

Development of screening model For *Psidium guajava* cultivars for their nutritive potential

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DECLARATION

This is to certify that the thesis entitled, "**Development of screening model for *Psidium guajava* cultivars for their nutritive potential**" submitted for the degree of M.Sc., in the course of Biotechnology, Thapar Institute of Engineering & Technology, Patiala, is a research work carried out by Uma Verma (301701033), no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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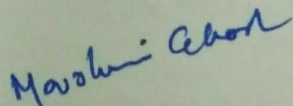
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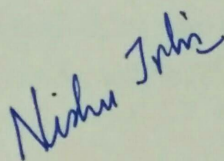
CERTIFICATE

This is to certify that the thesis entitled, "**Development of screening model for *Psidium guajava* cultivars for their nutritive potential**" submitted by **Uma Verma** admission No. **301701033** to the Thapar Institute of Engineering & Technology, Patiala, in partial fulfillment of the requirement for degree of M.Sc.in the course of Biotechnology is an authentic work carried out by her under our supervision and guidance.

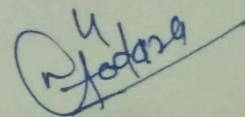
To the best of our knowledge, the matter embodied in this dissertation has not been submitted to any other university/institute for award of any Degree or Diploma.



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LIST OF ABBREVIATIONS

| Sr. no | Abbreviation | Full form |
|---------------|---------------------|--|
| 1. | µg | Microgram |
| 2. | µm | Micrometre |
| 3. | µs | Microsecond |
| 4. | IC | Integrated circuit |
| 5. | µl | Microlitre |
| 6. | mg | Milligram |
| 7. | ml | Millilitre |
| 8. | °C | Degree Celsius |
| 9. | TLC | Thin layer chromatography |
| 10. | min | Minutes |
| 11. | Rf | Retention factor |
| 12. | MEMS | Micro Electro Mechanical System |
| 13. | DMSO | Dimethyl Sulfoxide |
| 14. | HPLC | High performance liquid chromatography |
| 15. | TPC | Total phenolic content |
| 16. | TFC | Total flavonoid content |

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ABSTRACT

In the present study the two guava cultivars Shweta & Allahabad safeda were studied for their (Total phenolic content) TPC & (Total flavonoid content) TFC as they have direct correlation with the antioxidant activity and due to the metal chelation property of flavonoids, metal flavonoids complex have more potential to show the antioxidant activity. The maximum TPC (13.58 ± 0.02) TFC (6.51 ± 0.02) was found in the acetone extract of Shweta guava cultivar than the acetone extract of Allahabad safeda, TPC (10.22 ± 0.14) and TFC (6.33 ± 0.04) and the methanol extract of both the cultivar showed the minimum phenolic and flavonoid content. The maximum antioxidant activity in acetone extract of Shweta guava cultivar (94.50%), acetone extract of Allahabad safeda guava cultivar (92%) than the methanol extract of both the guava cultivars. All the above maximum results were obtained at 40 minutes of the treatment time by using the probe tip ultrasonication (20 kHz, 100W) extraction method. Whereas, comparatively low results were found with the bath ultrasonication (33 kHz, 100W) extraction technique used for 40 minutes of treatment time for both the guava cultivars. For the identification of possible flavonoids present in guava cultivars, TLC was performed by using mobile phase (Toluene: ethyl acetate: formic acid) (5:4:0.2). After the identification of possible flavonoids the HPLC analysis of each extract prepared was done against the standard quercetin by using solvent system (acetonitrile: methanol: water) (15:40:45) In which the maximum amount of quercetin was detected in acetone extract of Allahabad safeda guava cultivar ($103.515 \mu\text{g/ml}$) and Shweta guava cultivar ($101.047 \mu\text{g/ml}$) and minimum amount of quercetin was detected in methanol extract of both the cultivars. The characteristic property of complexation of flavonoids with metals were studied with the analytical model proposed by using the MEMS technology which showed that when the mass of the quercetin metal complex added on the microcantilever beam, the tip displacement (μm) was observed with respect to time (μs) against the standard quercetin and hence change in capacitance between the movable and fixed electrode of microcantilever beam was observed.

Keywords: Flavonoids, Metal flavonoids complexation, MEMS, Open loop control technique, Capacitance.

Chapter 1

INTRODUCTION

Quality food is the most important need for sustainably for humankind in the current era. The quality of the food being consumed is assessed by many factors viz: taste, texture, nutrients, vitamins, minerals, fibers, antioxidants, etc. There is a plethora of literature explains the importance of the nutritional components i.e. vitamins and minerals. Amongst the nutritional factors stated antioxidants have turned out to answer to diseases like cancer, stress borne diseases that are a menace for the existence of the human race. Flavonoids have several subgroups, which include chalcones, flavones, flavonols and isoflavones (Souza *et al.*, 2004). These subgroups have unique major sources. For example, onions and tea are major dietary sources of flavonols and flavones. Amongst the antioxidant 6000 types of flavonoids have been reported to be compounds which have antimicrobial, medicinal, nutraceutical and preservative properties associated to them. (Panche *et al.*, 2016)

In nature, flavonoids are found in fruits and parts of the plants bearing fruits. The agricultural institutes try their best to develop new cultivars better and best among existing cultivars of one species.

Guava is an important fruit crop of Punjab and ranks third after citrus and mango. It occupies an area of 8,183 hectares with an annual production of 1,55,526 tonnes. ⁽³³⁾ In Punjab Guava grows well in almost all the districts of the state. It is highly nutritious fruit and contains vitamin C, Carotenoids, Flavonoids, Phenolic compounds, lycopene, β - carotene, etc. The different guava cultivars in Punjab are L-49, Allahabad safeda, Banarsi, Chittidar, Red fleshed & Shweta. As it is known that guava is a fruit which has very high nutritional potential, it has more vitamin C content than citrus fruits also it contains a very good amount of phenolic and flavonoid compounds.

Flavonoids are widely occurring polyphenolic compounds of plant origin with multiple biological and chemical activities. Due to the presence of carbonyl and hydroxyl groups in their structures they can coordinate metal ions and form complexes. The complexes of metal flavonoids are known to have important properties e.g. antiproliferative and antimicrobial. So, as to elucidate the cultivar potency with respect to its biological makeup a series of biochemical reactions are

required to make it evident for presence of one specific flavonoid, in order to avoid the steps of rigorous and time-consuming biochemical tests, our endeavor is to model a MEMS capacitive based biosensor which would be able to detect the presence or absence of characteristic flavonoid. The MEMS are integrated devices or systems that combine mechanical and electrical components made by using the technique of microfabrication. MEMS sensors have a broad application field in various domains such as automotive, aerospace and medical field (Youunis *et al.*, 2001). The significance of detection and calculation techniques for mass at microscale has led to the development and design of sophisticated MEMS sensors. The characteristic property of complexation of flavonoids with metals were studied with the analytical model proposed by using the MEMS technology which showed that when the mass of the quercetin metal complex i.e. copper quercetin complex, iron quercetin complex and aluminum quercetin complex were added on the microcantilever beam, the tip displacement (μm) was observed with respect to time (μs) against the standard quercetin.

Quercetin, a plant flavonol having molecular weight 302.236g/mol is a member of the flavonoid group of polyphenol, is found in many fruits, vegetables, leaves, and grains. Quercetin is soluble in ethanol, methanol, (dimethylsulfoxide) DMSO & Dimethyl formamides. Red onions and kale are the foods that contain appreciable content of quercetin. It has a bitter flavor and is used as an ingredient in dietary supplements, beverages, and foods. Quercetin is one of the most abundant dietary flavonoids. The most existing form of quercetin is the aglycone of a number of other flavonoid glycosides, such as rutin and quercitrin. It forms the glycosides quercitrin and rutin together with rhamnose and rhamnose sugars. Like many other flavonoids quercetin has the property of metal chelation due to its chemical structure. The chelating biochemical properties of flavonoids to complex with transition metal ion (Cu^{2+} , Fe^{+} , etc.) In this study, we considered quercetin as the main model for the complexation with metal ions like copper, aluminum, and iron. Our aim is to develop a screening model for guava cultivars for their nutritive potential. In which the change in capacitance was studied by using metal flavonoid complex.

Kasprzak *et al.*, 2012 has reported specific compound formation in accordance with the specific molar ratio of metal: flavonoid and having characteristic molar mass. In natural forms also the flavonoids occur in complex forms with sugars and metal ions.

So keeping in view the above characteristic properties of flavonoids the following achievable objectives were worked upon –

- a) Standardization and estimation of flavonoids from two guava varieties Allahabad safeda and Shweta using ultrasonication technique.
- b) Identification of possible flavonoids using chromatography technique. (TLC & HPLC)
- c) Model a biosensor for the identification of standard state flavonoids found in above-mentioned guava varieties.

Chapter 2

REVIEW OF LITERATURE

The Present-day scenario undertakes the various variety of health ailments due to the changing lifestyles for the same the fruits, vegetable consumption is encouraged to get compensate the health issues. The current review of literature focused on the nutritional status of different cultivars of guava and the literature towards the development of biosensor model.

2.1 Flavonoids

Flavonoids are a group of natural substances with different phenolic structures. They are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine. They are used in various nutraceuticals, pharmaceuticals, medicinal and cosmetic. They have anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties along with their capacity to modulate key cellular enzyme function. It has been found that flavonoids have the potential to combat against chronic diseases. Many fruits and vegetables contain flavonoids like citrus juices, Mango legumes, red wine, apples, tea, soy products blueberries cabbage onions strawberries, chocolate, banana, pineapple, guava, Avocado, and many more fruits and vegetables. For good health, flavonoids should be included in health. According to the Panche *et al.*, 2016 there is a need to identify the molecules with flavonoid potential to be used in the treatment of various diseases for the health system.

2.1(a) Guava

Guava also is known as *Psidium guajava L.* belongs to the Myrtaceae family of the plant kingdom, commonly known as guava. It is an important crop and has medicinal properties, which is grown in tropical and subtropical countries. It is extensively used in the food and in folk medicine in the world. It is commercially grown in India for its consumable and pleasant fruits. The metabolites obtained from it shown to have biological activities mainly phenolic compounds (Jaydeep *et al.*, 2011). Guava is one of the best fruit found in various states of India fruit which has high nutritional value, possesses a high amount of vitamin c content (Khalid *et al.*, 2010) than citrus fruits and also rich in a large group of polyphenols and flavonoid content. It has been emphasized that (P. Mary *et al.*, 2015) total polyphenolic and flavonoid contents in

guava are more than the papaya, high polyphenolic and flavonoid content is directly related with the antioxidant activity which is the cure of many human diseases. The highest antioxidant activity has reported in the white guava after the red guava than the papaya and other fruits. Besides the antioxidant activity, it has antiseptic and antimicrobial properties.

