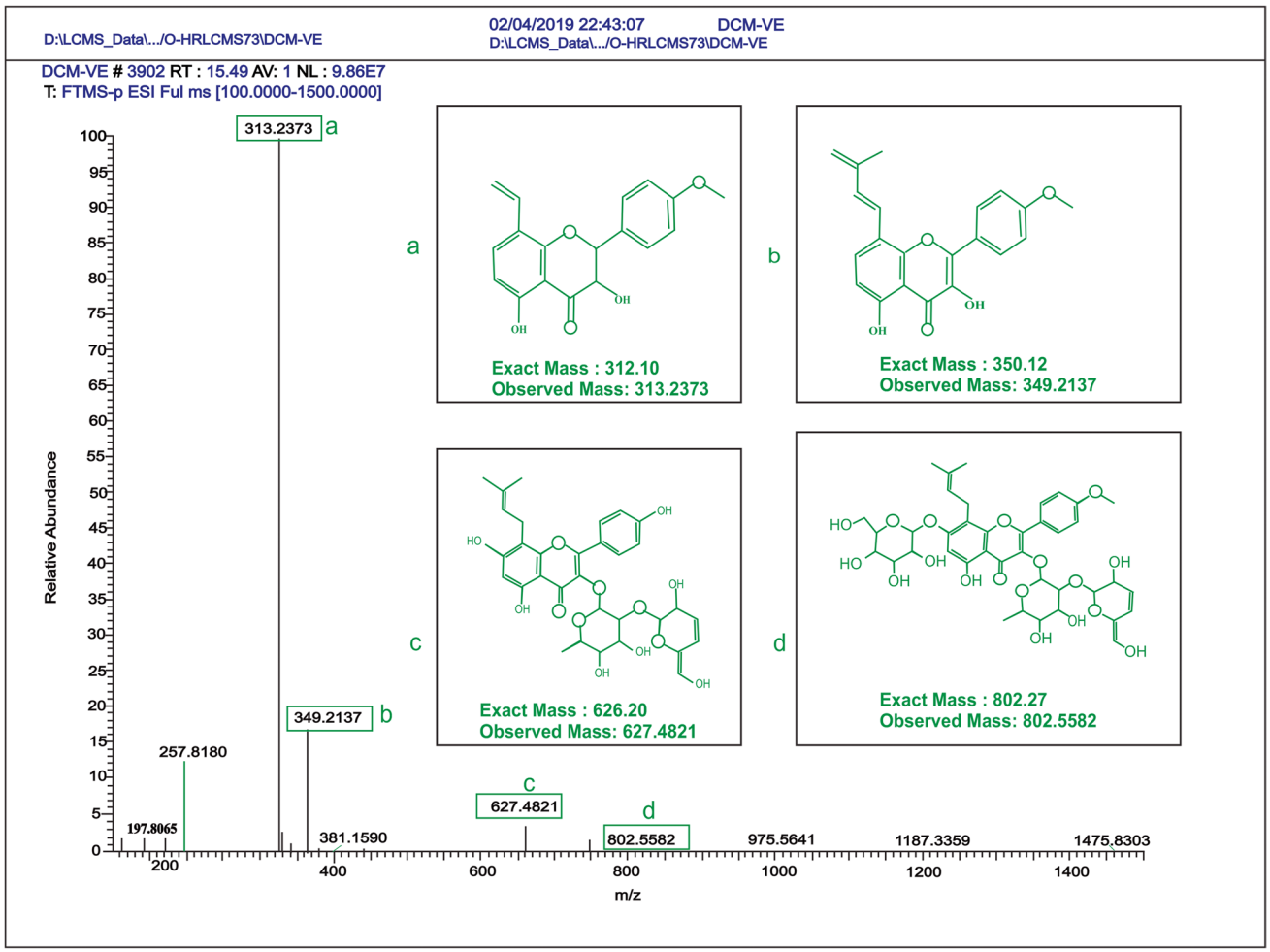


Fig. 7 (continued)

banana flower showed activity with IC_{50} less than 10 µg/mL for HeLa and CHO cell lines (Nadumane and Timsina 2014), banana peel showed upto 33% antiproliferative activity for MCF-7 cell lines (Zawawy 2015). Although banana sap based anti-cancer activity has not been clearly demonstrated in cell lines or animal models but the presence of flavones and flavanol indicates its anti-cancer effect (Pothavorn et al. 2010). However, in our studies we have proved anticancer potential of the dichloromethane extract of the banana sap.

