Wild ber (*Ziziphus nummularia*) seeds: Extraction & determination of proximate, functional, antioxidant & phenolic properties

A thesis submitted in partial fulfilment of the requirement for the award of the degree of

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

Submitted by: Nidhi Nain

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Under the supervision of

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TIET, PATIALA

July 2022

Certificate

This is to certify that the thesis entitled **"Wild ber (*Ziziphus nummularia*) seeds: Extraction & determination of proximate, functional, antioxidant & phenolic properties"** submitted by Ms. Nidhi Nain in the partial fulfillment of the requirement of the award for the degree of Master of Science in Biotechnology, Thapar University, Patiala is a record of the student's own work carried out under my supervision and guidance. This work has not been submitted for the award of any other degree or certificate in this or any other institute or university.

Dr Ovais Shafiq Qadri

Supervisor

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Declaration

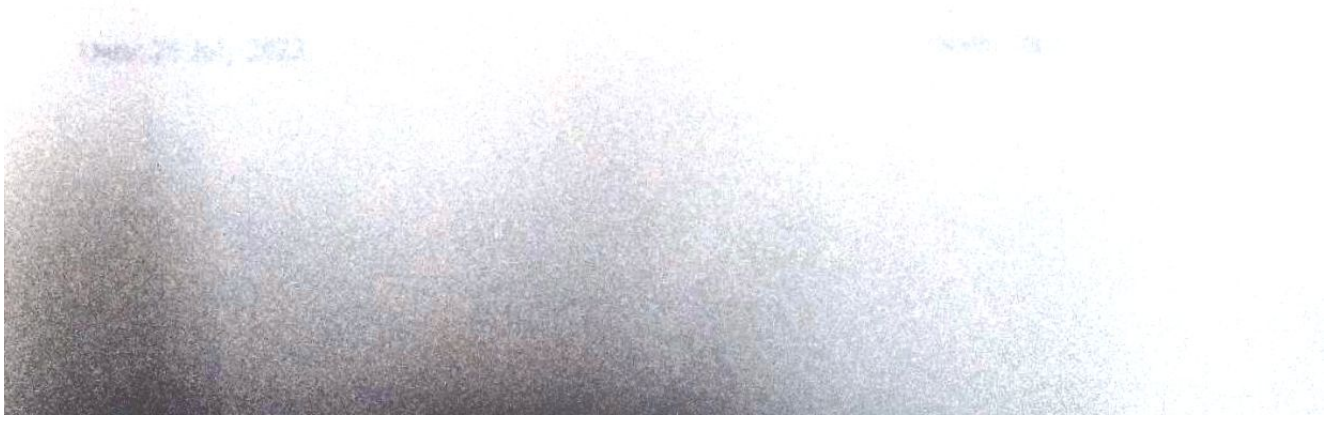
I hereby declare that the work that has been presented in thesis "Wild ber (Ziziphus nummularia) seeds: Extraction & determination of proximate, functional, antioxidant & phenolic properties" " submitted by Ms. Nidhi Nain in the partial fulfillment of the requirement for the award for the degree of Master of Science in Biotechnology, Thapar University, Patiala, is an original record of my own work done during the period from January 2022 to July 2022, carried under the supervision of Dr. Ovais Safiq Qadri.

Place : Patiala

Date: 29 july 2022

Nidhi

(Nidhi Nain)



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Nidhi Nain

Date: 29 July 2022

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Introduction Chapter 1

Ziziphus nummularia, also known as jhahrberi or wild jujube, is a plant native to India. It is one of the oldest continuously grown fruit trees in north Indian plantations with widely divaricating, flexuous, pale-purple stems, and pairs of grey velvety stipular prickles, *Ziziphus nummularia* is a prickly little bush or shrub that is 6-8 meters tall. Widely divergent, velvety-surfaced branches with a violet color. Deep and wide-ranging lateral roots system. Simple, alternating, 2.5 cm long, oval or orbicular, thickly tomentose underneath, and white, serrate, 3- to 5-veined leaves with three to five veins from the base. One stipule is small, hooked, and curved downward while the other is 1 cm long and straight. Stipules are typically spinescent and dark brown. minuscule, bisexual, pentamerous, and light-yellow flowers.

Ziziphus nummularia is dispersed from Iran to India. nevertheless, this species is extensively present in India, ranging from Punjab, Rajasthan, Gujrat, and U.P, as well as in the drier portions of the peninsular area from south to Maharashtra. Due to its perennial hardiness, ber provides some revenue to farmers who lack resources, even during extreme drought. It can be planted on marginal land or infertile soil when most fruit trees fail to thrive or perform extremely poorly. This rare fruit crop can produce well even under rainy weather. It may be cultivated in a wide range of soil types and climates, from subtropical to tropical. It is a plant that serves various purposes, prized for its delicious fruits, leaves for foraging, wood for the fire, building, furniture, and traditional medicine. This species' leaves are utilized as feed for livestock(Shankar 1980), and the fruit is consumed as food(Bhandari 1974), especially during times of food shortage in several parts of the country. The health advantages of jhar ber, including its anti-inflammatory, antidiabetic, antibacterial, antioxidant, anticancer, and immune system stimulating capabilities, have been widely recorded.

Even though the newly harvested fruits are significantly smaller, rounder, sweeter, and sourer than those of *Ziziphus mauritiana*, the lower classes nevertheless like them, especially during times of scarcity. The ber fruit has a good number of mineral components and is high in vitamin C and sugar. In terms of protein, calcium, phosphorus, carotene, and vitamin C, ber are more abundant than apples. They also outperform oranges in these nutrients as well as in iron, carbs, and Vit C. The FAO/WHO recommends that an adult man's daily diet contain 30 mg of ascorbic acid. This need may be fulfilled by consuming three ber each day. Occasionally, mature fruits will be grounded into a fine powder, pureed, and then consumed either by themselves or with

sugar or "jaggery." People of all ages like the healthy, fibre- and vitamin-rich paste made from this that is known as "Borakuti."(Rathore 2009). The fully developed fruits can be rehydrated and eaten straight away or used to make "chutney" when efficiently air-dried and preserved. Many culinary recipes may be colored and given a sweet-sour flavor by adding the powder made from dried maroon fruit pericarp. These goods with extra value can be used right now or put away for later.

The medical benefits of the genus Ziziphus include acting as an immune system stimulant, liver protector, hypotensive, hypoglycaemic, anti-inflammatory, antibacterial, antioxidant, and anticancer agent(Jain and tarafder 1970). Madhya Pradesh's indigenous people utilize the herb to treat diarrhea. Colds and coughs are treated by inhaling smoke made from dried leaves(wealth of India 1984). The fruit can be administered topically to treat wounds and treat pregnancy-related stomach discomfort as well as aconite toxicity(council of science and industrial research 1984). The ethanol extract has anti-inflammatory and antipyretic effects but no spermatotoxic ones. The bark possesses nematocidal and anthelmintic effects(Zafar et al. 2009).

Increased fruit and vegetable diet has been shown to lower the risk of chronic illnesses. These beneficial effects may be due to non-nutritive phytochemicals such as alkaloids, carotenoids, minerals, vitamins, and phenolics. flavonoids, and other phenolics. Numerous of these phytochemicals have antioxidant properties that guard the body against harm from free radicals. Oxidative stress caused by reactive oxygen and nitrogen species contributes to the body's degenerative damage. Polyphenolic substances originating from plants have antioxidant characteristics, and these qualities are strongly correlated with their health-promoting and/or disease-preventing benefits, according to epidemiological and laboratory research. Flavonoids, lignins, and tannins are only a few of the many structurally varied substances that make up the huge category of secondary plant metabolites known as polyphenols. It has been acknowledged that Z. nummularia is an underused plant that merits more study. Especially there has been very less work done on the seed part due to its rigidity. So, this whole study is designed based on the characterization of the seeds and discovering their nutritional, antioxidant, and antibacterial properties.

Review of Literature Chapter 2

2.1 A review study on *Ziziphus nummularia*:

Plant classification:

Kingdom	:	Plantae
Order	:	Rosales
Family	:	Rhamnaceae
Genus	:	Ziziphus
Species	:	Z. nummularia
Binomial name	:	*Ziziphus nummularia*
Synonyms	:	*Ziziphus rotundifolia*

Ziziphus nummularia, also known as jhahrberi or wild jujube, is a plant native to India. It is one of the oldest continuously grown fruit trees in north Indian plantations with widely divaricating, flexuous, pale-purple stems, and pairs of grey velvety stipular prickles, Ziziphus nummularia is a prickly little bush or shrub that is 6-8 meters tall. Due to its perennial hardiness, ber provides some revenue to farmers who lack resources, even during extreme drought. It can be planted on marginal land or infertile soil when most fruit trees fail to thrive or perform extremely poorly. This species' leaves are utilized as feed for livestock (Shankar 1980), and the fruit is consumed as food (Bhandari 1974), especially during times of food shortage in several parts of the country. The health advantages of Jhar ber, including its anti-inflammatory, antidiabetic, antibacterial, antioxidant, anticancer, and immune system stimulating capabilities, have been widely recorded.

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2.2 Ziziphus nummularia Phytochemistry :

The presence of alkaloids, fatty acids, saponins, flavonoids, and triterpenoids, has been discovered in Ziziphus nummularia. The structure of nummularine-T, a 13-membered - formylcyclopeptide alkaloid, was determined by spectroscopic and chemical techniques after it was isolated from the bark of Ziziphus nummularia (Singh and Pandey 1995). Along with the well-known alkaloid nummularine-B, Ziziphus nummularia has produced the two novel peptide alkaloids nummularine-N and nummularine-M, whose structures have also been determined. In contrast to nummularine-N, which has 13 members like nummularine-B and is of the integerrinine type, nummularine-M is a 14-membered cyclopeptide(Pandey *et al*. 1984). Nummularogenin, (25 S)-3 alpha-hydroxy-5 alpha-spirostane-2, 12- dione, a novel (25 S)-spirostane, was discovered and described(Shrivastava 1984).

2.3 Ziziphus nummulareia's pharmacological properties:

2.3.1 Antitumor Activity

Lapachol was initially isolated from the Ziziphus nummularia plant as 2-hydroxy-3-(3-methy1-2-buteny1)-1, 4-naphthoquinone. In female Swiss albino mice, aged 6 to 8 weeks, carrying sarcoma-180 (S-180) ascetic tumour cells, its anti-tumour properties were assessed both alone and in conjunction with radiation. S-180 viable ascetic cells were intraperitoneally delivered to female mice at a dose of 2x105. It has been observed that naphthoquinones and their analogues exhibit anticancer properties against murphy sternum lymphosarcoma and rat tumour walker 256 carcinosarcoma (Kumar *et al*. 2002).

2.3.2 Anthelmintic activity

The adult motility assay, the egg hatch test, and the larval development assay were used to investigate the plant's crude methanolic extract's (CME) in vitro anthelmintic activity against Haemonchus contortus. By giving escalating dosages of crude powder (CP) and CME (1.0-3.0g/kg), sheep naturally infected with gastrointestinal nematodes had their in vivo anthelmintic activity assessed (Bachaya *et al*. 2009).

2.3.3 Antibacterial activity

Plant aqueous and ethanol extracts that have been tested against a small number of medically significant bacteria create an antibacterial zone (B.cereus). 10g of each plant were used for aqueous and ethanol extraction after various plant components were collected, air dried, and ground in a homogenizer. For 6 hours at low heat, distilled water was used for the aqueous extraction. For use in antimicrobial assays, the extract was concentrated to 1/5th of its original volume. The material was extracted in ethanol and left on a rotary shaker overnight for ethanol extraction. At 5000 rpm, the filtrate was collected and centrifuged. For the antimicrobial assay, the extract was concentrated to 1/5th of its original volume (Broberg and Mziray 2005).

9 strains' development was suppressed by the ethanol extract of Ziziphus nummularia. Compared to ethanol extracts, aqueous extracts had less action, presumably because:

I. water extracts included the same active ingredients but in fewer amounts.
II. Organic solvents could dissolve the active ingredients, which meant that water extracts were devoid of them. Particularly in the treatment of infectious disorders brought on by resistant microbes, plant extracts offer considerable promise as antimicrobial agents (Nair and Chanda 2006).

2.3.4 Abortifacient and Antifertility activity:

Consuming 3-5 g of powdered Ziziphus nummularia root bark twice daily with milk results in an abortion. 56.75 % of them cause abortion, 35.13 % prevent pregnancy, and 8.1 % make men sterile. The aforementioned plant species are used for a variety of medical purposes, however, reports from various regions and nations have also shown that several of the species contain abortifacient and antifertility effects (Shah *et al*. 2009).

2.3.5 Other medicinal uses:

Numerous ancient medical practices still exist today, many of which are not necessarily supported by an understanding of the ingredients. Z. nummularia has a bitter, cooling root that is used in Ayurveda to treat headaches, biliousness, and coughing (Singh *et al*. 2010). The bark is effective for treating dysentery, and diarrhea, and boils for treating common fevers and treating vomiting, Chhattisgarh's traditional healers in India combine fruit with sugar and bar sprouts (Ficus benghalensis). Dry leaves and powdered bark are applied to wounds by the region's traditional healers in Bastar (Nandkarni 1982). They are also effective in treating blood disorders and TB. The seeds are excellent for treating leucorrhoea and curing eye disorders. The leaves have antipyretic properties and help with weight loss. The fruit is laxative, aphrodisiac, digestible, cooling, and relieves biliousness, burning feelings, vomiting, and thirst (Chopra 1956).