2.1(b) guava leaves and flower

There has been excessively work has been done on the organ of guava plant called leaf due to its medicinal properties. *P. guajava* is a well-known traditional therapeutic plant used in various indigenous systems of India. Guava leaves are astringent, anodyne, febrifuge, antispasmodic and tonic (Batubara *et al.*, 2017) *P. guajava* (Kaneria and Chanda *et al.*, 2011) flowers are also cooling, laxative, tonic, and used in bronchitis, colic and ophthalmodynia like many more diseases. Guava (*Psidium guajava*) is a tropical fruit and its leaves have medicinal properties can be used to treat various ailments like wounds, diarrhea, cough, mouth sores, and dengue fever. The studies have shown guava to have a have antioxidant, anti-inflammatory and anticoagulants activities. The guava leaves extract contains compounds such as Ferulic acid, Gallic acid along with catechins, quercetin, and kaempferol.

Quercetin and kaempferol have found to be effective against microbes, viruses, inflammation, oxidants, and diabetes. The Brine Shrimp Lethality Test (BSLT) is the preliminary test which is used to determine the activities of kaempferol and quercetin. This test used to find the pharmacological potential of natural components. It is cheaper than the other methods like cytotoxicity assay. The synergistic effect has been observed with the combination of quercetin and kaempferol when tested on human cell lines.

2.1 (c) Other fruits

Flavonoids are present naturally in different fruits, vegetables, and certain beverages that have various advantageous effects on health. Abida *et al.*, 2014 studied different vegetables including tomato, onion, garlic, green chilies and potato for their flavonoid content due to their easy availability. All of the vegetables are consumed in daily diet. Fruits like Mango, orange, pineapple, *Grewia asiatica*, and strawberries were also tested for their flavonoid content because of the easy availability of these fruits. The studies were done in order to compare the local market fruit and vegetables with the fresh ones.

In fruits *Grewia asiatica* (phalsa) juice, mango and strawberry jam, pineapple juice and tang orange were used and among vegetables garlic paste, onion paste, chili paste, tomato ketchup, and potato chips were used to compare and from study it was found that highest flavonoid was found in tomato which was 183.08 mg/kg among fresh vegetables and among fruits in strawberries (80.0 mg/kg). While on the other hand, among preserved products it was found in the strawberry jam (85.0 mg/kg) and garlic paste (177.0 mg/kg) respectively. It can be concluded from a study that in tomatoes flavonoid content decreased due to processing whereas the opposite was observed in strawberries where additives increased the flavonoid content. Among the selected products highest content was found in garlic paste.

2.2 Extraction of flavonoids

Various new methods have been established till now along with the conventional methods but no single method is standard for the extraction of the bioactive compounds from plants. The efficacies of both conventional and non-conventional extraction method depend on the critical input parameters, the chemistry of bioactive compounds, knowing the nature of plant matrix and scientific expertise (Azmir *et al.*, 2013). The various extraction techniques have been used are homogenization, shaking, sonication, magnetic stirring, maceration, ultrasonication (Hamid *et al.*, 2013). The solvents used are methanol, ethanol, and acetone. It has been reported that ultrasonication is the best technique for the extraction of total phenols and total flavonoids from guava fruit. Other techniques like Soxhlet extraction, Microwave-assisted extraction, Ultrasound-assisted extraction, Accelerated solvent extraction, Supercritical-fluid extraction have also been reported but there are no universal extraction methods as every extraction method is different to the plants (NN, 2015). According to Hamid *et al.*, 2013 ultrasonication is a superior technique for the extraction purpose.

2.3 Complexation of flavonoids with transition metal ions

It was reported by Maria *et al.*, 2012 that flavonoids have the tendency to form metal complexes with metal ions due to their chemical and structural properties. Flavonoids like Kaempferol, quercetin, and catechin interact with different metal ions like Al^{3+} , Ca^{3+} , Cd^{2+} , Co^{2+} , Cu^{2+} , Fe^{3+} , Fe^{2+} , La^{3+} , Ni^{2+} , Mg^{2+} , Pd^{2+} in different molar ratio of metal & ligand under different pH conditions results in the formation of high potent

complex molecules that possesses much more high antioxidant property as compared to the flavonoid alone (Rubens *et al.*, 2013). Metal coordination to the Penta-hydroxyflavone quercetin, the most common flavonoid, can occur *via* the 3-hydroxyl and 4-carbonyl group of the C ring (3-4 site), the 4-carbonyl-5-hydroxyl site of the A and C rings (4-5 site) or *via* the catechol moiety of the B ring (3'-4' site). The preferred binding site depends on the flavonoid, the metal ion and on the pH value. Hence they are the subcategory of a large group of polyphenolic compounds having many positive health effects. Flavonoids as polyphenols are weak acids that undergo deprotonation which strongly affects the coordination of metal ions (Markovic *et al.*, 2011). It is generally agreed that in alkaline solution Fe^{3+} , Cu^{2+} and Al^{3+} ions have the strongest affinity for the ortho-dihydroxyl group of quercetin. These flavonoids have the capability to form complexes with various cations, which have antioxidant and antitumor activity. They also offer anti-inflammatory properties along with DNA protection. This property of flavonoid to form complexes can be used for the detection of particular flavonoid in fruit or vegetable. The main objective of the project is sensing and comparing the mass change of free flavonoid and the flavonoid present in the complexed form with a metal ion.

2.4 Computer aids for fruits sorting

The buyers demand the fruits of high nutritional potential is increasing and this has given rise to the evolution of mechanical, optical & acoustic sensors that can help to determine the fruit quality (Olmos *et al.*, 2014). This has led to the possibility of enhancing the quality of horticulture products for healthier consumption of fruits and vegetables. Still better electrical characterization of the dielectric properties of fruits and vegetables is needed for this purpose (Bhargava & Bansal, 2018). The computer vision systems have taken over the manual inspection to identify external characteristics like size, color, shape, texture, and defect. This particular technology is equitable, nondestructive and has an authentic rating. Computer-based detection includes four steps for quality detection which are acquisition, segmentation, feature extraction and classification for these various methods along with algorithms proposed in each step by the researchers. As the traditional computer vision system recognizes the colors red, green, blue (RGB), so the image acquired by RGB color cameras are centered at RGB wavelength. As the traditional computer vision system accounts primary colors: red, green, blue (RGB). By using computers various

characteristics of fruits can be inspected. Hyperspectral computer vision system involves both imaging and spectral techniques for the image. The images of fruits and vegetables are taken mostly from the one direction but it should be captured from a different direction for more improved inspection (Gao *et al.*, 2010). In this way fruits and vegetable grading, sorting and disease recognition are done on single fruit but still a robust system for the inspection of the internal and external quality of fruits and vegetables with improved performance is required to be built.

Artificial neural methods (ANN) were also used in fruit detection. In this, proposed automated system which is designated to surpass the problem of manual technique. The system has several steps like extraction, categorization, and grading. It is designed to mix several processes such as image acquisition of sample of fruit than the extraction of features, sorting of them and lastly grading of fruit sample. Features like fruit color, fruit shape, fruit size are extracted. Size features are extracted in height and width. Extracting the size of fruit is known as grading. Image of fruit Captured by the camera using MATLAB it is uploaded and fruit sample features extracted and trained by the neural network, hence the image of the sample for testing get selected and with the help of training module button it is tested. These type of systems can be used for juice plants, fruit and vegetable farms, packaging, etc. This detection is based on the ANN should be compared with other mechanical and automated technique (Kaur & Sharma *et al.*, 2015). The market has a high demand for non-destructive methods for fruit quality detection. The method has both advantages and disadvantages as the method of optical property can only detect the surface injury of the fruit, not the internal damage. The detection based on Optical properties, sonic vibration, machine vision technique, nuclear magnetic resonance (NMR) has a distinct advantage over other methods like instrumental analysis and chemical analysis methods. The major disadvantages of traditional methods are high cost, hard sledding and time of the process. The other techniques for detections are electronic noses, electrical properties, and computed tomography. Other methods based on sonic properties and NMR detection are only applicable on a laboratory scale and not been applied on a commercial scale.

2.5 MEMS (Micro-electromechanical system)

Over the last two decades, the mass detection of micro components using micro-electro-mechanical systems (MEMS) has gained increasing attention (Gupta *et al.*, 2004; Bedair *et al.*, 2009; Bouchaala *et al.*, 2016). MEMS sensors have a broad application field encompassing various domains, such as automotive, aerospace and medical (Youunis *et al.*, 2001). The significance of detection and calculation techniques for mass at microscale has led to the development and design of sophisticated MEMS sensors. Although, in the field of mass sensing techniques, various methods have been proposed, but MEMS sensors based on tracing the response frequency shifting is most widely used technique (Bouchaala *et al.*, 2016) In this technique added mass results into downshifting of resonant frequency (Iiic *et al.*, 2004; Jensen *et al.*, 2008). One can correlate the frequency shift of corresponding vibration mode to the quantity of added mass at known positions. In the field of medical and food supply monitoring, this method has been employed to develop a mass sensor to detect pathogenic bacteria (Iiic *et al.*, 2004) and biological mass detection in fluid flow (Burg *et al.*, 2006). Burg *et al.*, 2006 designed an electrostatically driven microcantilever comprising a microchannel suspended on its surface. The frequency shift is measured when the added microparticle reaches the tip of the microcantilever. Further, determination of added mass at the unknown positions is the more challenging and key factor and it requires highly accurate detection technique. In this direction, many researchers proposed analytical techniques (Schmid *et al.*, 2010). A closed-form expression, to quantify the added mass on a string was proposed by Schmid *et al.*, 2010) and validated experimentally through measuring the frequency shift of the first and second vibrational modes. In resonant frequency based mass sensors, ultra-high frequency sensors are necessary to track changes in frequencies resulting due to the addition of a minute mass. Yang *et al.*, 2006, presented a mass detection technique using an ultra-high frequency nanoelectromechanical for zeptogram mass detection. The frequency shifting technique is well studied and has been presented by many researchers (Dai *et al.*, 2006; Rhoads *et al.*, 2010; Zhang *et al.*, 2005). The Bio-MEMS based detection of fruits and vegetable nutrient content have scantily reported. In this project work, the orientation of the aim will be to develop biomems based mathematical model on the basis of which the real-time studies will be carried out in the future to develop the

biomems based sensor which can detect the nutritional potential of the fruits and vegetables.

Chapter 3 (a)

MATERIALS AND METHOD

For the proposed study the sample was procured from the Department of Horticulture, Baradari, Patiala (Punjab). The two varieties of guava used for the extraction purpose were Allahabad safeda & Shweta for the preparation of extracts the samples of above said varieties were macerated in two solvents acetone and methanol. Ultrasonic waves were used to enhance the extraction following various biochemical tests were performed to determine the presence of flavonoids, polyphenols and antioxidant activity (DPPH assay). TLC (Thin layer Chromatography) was performed for both varieties of guava to detect the presence of possible flavonoids with quercetin as standard. The optimized extracts were used for High-performance liquid chromatography (HPLC) along with standard quercetin.



