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High-resolution accurate mass-spectrometry assay of bioactive metabolites in functional dairy dessert fortified with mahua flowers (*Madhuca longifolia* (L.) J.F. Macbr.)

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Abstract:
This study introduces a novel functional sugar-free dessert fortified with mahua (*Madhuca longifolia* (L.) J.F. Macbr.) flowers. Mahua flowers are rich in valuable nutrients that may provide functional status and give added value to traditional desserts. In this research, we modified a traditional dairy *kheer* dessert by substituting some ingredients with roasted mahua flowers. The research included a control sample (a traditional dessert) and four experimental ones (desserts with 150, 200, 250, and 300 g of mahua flowers). The bioactive profile and metabolomics of the desserts were described using the method of high-resolution mass-spectrometry. The sample with 250 g of mahua flowers had higher organoleptic characteristics compared to the other experimental samples and was selected for further study. This sample demonstrated higher total phenolics, total flavonoids, and DPPH compared to the control dessert. We detected a total of 39 major bioactive metabolites in the dessert with mahua flowers, including rhusflavanone, miquelianin, catechin, quercetin, rutin, robinetinidol 3-O-gallate, eriocitrin, ferulic acid, and kaempferol 3-apioside-7-rhamnosyl-(1- > 6)-(2"-(E)-caffeoyl glactoside). All these substances are associated with numerous health benefits. As mahua flowers have antioxidant, antibacterial, and anti-inflammatory properties, they can be used in commercial production of functional food products.

Keywords: Phytochemicals, bioactivity, *Madhuca longifolia*, mahua flowers, metabolomics, flavonoids, dessert, functional food

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INTRODUCTION

Mahua flowers (*Madhuca longifolia* (L.) J.F. Macbr.) are white to yellowish in color and contain about 70% of moisture. They have good prospects as a food additive; however, their stamens may give the final product a harsh taste that consumers find objectionable. Therefore, before making value-added food products, stamens must be taken out from the flower [1–3]. Dried mahua flowers have a longer shelf-life and are more palatable [4]. Although they are abundant in rural areas, they have never been a major source of nourishment because they are not especially liked as food. They enter the local diet mostly as part of liquor distillation. Mahua flowers also demonstrate some medicinal properties as a diuretic, tonic, and analgesic agent. They also can be unitized as a carbon source of fumaric acid [5–9].

M. longifolia seems to be an opportunity missed by India's food industry and pharmacy since myriads of mahua trees grow in the states of Andhra Pradesh, Madhya Pradesh, Uttar Pradesh, Orissa, West Bengal, Chhattisgarh, Jharkhand, Karnataka, Maharashtra, Rajasthan, Bihar, and Gujarat [10, 11]. This situation makes mahua flowers one of the most neglected national sources of nutrients. In addition to their high nutritional and medicinal properties, they are a good source of polysaccharides, proteins, fats, fiber, minerals, amino acids, vitamins, enzymes, and such fatty acids [2, 11]. They are also rich in reducing sugars, e. g., glucose, sucrose, arabinose, maltose, rhamnose, and fructose [12]. Mahua flowers contain such minerals as calcium, sodium, magnesium, and potassium. They are known for their total phenolics, total flavonoids, and antioxidant activity [13–15]. Their nutraceutical potential includes antioxidant and anti-

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cancerogenic properties [10, 16]. Despite their great nutritional content and availability, mahua flowers seldom enter food formulations [17, 18]. Some studies, however, report roasted or powdered mahua flowers as a high value-adding ingredient [19].

In this respect, mahua flowers could be assessed as a prospective component for functional desserts. Traditional desserts have a creamy consistency and include high amounts of fat and sugar [20]. However, the public attitude to traditional desserts has been changing ever since high-calorific foods and sedentary lifestyle made obesity a global issue. The growing health-consciousness presses on the food industry to decrease the quantity of fat, salt, and sugar in food formulations. As a result, food scientists have to come up with new, healthier formulations for traditional dairy desserts. Yet, no study has so far featured desserts fortified with mahua flowers. While some studies introduce novel foods fortified with *M. longifolia*, a certain scientific gap can be observed in metabolic profiling of functional desserts with mahua flowers.

We developed a special method to replace such traditional high-calorific *kheer* components as sugar and rice with mahua flowers while maintaining the typical flavor and texture of the original product. This article describes the phenolic compounds, mineral composition, functional groups, and secondary metabolites in an experimental functional dessert fortified with mahua flowers.

STUDY OBJECTS AND METHODS

Harvesting mahua flowers. Fully ripened mahua flowers (*Madhuca longifolia* (L.) J.F. Macbr.) were picked in the morning in March-April, 2023. They were gathered onto plastic sheets spread beneath the trees and transferred to the laboratory in clean plastic bags. After washing them in tap water, we removed stamens to prevent them from deteriorating the sensory properties of the finished product.

Chemicals and reagents. All of the chemicals and reagents used in this research were purchased from Sigma-Aldrich in Bangalore, India. Fisher Scientific (Bangaluru) provided the polypropylene falcon tubes.

Preparing the mahua flower *kheer* dessert. Having removed the stamens, we roasted 500 g of clean fresh mahua flowers in ghee (5 g) at low flame (60°C) until they turned light golden brown. Milk was boiled at medium heat for 10–12 min with constant stirring in a heavy-bottomed pan. After that, we added the roasted mahua flowers mixed with cashew and almond nuts. After cooling, the mahua dessert was stored in a refrigerator for further analysis. A control sample was a traditional dessert without mahua flowers. Experimental samples included four dessert samples with various amounts of mahua flowers (Table 1).

