DEVELOPMENT OF VALUE ADDED FOOD PRODUCTS FROM MAHUA FLOWERS AND THEIR NUTRITIONAL ANALYSIS

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CERTIFICATE

This is to certify that the research work entitled ***"DEVELOPMENT OF VALUE ADDED FOOD PRODUCTS FROM MAHUA FLOWERS AND THEIR NUTRITIONAL ANALYSIS"*** *submitted by* ***Monika Mishra*** *at* ***Sambalpur University****,* ***Odisha, India*** *is a bonafied record of her original work carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for any degree or diploma. I recommended this thesis in fulfilment for the award of degree of* ***Doctor of Philosophy*** *in* ***Biotechnology****.*

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DECLARATION

I hereby declare that, the work reported in the Ph.D thesis entitled "**DEVELOPMENT OF VALUE ADDED FOOD PRODUCTS FROM MAHUA FLOWERS AND THEIR NUTRITIONAL ANALYSIS**" submitted at Sambalpur University is the original report of my research, under the guidance of **Dr. Pradeep Kumar Naik**, Professor, Dept. of Biotechnology an Bioinformatics, Sambalpur University. I have not submitted this work previously to any other organization for any degree or professional qualification. I have confirmed the norms and guidelines given in the ethical code of conduct of the university. Whenever I have used materials (data, theoretical analysis and text) from other sources, I have given due credit to them by citing them in the text of the thesis and given their details in the references.

Date: **(Monika Mishra)**

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ABSTRACT

Mahua (*Madhuca indica*) is an indigenous tree species widely distributed across the tropical and subtropical regions of India and other parts of South Asia. Known for its multifaceted utility, Mahua has emerged as a significant non-timber forest product (NTFP) that supports the livelihoods of rural and forest-dependent communities. The tree produces edible flowers, seeds, and fruits, all of which have diverse uses in food, medicine, and industry. The flowers are particularly valuable, being harvested for their sweet nectar and fermented into alcoholic beverages, while also being used in traditional medicines to treat a variety of ailments. Beyond its nutritional and medicinal value, Mahua has cultural significance, particularly in tribal communities, where it is integral to various customs and rituals. The seeds contain oil that is extracted and used for cooking, lighting, and as a raw material in soap production. The tree's timber and other parts also find uses in construction, handicrafts, and as fodder. As a major NTFP, Mahua plays a vital role in ensuring food security, income generation, and poverty alleviation for marginalized communities. It also has potential for sustainable forest management, promoting biodiversity conservation while meeting the socio-economic needs of local populations. However, the over-exploitation and lack of proper management practices pose a risk to the species' long-term sustainability. This highlights the need for integrated policies that promote the conservation, sustainable harvesting, and processing of Mahua, ensuring that it continues to provide socio-economic benefits while maintaining ecological balance. Mahua is a tropical tree species that holds significant cultural, nutritional, and economic importance in many parts of India and South Asia. The tree's flowers, seeds, and fruits are rich in nutrients and have long been used as food and medicine by indigenous communities. While traditionally consumed in raw or minimally processed forms, Mahua has gained increasing attention as a potential source for value-added food products due to its versatile applications in the food industry. The most notable part of the Mahua tree is its flowers, which are typically harvested and consumed in fresh or dried forms, or fermented to produce traditional alcoholic beverages. However, Mahua flowers can also be processed into a variety of value-added food products such as syrups, jams, candies, and energy bars. The flowers are rich in sugars, vitamins, and minerals, offering health benefits such as improved digestion and anti-inflammatory properties. Additionally, Mahua seeds are a source of edible

oil, which can be utilized in cooking, as well as in the production of margarine and other processed food products. This thesis explores the potential of Mahua as a raw material for value-added food products. It reviews various methods of processing Mahua flowers and seeds, the nutritional benefits of Mahua-based food products, and the commercial opportunities these products offer to local economies. The value-added products derived from Mahua can contribute to improving food security, reducing post-harvest losses, and providing income-generating opportunities for rural and tribal populations. The study also addresses the challenges of scaling up Mahua-based food production, including issues of standardization, quality control, and consumer acceptance. Sustainable harvesting practices, coupled with appropriate processing techniques, are key to ensuring that Mahua can be developed as a commercially viable and nutritionally beneficial food source. In conclusion, Mahua holds significant promise as a value-added food product that can contribute to sustainable agriculture, economic development, and improved nutrition. This paper highlights the potential for innovation in the use of Mahua in the food sector and underscores the need for further research and development to unlock its full potential. Mahua flowers are a vital resource in many parts of India and South Asia, offering both nutritional and pharmacological benefits. Rich in essential nutrients, Mahua flowers have traditionally been consumed by local communities as a food source, while also being used for their medicinal properties. This review aims to highlight the nutritional composition, including protein, carbohydrates, vitamins, minerals, and sugars, as well as the pharmacological importance of Mahua flowers. Nutritionally, Mahua flowers are a valuable source of carbohydrates, particularly sugars, which provide a quick source of energy. The flowers also contain moderate amounts of protein, contributing to daily dietary requirements, especially in regions where protein-rich foods are limited. Mahua flowers are rich in essential vitamins such as Vitamin C, which plays a crucial role in immune function, and various B vitamins, which are important for metabolic processes. Additionally, the flowers are a good source of minerals such as calcium, phosphorus, iron, and potassium, which are essential for bone health, blood circulation, and overall physiological functions. The high sugar content, including natural sugars like glucose and fructose, makes Mahua flowers a natural source of energy, commonly used in traditional preparations like syrups, sweets, and fermented beverages. Pharmacologically, Mahua flowers possess significant therapeutic potential, supported by traditional and modern research. The flowers are known for their anti-inflammatory, antioxidant, and antimicrobial properties, making them useful in treating various ailments such as fever, digestive disorders, and skin infections. The bioactive compounds found in Mahua flowers, including flavonoids and phenolic acids, contribute to their antioxidant and anti-inflammatory effects, potentially aiding in the prevention of chronic diseases like heart disease and cancer. In addition, Mahua flowers have been traditionally used to treat respiratory conditions, liver disorders, and to promote wound healing, further emphasizing their diverse pharmacological value. This thesis underscores the dual importance of Mahua flowers as both a nutritious food source and a natural remedy. With growing interest in functional foods and plant-based medicines, Mahua flowers offer an opportunity for the development of nutraceutical products that combine health benefits with culinary applications. However, further scientific studies are needed to fully explore and validate the pharmacological properties and potential health benefits of Mahua flowers. In conclusion, Mahua flowers represent a unique combination of nutritional richness and medicinal value, offering significant potential for both food and pharmaceutical industries. Sustainable harvesting and further research into their health benefits could contribute to improving nutrition and healthcare in rural and underserved communities. Mahua flowers, traditionally used in various medicinal applications, have recently garnered attention for their potential therapeutic effects, particularly in the management of anemia. Anemia, a condition characterized by a deficiency of red blood cells or hemoglobin, affects a significant portion of the global population, particularly in developing regions. This review explores the anti-anemic activity of Mahua flowers, focusing on their nutritional composition, pharmacological properties, and mechanisms through which they may help in combating anemia. Mahua flowers are rich in essential nutrients, including iron, calcium, phosphorus, and vitamins such as Vitamin C, all of which play a critical role in the prevention and treatment of anemia. Iron is a key component in the synthesis of hemoglobin, and adequate intake is essential for preventing iron deficiency anemia. The presence of Vitamin C in Mahua flowers enhances the absorption of non-heme iron from plant-based sources, thus improving its bioavailability. Additionally, the high levels of other essential minerals and vitamins in the flowers contribute to overall health, supporting the body's ability to produce red blood cells. Pharmacologically, Mahua flowers exhibit promising anti-anemic effects due to their rich antioxidant and anti-inflammatory properties. Studies suggest that the flowers contain bioactive compounds such as flavonoids, phenolic acids, and alkaloids, which may contribute to increased hemoglobin levels and overall red blood cell count. These compounds are believed to promote the production of erythropoietin, a hormone that stimulates the production of red blood cells, and enhance the absorption of iron from the digestive tract. Additionally, the anti-inflammatory properties of Mahua flowers may help reduce oxidative stress, which is known to impair iron absorption and utilization in the body. In traditional medicine, Mahua flowers have been used to treat symptoms associated with anemia, including fatigue, weakness, and dizziness. Recent experimental studies have also supported their use as a natural remedy for anemia, further emphasizing their potential as a cost-effective, accessible alternative to synthetic iron supplements. However, further clinical studies are required to fully elucidate the mechanisms and efficacy of Mahua flowers in the treatment of anemia. In conclusion, Mahua flowers hold significant potential as a natural anti-anemic agent, owing to their rich nutritional profile and bioactive compounds. Their inclusion in traditional and modern medicinal practices could offer a sustainable, accessible approach to combating anemia, especially in regions where iron deficiency is prevalent. Further research is essential to confirm the therapeutic efficacy and safety of Mahua flowers in the management of anemia. The Mahua flower, a common flowering plant to India, had been traditionally applied for medicinal usage and for beverage and food stuff preparation. On the other hand, the exploration of Mahua flower concentrate into value-added raw materials for potential food products preparation is very minor. The intent of this investigation is to uncover the feasibility of Mahua flower concentrate as a potential functional ingredient towards the preparation of innovative nutritious foods. It comprises an intensive analysis of nutritional contents that includes sugar contents, amino acids, vitamin levels, shelf stability, and the organoleptic acceptance of foods formulated with Mahua flower concentrate. The nutritional analysis shows that Mahua flower concentrate is a rich source of essential sugars, including glucose and fructose, which provide natural sweetness and energy. Amino acid profiling identifies several essential amino acids such as leucine, lysine, and valine, crucial for human health and growth. The extract also shows a rich presence of vitamins, namely vitamin C, vitamin A, B-complex vitamins that include folic acid and riboflavin. Such vitamins can further add health benefits like immune support and antioxidant properties. Shelf life study: During the period of stability study, Mahua flower concentrate was evaluated with regard to various storage conditions, such as ambient temperature, refrigeration, and freezing. Results have shown that, though the nutrient integrity remains for several months, refrigeration and freezing proved to be most useful for shelf life extension and degradation of vital nutrients. This will be essential for the commercial viability of Mahua-based food products in terms of production and distribution. Sensory evaluation of food products developed from Mahua flower concentrate, such as drinks, energy bars, and syrups, was undertaken by a trained panel of evaluators. The results showed the products had high acceptability with consumers endorsing the flavor, texture, aroma, and overall likability of the products. The Mahua flavor food products were very well-received for their mild sweetness, a unique floral aroma, and a good smooth texture with no off-flavors or undesirable sensory attributes associated with these products. The sensory characteristics were found to be on par with existing commercial products, making Mahua-based foods an attractive addition to the market. This study shows the multifaceted potential of Mahua flower concentrate in the development of value-added food products. The nutritional profile of the concentrate is impressive, with a longer shelf life and favorable sensory properties, which may make it a sustainable and functional ingredient in different food applications. Its use will also contribute to diversifying food offerings while improving their nutritional value, supporting local economies, and promoting the utilization of under-explored natural resources.