Figure 1: Two guava cultivars used for the analysis.

3.1 Sample Extraction

The extraction was optimized with respect to different solvents extraction method (Methanol and Acetone). The two methods used for the sample extraction was bath ultrasonication (33 kHz, 100 W) and probe tip ultrasonication (20 kHz, 100 W) with treatment time for 20, 30, 40, 50 minutes respectively in order to break the guava fruit cells, to release of natural chemical compounds present in them.

3.2 Analytical methods

The procedure followed for the sample preparation for two varieties Allahabad safeda & Shweta as follows: fresh guava fruits were taken (15 days of harvest). They were washed thoroughly with distilled water after removing the calyx. Then the small pieces of guava were made by knife cutting. The weighed samples were transferred into pestle & mortar and macerated. Each macerated sample was sonicated in two types of solvents which were methanol (12.5 ml) and acetone (12.5 ml). In order to obtain the biochemical estimation within the range, weight of guava: the solvent ratio was optimized as 2g/12.5 ml of solvent used. The clear filtrate was obtained in a beaker through passing the sample solution through the muslin cloth and stored at -4°C for its estimation.

3.3 Total phenolic content determination

Assay by the singleton & Rossi (1965) was followed to determine the total phenolic content in both varieties of guava. With respect to the Gallic acid standard curve (1mg/ml) the total phenolic content was calculated (Concentration range: 0-12µg/ml). To each 50µl diluted extract of the sample added 3950µl of distilled water and then 250µl folin ciocalteu reagent (Merck). 20% of 1 ml sodium carbonate was added after 3 min, before the incubation of 40 min, 40°C in a water bath test tubes were vortexed properly. At 685 nm the readings of blue-colored solution were obtained against the standard blank. With 80% of methanol, a blank of 50µl was prepared in place of fruit extract. Expression of total phenol content results was given as mg/ml extract of fresh fruit.

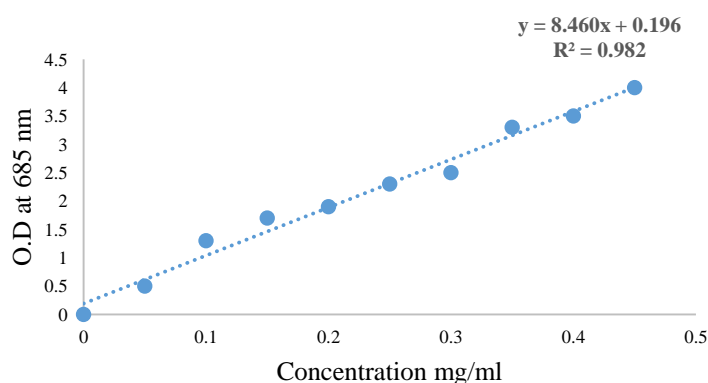


Figure 2: Gallic acid standard graph for total phenolic content.

3.4 Total flavonoids content determination

For the determination of flavonoids, colorimetric assay from Zhishen et.al (1999) was performed. To the Methanol extract of Allahabad safeda and Shweta (2ml) & Acetone extract of Allahabad safeda (500 μ l) & Shweta (1ml). Added 5% 150 μ l NaNO₂ (Sodium nitrate) and 10% 150 μ l aqueous AlCl₃ (Aluminium chloride) after 5 min. After one minute of incubation the 1M, 1ml NaOH (Sodium hydroxide) was added. Vortexed all the test tubes for better mixing of all the chemicals added. The absorbance was measured against blank at 510 nm. With 80% methanol reagent blank was prepared in place of fruit extract. With respect to Quercetin standard curve (1mg/ml) total flavonoid content was calculated, (Concentration range: 50-200 μ g/ml). Expressions of total flavonoid content results were given as mg/ml extract of fresh fruit.

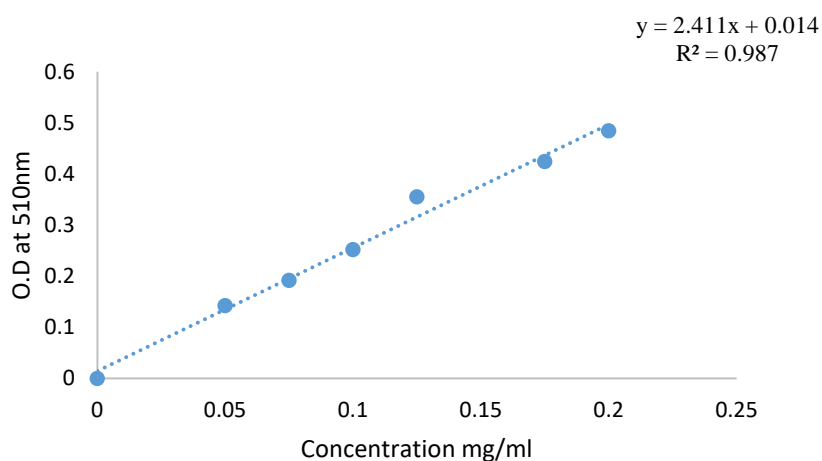


Figure 3: Quercetin standard graph for total flavonoid content

3.5 Antioxidant activity

DPPH assay was used to determine the antioxidant activity in the guava extract prepared in methanol as well as acetone for both varieties of guava treated with probe tip ultrasonication and bath ultrasonication. Methanol was taken as blank & 1mg/ml solution of DPPH was used to observe the antioxidant activity in all samples. The standard Ascorbic acid used as a positive control and DPPH as a negative control. In each 20 μ l of guava extract 2980 μ l DPPH used. The optical density was taken at 515nm. The decrease in value of readings shows high antioxidant activity. Antioxidant activity % = $\frac{\text{O.D of control} - \text{O.D of sample}}{\text{O.D of control}} \times 100$

3.6 TLC (Thin layer chromatography) for flavonoid identification in the extract Allahabad safeda and Shweta guava cultivar prepared in acetone and methanol by using probe tip ultrasonication.

The fresh TLC plates were prepared. The slurry of silica gel G was made by dissolving 30g of silica gel G in 60ml of distilled water. Mixed it well and then kept the mixture at rest for 5 min. The mixture was then poured on the glass plates uniformly such that the formation of air bubbles could be avoided which can affect the separation of the sample mixture. The plates were left at room temperature for 10-15 min. so that silica gel can solidify on plate surface properly and after that to dry & activate the pores of silica gel the plates were kept in an oven at 100°C for 2 hours. The mobile phases of 4 different solvent systems of varying ratios were made: solvent system A - Chloroform (28): methanol (1.5): formic acid (1.3) for aglycone. Solvent system B – Ethyl acetate (20): formic acid (2): acetic acid(2):water(5) for glycones(Boji *et al.*, 2013). Solvent system C–n-hexane (0.18): Ethyl acetate (20.58) (Batubara *et al.*, 2017) & Solvent system D toluene(5):Ethyl acetate (4):formic acid(0.2)(Sajeeth *et al.*, 2010). All these solvent systems were allowed to get saturated in a jar for 10-15 minutes before using it as mobile phase. The plates were carried out of the oven after 2 hours and were cooled down for a few minutes. The flavonoid standard quercetin freshly prepared with a concentration of 1mg/ml and diluted to 10 times with LR grade methanol. With the help of capillary tubes, the spotting of each sample (100µl) along with standard was done. It is a necessity that spot should be visible under a UV lamp and the color of spot most of the time is purple. After spotting samples in proper manner plates were placed in the jar containing the mobile phase, the plates were run for approximately 45 min. upto 3/4th of total length of the TLC plate. TLC plates were removed from the jar, dried at room temperature and bands were visualized under a UV lamp at 366nm.

3.7 Reverse Phase - High-Pressure Liquid Chromatography (RP-HPLC) Analysis

To confirm the presence of Quercetin in the samples the HPLC analysis was done for the samples prepared in acetone with probe-ultrasonication treatment for the varieties, Allahabad safeda, and Shweta. The composition of the solvent system was made in the ratio of 15:40:45 with Acetonitrile, methanol, and water with the flow rate of 1ml/minutes. The separation process was initiated after 30 minutes of purging by using C-18 column at the wavelength of 370nm (UV detector). The amount of volume

20 μ l, of the sample, was injected into the injection port before injecting the sample into the injection port the sample was passed through the filter of size 0.45 μ m. The area of peak of each sample obtained on the computer screen was compared with the reference peak area, quercetin.

3.8 MATLAB (Matrix Laboratory) Analysis

The quantitative estimation of the mass of metal flavonoid complex attached to the surface of the microcantilever beam, a modal superposition technique is used to simulate the dynamic response of the microcantilever. For this, firstly, an input shaping technique is employed to stabilize the microbeam at the specified position and then voltage perturbation is induced to make the system sensitive to inertial change. A parasitic mass expressed as a fraction of the total mass of the beam is then added resulting in an increase in the amplitude of vibration and hence an alteration in the capacitance. An empirical relation between the added mass and the change in capacitance is proposed. This parasitic mass is a mass of compound formed at the tip of the microcantilever beam. Using this methodology the quantification of the mass of the formed compound is predicted through recorded change in capacitance. Afterward, a compound associated with the quantified mass change is predicted. We proposed a novel capacitance-based mass detection technique employing an open-loop controlled microcantilever beam. The microcantilever first brought to a stagnation state by applying a series of step voltages. A small voltage perturbation is then added to make the beam sensitive to inertial changes. Upon introduction of a tip mass, the amplitude of vibration of the beam changes significantly which results in a concomitant change in the capacitance. This change in the capacitance is then correlated with the tip mass added to the cantilever. Chapter 3 (b), Section 3.9 describes the mathematical formulation together with the terminologies used throughout the following discussion. A command-shaping scheme and modal superposition method for simulating the electromechanical response is presented in the former half of Section 3.10. In the latter half, a mass detection technique using the capacitance variation is proposed by considering the actuation of a microbeam using a single Heaviside step voltage.

Chapter 3 (b)

A NOVEL CAPACITIVE MASS SENSOR USING AN OPEN-LOOP CONTROLLED MICROCANTILEVER

In this chapter, for quantitative estimation of the mass of an entity attached to the surface of an electrostatically actuated clamped-free micro beam implemented as a mass sensor, the analytical model was proposed. For this investigation, the microbeam is modeled as an Euler-Bernoulli beam taking into account the effects of electrostatic nonlinearity, viscous energy dissipation together with the effect of fringing field capacitance. A modal superposition technique is used to simulate the dynamic response of the microcantilever, a novel capacitance-based mass detection technique employing an open-loop controlled microcantilever beam. Which include the mathematical formulation together with the terminologies used, a command-shaping scheme & modal superposition method for simulating the electromechanical response and a mass detection technique using the capacitance variation was proposed by considering the actuation of a microbeam using a single Heaviside step voltage.