Plants are the key sources of natural phenolics, such as different types of phenolic acids, including a group of hydroxybenzoic acids (Sarker and Oba 2020a) and hydroxycinnamic acids (Sarker and Oba 2018), flavonoids, including flavonols (Sarker and Oba 2019), flavones (Sarker and Oba 2020b) flavanols (Sarker and Oba 2020c) flavanones (Sarker et al. 2020) isoflavones, anthocyanins, chalcones, and nonflavonoids, including tannins, lignans, and stilbenes (González-Sarrías et al. 2020). Major phytochemicals compounds reported from the banana sap can be grouped as alkaloids, phenolics, saponins, lignins, coumarins and cardiac glycosides (Onyema et al. 2016; Pothavorn et al. 2010). Furthermore, phytochemical analysis of banana sap varieties such as *Musa balbisiana, Musa laterita, Musa ornate and Musa acuminate* shows that sap is rich in flavones and flavanols (Pothavorn et al. 2010). HPLC based identification has shown that apigenin glycosides (which inhibits many types of cancer cell lines by promotion of cell cycle arrest and apoptosis), naringenin glycosides are very abundant in sap. Flavanols such as myrecetin glycoside, querecetin glycosides and kaempferol are also detected in high amounts in sap. Other than flavanols and flavones components such as dopamine and N-acetyl serotonin are also detected in sap. Flavanols and flavones are known anti-cancer compounds (Pothavorn et al. 2010). Nguyen et al. (2017) reported several antidiabetic compounds such as lupeol, ferulic acid, vanillic acid, trans-cinnamic acid, p-hydroxybenzoic acid, p-coumaric acid, rutin, catechin/epicatechin, chlorogenic acid, gallic acid, caffeic acid and nicotiflorin from the banana stem juice of *M. paradisiaca.* In our study LC–MS data detected the presence of alkaloid

and flavonoids as major compounds such as rescinnamine derivative, dihydrorescinnamine and epimedin A in DCM extract of banana sap.

The presence of rescinnamine has not been reported from any banana plant or cultivar previously. Alkaloids are largest group of secondary chemical constituents comprising nitrogen-containing naturally occurring chemicals that have been shown to have antibacterial effects owing to their tendency to intercalate with genetic material of the microorganisms (Ramu et al. 2015; Ogbonna et al. 2016). Furthermore, alkaloids have analgesic, anti-spasmodic and bactericidal effects and this is the basis for their use as basic medicinal agents (Okwu 2004). Rescinnamine is an anti-hypertensive drug which inhibits coronavirus binding to a receptor in the cell surface of human cell (Wu et al. 2020). Based on this, we can say that observed antimicrobial activity of banana sap in our study is due to the presence of the alkaloid compounds such as rescinnamine, dihydrorescinnamine. Likewise, epimedins have not been reported till date in banana. In fact, there are no reports on presence of glycosylated flavonoids in banana. Moreover, flavonoids are powerful antioxidants with anti-inflammatory, anti-neoplastic, anti-cancer, anti-allergic, anti-viral and hepatoprotective properties (Lewis et al. 1999; Xi et al. 2014; Xie et al. 2015; Ogbonna et al. 2016). According to data from *in vitro* and *in vivo* investigations, epimedin compounds have strong anticancer effect against a wide spectrum of cancer cells via a variety of pathways including apoptosis, cell cycle regulation, anti-angiogenesis, anti-metastasis, and immunological modulation (Tan et al. 2016; Lone et al. 2018). In another investigation on *E. koreanum*, all four Epimedium species markers, epimedin A, epimedin B, epimedin C and icariin showed high anticancer potential (Yasukawa et al. 2016). As a result, Epimedium herbs might be beneficial in cancer prevention. Our study reported the epimedin A in banana sap and also showed the presence of anti-oxidant, anti-cancer, antimicrobial activities in the banana sap. Therefore, our study is in agreement with the findings of Yasukawa et al. (2016).