Sensory evaluation. The sensory evaluation of the experimental mahua dessert involved color, appearance, aroma, taste, body, texture, and overall acceptability.

The results obtained were used to develop an optimal formulation for a dessert fortified with mahua flowers.

Total phenolic content. We used Folin-Ciocalteu's phenol reagent to measure the total phenolic content in the experimental mahua flower dessert. The results were expressed as mg GAE/g [21]. To measure the total phenolic content, we mixed 2.5 mL of 0.2N Folin-Ciocalteau's reagent with 0.5 mL of diluted extract. After letting it to settle for 5 min, we added 2 mL of 75 g/L Na_2CO_3 and incubated at room temperature for 2 h. To measure the absorbance, we used a 1-cm cuvette UV-1800 spectrometer (Shimadzu, Japan) at 760 nm. The calibration curve was based on gallic acid (0–800 mg/L). Following the application of the dilution factor, the total phenolic content was reported in mg of gallic acid equivalent as GAE/100 mL extract.

Total flavonoid content. The total flavonoid content was measured using the aluminum chloride colorimetric method as in article [3] and expressed as mg QCE/g.

Antioxidant activity (DPPH assay). To determine the free radical scavenging activity, we modified the methods developed by Dawidowicz *et al*. [22]. We mixed 100 mL of aliquoted samples or control (80% methanol) with 3.9 mL of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanolic solution. The mix was then thoroughly vortexed and kept in the dark for 30 min to measure the absorbance at 515 nm against a blank of methanol. The results were expressed as a percentage of inhibition of the DPPH, radical in equation:

$$\text{Inhibition} = 100 \times (A_0 - A)/A_0$$

where A_0 is the absorbance of the control and A is the absorbance of the product sample.

Profiling the optimized dessert. ***Microstructure analysis* (*Scanning Electron Microscopy*)**. This morphological analysis involved scanning electron microscopy equipment (Tescan Mira 3 LMU) with 1 nm resolution at 15 kV (100× and 200× magnification). The mineral content analysis involved energy-dispersive X-ray (EDX) spectroscopy.

Table 1 Formulations for experimental mahua *kheer* dessert

Sample	Milk, mL	Sugar, g	Rice, g	Almonds, g	Cashews, g	Mahua flowers, g	Cardamom powder, g
Control	500	25	12	5	5	–	0.2
Sample 1	500	–	–	5	5	150	0.2
Sample 2	500	–	–	5	5	200	0.2
Sample 3	500	–	–	5	5	250	0.2
Sample 4	500	0	0	5	5	300	0.2

***Fourier-Transform Infrared Spectroscopy* (*FTIR*)**. The optimized sample (with 250 g of mahua flowers) and control underwent Fourier-Transform Infrared Spectroscopy in a Shimadzu 8400S KBr beam splitter (Japan) with a resolution of 0.85 cm^{-1} and a spectral range of 400–4000 cm^{-1}.

High Resolution Mass Spectrometry. This test carried out by using an Orbitrap Eclipse Tribrid Mass Spectrometer (Thermo Fischer Scientific, USA). The compounds were separated in a Dionex UltiMate 3000 RSUHPLC System. Solvent A had 80% acetonitrile and 0.1% formic acid; Solvent B had 100% methanol and 0.1% formic acid; Solvent C had 100% water and 0.1% formic acid. For the chromatographic separation, we used a Hypersil GOLD™ C18 selectivity HPLC column of 100 mm in length, 2.1 mm in diameter, and 1.9 µm in particle size. The databases came from Compound Discoverer 3.3.3.200 with default parameters.

Sample preparation (High-resolution mass-spectrometry) and instruments. Using an orbital shaker, we mixed 500 mg of the sample with 4 mL *n*-Hexane and agitated the mix for 4 h at ambient temperature. After a 5 min centrifugation at 25°C and 5,000 rpm, the resulting liquid was subjected to extraction. To do that, we added 700 µL of 33% methanol mixed with acetonitrile to the same quantity of the dessert sample homogenized in an orbital shaker. After another 5 min of centrifugation at 4°C and 10,000 rpm, we filtered the supernatant using a 0.22-micron syringe in a LCMS vial. After that, the sample was used for the mass-spectrometry analysis.

Statistical analysis. All the experiments were performed in triplicate (n = 3) and went through the one-way analysis of variance (ANOVA) using SPSS Ver.12 ($p < 0.05$).

RESULTS AND DISCUSSION

The sensory evaluation of the experimental mahua dessert (Table 2) was carried out by 25 random semi-trained panelists. The panelists showed clear preference for Sample 3, which contained 250 g of mahua flowers. This sample was selected for further study. Sample 4 with 300 g of mahua flowers was rejected for thick consistency.

Total phenolics, total flavonoids, and % DPPH. The phytochemical analysis of a plant extract provides an overview of the bioactive compounds it contains. Plant phenols are common natural products that are present in many plant-based foods. They claim many health-related advantages; for example, they reduce the risk of heart diseases and inhibit oxidative stress. Phenolic compounds have received much scientific attention in recent decades due to their cardioprotective and antioxidant properties [14].