3.9 Mathematical Formulations

Figure 4 shows the schematic of electrostatically actuated beam-type MEMS mass sensor made up of a linear elastic material having Young's modulus \hat{E} , Poisson's ratio ν , and mass density $\hat{\rho}$. Here \hat{L} and \hat{h} denote the length and constant thickness of microbeam, respectively. Furthermore, \hat{b} and \hat{I} respectively denote the width and the area moment of inertia of the micro beam's cross-section. Initially, the movable electrode and substrate are separated by a distance equal to \hat{g}_o and the permittivity of the medium between them is $\hat{\epsilon}_0$. The equivalent viscous damping coefficient is denoted by \hat{c} . The Young's modulus of the microbeam material is dependent on the thickness to width ratio of the beam and its effective value is represented by \hat{E} . Following the Euler-Bernoulli theory of beams, the governing differential equation of the transverse deflection of an electrostatically driven microbeam is written as (Godara *et al.*, 2015)

$$\hat{E}\hat{I} \frac{\partial^4 \hat{w}}{\partial \hat{x}^4} + \hat{c} \frac{\partial \hat{w}}{\partial \hat{t}} + \hat{\rho} \hat{b} \hat{h} \frac{\partial^2 \hat{w}}{\partial \hat{t}^2} = \frac{\hat{\epsilon}_o \hat{b} \hat{V}^2}{2(\hat{g}_o - \hat{w})^2} \left(1 + \frac{2(\hat{g}_o - \hat{w})}{\pi \hat{b}} \right). \quad (1)$$

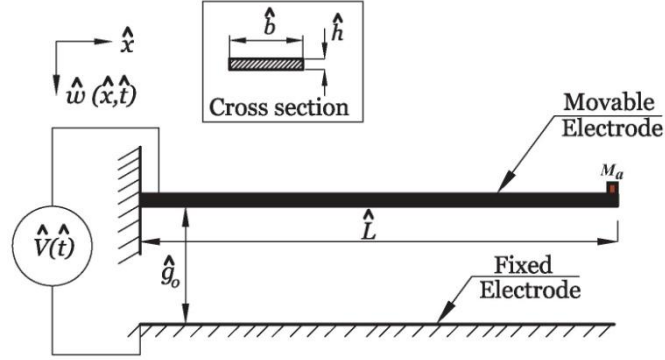


Figure 4: Schematic of a capacitance-based electrostatic beam-type MEMS mass sensor with tip mass M_a .

The electromechanical model (Eq. 1) incorporates the effect of fringing field capacitance and electrostatic nonlinearity. M_a represents the parasitic mass in the form of formed compound added at the tip of the microcantilever beam. The compound mass changes the shear force boundary condition at the free end of the beam. Consequently, the electromechanical model subjected to the following boundary conditions:

$$\begin{aligned} \hat{w}|_{(0,i)} = 0, \quad \frac{\partial \hat{w}}{\partial \hat{x}} \Big|_{(0,i)} = 0, \\ \frac{\partial^2 \hat{w}}{\partial \hat{x}^2} \Big|_{(\hat{L},i)} = 0, \quad \hat{E}\hat{I} \frac{\partial^3 \hat{w}}{\partial \hat{x}^3} - M_a \frac{\partial^2 \hat{w}}{\partial \hat{t}^2} \Big|_{(\hat{L},i)} = 0. \end{aligned} \quad (2)$$

In order to generalize the forthcoming analysis, we introduce the following dimensionless variables:

$$w = \frac{\hat{w}}{\hat{g}_o}, x = \frac{\hat{x}}{\hat{L}}, \tau = \frac{\hat{t}}{T}, T = \sqrt{\frac{\hat{\rho}\hat{b}\hat{h}\hat{L}^4}{\hat{E}\hat{I}}}, \kappa = \frac{M_a\hat{L}^3}{\hat{E}\hat{I}T^2} \quad (3)$$

$$c = \frac{\hat{c}\hat{L}^4}{\hat{E}\hat{I}T}, V = \sqrt{\frac{\hat{\varepsilon}_o\hat{b}\hat{L}^4\hat{V}^2}{2\hat{E}\hat{I}\hat{g}_o^3}}, \zeta = \frac{\pi\hat{b}}{2\hat{g}_o}, C_p = \frac{\hat{\varepsilon}_o\hat{A}\hat{L}}{\hat{g}_o},$$

On substitution of Eq. 3 into Eq. 1, we obtain the nondimensional form of the equation governing the transverse deflection of the microbeam as

$$EI \frac{\partial^4 w}{\partial x^4} + c \frac{\partial w}{\partial \tau} + \frac{\partial^2 w}{\partial \tau^2} = \frac{V^2}{(1-w)^2} \left(1 + \frac{(1-w)}{\zeta} \right). \quad (4)$$

The four boundary conditions are expressed in their dimensionless form as follows

$$w|_{(0,\tau)} = 0, \quad \frac{\partial w}{\partial x}|_{(0,\tau)} = 0, \quad (5)$$

$$\frac{\partial^2 w}{\partial x^2}|_{(1,\tau)} = 0, \quad \frac{\partial^3 w}{\partial x^3} - \kappa \frac{\partial^2 w}{\partial \tau^2}|_{(1,\tau)} = 0.$$

Here, κ denotes the dimensionless mass factor, which is the ratio of added mass at the tip of the microcantilever to the total mass of beam. The solution methodology to simulate the electromechanical behavior using the above-described model is presented in the following.

Method of solution

In this section, we discuss the Galerkin's modal superposition technique to simulate the electromechanical response of electrostatically actuated beam-type MEMS microsensor. Subsequently, the methodology of the proposed mass detection technique is provided.

Modal superposition method

To simulate the response of microsensor a reduced-order model using the Galerkin's modal superposition method is developed. The transverse deflection of beam type microsensor is approximated by the following expression

$$w(x, \tau) = \sum_{i=1}^N \phi_i(x) \eta_i(\tau), \quad (6)$$

where $\phi_i(x)$ is the known i^{th} undamped eigenmode of the microcantilever beam carrying a tip mass and $\eta_i(\tau)$ is represents the unknown time-varying generalized modal coordinate. The closed-form expression for mode shapes are given as

$$\phi_i(x) = \cosh(\lambda_i x) - \cos(\lambda_i x) - \beta_i (\sinh(\lambda_i x) - \sin(\lambda_i x)), \quad (7)$$

here, β_i is obtained from

$$\beta_i = \frac{\cosh(\lambda_i x) + \cos(\lambda_i x) + \lambda_i \kappa (\sinh(\lambda_i x) - \sin(\lambda_i x))}{\sinh(\lambda_i x) - \sin(\lambda_i x) - \lambda_i \kappa (\cosh(\lambda_i x) + \cos(\lambda_i x))}. \quad (8)$$

The values of λ_i , are obtained by solving the following characteristic equation:

$$\cosh(\lambda_i) + \cos(\lambda_i) + 1 + \lambda_i \kappa (\cos(\lambda_i) \sinh(\lambda_i) - \sin(\lambda_i) \cosh(\lambda_i)) = 0. \quad (9)$$

After putting, the parameter κ equals to zero, Eqs. 7 and 8 are analogs to the mode shape and frequency equations of cantilever beam without tip mass. To obtain the reduced-order model, we first substitute the Eq. 6 into Eq. 4, and then the resulting equation is multiplied by $(1-w)^2$. This avoids the displacement to occur in the denominator term and hence reduces the computational cost. Then the resulting equation is multiplied by $\phi_k(x)$ and integrated the outcome over the domain. Applying the orthogonality conditions of mode shapes, the following N second-order nonlinear coupled differential equations are obtained:

$$\int_0^1 \left(1 - \sum_{i=0}^N \phi_i \eta_i \right)^2 \left(\sum_{j=1}^N \phi_j'''' \eta_j + c \sum_{j=1}^N \phi_j \dot{\eta}_j \right) \phi_k dx = V^2 \int_0^1 \left(1 + \frac{\left(1 - \sum_{i=0}^N \phi_i \eta_i \right)}{\zeta} \right) \phi_k dx \quad (10)$$

Here, the prime ($\dot{}$) denotes the differentiation with respect to the spatial coordinate while the dot ($\dot{}$) denotes a temporal derivative. Equation 10 can be written in the matrix form as follows

$$[M]\{\ddot{q}_k\} + [C]\{\dot{q}_k\} + [K]\{q_k\} = \{F_k\}. \quad (11)$$

Once the system parameters have been defined, Eq. 11 can be numerically integrated with time using Runge-Kutta technique in MATLAB to calculate the time evolution of modal coordinate $\eta_i(\tau)$. In the following, we present a detailed discussion on the development of the proposed mass detection technique.

Design of mass detection technique

In this section, we discuss the rationale of the proposed mass detection technique. The proposed technique relies on bringing the microcantilever to a near-stagnation state by applying a series of step voltages at pre-determined magnitude at a pre-determined time. This is achieved by a feedforward control strategy, which has been detailed elaborately in (Godara *et al.*, 2015). Following the analysis in (Godara *et al.*, 2015; Godara *et al.*, 2016), the beam is first stabilized at a particular deflection by applying the following voltage waveform

$$V(\tau) = \begin{cases} V_r & \tau \leq \tau_r \\ V_1 & \tau_r < \tau \leq \tau_1 \end{cases} \quad (12)$$

Note that, due to the energy dissipation as well as the electrostatic nonlinearity involved, the values of V_r and τ_r are required to be extracted numerically. Here τ_r and τ_1 are the applications time durations for the step voltages V_r and V_1 , respectively. By setting the temporal derivatives in Eq. 11 to zero, one can arrive at the value of the voltage V_1 for the considered this equilibrium configuration $w_d = 0.1$.

In the present investigation, we consider illustrative examples involving exemplary dimensions and parameters reported previously in the literature (Joglekar *et al.*, 2011). Accordingly, the following dimensions of the microbeam are considered in the analysis:

Table 1: Microbeam dimensions and properties

| Beam Parameters | Values | Units |
|-----------------------------------|------------|--------------------|
| Length (L) | 300 | Mm |
| Width (b) | 50 | Mm |
| Thickness (h) | 3.0 | Mm |
| Young's Modulus (E) | 77 | GPa |
| Density (ρ) | 2648 | Kg m ⁻³ |
| Initial separation (g_0) | 1.0 | Mm |
| Poisson's ratio (ν) | 0.06 | - |
| Mass of the beam ($\rho b h L$) | 1.1916e-10 | Kg |

With reference to the data provided in (Khatar *et al.*, 2011) the quality factor Q corresponding to an equivalent viscous dissipation mechanism is set as 250. The dimensionless damping coefficient corresponding to the adopted value of the quality factor is equal to 0.014. In order to design the mass-sensing technique, we consider that the mass is anticipated to be stabilized at $w_d = 0.1$. For this position, we obtained the shaped input waveform as shown in Fig. 5(a).