Conclusions

Our present study has provided an insight on the potential of banana pseudo-stem sap as a good source of bioactive compounds which may possess a host of pharmacologically relevant properties beyond antioxidant, antimicrobial and anticancer activities. These bioactive properties may be associated with the presence of rescinnamine derivative, dihydrorescinnamine and epimedin A. Our study is a primer of opening up metabolomic studies under non-oxidized state of banana sap with a possibility of their applications in the herbal cosmeceutical and nutraceutical preparations thereby valorising the banana sap which is currently attributed as a waste product.

Acknowledgements The authors are thankful to IIT Bombay for helping us in the LC-MS analysis and Professor Manmohan Chhibber, School of Chemistry and Biochemistry, TIET for helping in predicting the bioactive compounds. We are also thankful to Thapar Institute of Engineering and Technology, Patiala for providing the necessary infrastructure and support to carry out the research.

Author contributions GG collected the plant material, prepared extracts; SS, MBW and MSR supervised the study; GG performed the experiments, made the statistical analysis of the data and prepared the manuscript. All authors revised the manuscript.

Declarations

Conflict of interest: The authors declare that they have no conflict of interest.

References

Alothman M, Bhat R, Karim AA (2009) Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chem 115:785–788. https://doi.org/10.1016/j.foodchem.2008.12.005

Ampasavate C, Okonogi S, Anuchapreeda S (2010) Cytotoxicity of extracts from fruit plants against leukemic cell lines. Afr J Pharm Pharmacol 4:013–021. https://doi.org/10.5897/AJPP.9000092

Asuquo EG, Udobi CE (2016) Antibacterial and toxicity studies of the ethanol extract of Musa paradisiaca leaf. Cogent Biol 2:1219248. https://doi.org/10.1080/23312025.2016.1219248

Borges MH, Alves DLF, Raslan DS, Piló-Veloso D, Rodrigues VM, Homsi-Brandeburgo MI, De Lima ME (2005) Neutralizing properties of Musa paradisiaca L. (Musaceae) juice on phospholipase A2, myotoxic, hemorrhagic and lethal activities of crotalidae venoms. J Ethnopharmacol 98:21–29. https://doi.org/10.1016/j.jep.2004.12.014

Budi HS, Juliastuti WS, Christy BR (2020) Antimicrobial Activity of Musa paradisiaca var. sapientum on Enterococcus faecalis Viability. Mal J Med Health Sci 16:17–21

Dahham SS, Mohamad TA, Tabana YM, Majid AMSA (2015) Antioxidant activities and anticancer screening of extracts from banana fruit (Musa sapientum). Acad J Cancer Res 8:28–34. https://doi.org/10.5829/idosi.ajcr.2015.8.2.95162

Dikshit P, Tyagi MK, Shukla K, Gambhir JK, Shukla R (2016) Antihypercholesterolemic and antioxidant effect of sterol rich methanol extract of stem of Musa sapientum (banana) in cholesterol fed wistar rats. J Food Sci Technol 53:1690–1697. https://doi.org/10.1007/s13197-015-2096-5

Ehiowemwenguan G, Emoghene AO, Inetianbor JE (2014) Antibacterial and phytochemical analysis of Banana fruit peel. Iosr J Pharm 4:18–25

El Zawawy NA (2015) Antioxidant, antitumor, antimicrobial studies and quantitative phytochemical estimation of ethanolic extracts of selected fruit peels. Int J Curr Microbiol Appl Sci 4:298–309