Mahua flowers (*Madhuca longifolia* (L.) J.F. Macbr.) are rich in phenolics and flavonoids known for their antioxidant properties. Thus, adding mahua flowers to traditional desserts may dramatically boost the concentration of phenolics and flavonoids in the final product. The amount of phenolic, flavonoid, and antioxidant chemicals increased significantly ($p < 0.05$) in the dessert fortified with mahua flowers as compared to the control sample. We evaluated the antioxidant properties of the fortified dessert by measuring the total phenolics, flavonoids, and % DPPH. The sample with mahoa flowers had 32.59 ± 0.41 mg GAE/g total phenolics and 15.38 ± 0.43 mg QCE/g total flavonoids (Table 3). The bioactive compounds in mahua flowers enhanced the functional attributes of the dessert.

Our results were in accordance with those described in [23, 24], where mahua flowers were reported to contain flavonoids, tannins, alkaloids, steroids, phenolics, and saponins with anticancer, antioxidant, antitumor, and anti-inflammatory activities. Singh *et al.* [6] reported 25,361.0 mg GAE/g phenolics in fresh mahua flowers. The *nutra* beverage prepared from mahua flowers contained 431 mg GAE/L phenolics [23]. Arun *et al.* [25] studied the antioxidant activities of various medicinal plants using DPPH and obtained a mahua flower extract with stronger DPPH properties (62.45 mg/L) than other medicinal plants. Sinha *et al.* [3] studied the total phenolic content in various concentrations of mahua flower juice at 20, 40, and 60°Brix. They reported 1.42 ± 0.03, 2.25 ± 0.05, and 6.15 ± 0.23 mg/mL, respectively.

Table 2 Sensory evaluation of experimental mahua *kheer* dessert

Sample	Color and appearance	Body and texture	Flavor	Overall acceptability
Control	6.50 ± 0.52	6.60 ± 0.69	6.80 ± 0.78	6.63 ± 1.07
Sample 1	6.50 ± 0.84	6.70 ± 0.67	6.60 ± 0.69	6.60 ± 0.51
Sample 2	6.80 ± 0.78	6.70 ± 0.67	6.90 ± 0.73	6.80 ± 0.82
Sample 3	7.30 ± 0.82	7.30 ± 0.48	7.90 ± 0.87	7.50 ± 0.73
Sample 4	6.50 ± 0.68	6.00 ± 0.47	6.80 ± 0.43	6.50 ± 0.52

Table 3 Antioxidant properties of experimental dessert fortified with mahua flowers

Components	Control (*kheer* white rice)	Dessert with mahua flowers
Total phenolics, mg GAE/g	28.63 ± 0.41	32.59 ± 0.41
Total flavonoids, mg QCE/g	11.68 ± 0.43	15.38 ± 0.43
DPPH, %	47.90 ± 0.43	80.78 ± 0.43

The values are expressed as a mean of three replicates ± standard deviation ($p < 0.05$)

Pinakin *et al*. [10] studied the phytochemical properties of mahua flowers using the HPLC method and reported 62.40 ± 0.98 of gallic acid, 32.04 ± 0.63 synergic acid, 105.34 ± 0.90 ferulic acid, and 222.36 ± 0.51 chlorogenic acid in mahua flowers. Parnika *et al*. [26] studied mahua flowers harvested in Chandrapur and reported 122.59 mg of gallic acid, 20.05 mg vanillin, 10.76 mg caffeic acid, 4.18 mg *p*-coumaric acid, and 12.7 mg tannic acid. The extract also contained such flavonoids as (+) – catechins (35.31 mg) and (–) – epi-catechin (39.51 mg), as well as (+) quercetin (11.7 mg) and vanillin (20.05 mg). Sinha *et al*. [3] revealed that ethanolic and methanolic extracts of mahua flowers had anthelmintic activity against *Pheretima posthuma*, with the methanolic extract being a more powerful anthelmintic agent. The variations in the total phenolic values of mahua flowers reported in different studies may be due to the variety, temperature, ripeness, environmental conditions, and measurement techniques [27].

In terms of antioxidant activity, our research yielded 80.78 ± 0.43% in the experimental sample. Soni & Dey [28] prepared a *nutra* beverage from mahua flowers and guava with a total phenolic content of 171.83 ± 5.21 mg GAE/L and recorded the highest antioxidant profile of 96.5 and 89.6% with ABTS and DPPH assays, respectively. Pinakin *et al*. [10] studied the antioxidant profile of dried mahua flowers and registered total phenolics of 307.5–715.1 mg/100 g, total flavonoids of 1,761–2,752 mg/100 g, and an antioxidant profile of 69.22–88.44%. In their case, the phenolic content, flavonoids, and antioxidant activity of the optimized sample were 417.71 mg/100 g, 2028.39 mg/100 g, and 71.64%, respectively. Similarly, Kuna *et al.* [29] also reported that mahua flowers contained 13.10 ± 1.56 μg/100 g total carotene, 6.92 ± 0.06 μg/100 g *β*-carotene, 1,076.11 ± 5.50 μg/100 g total antioxidant activity, 225.83 ± 1.06 mg GAE/100 g total phenolics, and 560.45 ± 3.46 mg QCE/100 g total flavonoids.

Flavonoids being a polyphenolic secondary metabolite, many studies have documented their preventive properties against inflammation, cancer, cardiovascular disorders, and platelet aggregation. In this study, the total flavonoid content in the dessert fortified with mahua flowers was 15.38 ± 0.43 mg QCE/g (Table 3). Similar results were reported in [30], where the total flavonoid content in mahua flowers varied from 2.03 ± 0.3 to 13.1 ± 0.3 mg QCE/g FW. Singh *et al*. [6] also reported 13.1 ± 0.3 mg QCE/g total flavonoids in mahua flowers.

Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray (EDX). An energy dispersive x-ray analyzer determines the elemental composition. In conjunction with Scanning Electron Microscopy, it provides elemental identification and quantification. Table 4 and Fig. 1 illustrate the mineral content in the experimental mahua dessert. It had 55.6% of carbon, 8.8% nitrogen, 33.1% oxygen, 0.7% calcium, 0.1% iron, 0.9% potassium, and 0.1% manganese (by weight). Singh *et al*. [15] reported such elements as carbon, iron, calcium, oxygen, potassium, sodium, magnesium, and nitrogen in mahua flowers. The method of Laser-Induced Breakdown Spectroscopy at 200–976 nm and Atomic Absorption Mass-Spectroscopy allowed them to measure the quantities of iron (90.00 ± 0.03 mg/100 g), magnesium (90.00 ± 0.02 mg/100 g), calcium 177.40 ± 0.47 mg/100 g), sodium 135.20 ± 0.02 mg/100 g), and potassium (480.00 ± 0.02 mg/100 g) in mahua flower extracts [15]. Das *et al*. [31] reported calcium, phosphorous, potassium, magnesium, sodium, and iron in mahua flowers. Table 4 clearly shows that that potassium was the most abundant mineral in our experimental dessert, followed by calcium and phosphorus. These results were in line with the previous findings reported in [32] for mahua fruit. Other elements were much less abundant, e. g., 0.2% for chlorine, 0.1% for manganese, and 0.1% for Fe^{+} (by weight), a similar trend reported in [33]. Meena & Meena [33] found 8% calcium and 2% phosphorous in mahua flowers. Gopalan *et al*. [34] measured 1.24 mg calcium and 8.064 g iron in 100 g of dried mahua flowers.

Fourier transform infrared spectroscopy (FTIR) analysis. Fourier transform infrared spectroscopy is a versatile and popular analytical technique that provides essential information about the molecular structure and composition of various substances. Figure 2 and Table 5

Table 4 Mineral content in experimental dessert fortified with mahua flowers

Element	Weight, %	Atomic, %	Net Intensity	Error, %	K ratio	Atomic number correction	Absorption correction	Fluorescence correction	Fluorescence excitation correction factor
Carbon	55.6	62.6	1,503.8	5.4	0.3454	1.0211	0.9898	0.6088	1.0000
Nitrogen	8.8	8.5	39.6	100.0	0.0084	0.9967	0.9997	0.0960	1.0000
Oxygen	33.1	28.0	501.2	10.4	0.0467	0.9756	1.0086	0.1446	1.0000
Phosphorus	0.4	0.2	52.2	9.5	0.0033	0.8495	1.0543	0.9240	1.0130
Sulfur	0.1	0.0	6.9	59.2	0.0004	0.8659	1.0592	0.9707	1.0192
Clorine	0.2	0.1	27.2	15.6	0.0019	0.8236	1.0638	1.0010	1.0278
Potassium	0.9	0.3	93.3	8.9	0.0078	0.8192	1.0721	1.0277	1.0440
Calcium	0.7	0.2	62.4	10.1	0.0063	0.8340	1.0758	1.0282	1.0469
Manganese	0.1	0.0	4.2	61.1	0.0007	0.7316	1.0888	1.0259	1.1950
Iron	0.1	0.0	2.5	61.1	0.0005	0.7428	1.0900	1.0241	1.2384

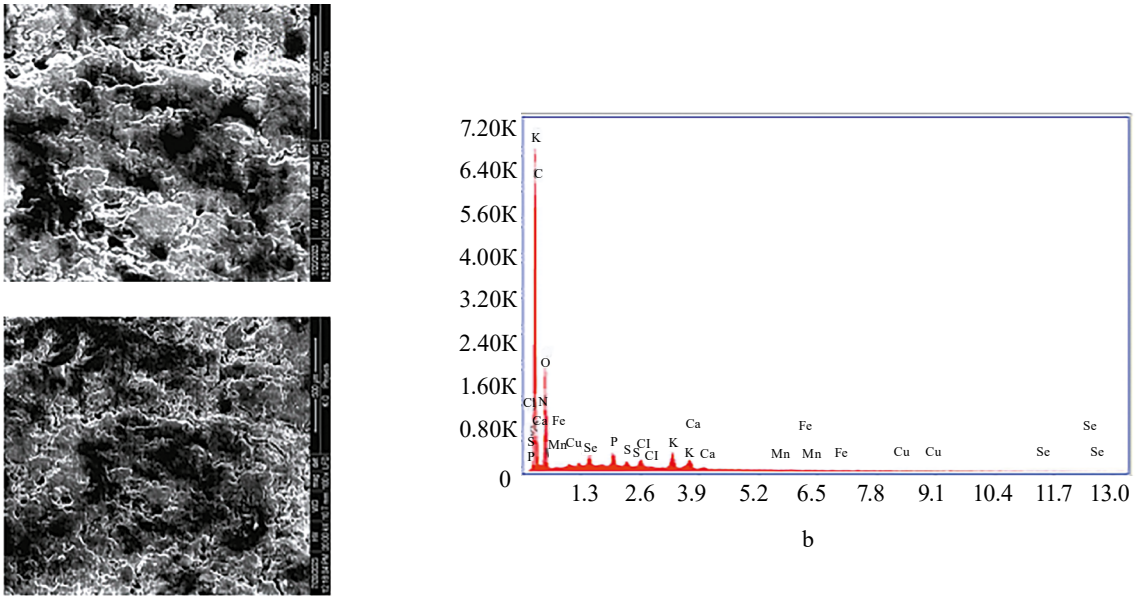

Figure 1 Mineral content in experimental dessert fortified with mahua flowers: (a) Scanning electron microscopy (100× and 200× magnification) and (b) Energy Dispersive X-Ray images