CHAPTER-I

INTRODUCTION

1. Background

India is the most populated country in the world and covers a forest area of 678,333 km^2 which is nearly 20% of the geographic area. Owing to its wide range of climatic conditions, the Indian forest is a rich source of flora and fauna. Nearly 275 million people living in rural areas are directly and indirectly dependent on forests for their livelihoods. The people who reside in the forest are identified as poor and as the most vulnerable or helpless groups of society. After Africa, India holds the second position in terms of having tribal populations. These groups of people are officially termed “Scheduled Tribes” (STs) and constitute approximately 8% of the total population. The majority of STs are dependent upon forests for their survival. The collection and selling of wood, timber, fuel, and some nonwood forest products (NWFP), commonly known as nontimber forest products (NTFPs), constitute the only source of income for them.

1.1 Non-Timber Forest Products (NTFPs)

NTFPs play crucial roles in the lives of rural and urban people worldwide (Shackleton et al., 2015). It plays a major role in supporting the livelihood of many poor people, such as providing food, fodder, fuel, shelter, medicines, and fibres (Saha and Sundriyal, 2012). These products are referred to as products that belong to the forest environment and are consumed within the household (Shackleton and Shackleton, 2004). These products provide 10–60% of the total household income. NTFPs also include drupes, medicinal plants, gums and resins, essences, bamboo grasses, leaves, seeds, mushrooms, honey, etc. NTFPs have gained global recognition, as they provide a source of income to the poorest people. A billion people are dependent on these products for their livelihood, and the remaining people are dependent on forests for several benefits, such as social, economic and environmental benefits, which also include rainfall and biodiversity (Pandey et al., 2016). The significance of NTFPs is that they provide adequate food, fodder, fuel, etc., for the growing population. In addition to meeting maintenance needs, NTFPs are good sources of income, provide employment during the leanest season of agriculture, and act as buffers against risk and household crises (FAO, 1995). Khare (1987) projected that forest-based initiatives provide 1,622.68 million person-days of employment annually. Out of these, 1,062.70 million days are created by small-scale forest-based initiatives that include the collection, gathering, and processing of NTFPs. Women undertake 517 million days of employment annually. Owing to the unorganized nature of this sector, reliable estimates of the number of children involved are not available. It is well known, however, that women and children constitute a significant part of those employed in NTFP collection and processing (Tewari, 1994). The forest and trees plays a major role in supporting the livelihood of the rural families. The nontimber forest products (NTFPs) are mostly obtained from the forest source, and it is used for the domestic consumption, and income of the people residing in the rural areas.

Though NTFPs play an important role in occupation for people, they normally put their expectations on potential advantages produced from the marketing of NTFPs for poverty mitigation. Furthermore, NTFPs straightly effect the financial status of rural people (Kumar & Meena, 2018). Collecting NTFPs can deliver a safety net or green social security to billions of poor people in the form of low-cost building materials, income, fuel, food supplements, and traditional medicines (Table 1.1). Between the 20% of NTFPs, only 0.8% have been commercially used (Maithani, 1994). NTFP deeds hold potentials for combined forms of development that yield advanced rural income and conserve biodiversity without challenging with cultivation (Sharma, 1992). *Madhuca indica*, frequently known as mahua, is a significant nontimber forest product (NTFP) that is typically found in Central and Eastern India, and it is directly related with tribal financial status in various ways. Though all parts of the mahua tree are used, mostly mahua seeds and flowers are gathered by tribes for self-consumption and for auction to increase money to uphold their daily life. Mahua pays to the monetary status of tribal civilisation. There is a strong linkage between numerous socioeconomic issues that affect the level of reliance of mahua in tribal society (Nayak & Sahoo, 2020).

1.2 Nutraceuticals with respect to Non-Timber Forest Products (NTFPs)

Nontimber forest products possess the most pure form of nutaceuticals, as they are collected directly from forests. The nutritive values and nutraceutical constituents of many nontimber forest products are well documented (Ferreira et al., 2007). Raghu et al. (2007) reported a number of fruits that presented several nutraceutical constituents that help fight several diseases. Different plant parts, such as the root, bark, leaves, flowers, fruits, and sometimes the whole plant, are known for the presence of active phytoconstituents. (Katalinic et al., 2006). Although numerous NTFPs have been scientifically investigated for their nutraceutical constituents and disease resistance properties and are being commercialized at an industrial scale, there is still a long list of edible NTFPs awaiting processing and value addition.

The flower of *Madhuca indica*, known as mahua in most parts of India, is a popular NTFP collected in large quantities in the central tribal belt of the country. This flower has been used as food and liquor since ancient times. However, detailed scientific investigations of value addition to this flower are still lacking.

Table 1.1: Annual Production of NTFPs in India (Shiva, 1994)

Sl. No	Name of the NTFPs	Approximate annual Turnover (in tonnes)
01	Myrobalans	132,250
02	Sal seeds	7,097,000
03	Mahua seeds	697,600
04	Mahua flowers	4,50,00,000
05	Neem seeds	115,000
06	Total essential oils	3,160
07	Gum karaya	15,000
08	Bamboos	4,716,600
09	Grasses other than oil-producing	80,000
10	Tendu leaves	360,000
11	Resin	45,000

1.3 Mahua (*Madhuca indica*): A Multipurpose Forest Tree

1.3.1 *Botanical description*

Taxonomic classification:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Ericales

Family: Sapotaceae

Genus: *Madhuca*

Species: *indica*

Figure 1 *Madhuca indica* (Mahua) tree

Mahua trees are distributed in different parts of India as well as Asian countries such as the Philippines, Pakistan, Sri Lanka and Australia. In central India, it is found near river banks and in semievergreen forests. Mahua trees are abundant in different states of India, such as Uttar Pradesh, Madhya Pradesh, Odisha, Jharkhand, Chhattisgarh, Andhra Pradesh, Maharashtra, Bihar, West Bengal, Karnataka, Gujarat, and Rajasthan. The annual production of mahua flowers is 45,000 million tonnes. The yield of every tree varies from 83 to 320 kg every year (Pinakin et al., 2018). Patel et al. (2012) reported that mahua is a medium-sized to large deciduous tree, usually with a short and large rounded crown found all over the green forest part of India up to an altitude of 1200 m, and the height of the tree is 1215 m. The bark is a dark brown color and is slightly cracked. The size of the leaves is 1030 cm, and the leaves are thick and leathery. According to Bisht et al. (2018), most of the leaves are pointed at the tip, clustescently glabrred near the end of branches, and epileptic or elliptic-oblong 7.523 cm. Flowers are small and fleshy, dull or pale white and define fascicles near the ends of branches. The corollas of the flowers are tubular, freshly pale, yellow aromatic and caduceus. Fruits are 26 cm long, fleshy, and greenish (Jha & Mazumder, 2018).