González-Sarrías A, Tomás-Barberán FA, García-Villalba R (2020) Structural diversity of polyphenols and distribution in foods. In Dietary Polyphenols: Their Metabolism and Health Effects, Wiley, pp 1–29. https://doi.org/10.1002/9781119563754.ch1

Gupta G, Baranwal M, Saxena S, Reddy MS (2019) Utilization of banana stem juice as a feedstock material for bioethanol

production. CLEAN–Soil Air Water 47:1–5. https://doi.org/10.1002/clen.201900047
Gupta G, Baranwal M, Saxena S, Reddy MS (2022) Utilization of banana waste as a resource material for biofuels and other value-added products. Biomass Conver Bioref: 1-20. https://doi.org/10.1007/s13399-022-02306-6
Jorgensen JH, Turnidge JD, Washington JA, Murray EJ, Pfaller MA, Tenover FC, Yolken RH (1999) Manual of clinical microbiology 7thed, American Society for Microbiology. ASM Press, Washington DC, pp 1526–1543
Jouneghani RS, Castro AHF, Panda SK, Swennen R, Luyten W (2020) Antimicrobial activity of selected banana cultivars against important human pathogens, including candida biofilm. Foods 9:435. https://doi.org/10.3390/foods9040435
Kar GA, Rout SK, Mishra D (2019) Evaluation of antidepressant Activity of methanolic Extract of Musa x paradisiaca Linn using animal models. Am J Pharm Tech Res 9:315–320
Karuppiah P, Mustaffa M (2013) Antibacterial and antioxidant activities of Musa sp. Leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection. Asian Pac J Trop Biomed 3:737–742. https://doi.org/10.1016/S2221-1691(13)60148-3
Khamboonruang P, Anuntasethakul T, Navephap S, LeGrand SM (2015) Evaluation of anti-gastric ulcer activity of ethanolic extract from pseudostem of Musa× paradisiaca in experimental animals. Thai J Sci Technol 4:310–320. https://doi.org/10.14456/tjst.2015.1
Kumar KS, Bhowmik D, Duraivel S, Umadevi M (2012) Traditional and medicinal uses of banana. J Pharmacogn Phytochem 1:51–63
Kumar PR, Srivastava S, Singh KK, Mathad C, Thin PS (2014) Study of Antioxidant and Antimicrobial Properties, Phytochemical screening and analysis of Sap Extracted from Banana (Musa acuminata) pseudostem. Int J Adv Biotechnol Res 5:649–658
Lewis DA, Fields WN, Shaw GP (1999) A natural flavonoid present in unripe plantain banana pulp (Musa sapientum L. var. paradisiaca) protects the gastric mucosa from aspirin-induced erosions. J Ethnopharmacol 65:283–288. https://doi.org/10.1016/S0378-8741(99)00005-7
Li F, Li S, Li HB, Deng GF, Ling WH, Wu S, Xu XR, Chen F (2013) Antiproliferative activity of peels, pulps and seeds of 61 fruits. J Funct Foods 5:1298–1309. https://doi.org/10.1016/j.jff.2013.04.016
Loganayaki N, Rajendrakumaran D, Manian S (2010) Antioxidant capacity and phenolic content of different solvent extracts from banana (Musa paradisiaca) and mustai (Rivea hypocrateriformis). Food Sci Biotechnol 19:1251–1258. https://doi.org/10.1007/s10068-010-0179-7
Lohia N, Baranwal M (2017) Immune responses to highly conserved influenza A virus matrix 1 peptides. Microbiolo Immunol 61:225–231. https://doi.org/10.1111/1348-0421.12485
Lone SA, Gupta AP, Manzoor MM, Goyal P, Hassan QP, Gupta S (2018) Epimedium elatum (morr & decne): a therapeutic medicinal plant from northwestern himalayas of India. Plant and Human Health, vol 1. Springer, Cham, pp 619–656
Lopes S, Borges CV, de Sousa Cardoso SM, de Almeida Pereira da Rocha MF, Maraschin M (2020) Banana (Musa spp.) as a Source of Bioactive Compounds for Health Promotion. Handbook of Banana Production, Postharvest Science, Processing Technology and Nutrition, Wiley, pp 227–244
Majhi S, Das D (2021) Chemical derivatization of natural products: semisynthesis and pharmacological aspects-A decade update. Tetrahedron 78:131801. https://doi.org/10.1016/j.tet.2020.131801
Mokbel MS, Hashinaga F (2005) Antibacterial and antioxidant activities of banana (Musa, AAA cv. Cavendish) fruits peel. Am J Biochem Biotechnol 1:125–131. https://doi.org/10.3844/ajbbsp.2005.125.131