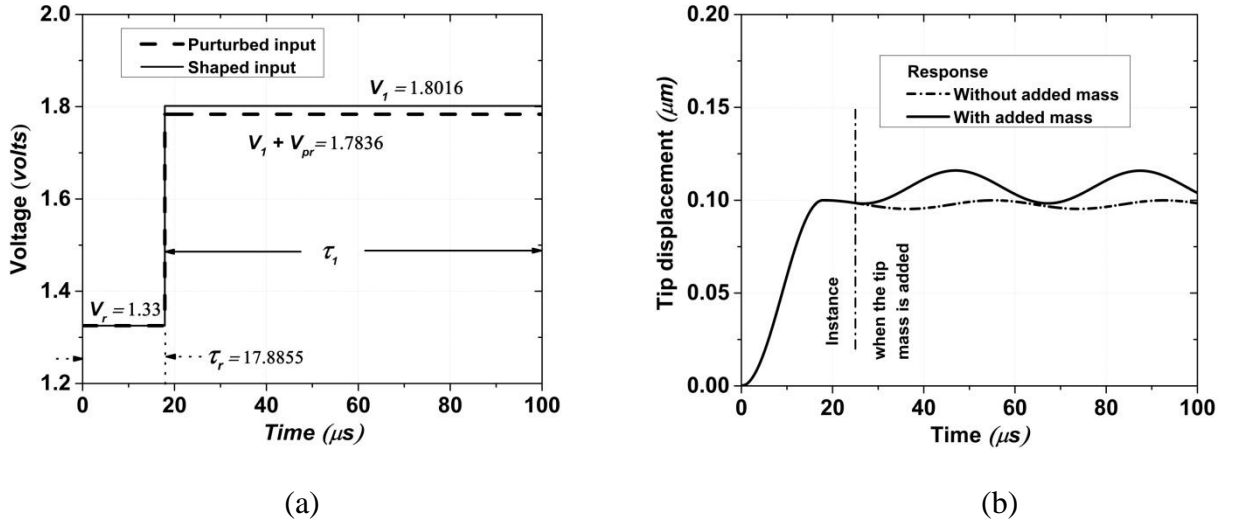


Figure 5: (a) Input voltage waveforms to stabilize the beam at considered deflection and after voltage perturbation, and (b) resulting responses indicating the effect of perturbation voltage and tip mass.

The application of shaped input stabilizes the microbeam at the desired position and hence, the microsystem cannot sense the change in the inertia, because at the stagnation stage the inertial force has no influence. Therefore, in order to create small amplitude oscillations, we perturb the shaped input waveform (Eq. 12) with a small amount of voltage signal, which is designated by V_{pr} . The perturbed input waveform is given as

$$V(\tau) = \begin{cases} V_r & \tau \leq \tau_r \\ V_1 + V_{pr} & \tau_r < \tau \leq \tau_1 \end{cases} \quad (13)$$

The perturbation in the input voltage considered as follows

$$V_{pr} = \pm Z * V_1. \quad (14)$$

Here the perturbation factor Z is taken as -0.01 and the obtained voltage waveform is shown in Fig.5 (a), which is represented by the perturbed input. Consequently, the static equilibrium is disturbed and hence, the microcantilever is no longer stabilized at considered deflection. As a result, beam starts to perform small amplitude oscillations about the equilibrium position, as shown in Fig. 5(b). Consequently, the microcantilever beam becomes sensitive to the inertial changes and hence to the added mass. When a mass is added at the tip of the beam the mode shapes and resonant frequency changes. Consequently, the amplitude of oscillations also changes. Now we calculate the change in vibrational amplitude of microcantilever after and before the addition of a mass at the tip of the beam. The corresponding capacitance calculated using the following expression

$$C(x) = \int_0^1 C_p \frac{1}{(1-w)} dx. \quad (15)$$

Now, as a defined amount of mass M_a is added at the surface of the microbeam the deviation in its transient response occurs, as shown in Fig. 5(b). Consequently, the capacitance between two electrodes, movable and fixed, changes. Further, the change in the capacitance before and after the addition of mass is measured as

$$\Delta C(\kappa) = C_i - C_f, \quad (16)$$

Here, C_i and C_f respectively, represent the capacitance before and after the addition of mass to the surface of the microsensor. It is found that this change in capacitance holds a functional relationship with the added mass. In this work, we proposed the empirical relation between change in the capacitance and the amount of added mass. In the upcoming section, we consider several test cases to demonstrate the applicability of the proposed mass-sensing technique to design the mass sensor. Subsequently, the development of proposed analytical expression for the calculation of added mass is discussed.

3.10 Representation of proposed microsensor to detect a change in the mass of metal flavonoid complex:

In order to develop a functional relationship between capacitance and added mass, we used the dimensionless mass scaling parameter, which is designated by κ . For this, we select 100 equal levels of mass scaling parameter in order to investigate the dependence of capacitance on them. The magnitude of the mass scaling parameters spanned over a range of zero to 0.1.

Using the technique described in the above, we numerically generate a database of capacitance for selected levels of mass scaling parameter. As the amplitude of oscillations is changed, the capacitance between two electrodes also changes. Figure 6 depicts the variation of the capacitance as the added mass parameter changes. It is inferred that the change in capacitance increases with an increase in added mass parameter's value, which suggests that the increase in the added mass results in oscillations with greater amplitude, as shown in Fig. 5(b), and hence, results in an increased change in capacitance.

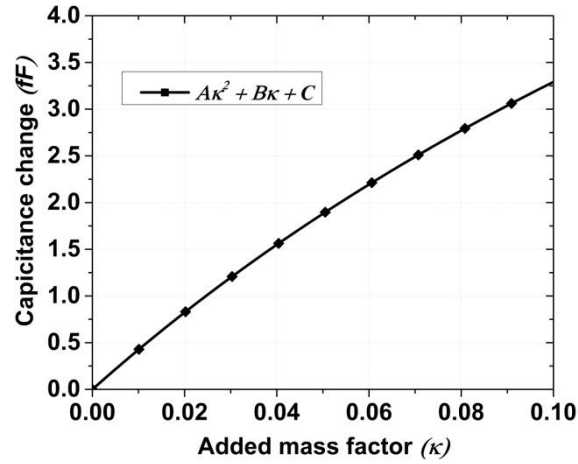


Figure 6: Variation of capacitance with respect to added mass factor

A critical examination of Fig. 6 suggests that the variation with respect to mass scaling parameter can be characterized by a quadratic function. With this observation, we propose the following quadratic function to describe the variation of the capacitance:

$$\Delta C(\kappa) = A\kappa^2 + B\kappa + C \quad (17)$$

In order to obtain the parameters in the above expression given in Eq. 17, we used a curve fit analysis in MATLAB and statistical measures of the obtained fit are indicated in Table 2.

Table 2: Coefficient of analytical expression obtained using the curve fit tool in MATLAB

| Coefficient of analytical expression (Eq. 18) | Values |
|---|------------------------|
| A | -6.4×10^{-14} |
| B | 3.9×10^{-14} |
| C | 7.8×10^{-17} |

In this analysis, κ represents the ratio of added mass to the mass of microbeam. The mass scale parameter can be converted into the added mass using the Eq. 3. The

amount of added mass at the tip of microcantilever corresponding to the value of the parameter κ is given in Table 3.

Table 3: Value of added mass at the tip of microcantilever corresponding to the mass scaling parameter

| Parameter κ | Added mass (pKg) |
|--------------------------------------|-----------------------------|
| 0.01 | 1.1916 |
| 0.05 | 5.9580 |
| 0.1 | 11.9160 |

In this work, we proposed the empirical relation between change in capacitance and added mass. This can find its potential application in designing a mass sensor. Next, have developed an analytical expression to calculate the mass of the nanogram scale using a simple capacitive based microsensors. For this, a capacitance-based electrostatically driven microcantilever beam is taken into consideration. An electromechanical model considering full order electrostatic nonlinearity is developed. The reduced-order model is obtained by employing the Galerkin's method. With reference to the increasing application of mass detecting MEMS devices, this investigation serves as a useful tool to obtain the change in mass of metal flavonoid complex of various fruit cultivars, summarized the salient inferences drawn from the investigation.

Chapter 4

RESULT AND DISCUSSION

Maceration

The samples of two guava cultivars, Allahabad safeda & Shweta (2g) were simply macerated with the help of pestle & mortar in two different solvents i.e. acetone (12.5ml) and methanol (12.5ml). Extracts of guava cultivars prepared by simple maceration were given two extraction treatments of ultrasonic waves i.e. probe tip ultrasonication and bath ultrasonication for the extraction of bioactive compounds.

5.1 (a) Bath ultrasonication treatment to the sample of guava cultivars Allahabad safeda and Shweta for the extraction of bioactive compounds:

The macerated sample of guava in solvent acetone and methanol were transferred into the test tubes directly from the pestle & mortar after that the volume of each test tube was made up to 12.5 ml with solvent acetone and methanol separately in test tubes. Then, the mixture of sample and solvent was undergone bath ultrasonication (33 kHz, 100 W) treatment for 20-50 minutes. As the treatment time completed all the samples were filtered through the muslin cloth and poured into the clean test tubes, airtight with test tube cap.

5.1(b) Estimation of total phenolic content & total flavonoid content with methanol and acetone extract of Allahabad safeda guava cultivar using bath ultrasonication extraction technique:

The total phenol content was estimated by using the folin ciocalteu reagent method. After following the procedure for total phenol content estimation, the absorbance of the blue-colored solution was recorded at 685 nm. The total flavonoids content was estimated by using Al (III)-flavonoid complexation reaction. After following the procedure for total Flavonoids content estimation, the absorbance of orange-red colored solution was recorded at 510 nm. Table 4 showed the results recorded during the estimation of total phenolic and total flavonoid content for Allahabad safeda guava cultivar. The total phenolic content in Allahabad safeda with methanol was obtained to be (4.70 ± 0.01) , with acetone (7.75 ± 0.34) ; total flavonoid content with methanol (2.89 ± 0.02) ; with acetone (4.50 ± 0.00) maximum at 40 minutes of treatment time.

5.1(c) Estimation of total phenolic content & total flavonoid content with methanol and acetone extract of Shweta guava cultivar using bath ultrasonication extraction technique:

The total phenol content was estimated by using the folin ciocalteu reagent method. After following the procedure for total phenol content estimation, the absorbance of the blue-colored solution was recorded at 685 nm. The total flavonoids content was estimated by using Al (III)-flavonoid Complexation reaction. After following the procedure for total Flavonoids content estimation, the absorbance of orange-red colored solution was recorded at 510 nm. Table 5 showed the results recorded during the estimation of total phenolic and total flavonoid content for Shweta guava cultivar. The total phenolic content in Shweta guava cultivar with methanol was obtained to be (7.98±0.01), with acetone (10.00±0.08); total flavonoid content with methanol (5.00±0.01); with acetone (6.01±0.02) maximum at 40 minutes of treatment time.