1.3.2 *Microscopy of mahua*

Yadav et al., 2011 did the microscopic study, in which he observed that the seeds were found to be dark brown and the seeds varies from 1-4. The stem is cylindrical in shape and are branched. The fruits seems to be fleshy, green and ovoid. The flowers are found numerously and are found in the tip of the branches, drooping on their pedicles. The colr of the corolla is yellowish-white and the tubes are fleshy. The stamen of the flowers range from 20-30 with the anthers being hispid on the back and covered in stiff hairs. The petiole is small, and the leaves are bunched at the branch tips. Both the leaves and petioles are young or tomentose. The leaves are coriaceous, elliptic or oblong-elliptic, with a short acuminate tip and a cuneate base.

1.3.3 Uses of different parts of mahua

(a) ***Flowers***

Liquor is derived from the corolla, due to its high content of fermentable sugar. According to Vimal and Tyagi (1984), one tonne of dry mahua flowers can yield 80,217 liters of ethyl alcohol. Moreover, Anonymous (1962) stated that the flowers are appropriate for distilling country liquor (Fig. 2,3&4).

(b) Fruits

They can be eaten either raw or cooked. At the ripening stage, fruits are rich in starch, which is converted to sugar after 23 days of plucking (Fig. 5)**(Anonymous, 1962).**

Figure 2 Mahua flower buds

Figure 3 Fresh mahua flowers

Figure 4 Dry mahua flowers

Figure 5 Mahua seeds

(c) Seeds

A typical mahua tree produces 100 kg of seeds annually. The seed kernels are composed of aspartic, glutamic, glycine, serine, proline, and alanine. The seed coat contains 2% quercetin and dihydroquercetin with some tannins and thioglucosides (Banerji & Mitra, 1996).

(d) Mahua oil

It can be used for washing soaps, manufacturing candles, lubricants, bathing oil, the production of steric acid, etc. (Douli et al. 1968a, 1968b).

(e) Cake

The roasted-deoiled cake comprises carbohydrates, proteins, and ash. Due to its toxic effects, it cannot be used as cattle feed uless it is detoxified which makes it safe for further comsupiton (Mitra & Misra, 1967). It is also applied as a wormicide and fish poison, but it does not decay or nitrify in the soil (Misra & Mitra, 1968). Consuming mahua seed cake can lead to hemolysis because of the saponins it covers (Birk, 1969). Saponin elimination was completed using various solvents such as water, ethanol, and isopropanol, and detoxification was carried out with minimal loss of protein and carbohydrates (Sen Gupta, 1980).

1.3.4 Traditional uses

For many years, there have been many traditional uses for each and every part of the mahua tree. Mahua plays a vital role in the treatment and prevention of many diseases. Different parts of the tree are used to treat different types of diseases. According to Patel et al. (2012), in Ayurveda mahua, flowers are used as a cooling agent for acute chronic tonsillitis, pharyngitis, ulcers, and bronchitis. The flowers are used as a tonic, analgesic, and diuretic. Jha and Mazumder (2018) clearly mentioned that flowers have shown some activity against swelling, itching, and fractures. According to Bisht et al. (2018), bark juice is taken orally for the treatment of diarrhea, and it is also used to treat chronic tonsillitis, leprosy, and fever. The bark of the tree is occasionally used to treat situations like rheumatism, bronchitis, and diabetes. Patel et al. (2012) distinguished that, in addition to this, the bark is effective in the treatment of bleeding and spongy gums. Verma et al. (2014) emphasized the important role of mahua leaves in the management of chronic bronchitis and Cushing's disease. The leaves are also known for a diversity of therapeutic activities, including wound healing, hepatoprotection, antioxidant, antimicrobial, verminosis, gastropathy, thirst reduction, bronchitis relief, dermatopathy, rheumatism, headaches, and hemorrhoid treatment. Pinakin et al. (2018) stated that the fat extracted from mahua seeds has numerous medicinal uses, such as acting as an emollient, treating skin infections, rheumatism, headaches, acting as a laxative, easing piles, and sometimes functioning as a galactagogue.

According to Bisht et al. (2018), mahua is conventionally used in food production.

- Due to the occurrence of a high quantity of sugar, mahua is used as a sweetener in the preparation of several food products.

- The flowers are often dried in sunlight and boiled with tamarind and sal seeds for the consumption.
- The left over flowers after fermentation are used for the cattle feed to improve the helath of the animals.
- The flowers are used for the preparation of country liquor with the alcohol amount of 30-40%.
- The mahua seed oil is often used for cooking purposes.

1.3.5 Nutritional and phytochemical profiling

1.3.5.1 Nutritional analysis of mahua

- Sugars: The sugar contents of mahua flowers are dextrose, levulose, maltose, sucrose, etc. According to Fowler et al. (1920), when the flower is ready to fall it has high sugar content. In the flower, the amount of levulose is greater than dextrose. In the storage period, the amount of sucrose decreases, but in the shedding stage of corolla the amount of sucrose is high. Sutaria & Magar (1955a, 1955b, 1955c) have done a paper chromatographic experiment of unhydrolyzed mahua extract which revealed the presence of maltose, sucrose, arabinose, fructose, and rhamnose, but the hydrolyzed extract revealed the presence of galacturonic acid.
- Polysaccharide: The water-soluble polysaccharides were extracted and gel-filtered to obtain a homogeneous frac tion. D-galactose, L-arabinose, L-rhamnose, D-xylose, and D-glutaric acid were found in the first fractionation. The second fractionation was done by Sarkar & Chaterjee (1984), and they reported the presence of D-glucose, L-arabi nose, and D-glucuronic acid.
- Protein and amino acid: The nitrogen content of the underdeveloped flower is higher than the fully developed flower, that is, 0.65% 1.1%. The protein content of the flower fluctuates from 4.4% to 7% (Belavady & Balasubramanian, 1959; Jayasree et al., 1998). Sutaria & Magar (1955a) reported the presence of eleven amino acids like lysine, arginine, aspartic acid, glutamic acid, threonine, valine, tryptophan, phenylalanine, isoleucine, leucine, and proline. Jayasree et al. (1998) discussed that the protein content of mahua flower was superior when compared with groundnut protein.
- Fat and fatty acid: The fat content of mahua flower is very low. As reported by Jayasree et al. (1998) and Sutaria & Magar (1955a, 1955b, 1955c) the fat content differs from 0.09%

to1.3%. Sutaria & Magar (1955a) stated that mahua flowers contain linoleic, oleic, palmitic, and stearic acid.

- Vitamins: Different researchers have evaluated different vitamin contents of fresh mahua flowers. Ascorbic acid con tent varies from 36.99 to 7 mg. Thiamine content is 140.3 μg (Sutaria & Magar, 1955b). Riboflavin is found to be 878 μg(Belavady & Balasubramanian, 1959). Niacin was found to be 5.2 mg (Belavady & Balasubramanian, 1959), and folic acid content is 214 μg(Sutaria & Magar, 1955b).
- Minerals: Different minerals have been reported in mahua flowers such as Ca, P, Fe, Mg, Na, and K. Out of these, Na is 25.27 mg/100 g, K is 1.2 mg/100 g, and Zn is 0.926 mg/100 g (Sutaria & Magar, 1955a).
- Enzymes: Fowler et al. (1920) detected enzymes in different developmental stages of the flowers such as amylase, maltase, invertase, catalase, and oxidase.
- Saponins: It can be termed as an anti-nutritional factor, but it has both positive and negative consequences. In the positive aspects, it helps in lowering the serum cholesterol level in humans. On the negative side, it hinders the pro ductive performance of nonruminants. They also show the toxic effect that leads to death in the rats (Goodhart & Sahil, 1980; Mulky & Gandhi, 1977)

Table 1.2 Phytochemical screening of Mahua flower extract.