Nadumane VK, Timsina B (2014) Anticancer potential of banana flower extract: An in vitro study. Bangladesh J Pharmacol 9:628–635. https://doi.org/10.3329/bjp.v9i4.20610
Nagarajaiah SB, Prakash J (2011) Chemical composition and antioxidant potential of peels from three varieties of banana. As J Food Ag-Ind 4:31–46
Naikwade PV, Gaurav S, Sharayu D, Kailas J (2014) Evaluation of antibacterial properties of Musa paradisiaca L. Leaves. In: Proceeding of the national conference on conservation of natural resources & biodiversity for sustainable development, pp 80–84
Nguyen D, Novakova A, Spurna K (2017) Antidiabetic compounds in stem juice from banana. Czech J Food Sci 35:407–413. https://doi.org/10.17221/172/2017-CJFS
Ogbonna OA, Izundu AI, Okoye NH, Ikeyi AP (2016) Phytochemical compositions of fruits of three Musa species at three stages of development. IOSR J Pharm Biol Sci 11:48–59. https://doi.org/10.9790/3008-1103044859
Okwu DE (2004) Phytochemical and vitamin content of indigenous spices of South Eastern Nigeria. J Sustain Agric Environ 6:30–34
Onyema CT, Ofor CE, Okudo VC, Ogbuagu AS (2016) Phytochemical and antimicrobial analysis of banana pseudo stem (Musa acuminata). J Pharm Res Int 10:1–9. https://doi.org/10.9734/BJPR/2016/22593
Padam BS, Tin HS, Chye FY, Abdullah MI (2012) Antibacterial and Antioxidative Activities of the Various Solvent Extracts of Banana (Musa paradisiaca cv. Mysore) Inflorescences. J Biol Sci 12:62–73
Pandey A (2021) A Glimpse into the Indian Traditional Medicine with Special Reference to Use of Hemidesmus indicus in Southern India: A review. Inter J Theor Appl Sci 13:73–79
Pothavorn P, Kitdamrongsont K, Swangpol S, Wongniam S, Atawongsa K, Svasti J, Somana J (2010) Sap phytochemical compositions of some bananas in Thailand. J Agric Food Chem 58:8782–8787. https://doi.org/10.1021/jf101220k
Ramu R, Shirahatti P, Zameer F, Lakkapa DB, Nagendra Prasad MN (2015) Evaluation of Banana (Musa sp. var. Nanjangud Rasa bale) flower and pseudostem extracts on antimicrobial, cytotoxicity and thrombolytic activities. Int J Pharm Pharm Sci 7:136–140
Saravanan K, Aradhya SM (2011) Potential nutraceutical food beverage with antioxidant properties from banana plant bio-waste (pseudostem and rhizome). Food Funct 2:603–610. https://doi.org/10.1039/C1FO10071H
Sarker U, Hossain M, Iqbal M, Oba S (2020) Bioactive components and radical scavenging activity in selected advance lines of salt-tolerant vegetable amaranth. Front Nutr 7:587257. https://doi.org/10.3389/fnut.2020.587257
Sarker U, Oba S (2018) Augmentation of leaf color parameters, pigments, vitamins, phenolic acids, flavonoids and antioxidant activity in selected Amaranthus tricolor under salinity stress. Sci Rep 8:10–1038. https://doi.org/10.1038/s41598-018-30897-6
Sarker U, Oba S (2019) Antioxidant constituents of three selected red and green color Amaranthus leafy vegetable. Sci Rep 9:1–11. https://doi.org/10.1038/s41598-019-52033-8
Sarker U, Oba S (2020a) Phenolic profiles and antioxidant activities in selected drought-tolerant leafy vegetable amaranth. Sci Rep 10:18287. https://doi.org/10.1038/s41598-020-71727-y
Sarker U, Oba S (2020b) Polyphenol and flavonoid profiles and radical scavenging activity in leafy vegetable Amaranthus gangeticus. BMC Plant Biol 20:1–12. https://doi.org/10.1186/s12870-020-02700-0
Sarker U, Oba S (2020c) Nutraceuticals, phytochemicals, and radical quenching ability of selected drought-tolerant advance lines of vegetable amaranth. BMC Plant Biol 20:564. https://doi.org/10.1186/s12870-020-02780-y
Sharma V, Lohia N, Handa V, Baranwal M (2017) Amomum subulatum seed extract exhibit antioxidant, cytotoxic and immune-suppressive effect. Indian J Biochem Biophys 54:135–139