The fruit is used topically in poultice and treatments for wounds as well as an antidote to aconite toxicity and pregnancy-related stomach discomfort. The kernels act as a tranquilizer and increase flesh and strength (council of scientific and industrial research 1985). The herb Ziziphus is effective in treating mental impairment. Until it is reduced to half, a handful of the dried fruit is cooked in a half-liter of water. Every night was taken before going to bed, add sugar or honey to your taste. Increasing the amount of glutamic acid in circulation improves brain function. Ziziphus is effective in reducing the frequency of cold and flu episodes. Colic, dysentery, and diarrhea can all be treated with the bark. A purgative used to treat constipation is an infusion of the bark's inner layer (Ayurvedam 2002). Scabies and boils are treated with leaf paste. Jujube is a tasty fruit and powerful herbal medicine. It promotes weight gain, strengthens the muscles, and boosts stamina. It is recommended as a tonic to support liver function in Chinese medicine. Jujube raises immune system resistance, according to Japanese studies. It can also be used as an expectorant, emollient, diuretic, and antidote. Saponins, triterpenoids, and alkaloids are present in dried fruits. They have the following properties: stomachic, styptic, tonic, anticancer, pectoral, refrigerant, sedative, and anodyne. They are said to improve digestion and cleanse the blood. Triterpenes, Saponins, alkaloids, and flavonoids, are only a few of the substances found in the seed that has been shown to have medicinal use. It has hypnotic, sedative, narcotic, tonic, and stomachic properties. It is taken internally to treat excessive sweating, palpitations, sleeplessness, nervous weariness, and

nocturnal sweats. The herb is used as a folk treatment for nephritis, anemia, hypertonia, and neurological disorders. In China, the plant is frequently employed as a burn treatment.

2.4 Review on study of different fruit seeds characterization:

Lima *et al*. (2014) concluded that flour made from Surinam cherry seeds has the greatest fiber content. High quantities of lipids were present in the cherry and peach seeds, which were classified as oleaginous. The fact that these lipids are derived from plants means that preparations created from these seeds can presumably be utilized as dietary supplements. The jackfruit seed flour is a strong option to replace commercial flours in baked items since it has a high concentration of resistant and accessible starch and is distinguished by its thermal stability. Compared to the identical dish made with normal flours, this substitute would offer more fibers.

Ocloo *et al.* (2010) concluded that Jackfruit seed flour has a 6.09% moisture content. On a dry matter basis, there were 2.70 % and 1.27% ash and fat levels, respectively. 13.50%, 3.19%, and 79.34% of the total calories were made up of protein, fiber, and carbohydrates, respectively. 382.79 kcal/100g were determined as the caloric value. The amount of calcium (3087 mg/kg), potassium (14781 mg/kg), iron (130.74 mg/kg), copper (10.45 mg/kg), sodium (60.66 mg/kg), and manganese (1.12 mg/kg) in the jackfruit seed flour is notable. 33%, 4.77%, and 25.34 %, respectively, were the results for foam stability, foam capacity, and swelling power. The flour generated may be used in food systems as a thickening and binding agent.

Silva *et al*. (2015) concluded that the pH of 6.36 and low acidity of 0.90 % in passion fruit seeds, respectively, place them close to neutral. When compared to soybean seeds, the number of lipids determined to be 30.22% shows that passion fruit seeds are a good source of oils. Due to its high level of unsaturated fatty acids, passion fruit seed oil has the potential to be used in the cosmetics sector as well as in the production of food for both humans and animals(Ferrari *et al*., 2004). The dietary fiber content in passion fruit seeds is substantial, at 67.23 %. Consuming more fiber is linked to improved gastrointestinal function, lower cholesterol levels, lower BP, decreased risk of coronary heart disease, improved weight management, improved glycemic control, and lower risk of some types of cancer.

Chodak *et al*. (2007) concluded that the peels of the sampion cultivar (7925 mg Trolox 100 g-1 d.w.) and white grapes (6944) and the seeds of the Idared cultivar and orange (7230 i 3423 mg Trolox 100 g-1 d.w.) of the fruits evaluated had the highest antioxidant activity. The grape seeds and skins have the greatest levels of polyphenols. Apple peels (1613 in the sampion cultivar and 1790 in the Idared one), kiwi fruit (1161), lemon (966), watermelon (969), and gooseberry, kiwi (1161) seeds all contained far lower levels of the polyphenol chemicals.

According to all the studies mentioned above genus ziziphus has lot of nutritional , functional and medicinal properties. Work has been done on few species of ziziphus but all these studies were around its fruit , bark , leaves ,etc. There were also few studies found on seeds but on other species but this area was not exploited much . So, in this study we will be working on Ziziphus nummularia seeds kernels .

Objectives of the design for the study of *Ziziphus nummularia* seed kernels

- To develop a method for extraction of the *Ziziphus nummularia* seed kernels
- To determine the nutritional and functional properties of *Ziziphus nummularia* seed kernels.
- To determine antioxidant and phenolic properties of Ziziphus nummularia seed kernels.

Materials and Methods chapter 3

3.1 Materials

3.1.1 Ziziphus nummularia

Ziziphus nummularia fruit (fig 1) was taken from the family farm which is located in the village Khanpur, district Fazilka, Punjab whereas seeds were extracted in the laboratory of the Thapar institute of engineering and technology, Patiala, Punjab.

Fig 1 *Ziziphus nummularia*

3.1.2 Chemicals

Petroleum ether, potassium sulfate, copper sulfate, sulphuric acid, Sodium hydroxide, distilled water, boric acid, bromocresol green, methyl red, ethyl alcohol, hydrochloric acid, methanol, DPPH(2,2-diphenyl-1-picrylhydrazyl), ascorbic acid, gallic acid, sodium carbonate, Folin–

Ciocalteu reagent, Quercetin, sodium acetate/ potassium acetate, E. coli, glacial acetic acid, sodium thiosulphate, potassium dichromate solution, starch, chloroform, potassium iodide solution, n-hexane, potassium hydroxide pallet, sodium sulfate anhydrous, d ionized water, spirit, wax parafilm.

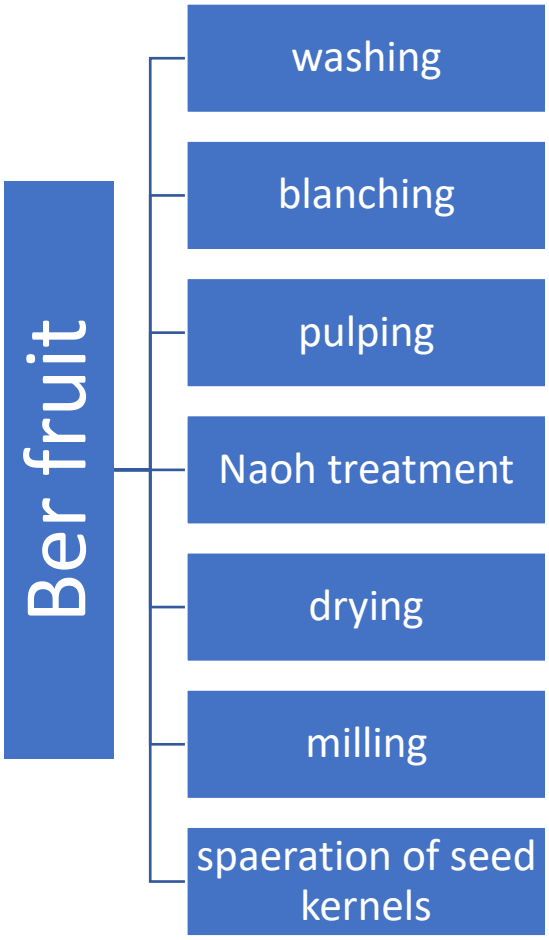
3.1.3. Apparatus Required

Soxhlet apparatus, Kjeldahl distillation digestion unit, pipettes, tips, centrifuge, hot air oven, magnetic stirrer, titration tube, test tubes, saponification flask, beakers, reflux condenser, syringe with filter, weigh machine, mile, gas, and stove, muffle furnace, GC-MS apparatus, spectrophotometer, laminar airflow, incubator.

3.2. Methods

3.2.1 Extraction of seed

To analyze the physical characteristics of Ziziphus seeds, several preliminary measures must be taken before further processing the seeds.



Washing: The fruit was washed under running tap water to remove any the superfluous material present.

Blanching: Blanching of the fruit was done in the hot water. It is done to inhibit the enzymatic activity in the fruit or also helps in the removal of the microorganisms on the surface of the fruit. It is done before further processing such as dehydration, freezing, thermal processing, or canning. Blanching the fruits included immersing them in boiling water for 10 to 15 minutes, varying according to the size of the fruit(boiling time must increase with the increase in the fruit size), and to minimize hot water damage, immediately wash with cool running water. Blanching loosens the skin and core of the fruit, allowing for simple peeling.

Pulping: It is carried out by meshing the fruit with the hands and then the seeds with debris will be immersed in the water. Seeds being denser than the debris will be settled on the bottom of

the container whereas debris will remain on the surface. Now discard the debris. Repeat the previous step until all the debris is removed and the seeds will be separated.

Sodium hydroxide treatment: debris was removed but some fibers cannot be separated from the seeds. to separate these fibers seeds would be treated with the 2% solution of sodium hydroxide. Seeds were immersed in the 2% solution of sodium hydroxide for 12- 15 min. after those seeds were rinsed under the running tap water properly.

Drying: For further processing, seeds would be dried. The drying seeds were kept in the hot air oven overnight at the temperature of 50^{o} C.

Fig 2 *Ziziphus nummularia* seeds.

3.2.2. Extraction of seed kernel

This is the most crucial part of the whole study. These seeds are small in size and have a stony outer covering that is difficult to break without crushing kernels inside the seed. To break this stone-like covering various methods were used such as breaking seed shells with the help of mortar & pestle and then separating the kernels via hand picking. This method was quite energy-consuming and not practically possible for the bulk quantity. So, another method opted

for crushing the seed's outer shell which was more efficient. A mill was used to crush the seed shell. Now this mill was set on its lowest intensity or in such a way that it will only crush the outer part and then kernels were hand-picked. Now, these kernels were used for further studies.

3.2.3. Proximate tests:

3.2.3.1 Preparation of sample:

To study the proximate properties of seeds kernels must be converted into flour consistency using a mortar and pestle.

3.2.3.2 Protein estimation:

Johan G.C.T. Kjeldahl, a Danish chemist, created this technique particularly to measure the nitrogen concentration of organic and inorganic compounds in 1883(AOAC 1984). The protein content of food is also estimated using this approach. The protein content is not directly assessed by the Kjeldahl technique; rather, a conversion factor (F) is required to convert the nitrogen concentration that was measured to a protein concentration. Based on the ratio of amino acids in each protein, the conversion factor varies. For nuts and seeds, use 5.18 to 5.46(FAO 1982).

Preparation of reagent:

40% NaOH: For the stock solution of 100ml, dissolve 40 g of NaOH pellets into 100ml of distilled water.

4% boric acid: Dissolve 4g of boric acid into 100ml of distilled water to prepare 100ml stock.

0.1N HCL: To take stock of 100 ml, add 800 microliters of HCl to 100ml of distilled water.

Mixed indicator Preparation: for preparation of 20 ml stock. Add 19.8 mg of bromocresol green, and 13.2 mg methyl red into 20 ml of ethyl alcohol.

Digestion: The digestion tube containing the flour sample (1 g) was filled with 25 ml of sulfuric acid, 10 g of potassium sulfate, and 1 g of copper sulfate. The digestion tube was then heated for 3hr at 350^{o}C.

Distillation: The machine was shut off after three hours, and 90ml of 40 percent NaOH and 50ml of distilled water were then poured into the tube. In a flask, a few drops of mixed indicator and 4% boric acid were then added. Run the distillation apparatus for 20 min. Finally, the green color was observed, which was our nitrogen content recovered from the sample and contains ammonia.

Titration: The ultimate result was pale pink after the solution had been treated with 0.1N HCl.

$$\text{Nitrogen (\%)} = \frac{((\text{Sample titer} - \text{Blank titer})) \times 14 \times N \times 100}{W \times 1000}$$

N = normality of standard HCL solution

W = weight of the sample

A conversion factor of 5.46 is multiplied to determine the amount of protein.

Protein(%)= Nitrogen(%) × 5.46

This whole process was carried out in replicates and the mean of the 2 replicates was taken.

3.2.3.3 Fat estimation

It was done via automated Soxhlet extraction using the Soxhlet apparatus(modal SCS 4 E: SOCS PLUS). Fats, waxes, and oils may be extracted from food samples quickly and accurately using equipment and processes. Take 2 g of sample into the thimble and place the thimble in the solvent holder with the help of a thimble supporting ring. Now pour non-polar solvent i.e., petroleum ether via thimble into the solvent holder to immerse the sample. Place the solvent holder into the apparatus on the heating plate and connect the solvent holder with the condenser. Before starting the apparatus make sure the water supply to the condenser is on. Run the automated apparatus for 2 hr. After the completion of the process, the maximum solvent is retained in the condenser and only extracted fat or oil remains in the solvent holder. To evaporate the remaining slightest amount of solvent, place the solvent holder into the hot air oven. When the solvent is evaporated calculate the amount of oil or fat extracted from the sample.

$$\text{Crude Fat (\%)} = \frac{\text{Initial weight of the sample}}{\text{The final weight of the extract}} \times 100$$

This whole process was carried out in replicates and the mean of the 2 replicates was taken

3.2.3.4 Moisture content:

It was done with the help of automated moisture analyzer apparatus. A moisture analyzer, which comprises a weighing unit and a halogen heating unit, is a device that measures the moisture content using the loss-on drying method. It is appropriate for quality assurance and manufacturing in the food, pharmaceutical, chemical, and other sectors. For this Aczet brand (model MB 50) moisture analyzer was used.

Place the seed kernels into the aluminum sheet of the apparatus and close the lead. run the apparatus and results will be displayed.