Sl. no	Phytoconstituents	Tests	Ethanolic extract	Methanolic extract	References
01	Proteins	Biuret test	+ve	-ve	Mishra & Usha (2019), Patel et al. (2019)
02	Carbohydrates	Molisch's test	+ve	+ve	
03	Amino acids	Ninhydrin test	-ve	-ve	
04	Volatile oil	Sudan test	-ve	-ve	
05	Alkaloids	Tannic acid test	+ve	+ve	
06	Flavonoids	Alkaline reagent test	-ve	-ve	
07	Tannins	Lactic acid test	+ve	+ve	

1.3.6 Comparative nutritional profile

Gopalan et al. (2004) and Jayasree et al. (1998) reported that the protein content of dry mahua flowers was 6.67%, which is much greater than that of other common fruits, such as apples, bananas, mangos, and raisins. Similarly, it has a lower amount of fat, that is, 0.09%. The carbohydrate content of fresh mahua flowers is 68%. The calcium content was found to be 139%. According to the different reviewers, fresh and dry mahua flowers contain all the essential nutritional compounds.

1.3.7 Effects of geographical distribution on flower composition

According to Elworthy (1887), there is an effect of climatic changes on the sugar composition of mahua flowers. The flowers were collected from Hyderabad, Jabalpur, Gujarat, and Mirzapur. The cane sugar % was found to be maximum, that is, 17.1% in the flower collected from Hyderabad. The inverted sugar % of the flowers collected from Gujarat was 45.3%. Dextrose % of the flowers collected from Mirzapur was 43.6%, whereas the total sugar % of the flowers from Hyderabad was 57.1%. According to Roy & Rao (1959), flowers collected from the Mandla district of Madhya Pradesh were found to contain reducing sugar (57.3%), nonreducing sugar (9.4%), starch (3.6%), protein (6.8%), ash (4.5%), and other components (18.4%), respectively. Phytochemical screening of mahua plant extract was studied by different researchers who have studied the presence or absence of different phytoconstituents. The detailed study of phytochemical screening of mahua flower extract is depicted in Table 7.1. Phytochemical profiling of different parts of mahua plant was done. In Table 7.2, reviewers have found the presence of various chemical constituents which were also responsible for different biological activities. GC-Ms analysis of seed, leaf, bark, and flower was done by Ranjana et al. (2018). Certain important phytochemicals were found such as hexylcyclohexane, octylcyclohexane, E-14-hexdecenal, pentadecan-8-one, 8-octadecanone, dibutyl phthalate (Table 1.3). Annalakshmi et al. (2013) did the GC-Ms and HPTLC analysis of leaf extract of mahua, and they found that there were several known and unknown bioactive compounds present which were mainly responsible for the treatment of different diseases.

Table 1.3: Phytochemical profiling of Mahua

Sl. no	Plant part	Chemical constituents	References
01	Leaves	D-glucoside, stigmasterol, β-carotene, xanthophylls, erythrodiol, palmitic acid, myricetin, 3-O-arabinoside, 3-O-L-rhamnoside, quercetin, 3galactoside; 3β-caproxy and 3β-palmitoxy-olean-12-en-28-ol, oleanolic acid, β-sitosterol, 3-O-β-D-glucoside, 3β-caproxyolcan-12-en-28-ol, β- carotene, n-octacosanol, sitosterol, quercetin, β-sitosterol-β-D- glucoside, n-hexacosanol. 3-O-arabinoside, xanthophylls, 3-O-L-rhamnoside, n-octacosanol, 3β-caproxyolcan-12-en-28-ol, β-sitosterol and 3-O-β-D-glucosideand sitosterol	Verma et al. (2014), Khare et al. (2018), Mishra and Pradhan (2013)
02	Seeds	Myristic, palmitic, and stearic acids, α-alanine, aspartic acid, cystine, glycine, isoleucine and leucine, lysine, methionine, proline, serine, threonine, myricetin, quercetin, Mi-saponin A, saponin B, arachidic, linolenic, oleic, quercetin	Verma et al. (2014), Khare et al. (2018), Mishra & Pradhan (2013)
03	Fruits	n-Hexacosanol quercetin and dihydroquercetin, β-sitosterol and its 3β-D-glucoside, α- and β-amyrin acetates	Verma et al. (2014), Mishra & Pradhan (2013)
04	Flower	Vitamins A and C	Verma et al. (2014)
05	Bark	α- and β-Amyrin acetates, 3β-monocaprylic ester of erythrodiol and 3β-capryloxy oleanolic acid, ethylcinnamate, α-terpineol, and sesquiterpene alcohol	Khare et al. (2018), Mishra & Pradhan (2013)
06	Nut-shell	n-Hexacosanol quercetin and dihydroquercetin, β-sitosterol and its 3β-D- glucoside	Mishra & Pradhan (2013)

1.3.8 Pharmaceutical uses and pharmacological importance

1.3.8.1 Industrial uses

Patel et al. (2019) observed a lot of traditional utilization of mahua tree. Mahua can be used industrially which can upgrade the economic status of the country. Flowers that are used for the preparation of country liquor can be fermented to obtain spirits, ethanol, acetone, lactic acids, vinegar, etc. Soon after the alcohol production, the remaining pulp and other ingredients are used for the production of biofertilizer. According to Mishra & Poonia (2019), mahua biofertilizer can be said as a good fertilizer as there is a presence of nitrogen, phosphorus, and potassium along with calcium. After the removal of oil from the seed, the seed cake may be used for cattle feed (Ramadan et al., 2016). Many researchers and reviews suggest that mahua flowers and mahua-deoiled cake can be used as cattle feed to provide proper nutrition to the cattle at an affordable rate. A cream formulated from mahua oil is nontoxic, less expensive, and biodegradable. So it could be used as an effective emulsifier for formulating cream (Mahajan et al., 2017). Because of lots of uses, mahua is giving employment and opportunity to the tribal people. They collect every part of the mahua tree and sell it in the local market, and in return they get money. But the amount is not sufficient for survival. Some government schemes should be provided for all such people who are completely dependent on mahua for their livelihood.

1.3.8.2 Biodiesel

Ghadge & Raheman (2006) optimized the biodiesel production using a surface methodology. The surface methodology process gave a yield of 98% mahua biodiesel that satisfies the properties of both American and European standards of biodiesel. Puhan et al. (2005) stated that mahua can be used as a source of renewable energy in India. They observed that in mahua oil methyl ester has lower emission compared with other esters. Raheman & Ghadge (2007) observed that the blend of mahua oil with high-speed diesel can be used as an alternative fuel that causes low pollution. Due to the increase in the price of oil, mahua oil can be used as a lubricant for maintenance purposes. Apart from the environmental benefits, Suhane et al. (2013) experimentally revealed that the addition of mahua oil with 90T oil showed good wear-riding traits. Goud et al. (2006) developed epoxides by using hydrogen peroxides using mahua oil. (Kapilan & Reddy, 2008) concluded that an engine ran effortlessly with methyl ester of mahua oil and B20, but there was a heavy emission when only mahua oil was used as a fuel.

1.3.8.3 Biological activity

- *Therapeutic property:* Mishra & Usha (2019) reported that from ages mahua has a lot of therapeutic potentials. Different parts of the plants have different therapeutic and pharmacological uses. Decoction of mahua flowers is used to quench the thirst. It is used as a cooling agent, and it is also used to treat tonsillitis, bronchitis, and inflammation. It has astringent properties so sometimes it is used in the treatment of piles, diarrhea, and colitis. The flowers are used to treat eczema, skin diseases, eye diseases, and arrest bleeding. It is also used to treat burning sensations, heart diseases, and ear complaints. The flower extract is used to increase the quantity of seminal fluids.
- *Anti-inflammatory activity:* The inflammation is caused due to the release of several chemicals like histamine and serotonin from the damaged cell. Inflammation is a self-protective mechanism of the body (Yadav et al., 2012). The vital mechanism of the anti-inflammatory drugs is reflected due to the inhibition of PG synthesis at the site of injury. According to Gaikwad et al. (2009), the potential of anti-inflammatory drugs is measured by their effectiveness to prevent COX. The anti-inflammatory activity is directly proportional to the inhibition of COX. The aerial parts of the *M. indica* are used in the action of inflammation. The aerial part was extracted by using Soxhlet apparatus. The extract was concentrated under vacuum-sounding apparatus for 30 minutes. The result was satisfactory when the solution was given to male Wistar rat.
- *Analgesic activity:* Pain killer can also be used for the word analgesic. Analgesic are the group of drugs that are used to give relief from pain without disturbing the consciousness. *Madhuca longifolia* exhibited the analgesic effect from both methanolic and ethanolic extracts. The activity was assessed on acetic acid writhing. Six animals were orally given the methanolic extract of *M. longifolia*. The number of writhing during the subsequent 30 minutes was witnessed after acetic acid injection (Verma et al., 2014). Anti-analgesia is expressed as the decrease in the number of abdominal constrictions between control animal and mice pre-treated with the extract. The analgesic effect was also screened through the tail flick, hot plate, and chemical graded doses of both aqueous and alcoholic extract of *M. longifolia* (4.0 64.0 mg/kg, i.m. for 3 days) produced a dose-dependent

analgesic effect in all the three nociceptive methods carried out either in rats or mice (Patel et al., 2012).