display various peaks associated with different functional groups. Figure 2 demonstrates the peaks, wavenumbers, and their associated functional groups for the experimental mahua dessert. Table 5 lists the functional groups present in the mahua dessert along with the bonding details that pertain to them.

In this research, we discovered such functional compounds as ester, alkanes, aromatics, amines, etc. The peaks of the absorption bands could be matched with some functional groups, e. g., hydroxyl, carbonyl, or aromatic. The sample with 250 g of mahua flowers demonstrated sharp peaks at 1163–1210 cm^{-1} with C-O stretch. The peak in the 1400–1000 cm^{-1} range represented the O-H functional group. The C=N group of imine/oxime functional group was represented by the peak range at 1690–1640 cm^{-1}. The peak at 1750–1735 cm^{-1} revealed ester and imine/oxime C-O stretch. The N=H stretch in amine salt compound was represented by the peak range at 3000–2800 cm^{-1}.

Identifying functional and bioactive compounds. In the last decade, mass-spectrometry has seen tremendous technological advancements that have improved its resolution, accuracy, and sensitivity. Advancements high-resolution mass-spectrometers are economically viable and can measure the mass-to-charge ratio up to fourth or fifth decimal place. They are fast, have a lot of options, and can detect a wide range of chemicals, which makes them very helpful in food research.

Secondary metabolites are natural organic compounds that are beneficial for living beings and can be part of medications, herbs, flavorings, etc. The photochemical profile of mahua flowers includes saponins, flavonoids, steroids, and sapogenin. A PubChem search showed that these phytochemicals possess proven antimicrobial, antioxidant, anticancer, and pharmacological properties. Following the metabolomic analysis, we obtained raw spectrum chromatograms of the experimental sample. Figure 3 shows the main peaks and their intensities based on the Total Ion Chromatogram (relative abundance vs. time) and Electrospray Ionization with Fourier Transform Mass-Spectrometry. Table 6 provides a detailed description for each compound and its biological activity. The bioactive compounds were further classified on the basis of different categories.

Ranjana *et al.* [35] identified 38 phytochemicals in a methanol extract of mahua flowers, including 2-furanmethanol, isosorbide, 2,4-dihydroxy-2,5-dimethyl-3(2H)-furanone, and 5-hydroxymethyl furfural. Kalaivani & Jegadeesan [36] reported antifungal activity in alcoholic extracts of mahua flowers caused by 2-furan methanol, 2,3-dihydro3,5-dihydroxy-6-methyl, 4H pyran 4-one, thiophene, 1,4-tetradecanediol, and 2-furancarboxyaldehyde-5-(hydroxymethyl).

Terpenoids and carotenoids. Terpenoids form a group of compounds of mostly plant origin that are known for their pharmacological properties. Caryophyllene oxide is known for its anti-inflammatory properties and analgesic effects. Sharma *et al.* [37] detected quercetin, betulinic acid, *β*-amyrin decanoate, *β*-amyrin, tannins, *β*-amyrin acetate, *β*-amyrin cinnamate, and stigma sterol. Betulinic acid demonstrates antitumor, anti-inflammatory, antiviral, and antidiabetic effects. It is also an effective neuroprotective substance. Kendre & Wakte [23] also found quercetin, *β*-amyrin acetate, stigmasterol, *β*-amyrin cinnamate, and betulinic acid in mahua flowers. Stigmasterol is a universal phytosterol that renders medicinal plants with anti-inflammatory, anticancer, and anti-arthritic properties [38]. Pinakin *et al.* [10] reported ascorbic acid, synergic acid,

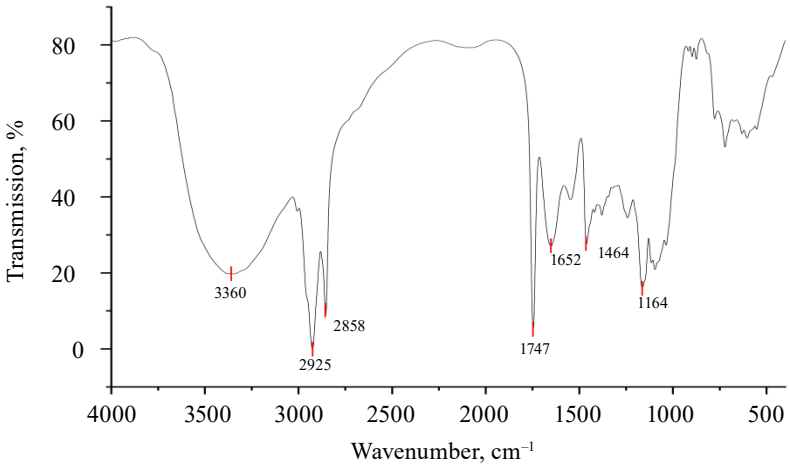

Figure 2 Fourier transform infrared spectroscopy of dessert fortified with mahua flowers

gallic acid, ferulic acid, and chlorogenic acid in dried mahua flowers while studying the effect of pre-treatment on HPLC profiling of ascorbic acid, sugars, and phenols.