3.2.3.5 Ash content determination:

Samples of each dried fruit seed flour, weighing about 2 g, were put into porcelain crucibles that had already been weighed. After being carbonized over a Bunsen burner, the samples were then put in a muffle furnace and heated to 550^{o} C. 2 hours were spent at this temperature, after which the material was moved to a desiccator comprising silica gel. The crucibles containing the samples were weighed once they had warmed to room temperature to calculate the ash content by comparison.

$$\text{Ash content (\%)} = \frac{\text{Total ash content weight}}{\text{Original sample weight}} \times 100$$

This whole process was carried out in replicates and the mean of the 2 replicates was taken.

3.2.3.6 Carbohydrate estimation:

The difference, as shown in Eq., where M is moisture (%), P is a protein (%), L is a lipid (%), A is ash(%), and was used to determine the total carbohydrate content of the flour samples:

Carbohydrate (%) : 100 – (M+P+A+L)

The sum of the amylaceous carbohydrates (available and resistant starches) and non-digestible carbohydrates account for the overall amount of carbs present in the samples (fibers) (lima et al. 2014).

3.2.4 Functional properties:

3.2.4.1 Preparation of sample:

To study the functional properties of seeds kernels were converted into flour consistency via using a mortar and pestle.

3.2.4.2 Water holding capacity:

The water-holding capacity (WHC) is commonly used to characterize the hydration qualities of seed flour and they relate to the quantity of water that can be retained per gram of sample material. In food processing water holding capacity is the key parameter(Farooq and Boye 2011). Low WHC materials may not be capable of holding water properly, whereas high WHC materials may cause food products to become crunchy and dry, especially when stored(Boye et al. 2010).

This was demonstrated using the techniques mentioned by (Beuchat 1977) with modifications. Kernels were crushed into flour using a mortar and pestle. one gram sample was weighed into conical centrifuge tubes with a graded volume of 25 ml and add 10 ml of water was into it. The mixture was kept at room temperature (37^{o}C) for a complete 1 hour. After one hour centrifuge the mixture at 2000rpm for 30 min. The amount of water on the suspended solids was quantified, and the amount that was absorbed was calculated as a percentage based on the weight of the original sample(Ocloo et al. 2010). This whole process was carried out in replicates and the mean of the 2 replicates was taken.

$$\text{Water holding capacity} = \frac{\text{Weight of wet sediment (g)}}{\text{Weight of dry sample (g)}}$$

3.2.4.3 Swelling capacity(SC) and Swelling Index(SI)

It was analyzed as demonstrated by(Okaka and potter 1977) with modifications. The sample was poured into a 100 mL graduated cylinder to the 10 mL mark. The cylinder was filled up to the 50 ml mark with distilled water. Tightly covered the top of the cylinder and mixing was done by inverting the cylinder. Let the mixture rest for 2 min and after that again mixed by inverting the cylinder. Let the mixture rest for another 8 min. The volume taken was recorded after the 8^{th} minute (Chandra et al. 2015). This whole process was carried out in replicates and the mean of the 2 replicates was taken.

$$SI = \frac{\text{Volume of the sample after soaking- the volume of the sample before soaking}}{\text{Weight of sample}}$$

$$\text{Swelling capacity} = \frac{\text{Weight of wet sediment} \times 100}{\text{Weight of sample}}$$

3.2.4.4 Oil Absorption capacity

The weight difference between flour before and after it has absorbed oil is known as the oil absorption capacity or OAC. They are crucial due to their storage stability, especially when it comes to the development of rancidity. This was demonstrated using the techniques mentioned by (Beuchat 1977) with modifications. 10 ml of vegetable oil(mustard oil, density-0.96 g/ml) and 1 gram of sample were kept in the 25 ml of graduated conical centrifuge tubes. At 2000 rpm centrifuge the suspension for 30 min. Record the volume of oil on sediment and the amount of absorbed oil was represented as a percentage of the original weight of the sample(Ocloo et al. 2010). This whole process was carried out in replicates and the mean of the 2 replicates was taken.

$$\text{Oil absorption capacity(OAC)} = \frac{\text{weight of oil absorbed (g)}}{\text{Weight of sample}}$$

.

3.2.5 Analysis of extracted Free fatty acid composition:

3.2.5.1 Gas chromatography (GC-MS with library search)

It was done via GC-MS with a NIST library search. For the GC-MS analysis the fatty acid sample was further converted into the fatty acid methyl ester molecules (FAME). The prepared sample was sent to the Sophisticated Analytical Instrumental Faculty which is located in IIT Madras, Chennai where the GC-MS with library search was carried out.

Preparation of fatty acid methyl ester

This process was carried out in 5 steps:

Step 1: Preparation of reagent:

0.5 M methanolic Potassium hydroxide (KOH: Take 2.8 g of potassium hydroxide and dissolve it into 100 ml of methanol. Shake the mixture properly and store it in a dark and cool place.

Methanolic HCl: To prepare methanolic HCL stock concentration must be 4:1 i.e., HCL: Methanol respectively. Mow 5 ml of Methanol is dissolved into the 20 ml of HCL and shake till the solution is mixed properly.

Step 2: Preparation of sample:

Take a dry saponification glass and tare its weight. Add 200 microliter of oil sample into the flask and note its weight. Add 4 ml of 0.5 methanolic potassium hydroxide into the flask and shake the flask to mix the content properly.

Step 3: Saponification:

Attach the condenser to the flask and heat at the boiling point in the water bath. Heating should be carried out with the periodic shaking of the flask. After 15 minutes of boiling add 1.6 ml of methanolic HCL solution into the flask and boil for 25 minutes. After 25 minutes detach the condenser and cool the flask at room temperature.

Step 4: Fatty acid methyl ester molecules (FAME) extraction:

Add 8 ml of deionized water and mix properly. Add 6 ml of n-Hexane and shake and rotate properly for one minute. Transfer the mixture into the clean glass tube and give a quick spin.

After the spin, two layers were observed. In the upper layer, hexane was separated. Collect the upper hexane layer into another clean glass vial. Take lower layer in flask again and add 5 ml of hexane and rotate properly. Again, two distinct layers were observed and the upper layer was extracted into the glass vial. Take lower layer in flask again and add 4 ml of hexane and rotate properly. Again, two distinct layers were observed and the upper layer was extracted into the glass vial. Store the vial with the sample at 4^{o}C before analysis.

Step 5: Filtration of FAME extraction:

Take the amount of sodium sulfate in the syringe and attach the syringe to the 0.4-micrometer syringe filter. Pour 3 ml of extracted FAME molecules into the syringe. Discarded the few drops. Now collect the filtered sample into the GC vial.

Note: 2 ml of the sample was sent for the GC-MS with library search analysis.

3.2.5.2 Peroxide test for free fatty acids

In this the sample is first allowed to treat in solution with acetic acid and an appropriate organic solvent mixture, followed by a potassium iodide solution. The released iodine is titrated using a typical sodium thiosulfate solution. Either milliequivalents of peroxide/kg or millimoles of peroxide/L are used to express peroxide levels. Approximately 3 g of the sample, carefully weighed, should be transferred into a 250 mL flask from the center of the sample. Add 50 mL of the necessary solvent combination. Add 1 mL of newly made saturated potassium iodide solution, and let it react for 60 seconds plus one second while rapidly stirring the mixture manually at least twice. Add 100 mL of water, then stir. Utilizing a 1 mL starch solution indicator, titrate the 0.01N sodium thiosulfate solution from a purple to a faint yellow or colorless endpoint.

$$\text{Peroxide value}(\ mEqKg^{-1}) = \frac{\text{Titer value} \times N \times 1000}{\text{Weight of oil sample in grams}}$$

Where N stands for normality of sodium thiosulphate solution.

This whole process was carried out in replicates and the mean of the 2 replicates was taken.

3.2.6 Biochemical analysis

3.2.6.1 Preparation of extract:

For the Biochemical analysis of ber seeds, methanolic extract was prepared(Santos et al. 2012). 2 g of seed kernels were grounded and converted into fine powder. Add methanol (1:10 w/v) into the sample and mix with the magnetic stirrer for 2 hours at 25 ^{o} C temperature. The extracts were then centrifuged at 4000 rpm for 10 min and the supernatant was obtained and used for further experiments.

3.2.6.2 Antioxidant properties:

3.2.6.2.1 Dpph assay (1,1-diphenyl-2-(2,4,6-trinitrophenyl)hydrazine)

The first method for assessing the antioxidant capability of a substance, an extract, or many other biological sources is the (DPPH) free radical scavenging method**.** The potential chemical or extract is combined with DPPH solution in this process, and then absorbance is measured after a certain period. The substances known as antioxidants work to neutralize free radicals by interfering with any one of the three main stages of the oxidative process that are mediated by free radicals: initiation, propagation, and termination. (Cui et al. 2004). The method is based on the assessment of the antioxidants' ability to scavenge it. By obtaining a hydrogen atom from antioxidants and converting it to the equivalent hydrazine, the odd electron of the nitrogen atom in DPPH is reduced(Contreras-Guzman and Srong 1982).

for Dpph 1mg /25ml stock was prepared where 1mg Dpph was dissolved in 25 ml of methanol. For ascorbic acid 1 mg/ml stock was prepared where 25 mg ascorbic acid was dissolved in 25 ml of distilled water.

Two reactions were prepared. Ascorbic acid was used as standard and methanol was used as control.

Reaction 1: take 40 µL of ascorbic acid in a test tube and add 600 µL Dpph + 160 µL methanol.

Reaction 2: take 40 µL of methanolic extract from the sample in a test tube and add 600 µL of Dpph + 160 µL methanol.

Now incubate in the dark for 30 min and take absorbance at 517 nanometers(Ma et al. 2011). The ability of the test materials to scavenge DPPH free radicals reveals their antioxidant capability and demonstrates their efficacy in the prevention, detection, and healing of damage to biological systems.

This whole process was carried out in replicates and the mean of the 2 replicates was taken.

$$I\ (\%) = \frac{[\ \text{control} - \text{sample}\ ] \times 100}{\text{control}}$$

3.2.6.3 Total phenolic content analysis:

The method to determine the quantity of phenolic material in the samples is TPC activity. Plants contain phenolic chemicals that have redox characteristics that allow them to serve as antioxidants. Folin-Ciocalteu test was used to assess the total phenolic content of the extracts. A test based on electron transfer, the Folin-Ciocalteu technique provides reducing capability, which is represented as phenolic content.

3.2.6.3.1 Standard Gallic Acid Preparation for Calibration Curve:

The Folin-Ciocalteu colorimetric technique, as reported by (Singleton et al. 1999) with modifications was used to measure the total phenolic contents (TPC) in the fruit, seed, and bark extracts. 10 mg of standard gallic acid was dissolved in 10 mL of methanol (1 mg/mL) to prepare the solution. From the standard solution, gallic acid solutions in methanol at various concentrations (25, 50, 75, and 100 g/mL) were created. A final volume of 10 mL was created by adding 5 mL of 10 % Folin-Ciocalteu reagent (FCR) and 4 mL of 7 % Na_2CO_3 to each concentration. The resulting blue-colored liquid was well agitated and incubated in a water bath for 30 minutes at 40°C. Then, against a blank, the absorbance at 760 nm was measured.

A UV-visible spectrophotometer is used to quantify the dark blue hue that results from the FCR reagent's oxidation of the phenols in plant extracts. The calibration curve was drawn using the average absorbance readings at various gallic acid concentrations that were acquired from all of the tests, which were all completed in duplicates

3.2.6.3.2 Total phenolic content sample preparation:

The extracts were produced in a range of concentrations (25, 50, 75, and 100 g/mL). A final volume of 10 mL was created by adding 5 mL of 10 % Folin-Ciocalteu reagent (FCR) and 4 mL of 7 % Na_2CO_3 to each concentration. Record the absorbance of extract at each concentration at 760 nm. To determine the concentration of phenolics in the extracts, the samples were made in duplicates for each measurement, and the calibration curve was plotted using the average value of absorbance. Gallic acid equivalents (GAE) per gram of material in dry weight (mg/g) were used to represent the total phenolic content of the extracts.

$$C = c\frac{v}{m}$$

C = total phenolic content

c = concentration of gallic acid obtained

v = volume of extract in ml

m = mass of extract in g

Result and Discussion Chapter 4

4.1 Extraction of seeds kernels:

For the extraction of the seeds kernels seeds were dried in the hot air at night at the 50° C temperature. Later on, to extract the kernels, seed shell was broken by passing the seeds through mini laboratory mill and after that kernel were manually separated from the seed shells.

Fig 1 extracted *Ziziphus nummularia* seed kernels

4.2 Proximate analysis:

4.2.1 Protein analysis:

As shown in table 1 the protein contents found in *Ziziphus nummularia* was 38.7%. The amount of protein content present in *Z. nummularia* is comparable to *Z. mauritiana* which is 36.4%. although it has much higher than the *Amaranthus sp.* Seeds which are 10.8 to 18.3 % ((Dhan and Pal, 1992), melon seed (unfermented) i.e., 33.8% (Afam and Jacob, 1993), *Digitaria exilis*

i.e 1.3%. It also has a comparable amount of protein present in the orange seed which is 33% but relatively higher than jack fruit seed which is 20 % (Lima *et al.* 2014). However, this study shows that fruit seed flours have far more protein than industrial flours (such as maize, wheat, or potato starch flour). With a protein content of around 10%, wheat flour is the commercial flour with the greatest level; the other flours examined had levels between 0.5 % and 1.5 % (TACO 2011).