- *Antipyretic activity:* Antipyretic can be defined as a term that works against fever. The prostaglandin is responsible for fever, and the antipyretics causes the hypothalamus to override the prostaglandins which results in a reduction in fever (Khare et al., 2018). In animal experiments, the mahua extract was found to reduce fever (Patel et al., 2019).
- *Antihyperglycemic activity:* The important hypoglycemic effects of Mahua bark in diabetic rats specify that this effect can be facilitated by stimulation of glucose consumption by peripheral tissues. The consequences of the study specified the ethanolic extract of Mahua bark to have a hypoglycemic effect on STZ-induced diabetic rats. In all groups except for glibenclamide, at 30 minutes of initiating the glucose tolerance test, blood glucose concentration was higher than at zero time but decreased significantly from 30 to 120 minutes (Yadav et al., 2012). Methanolic extracts were improving glucose operation; thus the blood glucose level was expressively reduced in glucose-loaded rats. Methanolic extract of Madhuca has considerably reduced the serum glucose level in streptozotocin and STZ NIC-induced diabetic rats. According to Patel et al. (2012), the crude methanolic extract of Mahua leaves confirmed dose-dependent reductions in serum glucose level succeeding administration in glucose-loaded mice. The serum glucose levels were found to be considerably reduced at doses of 100, 250, and 500 mg extract per kg body weight.
- *Antiulcer activity:* Ulcer is considered as a common complaint of gastrointestinal tract (Patel et al., 2019). Due to the imbalance in the defensive and attacking factor of GIT, there is a result in ulcer. The alcoholic extract of mahua flower is used to study the antiulcer activity. The experiment was conducted with the help of Wistar rats by pylorus ligation method, and the standard drug used was ranitidine. The alcoholic extract of mahua and the standard drug were given simultaneously 2 days before pylorus ligation, and the gastric juice was measured. The increase in the amount of gastric juice could be due to the inhibition of histamine which exaggerates acid release (Mohod & Bodhankar, 2013).
- *Antioxidant activity:* Free radicals are the cause of various diseases such as aging, cancer, heart disease, etc. Generally, antioxidants protect our cells from free radicals (Verma et al., 2013). Higher the phenolic content, more the antioxidant activity because the phenol groups donate the hydrogen atom. The antioxidant power of a drug depends upon two mechanisms, first to prevent oxidation by oxidizing itself or second by generating a layer

of protection over the material. The methanolic bark extract of mahua showed antioxidant activity, that is, capable of donating hydrogen atoms (Khare et al., 2018).

- *Antifertility activity:* Some studies revealed that mahua has antifertility activity. The term antifertility means a sub stance that inhibits the ability to produce offspring (Verma et al., 2014). Male and female mice were taken for the study, and the study revealed that the percentage of fertility in the case of males and females has decreased in atropine-induced mice. It decreases the sperm count and reduced the spermatozoa density (Patel et al., 2012).
- *Dermatological activity:* As the condition of the environment is changing, there are a lot of skin-related problems people are facing in their daily life (Verma et al., 2014). There are several marketed cosmetic products which sometimes cause skin irritation, itching, etc. Mahua seed oil could be used as an alternative for the treatment of various skin-related problems. Traditionally, the tribes of many states use the mahua oil as a lotion (Patel et al., 2019).
- *Hepatoprotective activity:* To study the hepatoprotective activity, the albino rats were injected with CCl4 or carbon tetrachloride. Mahua methanolic extract has shown hepatoprotective activity (dose-dependent 300 mg/kg body weight) (Patel et al., 2019). The methanolic extract was proved to be effective against lowering the serum level of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphate (ALKP), and total bilirubin. It has also increased the level of total proteins and albumin (Yadav et al., 2012).
- *Antibacterial activity:* According to Khare et al. (2018), the flower extract shows antibacterial activity against *Escherichia coli* and has the ability to resist rice pest disease. By disk diffusion method, the methanolic extract of dried bark shows a significant effect against *Bacillus subtilis, Staphylococcus aureus, Staphylococcus, E. coli*. Patel et al. (2019) also informed that the ethanolic extract showed to be having good antibacterial activity. For the treatment of diarrhea, the methanolic extract of mahua flowers showed effective results against *S. aureus, Salmonella typhi, Bacillus pumilus, Proteus vulgaris, E. coli, B. subtilis, Candida albicans, Pseudomonas aeruginosa, and Micrococcus luteus*. The methanolic extract of mahua was used to study the antimicrobial study by disk diffusion method in which three fungal strains and three bacterial strains were taken, that

is, *E. coli, Pseudomonas, S. aureus, Aspergillus niger, Penicillium spp., Scytalidium spp.* All of them showed positive results (Patel et al., 2012).

- *Antiepileptic activity:* Verma et al. (2014) and Patel et al. (2012) revealed that *M. longifolia* retains significant anti-epileptic activity as it amplified the onset time of seizure and reduces the period of seizures. The methanolic extract of mahua has shown antiepileptic activity. The mouse was induced with pentylenetetrazol-induced seizures (PTZ), and diazepam has been used as a standard drug. The researchers have successfully studied the involvement of benzo diazepine and opioid receptor.
- Antinociceptive and antidiarrheal activity: Khare et al. (2018) and Patel et al. (2019) studied that diclofenac sodium was used as a standard drug for the comparison of the response. The mice showed antinociceptive and antidiarrheal activity in the alcoholic extract of the bark. Mahua bark extract showed to be effective in monitoring the writhing reflex induced by acetic acid at a dose of 250 and 500 mg/kg of body weight by oral route.
- *Antihelminthic activity:* Mahua plant has numerous phytochemical constituents in it. It contains protein, starch, phenols, flavonoids, tannins, terpenoids, and many more (Khare et al., 2018). The study was done by in vivo method, and Pheretima posthuma (adult Indian earthworm) was used in the study. Metronidazole was used as a standard drug. The alcoholic and hydroalcoholic extract has shown antihelminthic activity due to the presence of tannins and other phytochemical constituents (Patel et al., 2019).
- *Immunomodulatory activity:* The alcoholic extract of mahua showed significantly good immunomodulatory activity. Cyclophosphamide-induced myelosuppression in mice was tested. The mahua extract improved the DTH response and antibody titer value and also triggered the renovation of total leukocyte count (TLC) and differential leukocyte count (DLC) (Khare et al., 2018).
- *Larvicidal and ovicidal activity:* Not only the mahua oil but also the mahua seed cake have shown larvicidal and ovicidal activity against Meloidogyne incognita, and they were also effective against larval growth from the egg sacs of cyst nematodes (Khare et al., 2018).
- *Antidiabetic activity:* Yadav et al. (2012) reviewed that the alcoholic extract of mahua leaf and bark was used for the study of the antidiabetic property. The albino Wistar rat was induced by streptozotocin, and after 30 days, it was compared with insulin-treated rat. The experiment showed a decrease in blood sugar level.

- *Spasmolytic activity:* Saponin is responsible for the spasmolytic activity in the alcoholic extract of mahua. The saponins extracted from mahua leaves influenced a major spasmolytic activity. The saponins existing in the leaves, and seeds of *M. longifolia* possessed spasmolytic property on isolated guinea pig ileum (Patel et al., 2019).
- *Spermicidal activity:* Khare et al. (2018) studied the steroid and triterpenoid saponins existing in *M. longifolia* seeds which influenced clear spermicidal activity.
- *Insecticidal and pesticidal activity:* Mahua cake influenced an important insecticidal and pesticidal action against phytonematode. Mahua has shown pesticidal activity against *Tetranychus urticae* (Khare et al., 2018).
- *Wound-healing activity:* According to Yadav et al. (2012) and Jha and Mazumder (2018), methanolic extract of mahua bark was used to treat the wound of the mice. Betadine was used as a standard drug. Mahua extract-treated animals showed a substantial decrease in wound area and period of epithelization. Mahua extract showed a faster wound-healing activity than the standard drug.
- *Nephroprotective activity:* Against acetaminophen-induced nephrotoxicity, the alcoholic extract of mahua possesses nephroprotective activity. There was an increase in the levels of serum urea, hemoglobin (Hb), total leukocyte count, creatinine, filled cell volume, DLC, mean corpuscular volume, and upraised body weight sideways with condensed levels of neutrophils, mean corpuscular Hb content, mean corpuscular hematocrit, granulocytes, uric acid, and plate let concentrations (Khare et al., 2018; Patel et al., 2019).
- *Neuropharmacological activity:* For the study of neuropharmacological activity, tramadol hydrochloride, diazepam, and chlorpromazine were used as a standard drug against Swiss albino male mice. The activity was measured via phenobarbitone sodium-induced sleep and their antagonistic regaining righting effects by mahua extract. Actophotometer was used for spontaneous motor activity. There was a decrease in sleeping time and a reduction in spontaneous motor activity with the use of actophotometer (Patel et al., 2019)
- *Rheumatic arthritis:* Patel et al. (2019) considered that arthritis can also be called as an inflammatory disorder which is usually caused by a lack of synovial fluid. Lack of synovial fluid can cause loss of lubrication which in turn may cause severe pain in the joints. Mahua oil was used for the action of rheumatic arthritis.
- *Anticancer activity:* According to Yadav et al. (2012), the cytotoxic activity against Ehrlich ascites carcinoma cell lines using different in vitro cytotoxic assays at 200 μg/mL

was shown by the alcoholic extract of mahua leaves. Patel et al. (2012) found that both extracts revealed important cytotoxic action, but greater cytotoxic activity was found in ethanol extract.