Isoflavones and flavonoids. Flavonoids are the polyphenolic compounds with a low molecular weight found in many plant-based foods credited for numerous biological activities. In this research we detected miquelianin, tiliroside, kaempferol 3-apioside-7-rhamnosyl-(1->6)-(2"-(E)-caffeoyl galactoside), rhusflavanone, and robinetinidol 3-O-gallate (Table 6). Rhusflavanone is a new biflavonone that shows strong inhibitory activities against elastase and tyrosinase but lower DPPH radical scavenging activities. Myint *et al.* [39] extracted 0.35 ± 0.04% of rhusflavanone from *Mesua ferrea* L. stamens

Miquelianin, also known as quercetin 3-O-glucuronide, is the most plentiful flavonoid [40, 41]. It reduces blood glucose and lipid levels. Tiliroside is a glycosidic flavonoid with hepatoprotective, anti-inflammatory, antioxidant, and anticarcinogenic properties. It was reported to reduce the amount of visceral fat in mice [42]. Mishra & Usha [43] reported total flavonoid content (1.217 mg QE), total phenolic content (107.00 mg GAE), total alkaloid content (12.28 mg AE), and total saponins (0.163 mg DE) per 1 g of dry extract of dried mahua flowers. Ramadam *et al.* [42], Verma *et al.* [30], and Annalakshmi *et al.* [44] detected tannins, alkaloids, saponins, proteins, carbohydrates in ethanolic extract of mahua flowers; tannins, alkaloids, and carbohydrates in methanolic extracts; as well as carbohydrates, flavonoids, proteins, and tannins in aqueous, ester, and acetone extracts. Robinetinidol 3-O-gallate is an oligomeric condensed polyphenol. According to Ogawa & Yazaki [45], robinetinidol 3-O-gallate possesses antioxidant qualities and may shield the brain from oxidative damage caused by acrolein. It also shields neurons from acrolein's harmful effects by reducing oxidative stress.

Tannins are compounds that act as primary antioxidants and free radical scavenger. In this study, we detected quercetin and ferulic acid (Table 6). Similar results belonged to Sinha *et al.* [3] and Sharma *et al.* [37], who reported quercetin, betulinic acid, *β*-amyrin acetate, *β*-amyrindecanate, stigmasterol, tannins, and *β*-amyrin cinnamate in mahua flowers, rendering them with analgesic, aphrodisiac, demulcent, and diuretic properties. Macbr *et al.* [46] mentioned alkaloids, glycosides, carbohydrates, phenols, saponins, reducing sugars, amino acids, terpenoids, flavonoids, and tannins in *M. longifolia*.

Table 5 Functional compounds detected by Fourier transform infrared spectroscopy in dessert fortified with mahua flowers

Wavenumber (optimized)	Functional groups	Wavenumber (control)	Functional groups
1163–1210	1164	C-O stretching	Ester
1400–1000	1464	O-H bending	Carboxylic acid
1690–1640	1652	C=N stretching	Imine/oxime
1750–1735	1747	C-O stretching	Ester
1750–1735	1747	C-O stretching	Lactone
3000–2800	2925	N=H stretching	Amine salt
3400–3300	3360	N-H stretching	Aliphatic primary amine

Fatty acids are carboxylic acids with long hydrocarbon chains. They are essential components of lipids, often known as fats and oils, and are required for the signaling system, cellular structure, and energy storage. The experimental dessert with mahua flowers proved to contain linoleic, salicylic, oleic, moroctic acids, 6-hydroxycaproic, and *β*-muricholic acids, as well as oleamide (Table 6). According to Dhingra *et al.* [47], mahua flowers are rich in free fatty oleic, arachidonic, linoleic, palmitic, and myristic acids.

Amino acids are made up of basic amino groups, acidic carboxylic groups, and organic groups. They give cells different structural components and help them adhere to tissues. We found DL-glutamine, L-isoleucine, L-(+)-arginine, DL-tryptophan, and L-phenylalanine in the experimental dessert (Table 6). Patel & Naik [48]