4.2.2 Fat analysis:

As shown in table 1 the lipide contents found in *Ziziphus nummularia* was 23.1%. It has a relatively high amount of lipid contents concerning jack fruit i.e., 1.7% (Ocloo *et al.* 2010), pearl millet i.e., 7.6%, pigeon pea flour i.e., 1.80% (Okpala and Mammah, 2001), quinoa i.e., 6.3% (Oshodi *et al.*, 1999) and wheat flour i.e., 3.10% (Okpala and Mammah, 2001). Depending on the features of the soil, environment, and germination/propagation, the energy store of seeds may take the form of lipids or glycids. As a result, seeds with high-fat concentrations during the latency stage typically have low starch contents. To give energy for germination, the lipids are then converted by exothermic processes into glycids, although in other species, there is little to no conversion of lipids to carbohydrates (Kozlowski & Pallardy, 1997).

4.2.3 Moisture content:

Moisture serves as a gauge for the amount of water and overall solids in the seed flour. Additionally, it measures the flour's storage stability. As shown in table 1 moisture content of *Ziziphus nummularia* seeds flour was 5% which is lower than the jack fruit i.e 6.7% (Ocloo *et al.* 2010), cherry (6.2%), peach (7.8%), melon (7.1%), orange (7.2%) (Lima 2014). The quality of flour increases with reduced moisture content because it has better shelf stability. The length of the drying process generally determines the moisture content of flour.

4.2.4 Ash content:

The organic residue left over after the organic matter has been burned off is what is referred to as the ash content. There could have been losses by volatilization or interactions between

constituents, thus it might not have the same composition as the mineral matter that was originally contained in the flour. The percent ash content found in *Ziziphus nummularia* was 3.5% which is slightly higher than jackfruit (2.70%) (Ocloo *et al.* 2010).

4.2.5 Carbohydrates :

The seed flour's main ingredient was carbohydrates. As shown in the table.1 the carbohydrate value obtained for the *Ziziphus nummularia* was 29.1%. Given the number of glycines in the seeds, the quantities of carbohydrates determined by difference were as anticipated.

4.3 Functional properties:

4.3.1 Water holding capacity:

The water holding capacity of the *Ziziphus* seed was 28% (2.8 g/g). The value obtained was higher than the jackfruit i.e., 25% (Ocloo 2010), and pumpkin i.e., 24% (Lazos 1992). The 3.4 ml/g given for raw camphor flour serves as a comparison, though (Odoemelam 2003). Under conditions of scarce water availability, the ability of flour to bind with water is described as water absorption capacity. However, the outcome is less favorable than what was anticipated by (Singh *et al.* 1991) 141%(Tulyathan *et al*. 2002) and 205% for jackfruit seeds without the brown spermoderm and complete jackfruit seed flour. The approach employed and the variations in the varieties might both be blamed for the discrepancies found. The outcome demonstrates that flour has high water-binding capabilities. This finding indicates that *Ziziphus* seed flour may be suitable for usage in the bread sector.

4.3.2 Oil absorption capacity:

the oil absorption capacity of the *Ziziphus* seeds was 23.8%. Because fats enhance food flavor and mouthfeel, fat absorption is a crucial component of food compositions (Kinsella, 1976). Fat can enhance flavor retention in processed simulated foods. The outcome is comparable to the reported raw jackfruit flour concentration of 2.8 g/ml (Odoemelam, 2005). For tiger nut flour, values between 1.07 -1.13 ml/g were found (Oladele and Aina 2007). winged bean levels of 1.2–1.4 ml/g were reported by (Narayana and Narasinga Rao 1982). Additionally, an African

yam bean value of 1.42 ml/g was recorded by (Eke and Akobundu 1993). The results show that *Ziziphus* is a high flavour retainer and could be used in the food industry.

4.3.3 Swelling capacity(SC) and Swelling Index(SI):

Swelling capacity (SC) and swelling Index (SI) were 4.067 (406.7%) and 0.88. This value is comparable to the jack fruit seed (4.77) but relatively more lover than the ginger-modified starch (Daramola and Osanyinlusi 2006). Because the measurement is based on the weight of swelled starch granules and the water that has been trapped inside of them, swelling power is a measure of hydration capacity. The retention of water in the enlarged starch granules is frequently linked to the quality of food consumed (Rickard *et al.* 1992).

Table 1 Proximate, functional, free fatty acid, antioxidant, and phenolic contents of *Ziziphus nummularia*. (Mean ± standard deviation, where n = 2)

Analysis	Result (% value)
Proximate analysis	
Protein content	38.7 ± 1.83
Lipid content	23.4 ± 1.90
Ash content	3.5 ± 0.77
Moisture	5
Carbohydrate	29.1 ± 4.52
Functional analysis	
Water holding capacity	28 ± 0.14
Swelling capacity	406.7 ± 11.78
Swelling Index	1.13 ± 0.35
Oil absorption capacity	23 ± 0.12
Free fatty acid analysis	
Peroxide value	58 ± 11.31
Antioxidant properties	
Dpph	76 ± 3.53
Phenolic properties	
Total phenolic content	32.5 ± 3.5

4.4 GC-MS analysis :

The crude fat's fatty acid content was determined by saponification, which was followed by the creation of their methyl esters. Latter these fatty acid methyl esters were analysed by the GC-MS with a library search. individual fatty acids were identified with the help of mass spectral deviation as shown in figure 1 and the compounds are listed below with given different parameters.

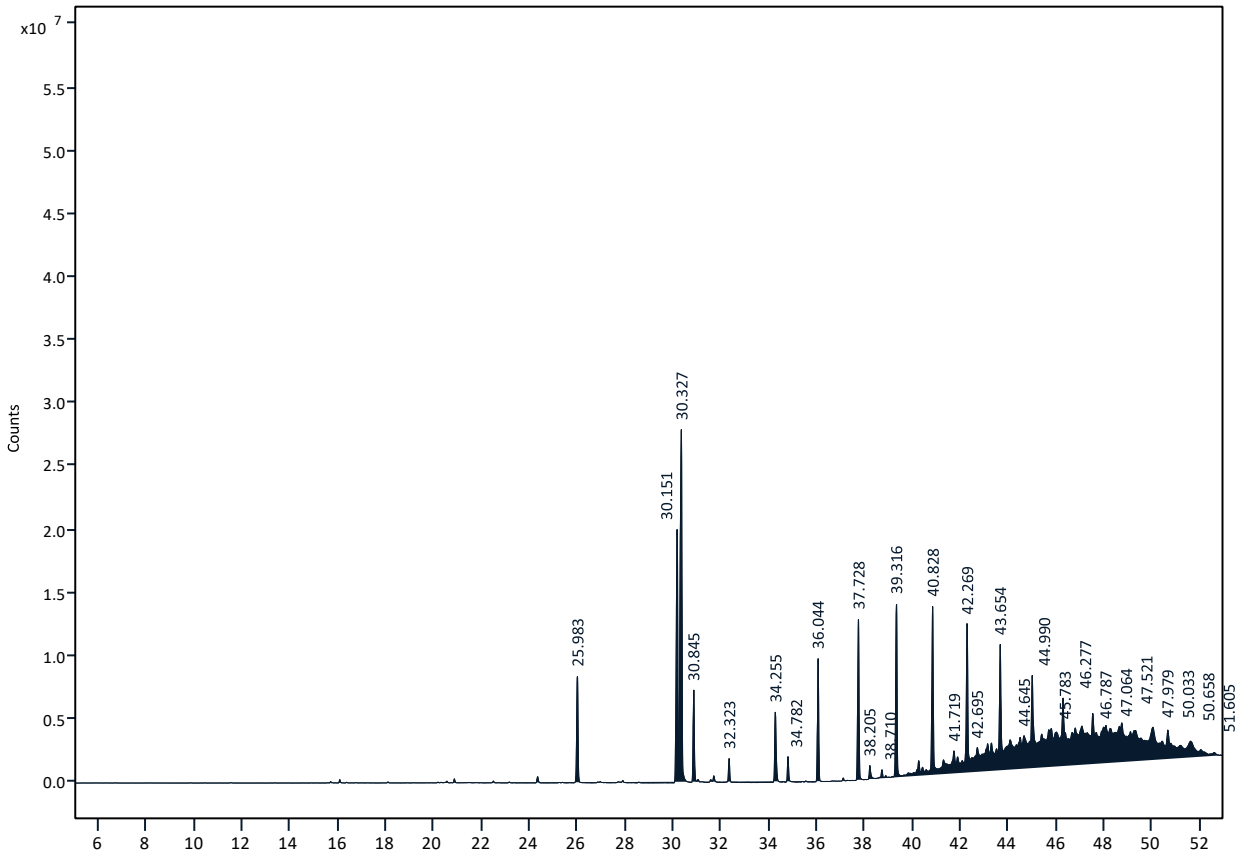


Fig.1

As shown in fig.1 different fatty acid compounds are identified in the FAME molecules of *Ziziphus nummularia.* There are different peaks shown in fig 1, each peak indicates the presence of different free fatty acid compounds with different concentrations.

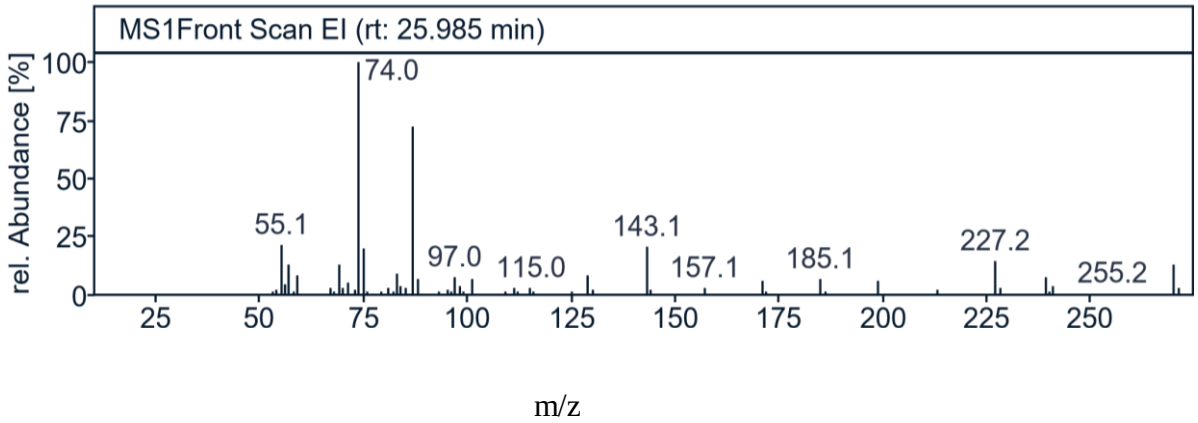


Fig 2 free fatty acid concentration at peak 25.985 min

Fig 2 is the representation of the presence of different compounds at a retention time (rt) 25.985 min. with different concentrations. Hexadecanoic acid, methyl ester; Pentadecanoic acid, 14methyl-, methyl ester; Pentadecanoic acid, methyl ester are the compounds that are found on this particular retention time with different concentrations as per shown in table 2.

Table 2 free fatty acid concentration at peak 25.985 min

S no.	Compound name	Concentration %
1	Hexadecanoic acid, methyl ester	88.7
2	Pentadecanoic acid, 14methyl-, methyl ester	5.53
3	Pentadecanoic acid, methyl ester	1.16

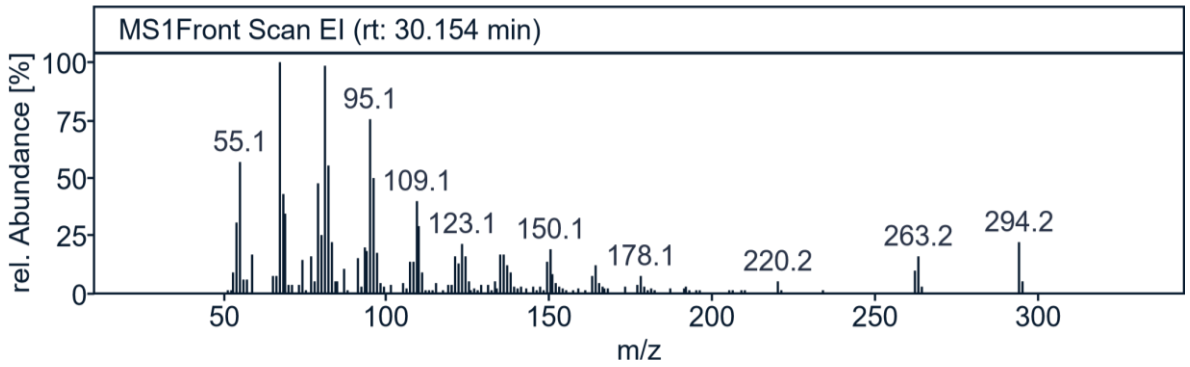


Fig 3 Table 3 concentration of free fatty acid at peak 30.154 min

Fig 3 is the representation of the presence of different compounds at a retention time (rt) 30.154 min. with different concentrations. 9,12-Octadecadienoic acid (Z, Z)-, methyl ester; Methyl 9-cis,11-transoctadecadienoate; 9,11-Octadecadienoic acid, methyl ester, (E, E)- are the compounds which are found on this particular retention time with different concentration as per shown in table 3.