- *Toxicity:* A combination of saponins from *M. longifolia* seed did not disclose any cholinergic action, though it was shaped at an advanced concentration (Patel et al., 2019). The saponin is tremendously toxic after it has been induced parentally, and LD50 by the IV route was found to be 50 70 times higher than the oral route. In the root of M. indi ca, the extreme quantity of phenol was detected, that is, 46.0 mg/g dry weight. These complexes showed an important part in the precursor of toxic constituents. Patel et al. (2012) also described having toxic biochemical such as aflatoxine in *M. indica* seed oil. Rajgor et al. (1986) took 24 albino rats and fed them with ordinary boiled and pressure-cooked mahua flowers which showed that there was no change in food intake. But the organ weight was significantly lower in the ordinary-fed group compared to the pressure cooker fed. Bora & Singh (1994) observed the feeding of dried mahua as a substitute for maize. They concluded that dried flowers can be incorporated into the gill ration as a substitute for maize. Kotwal (2000) studied the feeding diet of processed (without saponin) and unprocessed (with saponin) mahua syrup on the biochemical and histopathological status of albino rats. According to the findings, the unprocessed syrup showed some desirable changes in the parameters, but the processed syrup does not show any changes.

1.3.9 Mahua as a functional food

1.3.9.1 Processing of flowers

Collection of flowers

The primary collectors or usually the tribal families collect the fresh flowers in the early morning (Mishra & Poonia, 2019). A bamboo stick is used to pluck the flowers from the trees. Before the collection of flowers, the ground is cleaned, and the floor is cleared from leaves and grass (Bakhara et al., 2016; Chandel et al., 2018). The time required for the collection of flowers may vary because the mahua flowers are collected manually from the ground.

Preprocessing

The flowers are preprocessed to increase their shelf life (Mishra & Poonia, 2019). The preprocessing methods include washing, stamen removal, and blanching with preservatives. Sometimes, the stamen is removed manually or mechani cally. To reduce the rate of spoilage, the flowers are free from moisture which includes shed drying, sun drying, and tray drying (Kumari et al., 2018)

Drying

Before drying pretreatment was given to the mahua flower with 4.1 minutes of blanching, 1285 ppm KMS, and 0.77% citric acid concentration (Pinakin et al., 2018). The tribals used to dry the flowers by spreading them in a clean dry place for 3 4 days under the sun (Chandel et al., 2018). After the flowers are completely dried, the stamen is detached from the flowers manually. Stamen removal is necessary because it gives a bitter taste which is mostly disliked by the customers (Bakhara et al., 2016). Storage: The dried flowers are stored in dark rooms packed in gunny bags. But the flowers absorb moisture easily from the earthen floor or roof which is more prone to bacterial and fungal spoilage (Bakhara et al., 2016; Mishra & Poonia, 2019).

Postharvest spoilage of flowers

Due to the lack of storage facility, about 30% of the flowers get spoiled. Fresh flowers are highly nutritious and have high moisture content which leads to bacterial and fungal spoilage (Behera & Swain, 2013). The bacteria that involve in the spoilage are *Bacillus, Micrococcus, Siderococcus, Nocardia, and Pseudomonas,* and the fungi that are involved in the spoilage include *A. niger, Aspergillus flavus, Penicillium,* and *Rhizopus* (Mishra & Poonia, 2019). Due to bacterial and fungal spoilage, the amount of sugar and ascorbic acid decreases. Certain insects and larvae are also involved Exploring the role of Mahua as a functional food and its future perspectives Chapter | 7 117 in the spoilage of the flowers which belong to the family of Noctuidae, Anthocoridae, Cucujidae, Bostrychidae, Tephritidae, and Formicidae (Behera & Swain, 2013).

Methods of preservation

Since mahua is a multipurpose tree having high moisture content and is rich in nutritional compounds, it is more likely to have spoilage. One of the spoilage is caused by *Fusarium solani* (Verma et al., 2014). The spoilage of the flowers is mainly due to its high moisture content, lack of storage facilities, and inadequate knowledge of further processing.

- Moisture content of the flowers can be reduced by the use of modern machinery such as oven drying, hot air drying, tray drying, solar drying, cabinet drying, etc. Reduction in moisture content is an unfavorable environment for the growth of microorganisms. Humectants are used to keep moisture away from the flowers.

- To check the spoilage, the flowers are treated with UV and certain antibiotics. The flowers should be kept in a controlled atmosphere. Government warehouses should be built-in tribal areas so that the poor can avail the facilities and can keep their raw materials in a good condition.
- Das et al. (2010) found that the proper use of liquid nitrogen to powder the flowers can lead to a decrease in spoil age, and deep-freeze flowers are found to be less prone to microbial growth

1.3.9.2 Value-added food products

Sugar syrup is the oldest food product that is prepared by researchers. Sugar syrup was prepared by Abhyankar & Narayana (1942) in which they found that there is 75% 87% of reducing sugar present in the flowers. In the process of preparing the sugar syrup, they extracted 90% of the sugar with hot water. The syrup was clarified with the addition of slacked lime followed by superphosphate. The syrup was passed through activated charcoal and evaporated to a consistency of 70 75 Brix. Shrivastava et al. (1970) extracted the sugar syrup in two different ways. The first method includes the extraction process directly from fresh water, and the second method was the extraction done from the previous extraction. The sec ond method was more effective and economical that takes less time. Chand & Mahaptra (1983) prepared the sugar syrup from dried mahua flowers. The syrup was first clarified using lime, and pH was maintained by adding citric acid solution. The addition of 50 ppm of metabisulphite to the syrup has increased the shelf life. Since mahua is rich in sugar, it has the potential for making good-quality fermented products. Soni & Dey (2013) observed that the fermented mahua when added with guava improved the flavor of the wine. The addition of guava has increased the antioxidant activity. Singh et al. (2013) investigated that mahua flower juice has a maximum yield of eth anol at 25C with a pH 4.5 after 14 days of fermentation. The alcoholic beverage developed by Singh et al. (2013) has the characteristic flavor and aroma of mahua flowers. Dushing & Surve (2019) developed wine from mahua flower extract and pomegranate fruit juice. The result showed that S. cerevisiae (NCIM-3215) produced better results after 7 days of fermentation, and the wine was acceptable at a proportion of (20:80). Yadav et al. (2009) standardized a pre-treatment condition for mahua wine. They have optimized that a dip of KMS for 10 minutes leads to a decrease in microbial population, and heating at 100C and 500 ppm KMS sulphitation are required for self-stable mahua flower juice. Patel et al. (2016) introduced an antioxidant-rich beverage from mahua flower and amla. Several blends were pre pared, but a blend of 40 Brix mahua having 50% amla was best of all. Singh et al. (2018) developed cupcakes from mahua flower syrup which has 100% replaced sugar, and the product was also acceptable by the panelist. Likewise, Ravat & Dixit (2017) developed gluten-free biscuits incorporated with mahua powder as natural sugar. The gluten-free biscuits had a shelf life of 60 days. According to Pinakin et al. (2018), several researchers have developed value-added products from mahua flowers such as puree and sauces, juice, mahua jam, jelly, marmalade, mahua bar, mahua candy, mahua toffee, mahua cake, mahua squash, mahua ladoo, mahua RTS. The seed oil can be used as food supplements having proper health benefits (Yadav et al., 2011). The chemical composition of mahua oil is the same as other edible oils. Rukmini (1990) compared mahua oil with groundnut oil which showed maximum similarities in the nutritional properties.