5.2(a) Probe tip ultrasonication treatment to the sample of guava cultivars Allahabad safeda and Shweta for the extraction of bioactive compounds:

The macerated samples of two guava cultivars in solvent acetone and methanol were transferred into the test tubes directly from the pestle & mortar after that the volume of each test tube was made upto 12.5 ml with solvent acetone and methanol separately in test tubes. Then, the mixture of sample and solvent was undergone probe tip ultrasonication (20kHz, 100 W) treatment for 20-50 minutes. As the treatment time completed all the samples get filtered through the muslin cloth and poured into the clean test tubes, airtight with test tube cap.

5.2 (b) Estimation of total phenolic content & total flavonoid content with methanol and acetone extract of Allahabad safeda guava cultivar using probe tip ultrasonication extraction technique:

The total phenol content was estimated by using the folin ciocalteu reagent method. After following the procedure for total phenol content estimation, the absorbance of the blue-colored solution was recorded at 685 nm. The total flavonoids content was estimated by using Al (III)-flavonoid Complexation reaction. After following the procedure for total Flavonoids content estimation, the absorbance of orange-red colored solution was recorded at 510 nm. The total phenolic content in Allahabad safeda with methanol was recorded to be (8.06±0.06), with acetone (10.22±0.14);

total flavonoid content with methanol (6.00 ± 0.31); with acetone (6.33 ± 0.04) maximum at 40 minutes of treatment time.

5.2(c) Estimation of total phenolic content & total flavonoid content with methanol and acetone extract of Shweta guava cultivar using probe tip ultrasonication extraction technique:

The total phenol content was estimated by using the folin ciocalteu reagent method. After following the procedure for total phenol content estimation, the absorbance of the blue-colored solution was recorded at 685 nm. The total flavonoids content was estimated by using Al (III)-flavonoid Complexation reaction. After following the procedure for total Flavonoids content estimation, the absorbance of orange-red colored solution was recorded at 510 nm. Table 5 showed the results recorded during the estimation of total phenolic and total flavonoid content for Shweta cultivar. The total phenolic content in Shweta with methanol was recorded to be (9.40 ± 0.06), with acetone (13.58 ± 0.02); total flavonoid content with methanol (6.13 ± 0.01); with acetone (6.51 ± 0.02) maximum at 40 minutes of treatment time.

Table 4: Estimated TPC and TFC content in Allahabad safeda cultivar of guava using bath ultrasonication and probe tip ultrasonication extraction technique with methanol and acetone solvent

| Allahabad safeda | | | | | | | | | |
|----------------------|---------------------|---------------------|---------------------------|---------------------|----------------------|---------------------|---------------------|---------------------------|---------------------|
| Methanol | | | | | Acetone | | | | |
| Bath ultrasonication | | | Probe-tip ultrasonication | | Bath ultrasonication | | | Probe-tip ultrasonication | |
| Time (min.) | TPC content (mg/ml) | TFC content (mg/ml) | TPC content (mg/ml) | TFC content (mg/ml) | Time (min.) | TPC content (mg/ml) | TFC content (mg/ml) | TPC content (mg/ml) | TFC content (mg/ml) |
| 20 | 3.01±0.01 | 0.72±0.19 | 5.39 ± 0.23 | 4.83 ±0.00 | 20 | 6.61±0.07 | 3.39±0.44 | 6.80 ± 0.00 | 4.38±0.22 |
| 30 | 3.67±0.33 | 1.79 ±0.14 | 6.80 ± 0.14 | 5.54 ±0.31 | 30 | 6.75±0.35 | 3.59±0.64 | 7.15 ± 0.08 | 6.23±0.04 |
| 40 | 4.70±0.01 | 2.89 ± 0.02 | 8.06 ± 0.09 | 6.00 ±0.31 | 40 | 7.75±0.34 | 4.50±0.00 | 10.22 ±0.14 | 6.33±0.04 |
| 50 | 4.63±0.18 | 2.09 ± 0.14 | 7.99 ± 0.01 | 5.53 ±0.01 | 50 | 7.74±0.35 | 4.45±0.07 | 10.22 ±0.00 | 3.32±0.49 |
| Control | 0.22±0.05 | 0.09±0.01 | 0.25±0.08 | 0.10±0.01 | Control | 0.46±0.05 | 0.03±0.01 | 0.28±0.11 | 0.04±0.00 |

Experimental instrument specification:-Bath ultrasonication- 33kHz, 100W; Probe tip ultrasonication- 20 kHz, 100W.

Table5: EstimatedTPC and TFC content in Shweta cultivar of guava using bath ultrasonication and probe tip ultrasonication extraction technique with methanol and acetone solvent.

| Shweta | | | | | | | | | |
|----------------------|---------------------|---------------------|---------------------------|---------------------|----------------------|---------------------|---------------------|---------------------------|---------------------|
| Methanol | | | | | Acetone | | | | |
| Bath ultrasonication | | | Probe-tip ultrasonication | | Bath ultrasonication | | | Probe-tip ultrasonication | |
| Time (min.) | TPC content (mg/ml) | TFC content (mg/ml) | TPC content (mg/ml) | TFC content (mg/ml) | Time (min.) | TPC content (mg/ml) | TFC content (mg/ml) | TPC content (mg/ml) | TFC content (mg/ml) |
| 20 | 4.98±0.02 | 2.99±0.01 | 4.28±0.07 | 4.89±0.01 | 20 | 7.99±0.00 | 3.10±0.01 | 6.20±0.01 | 4.90±0.01 |
| 30 | 5.07±0.08 | 3.72±0.21 | 6.88±0.12 | 5.65±0.21 | 30 | 8.61±0.09 | 4.99±0.01 | 9.26±0.12 | 6.49±0.01 |
| 40 | 7.98±0.01 | 5.00±0.01 | 9.40±0.06 | 6.13±0.01 | 40 | 10.00±0.08 | 6.01±0.02 | 13.58±0.02 | 6.51±0.02 |
| 50 | 7.48±0.68 | 4.99±0.01 | 9.36±0.11 | 4.98±0.01 | 50 | 9.95±0.01 | 5.77±0.01 | 13.47±0.30 | 5.08±0.01 |
| Control | 0.08±0.01 | 0.07±0.00 | 0.93±0.01 | 0.07±0.00 | Control | 0.09±0.00 | 0.08±0.00 | 0.95 ± 0.01 | 0.08±0.00 |

Experimental instrument specification:-Bath ultrasonication- 33kHz, 100W; Probe tip ultrasonication- 20 kHz; 100W.

5.3 (a) Estimation of antioxidant activity percentage in Allahabad safeda cultivar of guava using bath ultrasonication and probe tip ultrasonication extraction technique with methanol and acetone solvent.

For the estimation of antioxidant activity, the DPPH assay was performed for Allahabad safeda guava cultivar treated with probe tip ultrasonication and bath ultrasonication in two different solvent systems acetone and methanol. Table 6 represented the results obtained in Allahabad safeda guava cultivar.

Table6: EstimatedAntioxidant activity in Allahabad safeda guava cultivar

| Allahabad Safeda | | | | | |
|---------------------------|----------------------------------|---------------------------------|----------------------|----------------------------------|---------------------------------|
| Probe-tip ultrasonication | | | Bath ultrasonication | | |
| Time (min.) | Antioxidant activity %(Methanol) | Antioxidant Activity %(Acetone) | Time (min.) | Antioxidant activity %(Methanol) | Antioxidant Activity %(Acetone) |
| 20 | 70.88 | 87 | 20 | 69 | 84.94 |
| 30 | 74..22 | 89 | 30 | 70 | 88.38 |
| 40 | 80.36 | 92 | 40 | 75 | 91.38 |
| 50 | 79.03 | 91 | 50 | 72 | 89.89 |

The antioxidant activity percentage obtained: Allahabad safeda guava cultivar treated with probe tip ultrasonication, in methanol 80.36%; in acetone 92% and Allahabad safeda guava treated with bath sonication, in methanol 75% & 91.38% in acetone, maximum in a sample treated for 40 minutes.

5.3 (b) Estimation of antioxidant activity percentage in Shweta cultivar of guava using bath ultrasonication and probe tip ultrasonication extraction technique with methanol and acetone solvent.

For the estimation of antioxidant activity, the DPPH assay was performed for Shweta variety of guava cultivar treated with probe tip ultrasonication and bath ultrasonication in two different solvent systems acetone and methanol. Table 7 represented the results obtained in Shweta guava cultivar.

Table7: EstimatedAntioxidant activity in Shweta guava cultivar

| Shweta | | | | | |
|---------------------------|----------------------------------|---------------------------------|----------------------|----------------------------------|---------------------------------|
| Probe-tip ultrasonication | | | Bath ultrasonication | | |
| Time (min.) | Antioxidant activity %(Methanol) | Antioxidant Activity %(Acetone) | Time (min.) | Antioxidant activity %(Methanol) | Antioxidant Activity %(Acetone) |
| 20 | 78.84 | 93.29 | 20 | 70 | 85 |
| 30 | 86.14 | 93.54 | 30 | 77 | 89 |
| 40 | 88.79 | 94.51 | 40 | 82 | 92 |
| 50 | 88.44 | 93.57 | 50 | 80 | 90 |

The antioxidant activity percentage found to be: Shweta guava treated with probe tip ultrasonication, in methanol 88.79%; in acetone 94.51% and Shweta guava treated with bath ultrasonication, in methanol 82% & 92% in acetone, maximum in a sample treated for 40 minutes.

5.4 Profiling of thin-layer chromatography

To separate the possible flavonoid like quercetin from the samples prepared in methanol and acetone the thin layer chromatography (TLC) was performed. A mixture of mobile phase with Toluene: ethyl acetate: formic acid (5:4:0.2) was found as the best solvent system for the separation of quercetin from the samples of two guava cultivars Allahabad safeda and Shweta.

Table8: TLC analysis depicting the Rf values for the extracts of Allahabad safeda and Shweta guava cultivars prepared with methanol and acetone solvent using probe tip ultrasonication extraction technique

| Sr. No. | Sample Name | Distance travelled by solvent | Distance travelled by standard | Distance travelled by sample | Rf value (Quercetin) | Rf value (cm) (sample) |
|---------|-----------------------------|-------------------------------|--------------------------------|------------------------------|----------------------|------------------------|
| 1 | Allahabad safeda (methanol) | 15.3 | 11.4 | 11.4 | 0.745 | 0.745 |
| 2 | Allahabad safeda (acetone) | 15 | 11.3 | 11.3 | 0.753 | 0.753 |
| 3 | Shweta (methanol) | 15.5 | 11.4 | 12 | 0.735 | 0.774 |
| 4 | Shweta (acetone) | 15.1 | 11.1 | 11.1 | 0.735 | 0.735 |

The above table8 showed that the Rf values of methanol extract of Allahabad safeda guava cultivar (0.745cm), acetone extract of Allahabad safeda guava cultivar (0.753cm), methanol extract of Shweta guava cultivar (0.774cm), acetone extract of Shweta guava cultivar (0.735cm). The possible flavonoid like quercetin was showed its presence in methanol extract of Allahabad safeda guava cultivar (0.745cm), acetone extract of Allahabad safeda guava cultivar (0.753cm) and acetone extract of Shweta guava cultivar (0.735cm) as their Rf values were found to be equal with the Rf value of the quercetin standard.