Table 3 concentration of free fatty acid at peak 30.154 min

S no.	Compound	Concentration %
1	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	42.42
2	Methyl 9-cis,11-transoctadecadienoate	9.35
3	9,11-Octadecadienoic acid, methyl ester, (E, E)-	7.9

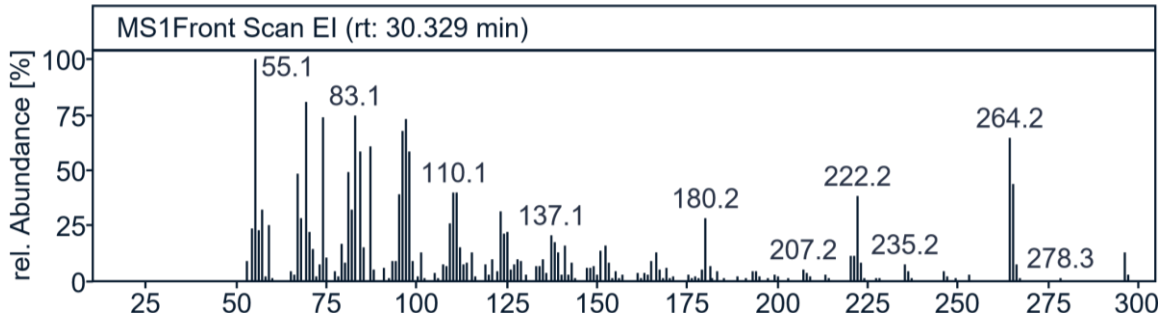


Fig 4 concentration of free fatty acid at peak 30.329 min

Fig 4 is the representation of the presence of different compounds at a retention time (rt) 30.329 min. with different concentrations. 9-Octadecenoic acid (Z)-, methyl ester; cis-13-Octadecenoic acid, methyl ester; 9-Octadecenoic acid, methyl ester, (E)- are the compounds that are found on this particular retention time with different concentration as per shown in table 4.

Table 4 concentration of free fatty acid at peak 30.329 min

S no.	Compound name	Concentration %
1	9-Octadecenoic acid (Z)-, methyl ester	26.84
2	cis-13-Octadecenoic acid, methyl ester	12.22
3	9-Octadecenoic acid, methyl ester, (E)-	11.28

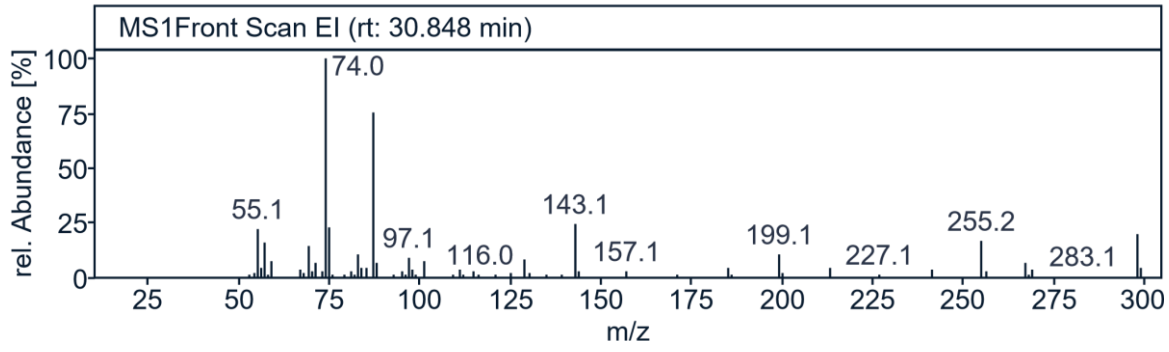


Fig 5 the concentration of free fatty acid at a peak of 30.848 min

Fig 5 is the representation of the presence of different compounds at a retention time (rt) 30.848 min. with different concentrations. Methyl stearate; Heptadecanoic acid, 16-methyl-, methyl ester; Heptadecanoic acid, 15- methyl-, methyl ester are the compounds that are found on this particular retention time with different concentrations as per shown in table 5.

Table 5. the concentration of free fatty acid at a peak of 30.848 min

S no.	Compound name	Concentration %
1	Methyl stearate	85.05
2	Heptadecanoic acid, 16-methyl-, methyl ester	7.04
3	Heptadecanoic acid, 15- methyl-, methyl ester	4.4

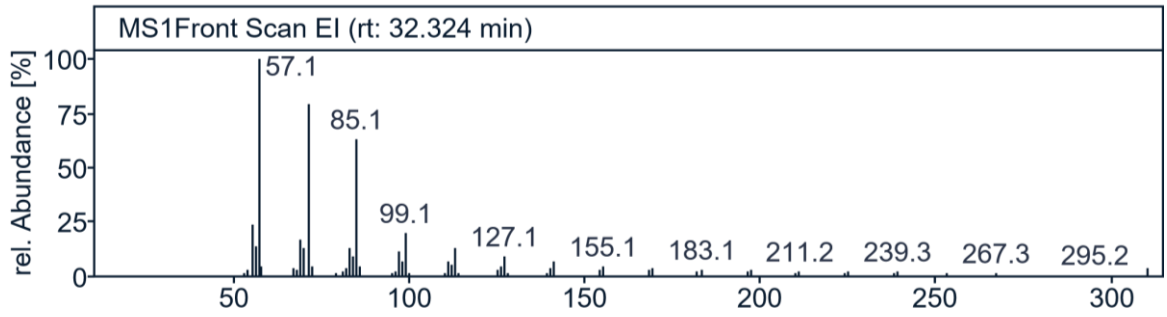


Fig 6 the concentration of free fatty acid at a peak of 32.324 min

Fig 6 is the representation of the presence of different compounds at a retention time (rt) 32.324 min. with different concentrations. Eicosane; Docosane; Heneicosane are the compounds that are found on this particular retention time with different concentrations as per shown in table 6

Table 6 the concentration of free fatty acid at a peak of 32.324 min

S no.	Compound name	Concentration %
1	Eicosane	18.87
2	Docosane	14.46
3	Heneicosane	7.89

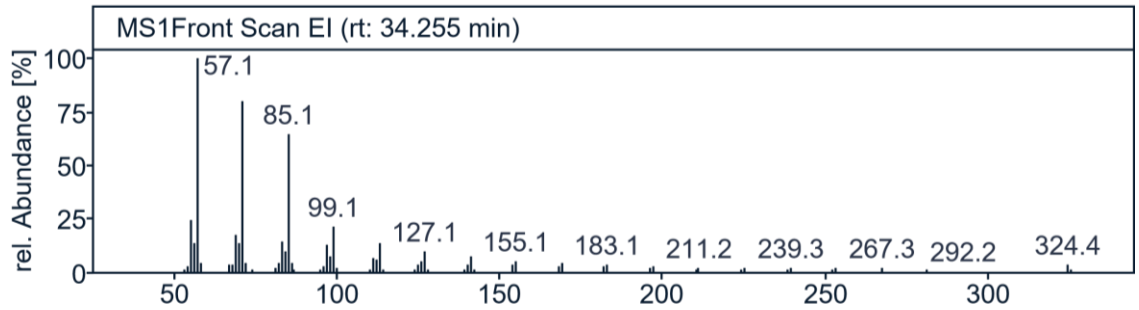


Fig 7 the concentration of free fatty acid at a peak of 34.255 min

Fig 7 is the representation of the presence of different compounds at a retention time (rt) 34.255 min. with different concentrations. Tricosane; Eicosane; Tetracosane are the compounds that are found on this particular retention time with different concentrations as per shown in table 7

Table 7 the concentration of free fatty acid at a peak of 34.255 min

S no.	Compound name	Concentration %
1	Tricosane	23.68
2	Eicosane	17.19
3	Tetracosane	8.35

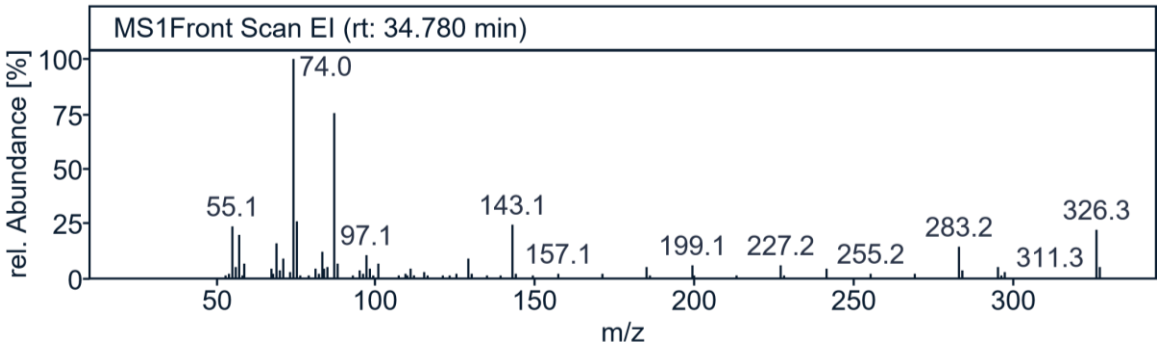


Fig 8 the concentration of free fatty acid at a peak of 34.780 min

Fig 8 is the representation of the presence of different compounds at a retention time (rt) 34.780 min. with different concentrations. Eicosanoic acid, methyl ester; Methyl 18-methylnonadecanoate; Nonadecaonoic acid, 10-methyl-, methyl ester are the compounds that are found on this particular retention time with different concentrations as per shown in table 8.

Table 8 the concentration of free fatty acid at a peak of 34.780 min

S no.	Compound name	Concentration %
1	Eicosanoic acid	75.34
2	Methyl 18-methylnonadecanoate	20.94
3	Nonadecaonoic acid, 10-methyl-, methyl ester	1.96

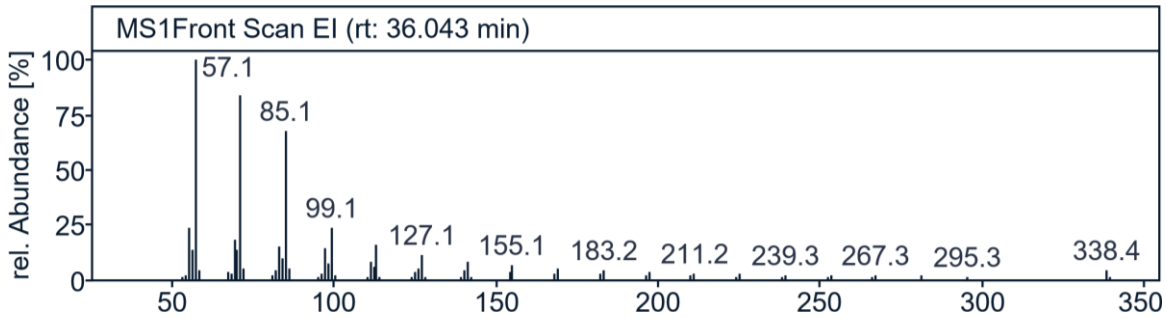


Fig 9 the concentration of free fatty acid at a peak of 36.043 min

Fig 9 is the representation of the presence of different compounds at a retention time (rt) 36.043 min. with different concentrations. Pentacosane; Eicosane; Tetracosane are the compounds that are found at this particular retention time with different concentrations as per shown in table 9.

Table 9 the concentration of free fatty acid at a peak of 36.043 min

S no.	Compound name	Concentration %
1	Tetracosane	34.68
2	Eicosane	8.7
3	Pentacosane	7.35

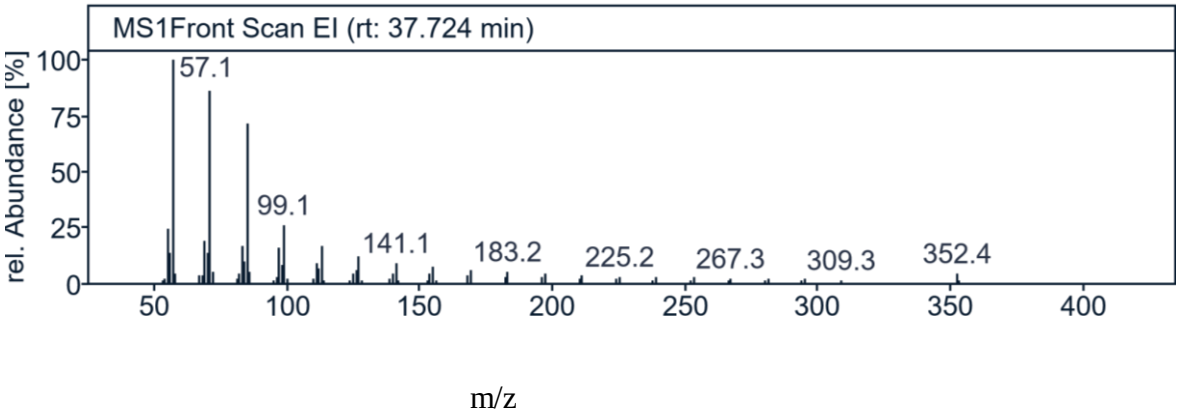


Fig 10 the concentration of free fatty acid at a peak of 37.724 min

Fig 10 is the representation of the presence of different compounds at retention time (rt) 37.724 min. with different concentrations. Pentacosane; Nonacosane; Triacontane are the compounds that are found in this particular retention time with different concentrations as per shown in table 10.