Other uses:

- Mahua yeast was extracted from the alcohol factory that contains some vitamins in it. The yeast extracted from mahua was far better than distillery yeast (Daver & Ahmed, 1944)
- A new type of yeast Zygosaccharomyces mahwae was isolated by Lender (1992).
- Organic manure was prepared by adding mahua to the waste organic matter (Fowler & Gilber, 1930).
- The role of molasses, mahua flower, and mannitol in nitrogen fixation was reported by Patel & Kibe (1951).
- Hasan et al. (1928) reported that when the cattle were given mahua there was an increase in the health status and quality of the milk.
- A new agar medium for the fungal culture was developed by Saha & Singh (1991)

1.3.10 Health benefits of mahua

According to Mishra & Pradhan (2013), there are several health benefits of mahua. It can be used to clear chronic bron chitis problems and can be used as a remedy for cough and tonsillitis. For testis inflammation, mahua leaves are used. A decoction of bark in water is taken orally for the relief of rheumatism. Sometimes oil is also used in the affected area to get relief. It can be used for the treatment of diabetes. Mahua seed oil has laxative properties so it can be used for constipation and piles. Mahua leaves are effective against eczema and bleeding gums. Mahua leaf ash is used to cure itching. The feeding mothers consume mahua flowers to increase lactation.

1.3.11 Current trends and future perspectives

After the study of all the information available, we can conclude that the mahua tree is a boon to our environment. Nature has gifted us with this tree which is a rich source of natural sugar and different phytochemical constituents. Mahua is a perfectly balanced and fully synchronized plant that gives traditional, pharmacological, and economic benefits. Besides its utilization as food, fodder, and fuel, it has antibacterial, anticancer, hepatoprotective, antihyperglycemic, analgesic activities, etc., with certain health importance which helps to treat bronchitis, cough, piles, eczema, bleeding gums. It has also certain important chemical constituents which include flavonoids, glycosides, alkaloids, tannins, and terpenoids. For a long time, mahua is being used in the production of country liquor, but if the tribes are trained with available food technologies, they can market the mahua food products which in turn can raise their standard of living. To increase the commercial utilization of the flowers, there should be an implementation of advanced technologies by which they can make different value-added food products. Government and NGOs should aware people of the utilization of mahua flowers by conducting different entrepreneurship programs by which there will be an enhancement of income sources among the poor people.

CHAPTER-II

REVIEW OF LITERATURE

2. Background

Mahua is a multipurpose tree with high economic importance, as it grows in the leanest season of agriculture and mainly fulfils the 3 major F's, i.e., food, demand and fuel. Excellent review articles have been published on different aspects of the tree in the past several years. However, since the present doctoral thesis is focused on the mahua flower, it is crucial to review all the former works that have focused on different aspects of flowers to determine their present status.

2.1 Distribution and Habitat

The different species of mahua grow in dry tropical and subtropical forests up to an altitude of 1200–1800 mt in India. For proper growth, the plant requires a temperature of 2–460°C, annual rainfall of 550–1500 mm and humidity of 40–90%. However, trees do not survive in waterlogged conditions (Bisht et al., 2018). They are considered hardy trees and are grown in the pockets of soil between the cervices of barren rocks (Bisht et al., 2018). It is well distributed in Asian countries such as India and the Philippines. Pakistan. Sri Lanka, etc. The plant is well grown in the pasture lands of Central India and in the river banks in semievergreen forests. In India, trees are mostly found in areas such as Odisha, Chhattisgarh, Uttar Pradesh, Andhra Pradesh, Maharashtra, Bihar, West Bengal, Karnataka, Gujarat, and Rajasthan. The annual production of mahua flowers is 45000 million tonnes. The yield of mahua flowers from each tree is approximately 80--320 kg (Pinakin et al., 2018). The complete geographical distribution of Mahua trees in India is given in Fig. 6.

Figure 6 The figure depicts the mahua production belt of India

2.2 Morphological studies

Fowler et al. (1920) studied the morphology of fresh flowers and described certain stages, as mentioned below:

- In the 1st stage, the flower bud is hard, and it is completely closed with the calyx.
- In the 2nd stage, the style is protruded, but the buds are still closed. Over time, the flowers became more flaccid and softer, and the calyx gradually separated into the sepals.
- In the 3rd stage, the lobes of the corolla are visible, the flowers are partially open, and the sepals are separated. During this stage, the anthers mature, and the pollen begins to shed.
- In the last stage, the flowers are fully mature and ripe and are ready to shed. In this stage, the corolla is more translucent than it was in the previous stage.

They also studied the presence of tannins in epidermal cells and reported that starch is absent in all four stages. Moreover, sucrose, fructose, glucose and maltose were present in the juice of fresh flowers.

Mehta et al. (2016) reported that the length, width, and weight of fresh and dry mahua flowers did not significantly vary within populations. The mean widths of the fresh and dry flowers were 19.82 ± 5.48 cm and 4.43 ± 1.06 cm, respectively, and the mean widths were 0.97 ± 0.12 cm and 1.37 ± 0.17 cm, respectively. The weights (in g) of the selected populations of *Madhuca indica* are listed in Table 2.1.

Table 2.1: Weights (g) of the selected populations of *M. indica*

Sl. No.	Populations	Weight of the flowers (in g)	
		Fresh Flowers	Dry Flowers
01	Annupur	21.36 ± 5.12	4.65 ± 0.94
02	Balaghat	19.35 ± 5.66	3.55 ± 0.65
03	Dindori	16.63 ± 4.34	4.84 ± 1.00
04	Jabalpur	20.25 ± 4.52	4.76 ± 0.86
05	Mandla	22.20 ± 6.22	5.11 ± 1.06
06	Seoni	17.33 ± 6.64	4.35 ± 1.31
07	Shahdol	19.75 ± 5.22	3.90 ± 0.99
08	Umariya	21.71 ± 3.71	4.30 ± 0.79

2.3 Biochemical investigations

The proximate compositions of fresh and dry mahua flowers have been studied and reported by various reviewers. Table 2.2 provides a detailed account of previous studies on these flowers.

2.3.1 Sugars

Reducing and nonreducing sugars, such as dextrose, levulose, maltose, sucrose, pentose, and cellulose, are sugars that have been identified by many researchers. On the basis of this review, it can be said that flowers contain the greatest amount of sugar when they are ready to fall. The amount of levulose is greater than that of dextrose when the flower is in the growing stage. During the shedding period of the corolla, the amount of sucrose increased, but during the storage period, it decreased. However, in the final stages, the quantity approximates but does not become equal.

Table 2.2: Sugar content of *Madhuca indica* flowers according to different researchers

Sl. No	Reviewers	Reducing sugar (%)	Non reducing sugar (%)	Total sugar (%)
01	Church,1886	52.4	3.2	-
02	Ruediger, 1913	-	-	70.8
03	Elworthy, 1887	40-45.3	4.6-17.1	46-57.1

04	Fowler et al., 1920	-	2.4-21.7	40-60
05	Sutaria and Magar, 1955	48-55	14-18	65-70
06	Roy and Rao, 1959	57.3	9.4	-
07	Belavady and Balsubramaniam, 1959	-	-	72.9
08	Jaysree et al., 1998	-	-	68
09	Gopalan et al., 2004	-	-	22.7

These results indicate that geographic location and climatic conditions are also responsible for sugar composition. The flowers collected from different locations, such as Hyderabad, Jabalpur, Gujarat, Mirzapur and Maharashtra, presented different percentages of reducing sugars, nonreducing sugars, and total sugars, as described in Table 2.3.

Table 2.3: Sugar contents of mahua flowers collected from different locations

Location	Cane sugar (%)	Invert sugar (%)	Reducing sugar (%)	Total sugar (%)
Hyderabad	17.1	40.0	-	57.1
Jabalpur	4.6	41.4	-	46.0
Gujarat	9.6	45.3	-	54.9
Mirzapur	6.7	-	43.6	50.3
Nasik	-	18.03	52.8	70.31
Nadiad	-	14.9	49.85	70.1
Godra	-	14.57	54.95	69.52
E. khandesh	-	16.51	49.21	65.72
Prantij	-	16.35	50.21	66.65
Himatnagar	-	17.27	49.85	67.12

2.3.2 Polysaccharides

As stated above, the sugar content of flowers varies across geographical locations and varieties of mahua flowers. In accordance with Patel and Naik, (2010), polysaccharides can be extracted via hydrolysed and unhydrolysed extraction methods. In unhydrolysed extraction methods, glucose, maltose, sucrose, arabinose, fructose, and rhamnose are found via paper chromatographic analysis. In the hydrolysed extraction method, polysaccharides such as rhamnose, glucose, fructose and arabinose are mostly found. On the basis of the data of Sarkar & Chatterjee (1983), dry mahua flowers contain water-soluble polysaccharides. Water-soluble polysaccharides include D-galactose, L-rhamnose, D-glucuronic acid, L-arabinose, L-xylose, etc.