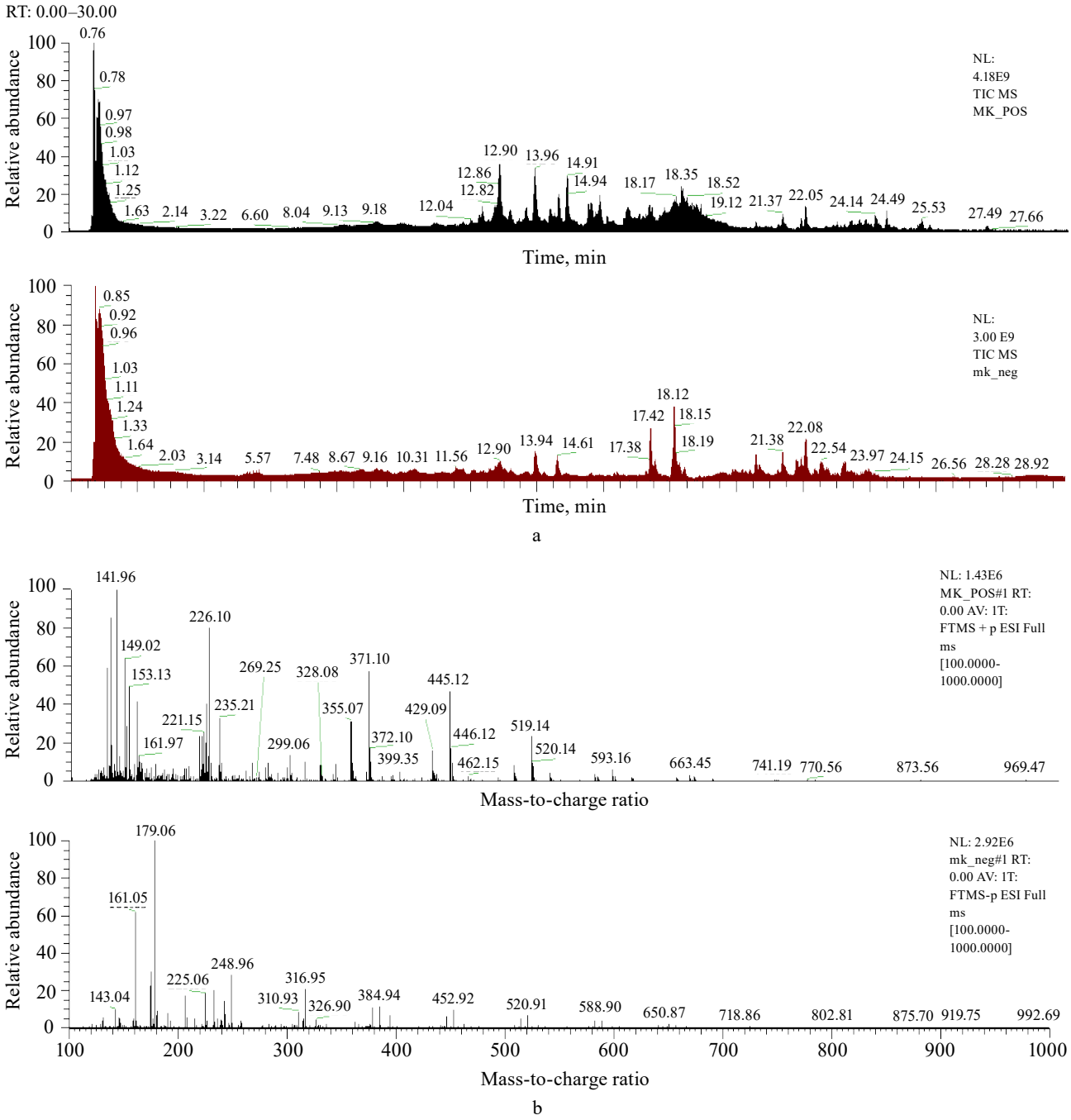

Figure 3 Chromatograms of metabolomic analysis for experimental mahua flower dessert: (a) the high-resolution accurate mass-spectrometry combined with total ion chromatogram (HRAMS TIC MS) and (b) the fourier transform mass-spectrometry coupled with probe electrospray ionization (FTMS pESI)

mentioned glutamic acid, lysine, aspartic acid, valine, threonine, leucine, phenylalanine, isoleucine, and proline.

Carbohydrates and sugars. Plants are a very important source of carbohydrates that provide energy and help in performing various biological activities. Table 6 includes such sugar compounds as D-raffinose, which promotes the growth of lactic acid bacteria while inhibiting the growth of pathogens. Similar results for mahua flowers were reported in [3, 49], which also included glucose, sucrose, maltose, fructose, and arabinose. Our results were in accordance with Singh *et al.* [15], who reported 934.0 ± 8.4 mg of raffinose and 978.0 ± 7.0 mg of inositol in 100 g mahua flowers. Similarly, Meena & Meena [33] also detected 54.24% of total invert sugar, 50.62% reducing sugar, and 54.06 % total sugar.

CONCLUSION

Mahua flowers (*Madhuca longifolia* (L.) J.F. Macbr.) are rich in antioxidants that provide various health advantages. As a result, their bioactive metabolites can turn traditional dairy desserts into functional foods. In this