Sheng Z, Dai H, Pan S, Ai B, Zheng L, Zheng X, Prinyawiwatkul W, Xu Z (2017) Phytosterols in banana (Musa spp.) flower inhibit α-glucosidase and α-amylase hydrolysations and glycation reaction. Int J Food Sci Technol 52:171–179. https://doi.org/10.1111/ijfs.13263

Sivasamugham LA, Nimalan V, Subramaniam G (2021) Antibacterial effects of Musa sp. ethanolic leaf extracts against methicillin-resistant and susceptible Staphylococcus aureus. South African J Chem Eng 35:107–110

Sulaiman SF, Yusoff NAM, Eldeen IM, Seow EM, Sajak AAB, Ooi KL (2011) Correlation between total phenolic and mineral contents with antioxidant activity of eight Malaysian bananas (Musa sp.). J Food Compos Anal 24:1–10. https://doi.org/10.1016/j.jfca.2010.04.005

Tan HL, Chan KG, Pusparajah P, Saokaew S, Duangjai A, Lee LH, Goh BH (2016) Anti-cancer properties of the naturally occurring aphrodisiacs: icariin and its derivatives. Front Pharmacol 7:191. https://doi.org/10.3389/fphar.2016.00191

The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 8.0, 2018. http://www.eucast.org

Vasundhara M, Baranwal M, Sivaramaiah N, Kumar A (2017) Isolation and characterization of trichalasin-producing endophytic fungus from Taxus baccata. Ann Microbiol 67:255–261. https://doi.org/10.1007/s13213-017-1256-4

Wu C, Chen X, Cai Y, Zhou X, Xu S, Huang H, Zhang L, Zhou X, Du C, Zhang Y, Song J (2020) Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med 180:934–943. https://doi.org/10.1001/jamainternmed.2020.0994

Xi W, Fang B, Zhao Q, Jiao B, Zhou Z (2014) Flavonoid composition and antioxidant activities of Chinese local pummelo (Citrusgrandis Osbeck.) varieties. Food Chem 161:230–238. https://doi.org/10.1016/j.foodchem.2014.04.001

Xie Y, Zheng Y, Dai X, Wang Q, Cao J, Xiao J (2015) Seasonal dynamics of total flavonoids contents and antioxidant activity of Dryopteriserythrosora. Food Chem 186:113–118. https://doi.org/10.1016/j.foodchem.2014.05.024

Yakubu MT, Nurudeen QO, Salimon SS, Yakubu MO, Jimoh RO, Nafiu MO, Akanji MA, Oladiji AT, Williams FE (2015) Antidiarrhoeal activity of Musa paradisiaca Sap in Wistar rats. Evid Based Complement Altern Med. https://doi.org/10.1155/2015/683726

Yasukawa K, Ko SK, Whang WK (2016) Inhibitory effects of the aerial parts of Epimedium koreanumon TPA-induced inflammation and tumour promotion in two-stage carcinogenesis in mouse skin. J Pharm Nutr Sci 6:35–42