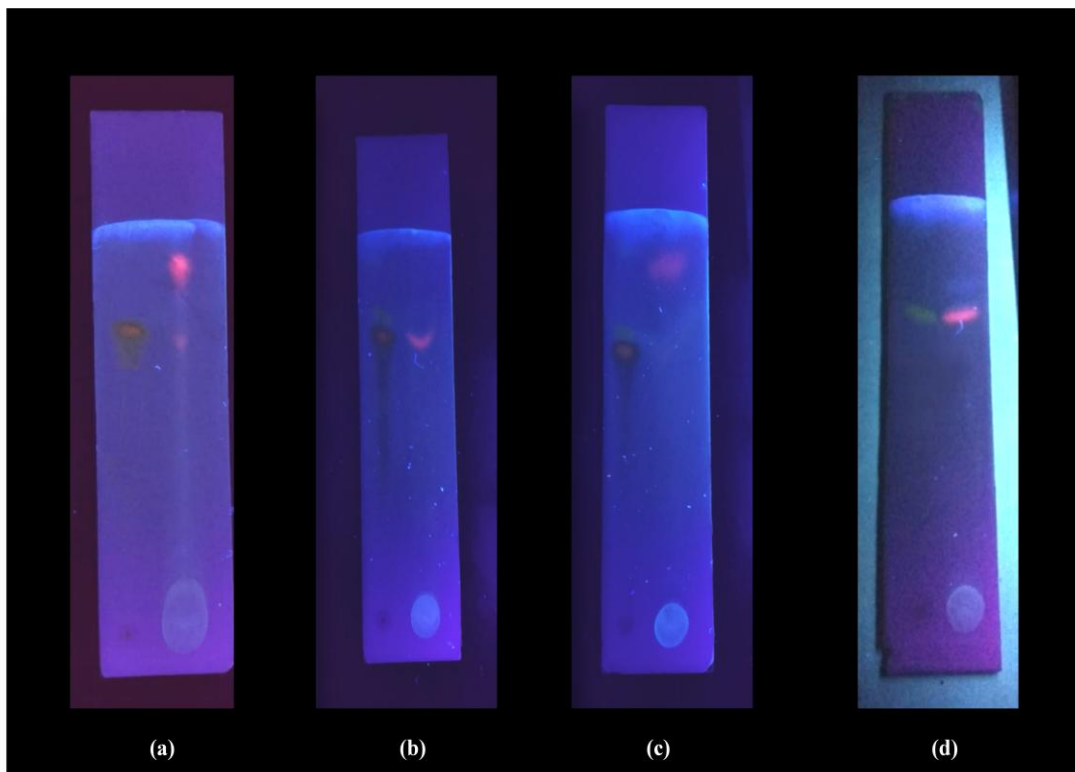


Figure 7: TLC analysis of two guava cultivars Allahabad safeda and Shweta.(Left- Quercetin standard; Right samples) a) Allahabad safeda (Methanol); b)Allahabad safeda (Acetone); c)Shweta (Methanol); d) Shweta (Acetone)

5.5 Reverse-Phase High-pressure liquid chromatography analysis

After depicting the probability of the presence of quercetin flavonoid, the HPLC analysis was also done as a confirmatory test. Table 9 given below shows the result obtained during the analysis against the standard Quercetin.

Table 9: HPLC analysis of an extract of two guava cultivars prepared in methanol and acetone probe tip ultrasonication, against the standard quercetin

| Sample name | Retention time(minutes) | Quercetin ($\mu\text{g/ml}$) |
|-----------------------------|-------------------------|--------------------------------|
| Allahabad safeda (methanol) | 3.025 | 48.34 |
| Allahabad safeda (acetone) | 3.115 | 103.515 |
| Shweta (methanol) | 3.109 | 0.36 |
| Shweta (acetone) | 3.120 | 101.047 |

The retention time of quercetin was recorded was 3.152 minutes after the run time of 5 minutes. The maximum amount of quercetin was detected in acetone extract of Allahabad safeda guava cultivar (103.515 $\mu\text{g/ml}$) and the minimum amount of quercetin was present in methanol extract of Shweta guava cultivar (0.36 $\mu\text{g/ml}$). The retention time of the methanol and acetone extract of Allahabad safeda guava cultivar (3.025 min), (3.115), the methanol and acetone extract of Shweta cultivar of guava was recorded as (3.109 min), (3.120 min).

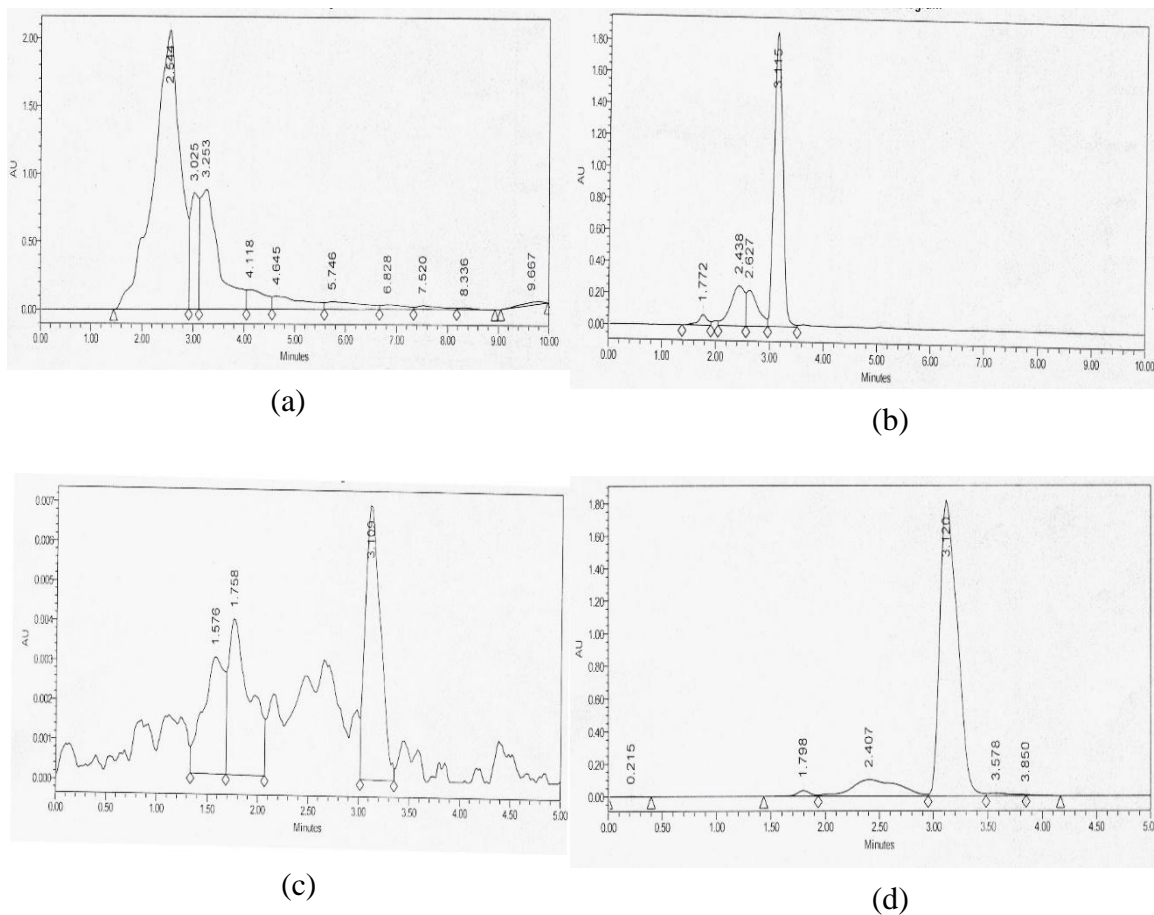


Figure 8: HPLC chromatogram of an extract of two guava cultivars treated with probe tip ultrasonication technique. a) The methanol extract of Allahabad safeda b) acetone extract of Allahabad safeda c) Methanol extract of Shweta d) acetone extract of Shweta

5.6 A proposed dynamic response of microcantilever

An input shaping technique is employed to stabilize the microbeam at the specified position and then voltage perturbation is induced to make the system sensitive to inertial change shown in figure 9(a), 9(b), 9(c) & 9(d). As at the tip of microcantilever beam mass, the of complex (copper-querctin complex; aluminum-querctin complex & iron-querctin complex) and querctin was added it resulted in the tip displacement for copper querctin complex- 0.13 μm , iron querctin complex- 0.14 μm , aluminum querctin complex-0.15 μm and for standard querctin-0.1 μm was recorded. As the molecular mass of the Querctin is 302.236g/mol in the free state but after the formation of the complex with copper, aluminum, and iron the original mass of querctin get changed. In the investigation (Souza *et al.*, 2004) the change in mass of the complexed querctin with the copper, iron, and aluminum has been reported. Change in mass showed (figure10) the deflection in the original position of the microcantilever. This change in the position of microcantilever caused the change in capacitance with respect to mass change of querctin metal complexes.