Table 10 the concentration of free fatty acid at a peak of 37.724 min

S no.	Compound name	Concentration %
1	Pentacosane	16.82
2	Triacontane	13.22
3	Nonacosane	9.33

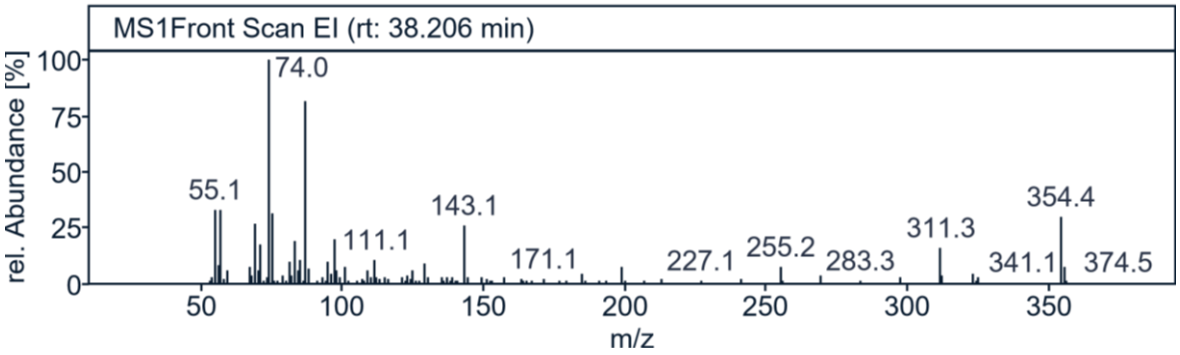


Fig 11 the concentration of free fatty acid at a peak of 38.206 min

Fig 11 is the representation of the presence of different compounds at a retention time (rt) 38.206 min. with different concentrations. Docosenoic acid, methyl ester; Methyl 20-methyl-, heneiosanoate; Octadecanoic acid, 17-methyl-, methyl ester are the compounds that are found on this particular retention time with different concentrations as per shown in table 11

Table 11 the concentration of free fatty acid at a peak of 38.206 min

S no.	Compound name	Concentration %
1	Docosenoic acid, methyl ester	65.96
2	Methyl 20-methyl-, heneiosanoat	26.07
3	Octadecanoic acid, 17-methyl-, methyl ester	1.11

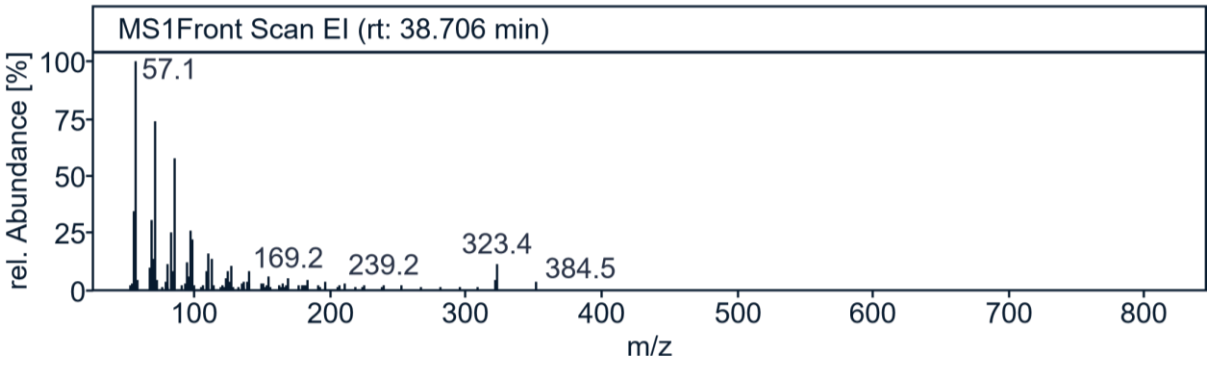


Fig 12 the concentration of free fatty acid at a peak of 38.706 min

Fig 12 is the representation of the presence of different compounds at a retention time (rt) 38.706 min. with different concentrations. Tetrapentacontane, 1, 54-dibromo-; 2-Methylpentacosane; Octadecane, 3-ethyl-5-(2-ethylbutyl)- are the compounds that are found on this particular retention time with different concentrations as per shown in table 12

Table 12 the concentration of free fatty acid at a peak of 38.706 min

S no.	Compound name	Concentration %
1	Tetrapentacontane, 1, 54-dibromo-	11.07
2	2-Methylpentacosane	7.59
3	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	5.66

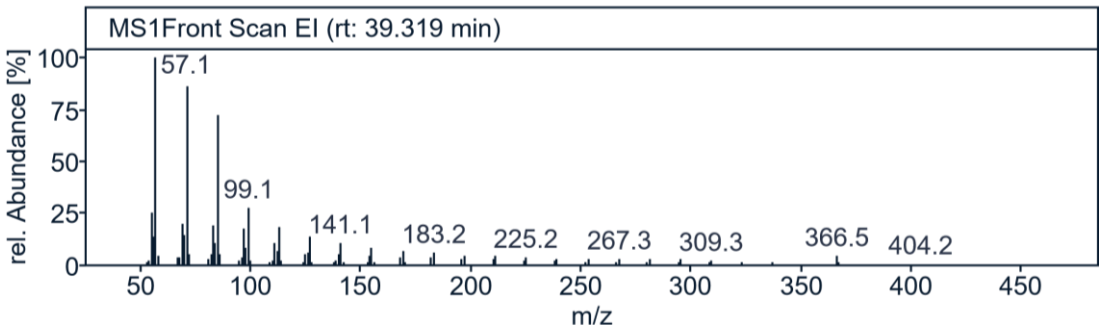


Fig 13 the concentration of free fatty acid at a peak of 39.319 min

Fig 13 is the representation of the presence of different compounds at a retention time (rt) 39.319 min. with different concentrations. Triacontane; Tetratriacontane; Nonacosane are the compounds that are found on this particular retention time with different concentrations as per shown in table 13

Table 13 the concentration of free fatty acid at a peak of 39.319 min

S no.	Compound name	Concentration %
1	Triacontane	15.35
2	Tetratriacontane	10.53
3	Nonacosane	9.3

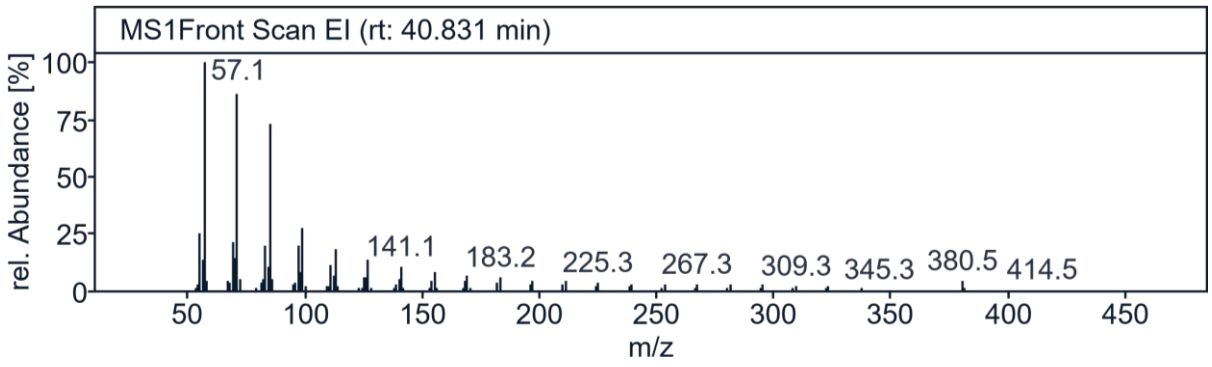


Fig 14 the concentration of free fatty acid at a peak of 40.831 min

Fig 14 is the representation of the presence of different compounds at a retention time (rt) 40.831 min. with different concentrations. Triacontane; Heptacosane; tetratriacontane are the compounds that are found on this particular retention time with different concentrations as per shown in table 14

Table 14 the concentration of free fatty acid at a peak of 40.831 min

S no.	Compound name	Concentration %
1	Triacontane	16.76
2	Hetacosane	12.17
3	Tetratriacontane	10.75

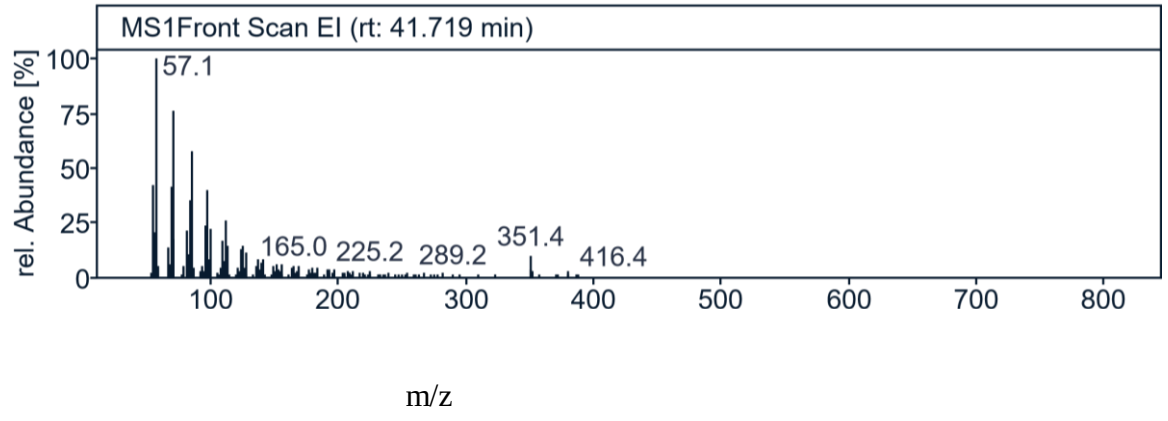


Fig 15 the concentration of free fatty acid at a peak of 41.719 min

Fig 15 is the representation of the presence of different compounds at a retention time (rt) 41.719 min. with different concentrations. Tetrapentacontane, 1, 54-dibromo-; are the

compounds that are found on this particular retention time with different concentrations as per shown in table 15

Table 15 the concentration of free fatty acid at a peak of 41.719 min

S no.	Compound name	Concentration %
1	Tetrapentacontane, 1, 54-dibromo-	38.07
2	17-Pentariactoene	13.9
3	tetracosane, 1-bromo-	3.27

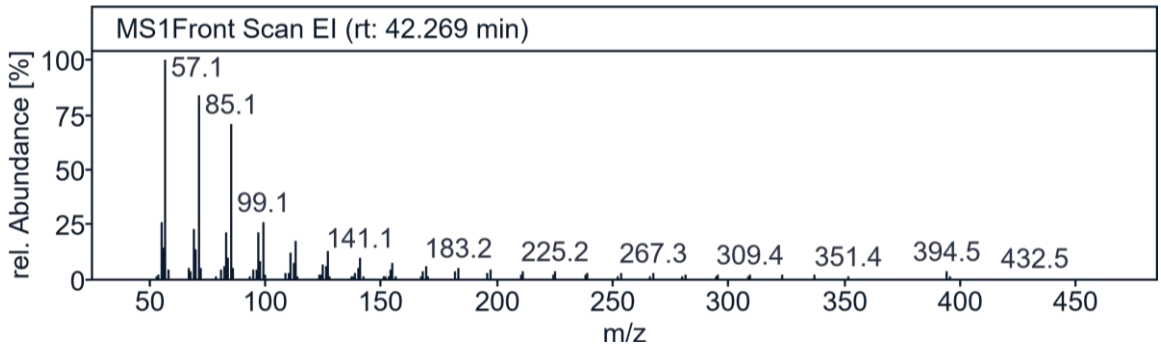


Fig 16 the concentration of free fatty acid at a peak of 42.269 min

Fig 16 is the representation of the presence of different compounds at a retention time (rt) of 42.269 min. with different concentrations. Tetratriacotane; Octacosane; Triacontane are the compounds that are found on this particular retention time with different concentrations as per shown in table 16

Table 16 the concentration of free fatty acid at a peak of 42.269 min

S no.	Compound name	Concentration %
1	Tetratriacotane	16.87
2	Octacosane	16.87
3	Triacontane	16.22

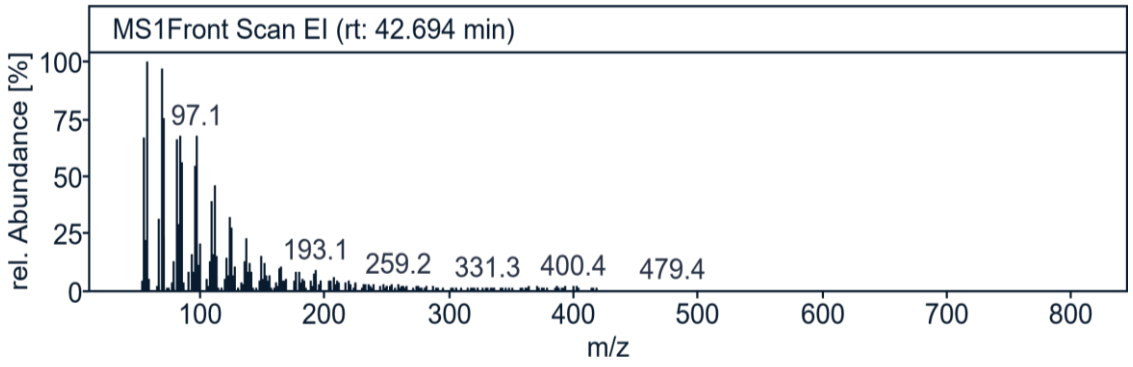


Fig 17 the concentration of free fatty acid at a peak of 42.694 min

Fig 17 is the representation of the presence of different compounds at a retention time (rt) 42.694 min. with different concentrations. Tetrapentacontane, 1, 54-dibromo-; 17-Pentatriacontane; 1-Hexacosane are the compounds that are found on this particular retention time with different concentrations as per shown in table 17

Table 17 the concentration of free fatty acid at a peak of 42.694 min

S no.	Compound name	Concentration %
1	Tetrapentacontane, 1, 54-dibromo-	20.84
2	17-Pentatriacontane	16.8
3	1-Hexaconsane	11.19

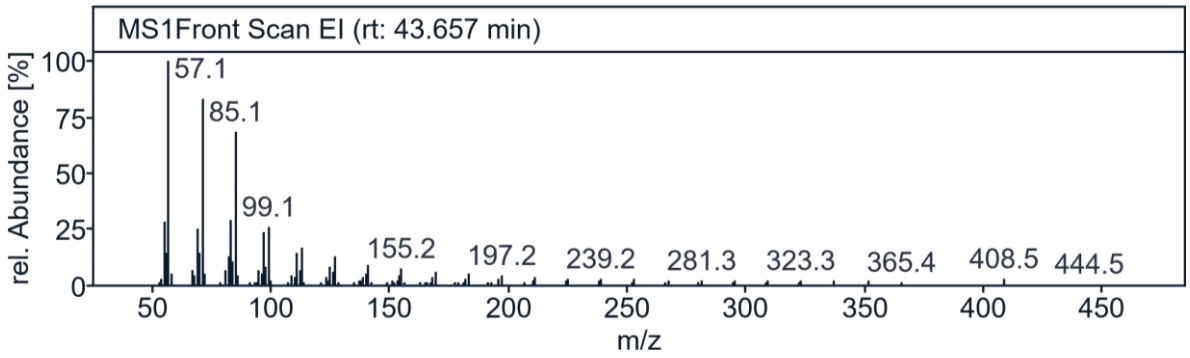


Fig 18 the concentration of free fatty acid at a peak 43.657 min

Fig 18 is the representation of the presence of different compounds at a retention time (rt) 43.657 min. with different concentrations. Tetratriacontane; Nonacosane; Tiacontane; are the compounds that are found on this particular retention time with different concentrations as per shown in table 18