Table 6 Bioactive compounds and metabolites in mahua flower dessert

Compounds	Molecular formula	Molecular weight	Mass-to-charge ratio	Retention time, min	Biological activity
Rhusflavanone	$C_{30}H_{22}O_{10}$	542.12358	543.13086	0.886	Antioxidant properties
Miquelianin	$C_{21}H_{18}O_{13}$	478.07459	477.0676	11.568	Antioxidant properties
D-(-)-Quinic acid	$C_7H_{12}O_6$	192.0636	191.05647	0.788	Antioxidant, antidiabetic, anticancer, antimicrobial, antiviral, anti-aging, antinociceptive, and analgesic effects
DL-malic acid	$C_4H_6O_5$	134.02174	133.01447	0.846	Enhances mineral absorption; prevents kidney stones
Catechin	$C_{15}H_{14}O_6$	290.07888	289.07172	7.477	Antioxidant properties
Tiliroside	$C_{30}H_{26}O_{13}$	594.13768	593.13116	16.119	Anti-inflammatory and anticancer effects
Phaseolic acid	$C_{12}H_{22}O_6$	262.14171	261.13443	12.736	Cardioprotective and anti-obesity effects
Oleamide	$C_{18}H_{35}NO$	281.27153	282.27881	24.485	Improves sleep and mood
Glycocholic acid	$C_{26}H_{43}NO_6$	465.30934	464.30206	19.526	Improves fat digestion and absorption, absorption of fat-soluble vitamins, and cholesterol metabolism
β-Muricholic acid	$C_{24}H_{40}O_5$	408.28767	407.2804	20.743	Cholesterol management, metabolic regulation
L-Threonic acid	$C_4H_8O_5$	136.03741	135.03014	0.785	Hair loss treatment
Moroctic acid	$C_{18}H_{28}O_2$	276.20888	275.2016	21.563	Anti-inflammatory, cardioprotective properties; brain health
Caryophyllene oxide	$C_{15}H_{24}O$	220.18247	203.17918	20.892	Anti-inflammatory, analgesic properties
Choline phosphate	$C_5H_{14}NO_4P$	183.06581	184.07309	23.387	Prevents neurological disorders and liver diseases
Benzothiazole	C_7H_5NS	135.01392	136.02119	14.696	Anticancer, antimicrobial, antidiabetic, anticonvulsant, anti-inflammatory, antiviral, antitubercular properties
D-Raffinose	$C_{18}H_{32}O_{16}$	504.16964	503.16235	0.993	Promotes lactic acid bacteria, suppresses pathogens
L-Isoleucine	$C_6H_{13}NO_2$	131.09462	132.10184	1.083	Improves hemoglobin synthesis, regulates blood sugar and energy levels
6-Hydroxycaproic acid	$C_6H_{12}O_3$	132.07876	131.07149	7.839	Therapeutic applications
Quercetin	$C_{15}H_{10}O_7$	302.04207	303.04935	11.216	Cardioprotective, anticancer, antitumor, anti-ulcer, anti-allergy, antiviral, anti-inflammatory, antidiabetic, gastroprotective, antihypertensive, immunomodulatory effects
Rutin	$C_{27}H_{30}O_{16}$	610.15375	609.14673	11.787	Cardioprotective, antidiabetic, anti-inflammatory, antimicrobial properties
Robinetinidol 3-O-gallate	$C_{22}H_{18}O_{10}$	442.08977	441.08249	10.066	Antioxidant properties
L(–)-Carnitine	$C_7H_{15}NO_3$	161.10498	162.11226	0.842	Prevents cardiovascular diseases
Linoleic acid	$C_{18}H_{32}O_2$	280.24064	279.23346	24.687	Anti-obesity, immunomodulatory properties; prevents hypercholesterolemia, improves bone metabolism
Oleic acid	$C_{18}H_{34}O_2$	282.25617	281.24902	25.563	Prevents cardiovascular diseases; skin repair
Salicylic acid	$C_7H_6O_3$	138.03186	137.02461	2.933	Bacteriostatic, fungicidal, keratolytic
Eriocitrin	$C_{27}H_{32}O_{15}$	596.17445	595.16718	7.942	Antioxidant properties
Monoolein	$C_{21}H_{40}O_4$	356.29233	339.28903	24.586	Antibacterial properties
Nicotinic acid	$C_6H_5NO_2$	123.03196	124.03924	0.9	Regulates blood pressure
Ferulic acid	$C_{10}H_{10}O_4$	194.05807	193.0508	9.979	Anticancer, cardioprotective, neuroprotective effects
Gentisic acid	$C_7H_6O_4$	154.02691	153.01964	1.616	Antidiabetic properties
DL-Tryptophan	$C_{11}H_{12}N_2O_2$	204.08961	188.07034	1.296	Prevents neuropsychiatric disorders
L-Phenylalanine	$C_9H_{11}NO_2$	165.07877	166.086	1.266	Regulates the biosynthesis of other amino acids; part of the structure and function of many proteins and enzymes
DL-Glutamine	$C_5H_{10}N_2O_3$	146.0691	147.0762	0.886	Immunomodulatory effect; improves brain function and digestion
Adenine	$C_5H_5N_5$	–	136.06161	0.862	Fundamental component of adenine nucleotides
L-Histidine	$C_6H_9N_3O_2$	155.06915	156.07643	0.746	Histamine production
Guanine	$C_5H_5N_5O$	151.04924	152.05652	0.901	Purine base
L-(+)-Arginine	$C_6H_{14}N_4O_2$	174.11191	238.12804	0.828	Antifatigue, immunomodulatory properties; prevents and treats cardiovascular and circulatory diseases
Kaempferol 3-apioside-7-rhamnosyl-(1->6)-(2”-(E)-caffeoyl glactoside)	$C_{41}H_{44}O_{22}$	888.22899	889.23627	18.936	Anti-inflammatory, anticancer, antidiabetic effects
(R)-Amygdalin	$C_{20}H_{27}NO_{11}$	457.1588	456.15152	1.259	Antitumor properties

research, we developed a functional *kheer* dessert with added value by fortifying it with 250 g of mahua flowers. The bioactive and phenolic value of the experimental dessert were proven using the method of high-resolution mass-spectrometry. In general, foods fortified with mahua flowers could be a good source of natural antioxidants and antibacterial or anti-inflammatory agents. Given the wide natural occurrence of *M. longifolia*, our research also encourages food security and offers an economical solution to national nutrition issues at commercial level.

CONFLICT OF INTEREST

The authors reported no conflict of interest regarding the publication of this article.

CONTRIBUTION

S. Singh collected and analyzed the data; A. Poonia developed the research concept, drafted the manuscript, and analyzed the data; A.T. Trajkovska Petkoska analyzed the data and proofread the manuscript.

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