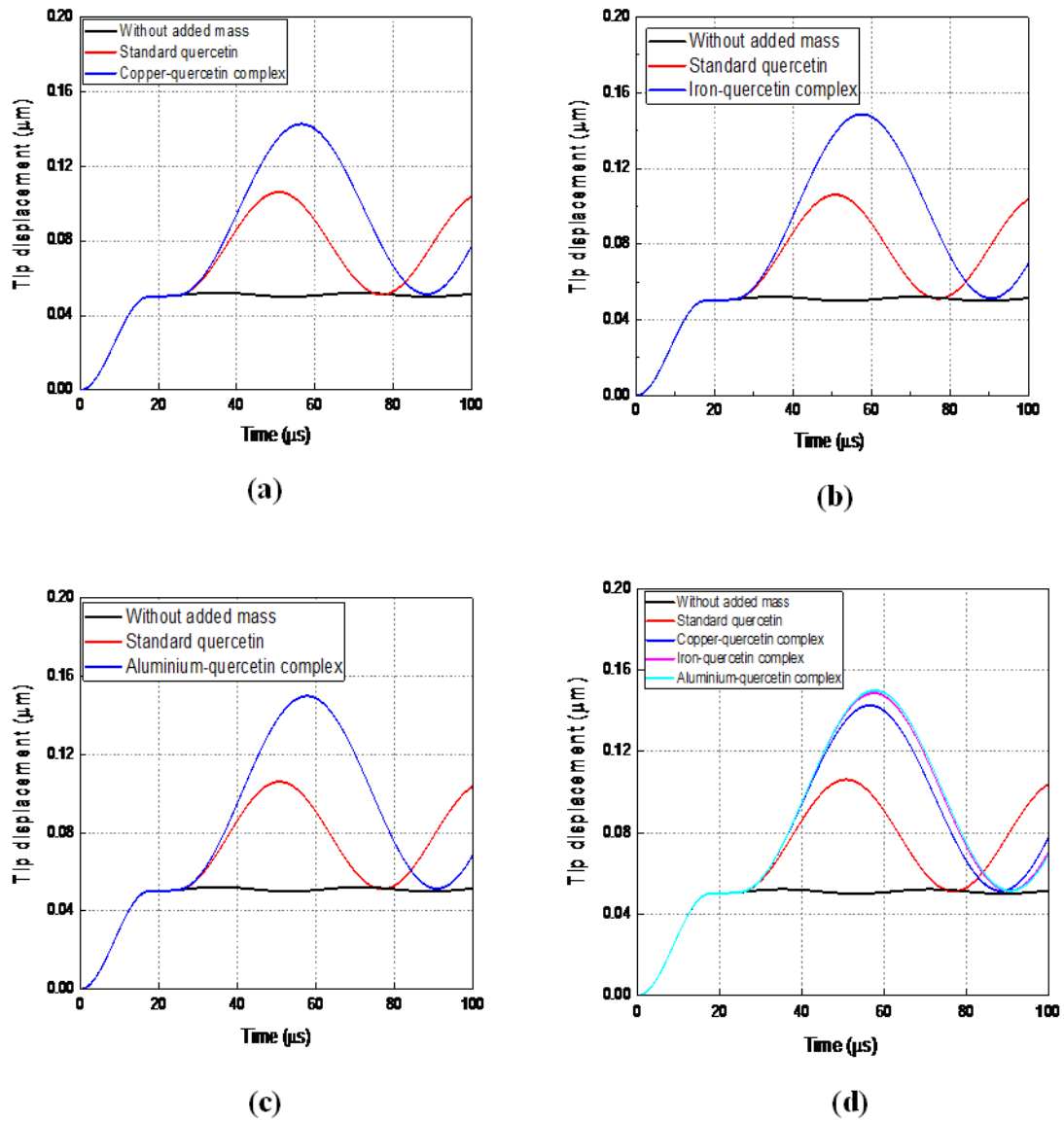


Figure 9: Response towards the tip displacement (μm) with respect to time (μs) after adding flavonoid Quercetin, complexed with three metal ions in comparison to standard or free quercetin (a) copper- quercetin complex; (b) Iron-quercetin complex; (c) Aluminium-quercetin complex; (d) combined response of copper, aluminium & iron quercetin complex in comparison of standard.

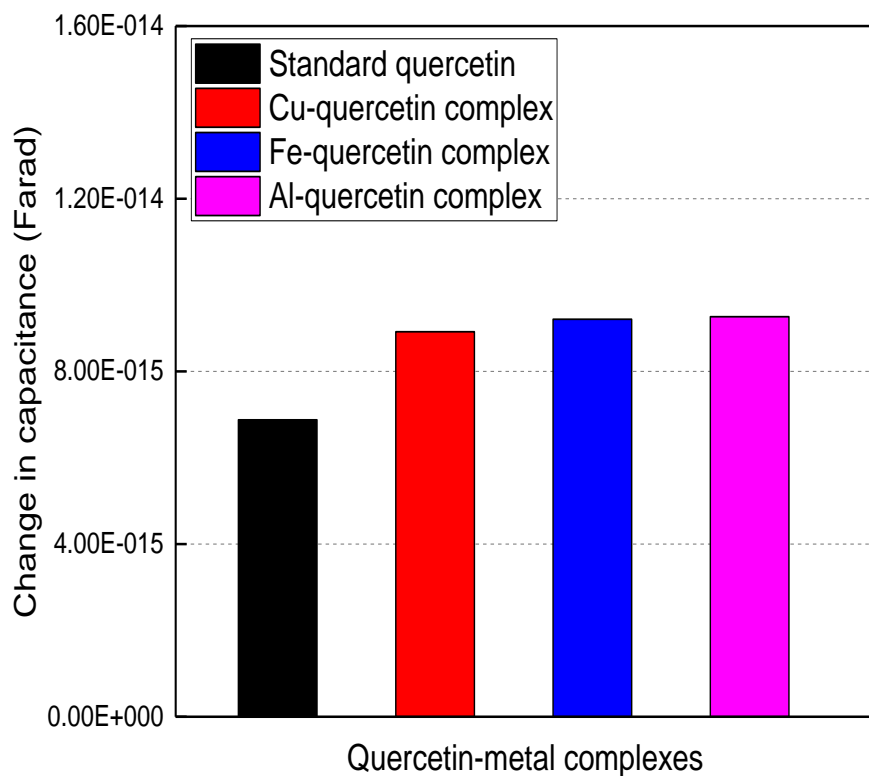


Figure 10: Change in capacitance with respect to quercetin metal complexes.

The respective tip displacement for copper quercetin complex, iron quercetin complex and aluminum quercetin complex is represented in figure 9, corresponding to a characteristic change in capacitance (Pico farad) is mentioned in figure 10 for the above-mentioned complexes sensing through modeled microcantilever beam biosensor.

The amount of total phenols and total flavonoids released in both the guava cultivars are mentioned in table 4 and table 5. It was analyzed that out of all the maximum amount of total phenols was found in the acetone extract of Shweta guava cultivar (13.58 ± 0.02) than the methanol extract of Shweta guava cultivar (9.40 ± 0.06). With this, the flavonoid content in acetone extract of Shweta guava cultivar (6.51 ± 0.02) was found maximum as compared to methanol extract of this variety (6.13 ± 0.01). Also, table 4 shows that the total phenolic content (10.22 ± 0.14) and flavonoid content (6.33 ± 0.04) was found maximum in acetone extract of guava cultivar Allahabad safeda than the methanol extract which is (8.06 ± 0.09) total phenol and (6.00 ± 0.31) total flavonoid content among all the extract prepared. It has been also reported in Tello *et al.*, 2018 for the maximum TPC and TFC content the acetone is the best solvent. As reported in Batubara *et al.*, 2017 the extraction technique and solvent used has a greater effect on the release of total phenol and flavonoid from the leaves of guava fruit cells. To release the possible bioactive compounds viz. polyphenol and flavonoids both ultrasonication and Probe-tip ultrasonication techniques were used. The best results were found in samples treated with probe tip ultrasonication than the bath ultrasonication. The best treatment time was 40 minutes, the treatment given for 50 minutes showed the low content of TPC and TFC this might be happened due to the destruction of the bioactive compounds at 50 minutes of treatment time. It has been emphasized Torres *et al.*, 2017 that the ultrasonic waves are much more efficient in damaging the plant cell wall to facilitate the release of bioactive compounds. Jongkwon *et al.*, 2013 worked on guava leaves for the estimation of a direct correlation between the antioxidant activity and polyphenols. They have found that the samples with high TPC and TFC content possessed the great potential to reduce the oxidative damage by quenching the free radicals formed in the body cells and it was estimated from table 6 & table 7 that the maximum antioxidant activity percentage was observed in the acetone extract treated with bath ultrasonication of Shweta 92% and Allahabad guava cultivars 91.38% than the methanol extracts treated with the same methods, methanol extract of Shweta 82% & methanol extract of Allahabad safeda 75%. Whereas, the maximum antioxidant activity percentage comparatively more was estimated in the acetone extract treated with probe tip ultrasonication of Shweta 94.51% and Allahabad safeda 92.0% guava

cultivars than the methanol extracts, Shweta methanol extract 88.79%, Allahabad safeda methanol extract 80.36% treated with the same methods. Antioxidants are very powerful molecules which have the great potential to reduce the chance of disease table 6 showed the antioxidant activity with respect to the treatment time of the fruit sample. It has been already reported in Musa *et al.*, 2011 that there is the highest positive correlation between the total phenol content and antioxidant activity. More is the phenolic content higher will be the antioxidant activity will be shown by the molecules. The sample treated with probe tip ultrasonication, for 40 minutes of time in acetone has the maximum antioxidant activity 94.51% in Shweta guava cultivar. In Figure 8 the Rf value of both guava cultivars was recorded equal to the Rf value of the standard quercetin except methanol extract of Shweta guava cultivar with red-colored Rf bands. The green-colored fluorescent gets blocked by the red-colored fluorescent compound which could be the isomer of Quercetin like structure as mentioned in the report of Batubara *et al.*, 2017 for the guava leaves.

Table 9 shows the similarity of retention time of each sample with the standard quercetin but not with the relative area percentage, this might be happened due to the less concentration of the sample solution. If the solution that has to be tested has the low concentration the area under the peak would be less although the retention time will be the same (Zu *et al.*, 2005). The maximum amount of quercetin was recorded in the acetone extract of Allahabad safeda and Shweta guava cultivar (103.515 µg/ml), (101.047 µg/ml).

The formation of copper-quercetin complex, Aluminium-quercetin complex and iron-quercetin complex highly depends upon the preferred binding site of flavonoid, properties of metal ion involving in the chelation process, pH conditions, the specific molar ratio of the metal and flavonoid. The specifications mentioned also showed the extent of complex stability. (Kasprzak *et al.*, 2015)

It was investigated by Yilmiz & Perkin, 2017 that the flavonoids with stoichiometry ratio of 1:1 was more stable complexes with the metals ions copper, aluminum and other metal flavonoid complexes. The stability of aluminum quercetin complex was predicted higher than the copper and iron quercetin complex. The stability of the metal flavonoid complex in particular stoichiometric ratio is important for the analysis purpose. If the complex has lesser stability then to sense the change in the mass of

complex become difficult. So, the molar ratio of the metal flavonoids complexes is important. There are many destructive, nondestructive techniques used to detect the fruits internal and external characteristics but the focus on the sensing of the nutritional value has not been considered that much. Hereby proposing the biomems based mathematical model the biomems sensors could be developed to detect the nutritional potential of fruit cultivars like guava due to the tip displacement of microcantilever beam after the addition of metal flavonoid complex mass and hence change in capacitance

CONCLUSION

In the present scenario, the use of biomems is expanding mainly in the medical field but very less in food industries. There are very fewer methods reported to detect the nutritional potential at the nanogram level. The work carried out for the proposed study, a mathematical model was developed based on that the bio-mems sensor could be fabricated, which would detect the different capacitive change for different metal-flavonoid complexation. By using the proposed analytical model a novel biosensor modeled to elucidate the nutritionally rich guava cultivars.

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