Table 18 the concentration of free fatty acid at a peak 43.657 min

S no.	Compound name	Concentration %
1	Tertratriacontane	12.35
2	Nonaconsane	8.97
3	Triacontane	7.05

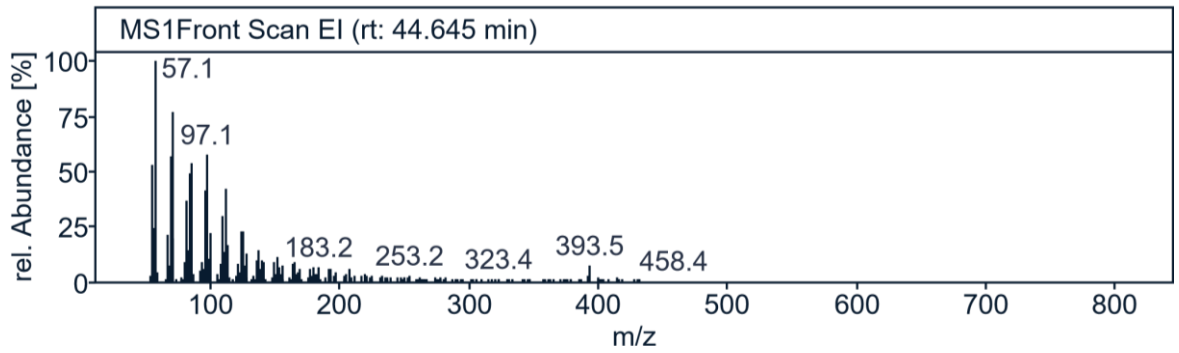


Fig 19 the concentration of free fatty acid at a peak 44.654 min

Fig 19 is the representation of the presence of different compounds at a retention time (rt) 44.654 min. with different concentrations. Tetrapentacontane, 1, 54-dibromo-; 17-Pentatriacontane; 1-Hexacosane are the compounds that are found on this particular retention time with different concentrations as per shown in table 19

Table 19 the concentration of free fatty acid at a peak 44.654 min

S no.	Compound name	Concentration %
1	Tetrapentacontane, 1, 54-dibromo-	38.38
2	17-Pentatriacontane	17.48
3	1-Hexacosane	4.48

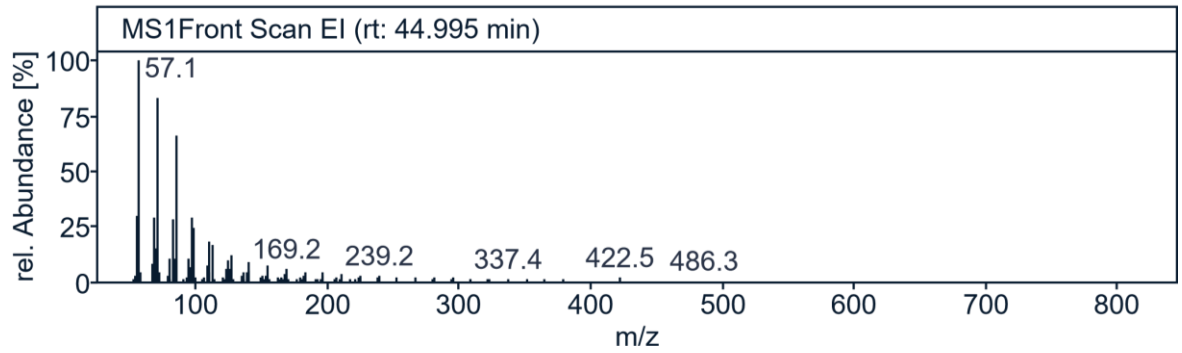


Fig 20 the concentration of free fatty acid at a peak 44.995 min

Fig 20 is the representation of the presence of different compounds at a retention time (rt) 44.995 min. with different concentrations. Tetrapentacontane, 1, 54-dibromo-; 17-Pentatriacontane; 1-Hexacosane are the compounds that are found on this particular retention time with different concentrations as per shown in table 20

Table 20 the concentration of free fatty acid at a peak 44.995 min

S no.	Compound name	Concentration %
1	Tetrapentacontane, 1, 54-dibromo-	11.91
2	17-Pentatriacontane	10.52
3	1-Hexacosane	6.37

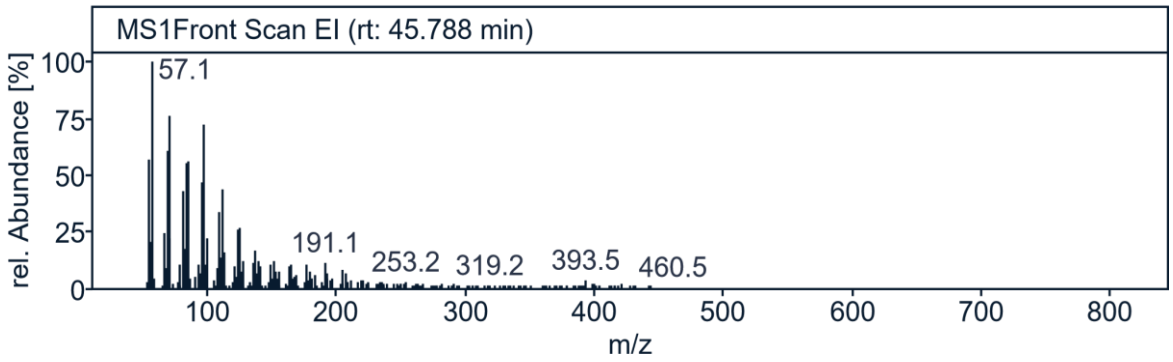


Fig 21 the concentration of free fatty acid at a peak 45.788 min

Fig 21 is the representation of the presence of different compounds at retention time (rt) 45.788 min. with different concentrations. Tetrapentacontane, 1, 54-dibromo-; 17-Pentatriacontane; 1-Hexacosane are the compounds that are found on this particular retention time with different concentrations as per shown in table 21

Table 21 the concentration of free fatty acid at a peak 45.788 min

S no.	Compound name	Concentration %
1	Tetrapentacontane, 1, 54-dibromo-	50.5
2	17-Pentatriacontane	13.76
3	1-Hexacosane	3.6

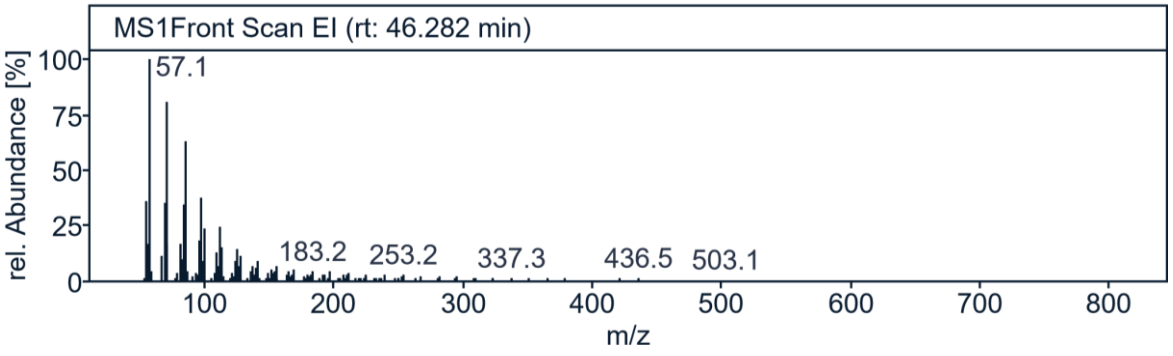


Fig 22 Table 22 the concentration of free fatty acid at a peak 46.288 min

Fig 22 is the representation of the presence of different compounds at retention time (rt) 46.282 min. with different concentrations. Tetrapentacontane, 1, 54-dibromo-; 17-Pentatriacontane; 1-

Hexacosane are the compounds that are found on this particular retention time with different concentrations as per shown in table 22

Table 22 the concentration of free fatty acid at a peak 46.288 min

S no.	Compound name	Concentration %
1	Tetrapentacontane, 1, 54-dibromo-	46.54
2	17-Pentatriacontene	11.15
3	1-Hexacosane	3.1

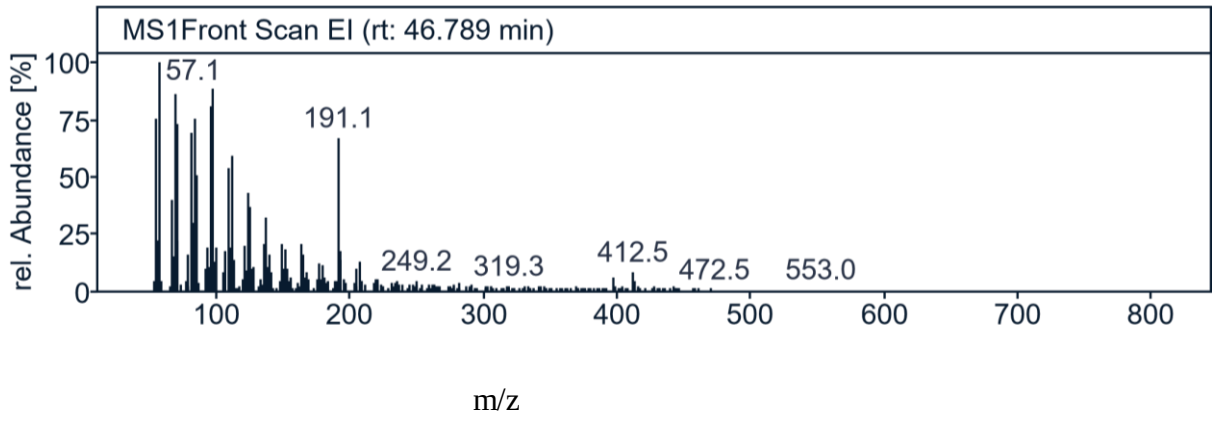


Fig 23 the concentration of free fatty acid at a peak 46.789 min

Fig 23 is the representation of the presence of different compounds at retention time (rt) 46.789 min. with different concentrations. 7,8,-Epoxylanostan-11-ol, 3-acetoxy-; Tetrapentacontane, 1,54-dibromo-; 17-Pentatriacontene are the compounds that are found on this particular retention time with different concentrations as per shown in table 23

Table 23 the concentration of free fatty acid at a peak 46.789 min

S no.	Compound name	Concentration %
1	7,8,-Epoxylanostan-11-ol	30.43
2	Tetrapentacontane, 1,54-dibromo-	9.28
3	17-Pentatriacontene	5.99

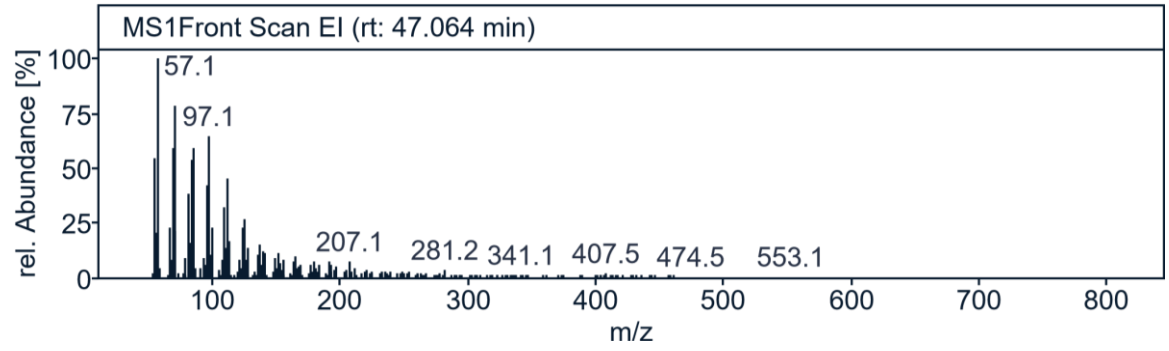


Fig 24 the concentration of free fatty acid at a peak 47.064 min

Fig 24 is the representation of the presence of different compounds at retention time (rt) 47.064 min. with different concentrations. Tetrapentacontane, 1, 54-dibromo-; 17-Pentatriacontane; 1-Hexacosane are the compounds that are found on this particular retention time with different concentrations as per shown in table 24

Table 24 the concentration of free fatty acid at a peak 47.064 min

S no.	Compound name	Concentration %
1	Tetrapentacontane, 1, 54-dibromo-	48.95
2	17-Pentatriacontene	16.4
3	1-Hexacosane	3.4

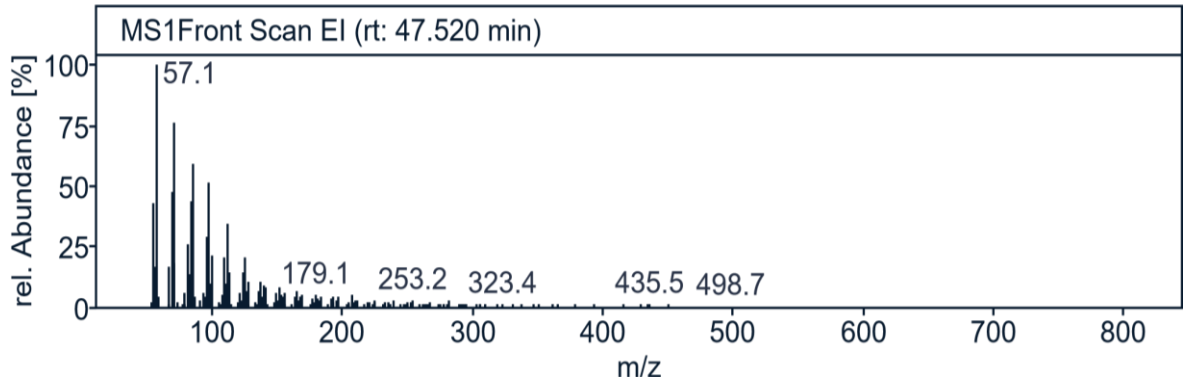


Fig 25 the concentration of free fatty acid at a peak of 47.520 min

Fig 25 is the representation of the presence of different compounds at retention time (rt) of 47.520 min. with different concentrations. Tetrapentacontane, 1, 54-dibromo-; 17-Pentatriacontane; 1-Hexacosane are the compounds that are found on this particular retention time with different concentrations as per shown in table 25

Table 25 the concentration of free fatty acid at a peak of 47.520 min

S no.	Compound name	Concentration %
1	Tetrapentacontane, 1, 54-dibromo-	53.39
2	17-Pentatriacontene	14.84
3	1-Hexacosane	4.04

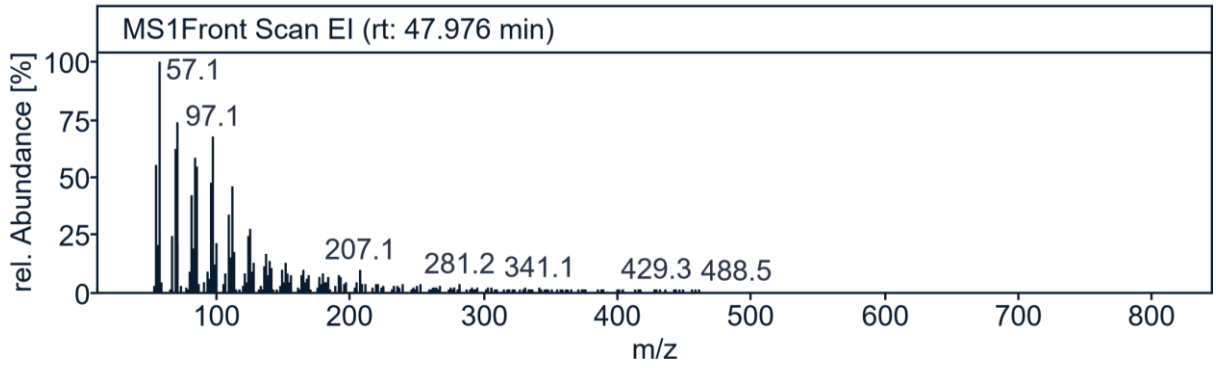


Fig 26 Table 26 the concentration of free fatty acid at a peak of 47.976 min

Fig 26 is the representation of the presence of different compounds at a retention time (rt) of 47.976 min. with different concentrations. Tetrapentacontane, 1, 54-dibromo-; 17-Pentatriacontane; 1-Hexacosane are the compounds that are found on this particular retention time with different concentrations as per shown in table 26

Table 26 the concentration of free fatty acid at a peak of 47.976 min

S no.	Compound name	Concentration %
1	Tetrapentacontane, 1, 54-dibromo-	44.8
2	17-Pentatriacontene	19.06
3	1-Hexacosane	4.49

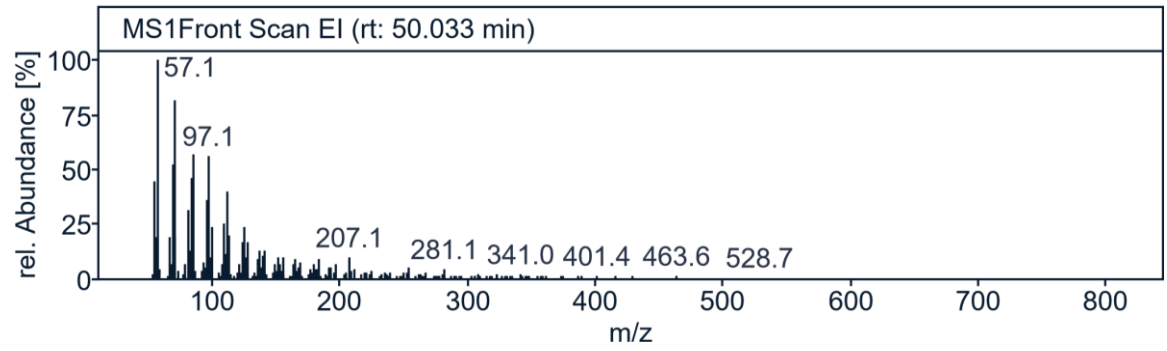


Fig 27 the concentration of free fatty acid at a peak of 50.033 min

Fig 27 is the representation of the presence of different compounds at a retention time (rt) of 50.033 min. with different concentrations. Tetrapentacontane, 1, 54-dibromo-; 17-Pentatriacontane; 1-Hexacosane are the compounds that are found on this particular retention time with different concentrations as per shown in table 27

Table 27 the concentration of free fatty acid at a peak of 50.033 min

S no.	Compound name	Concentration %
1	Tetrapentacontane, 1, 54-dibromo-	51.3
2	17-Pentatriacontene	14.81
3	1-Hexacosane	298

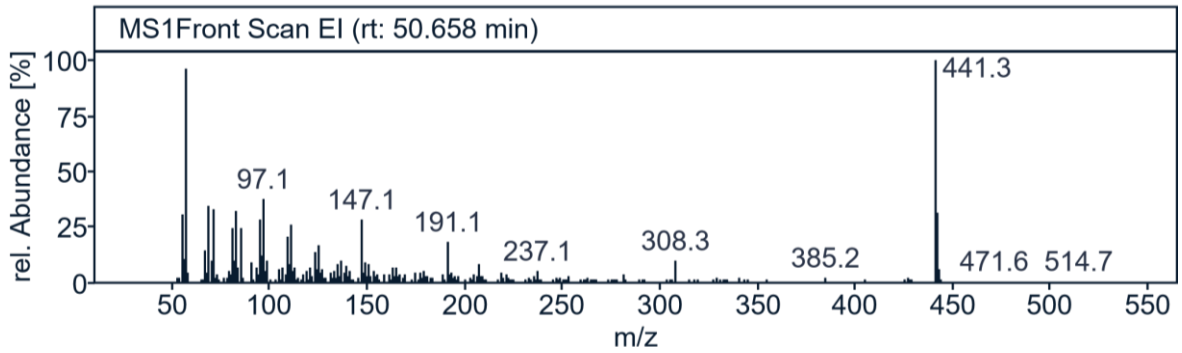


Fig 28 Table 28 the concentration of free fatty acid at a peak of 50.658 min

Fig 28 is the representation of the presence of different compounds at a retention time (rt) of 50.685 min. with different concentrations. 2',5'-Bis(tert-butyldimethylsilyl)oxy-4-methoxychalcone; Cardamonin, bis(tert-butyldimethylsilyl) ether; are the compounds that are found on this particular retention time with different concentrations as per shown in table 28

Table 28 the concentration of free fatty acid at a peak of 50.658 min

S no.	Compound name	Concentration %
1	2',5'-Bis(tert-butyldimethylsilyl)oxy-4-methoxychalcone	16.27
2	Cardamonin, bis(tert-butyldimethylsilyl) ether	11.16
3	9,12-Octadecadienoic acid, (2-phenyl-1,3-dioxolan-4-yl)meth	7.88

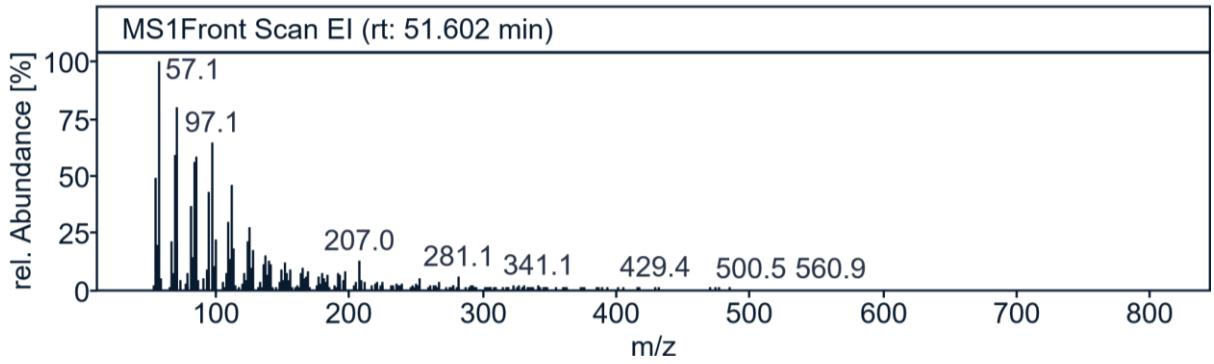


Fig 29 Table 29 the concentration of free fatty acid at a peak of 51.602 min

Fig 29 is the representation of the presence of different compounds at a retention time (rt) of 51.602 min. with different concentrations. Tetrapentacontane, 1, 54-dibromo-; 17-

Pentatriacontane; Oleic acid, 3-(octadecyloxy)propyl ester are the compounds that are found on this particular retention time with different concentrations as per shown in table 29

Table 29 the concentration of free fatty acid at a peak of 51.602 min

S no.	Compound name	Concentration %
1	Tetrapentacontane, 1, 54-dibromo-	31.98
2	17-Pentatriacontene	21.3
3	Oleic acid, 3-(octadecyloxy)propyl ester	4.17

4.5 Peroxide value of extracted fatty acid:

The peroxide value of extracted fat od *Ziziphus nummularia* was 58 ($mEqKg^{-1}$) milliequivalent of peroxide oxygen per kg of oil sample. Fats' peroxide values show how much of their primary oxidation has already occurred, as well as how likely they are to get rancid. An oil's quality and state of preservation are indicated by a lower peroxide value. According to (cox 1962) fresh edible oils have a peroxide value <10 $mEqKg^{-1}$, while rancid oils have a value > 20 $mEqKg^{-1}$. This clearly shows that fatty acids obtained are rancid and can be used in making biodiesel.

4.6. Antioxidant properties:

4.6.1 DPPH ((1,1-diphenyl-2-(2,4,6-trinitrophenyl)hydrazine) assay:

The antioxidant capacity of *Ziziphus nummularia* was 76% which is higher than the antioxidant capacity of *Z. Spina Christi* and *Ziziphus lotus* (68 % and 45 % respectively) (Maadien *et al.* 2020). ascorbic acid was employed as the standard. Ascorbic acid's IC50 value was 38.4 μg/mL. Lesser radical scavenging activity or lower antioxidant capacity is indicated by a greater IC50 value. Antioxidant constituents of *Ziziphus* are lesser than *Zanthoxylum armatum* which is 86.75 μg/mL and 94.49 μg/mL, respectively for a while and cultivated seeds (Karmakar *et al.* 2015). The inhibitory effects of oxidative stress are inhibited or prevented by natural antioxidants found in plants. Among many other assays, the DPPH assay is one of the practical ways to assess the antioxidant capacity of plants. The methanolic DPPH solution is reduced in the presence of antioxidant compounds with hydrogen-donating groups, such as flavonoids and phenols, as a result of the creation of nonradical (Mensor *et al.* 2011). Antioxidants are vital nutrients with the power to shield the body from the harm brought on by oxidative stress brought on by free radicals. Because their hydroxyl groups can donate

hydrogen, plant polyphenols have reducing and antioxidant properties (Aberoumand and Deokule 2008).

4.7 Total Phenolic content:

The findings demonstrated that there were differences in the overall polyphenol concentration between different species, parts, and extracts. Total phenolic content of ziziuphus nummularia was 32.45 mg GAE/gDW . *Ziziphus nummularia*'s phenolic concentration is higher than the *Z. Spina Christi* and *Z. lotus* which is (30.24-24.46 mg GAE/DW). It should be noted that the method utilized, the solvent used, the kind of compound used, the sample size, time and storage conditions, the reference standard used, and interfering factors can all have an impact on the detection of polyphenolic levels ((Bucić-Kojić *et al.* 2009). The fruits, seeds, and bark extracts of Z. armatum contain a sizable amount of phenolics, which may significantly contribute to their antioxidant effects. These characteristics may have led to the usage of this plant in several conventional herbal remedies.

Conclusion Chapter 5

This study concludes that *Ziziphus nummularia* seeds are a possible source of natural antioxidant value for their nutritional, sensory, and health properties since they have a comparatively greater ratio of oil content that seems to be rich in free unsaturated fatty acids. As it has a high oil absorption capacity it can be a rich flavor retainer and could be used in the food industry. It also has a high-water holding capacity which indicates that *Ziziphus* seed flour may be suitable for usage in bread. Swelling capacity of the *Ziziphus* seed is also good swelling power which is a measure of hydration capacity. Due to this quality of food consumed can be checked. Due to the high amount of protein concentration present in *Ziziphus* seed protein from plants could be investigated as a superior nutritional supplement, particularly in developing nations. The crude fat content was also in a very good amount and there were also a lot of free fatty acids identified. So as a result, ber seed oil demonstrated massive promise as a substitute source of phytochemicals with therapeutic and dietary value. There were also antioxidants and phenolic contents. It can be used in the pharma industry in the development of new drugs for several diseases.

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