2.3.3 Protein and amino acids

Jayasree et al. (1998) compared the amino acid composition of mahua flowers with that of ground nuts. This study revealed that the amino acid composition of mahua is superior. Table 2.4 shows the amino acid compositions of the mahua flowers and groundnuts. According to the analysis performed with an automatic amino acid analyser, the amounts of lysine, cysteine, and methionine were much greater. Mishra and Poonia, 2019 reported the presence of eleven different amino acids, such as lysine, arginine, aspartic acid etc.

According to Belavady & Balsubramanian, 1959, and Jayasree et al., 1998, the protein content of flowers varies from 4.4% to 7%. Bisht et al. (2019) and Pinakin et al. (2018) reported the presence of protein (6.05–6.37%) in mahua flowers.

Table 2.4: Amino acid composition of mahua flowers compared with groundnut cake flowers

Sl. No	Amino acid	Mahua flowers (g/100 g)	Groundnut cake (g/100 g)
01	Phenylalanine	6.4	5.2
02	Isoleucine	7.91	4.0
03	Cystine	3.35	1.3
04	Glysine	9.12	4.8
05	Glutamic acid	22.8	9.6
06	Lysine	4.67	5.1
07	Threonine	5.86	3.9
08	Proline	7.1	11.0
09	Tyrosine	3.94	3.7
10	Methionine	1.8	1.4
11	Valine	7.25	5.0

12	Histidine	3.63	2.4
13	Leucine	12.98	7.0
14	Arginine	4.54	6.3
15	Aspartic acid	20.28	12.8
16	Alanine	9.31	5.00
17	Serine	7.29	4.0

2.3.4 Fat and Fatty acids

On the basis of the literature, dry mahua flowers contain a minimal amount of fat, which varies from 0.09%-1.3% (**Jayasree et al., 1998, Sutaria & Magar, 1955a**). By the paper chromatography method, four different fatty acids, oleic acid, stearic acid, linoleic acid and palmitic acid, were detected. **Table 2.5** shows the detailed fatty acid composition of fat as studied by **Jayasree et al., 1998.** The data indicate that the fatty acids present in mahua flowers are similar to those present in kernel oil.

Table 2.5: Fatty acid composition of Mahua flowers

Sl. No	**Fatty acids**	**Percentage on total fat basis**
01	Capric 10:0	10.0 (11.3)
02	Lignoceric 24:0	4.0 (4.5)
03	Lauric 12:0	13.1 (14.8)
04	Behenic 22:0	2.4 (2.7)
05	Lauroleic 12:1	0.2 (0.2)
06	Linolenic 18:2	21.3 (24.0)
07	Myristic 14:0	3.6 (4.0)
08	Oleic 18:1	17.0 (19.2)
09	Palmitic 16:0	11.6 (13.1)
10	Myristoleic 14:1	0.5 (0.6)
11	Linolenic 18:2	21.3 (24.0)

2.3.5 Vitamins

According to reports, mahua is sweet in taste, as it is a rich source of sugars. However, the flowers of *Madhuca indica* are an ironic source of vitamins. The major vitamins found in the flowers, as shown in Table 9, include ascorbic acid, thiamine, riboflavin, niacin, folic acid, etc. The presence of ascorbic acid or vitamin C increases the antioxidant properties of the flowers (Table 2.6).

Table 2.6: Major vitamins found in Mahua flowers on a fresh and dry weight basis

Sl. No	Name of authors	Vitamin-C (mg)	Thiamine (µg)	Riboflavin (µg)	Niacin (µg)	Folic acid (µg)
01	Sutaria & Magar, 1955b[1]	29	110	660	1.413	167.8
02	Sutaria & Magar, 1955b[2]	36.99	140.3	841.8	1.802	214
03	Belavady& Balsubramanian, 1959[3]	7	841.8	878	5.2	-
04	Jayashree et al., 1998[4]	17	214	870	4.8	-

*** [1] on fresh weight basis, [2, 3, and 4] on fresh weight basis**

2.3.6 Minerals

The major role of minerals is to produce enzymes and hormones in the body. The consumption of an adequate amount of minerals keeps the bone, muscle and heart in operation properly. Major minerals, including calcium, potassium, iron, sodium, magnesium and phosphorous, are found in dry mahua flowers. According to Mishra and Poonia, 2019, the mineral composition of mahua flowers varies from 2.5–4.5 g/100 g. Table 2.7 provides the detailed compositions of the minerals present in Mahua flowers.

Table 2.7: Composition of minerals (in %) in Mahua flowers

Sl. No	Minerals (%)	Sutaria & Magar, 1955b[1]	Sutaria & Magar, 1955b[2]	Belavady& Balsubramanian, 1959[3]	Jayashree et al., 1998[4]
01	Calcium	0.25	-	0.14	0.14

02	Phosphorous	0.13	-	0.14	0.14
03	Iron	0.03	-	0.015	-
04	Magnesium	0.21	-	-	-
05	Sodium	-	0.025	-	-
06	Potassium	-	1.2	-	-

2.3.7 Enzymes

The key work of enzymes is to fasten the chemical reactions that are taking place in our body. Researchers have detected the presence of several enzymes, including oxidase, maltase, catalase, invertase and amylase (Kureel et al., 2009).

2.4 Storage study

As discussed in the introduction section, flower preprocessing is considered an important step. Ignoring the preprocessing step leads to spoilage of the flowers, as they are a rich source of sugar. The dry flowers are typically stored in gunny bags in an unventilated room. Mahua are hygroscopic in nature, so they absorb moisture. The high moisture content of flowers attracts microbes, and postharvest spoilage of flowers is initiated (Behera et al., 2012). Traditionally, the processing of fresh flowers is quite time-consuming. The fresh flowers are subjected to sun drying by spreading in the yards. When flowers lose approximately 80% of their moisture, they are further stored. Furthermore, the flowers are cleaned by beating with a wooden log to remove the stamen and corolla.

2.5 Comparative Nutritional Profile

Several researchers have compared the nutritional aspects of dry mahua flowers with those of different locally available fruits, such as mangos, rasins, bananas, and apples. These researchers have clearly reported that the nutritional value of dry mahua flowers is greater than that of other fruits. In dry mahua flowers, the protein content (per 100 g) was found to be highest, i.e., 6.67, the calcium content was found to be 139 mg/100 g; likewise, riboflavin and niacin were found to be the highest in dry mahua flowers. Interestingly, the fat percentage was found to be lowest in dry mahua flowers, i.e., 0.09%, as depicted in Table 2.8. Mandal & Bhattacharyya (1993) determined the carbohydrate, protein, lipid, nucleic acid, mineral, ash, moisture, calcium and magnesium contents of the pollens of mahua flowers.

Table 2.8: Nutritional profile of various fruits vs. mahua flowers (Gopalan et al., 2004)

Nutritional profile (per 100 g)	Apple	Banana (ripe)	Mango	Raisins	Mahua (ripe)	Mahua (Dry)
Moisture	84.6	70.1	81	20.2	73.6	11.61
Protein	0.2	1.2	0.6	1.8	1.4	6.67
Fat	0.5	0.3	0.4	0.3	1.6	0.09
Minerals	0.3	0.8	0.4	2.0	0.7	-
Fibre	1	0.4	0.7	1.1		1.9
Carbohydrates	13.4	27.2	16.9	74.6	22.7	68
Energy (Kcal)	59	116	74	308	111	-
Calcium (mg)	10	17	14	87	45	139
Phosphorous (mg)	14	36	16	7.7	22	139
Iron (mg)	0.66	0.36	1.3	2.4	0.23	137
Carotene (μg)	0	78	2743	0.07	307	-
Thiamine (mg)		0.05	0.08	0.19	-	-
Riboflavin (mg)		0.08	0.09	0.7	-	0.028
Niacin (mg)	0	0.5	0.9	-	-	0.87
Vitamin C (mg)	1	7	16	-	40	4.8
Choline (mg)	321	-	-		-	-

2.6 Traditional uses

Traditionally, mahua is used as a substitute for staple grains. Owing to the lack of knowledge about the nutritional aspects of mahua flowers, the tribals used flowers for the preparation of "*country liquor*", spirits, vinegar and animal feed. 2-Acetyl-1-pyrroline, a compound responsible for the pleasant smell of basmati rice, is found in fresh mahua flowers. Locally, the flowers are used for the preparation of various sweet dishes, such as halwa, kheer, and barfi, in different belts of India. However, the degree of postharvest loss is greatest in dry flowers because of a lack of proper knowledge and scientific investigations. In addition, the flowers also have various ethno-medicinal uses, as depicted in Table 2.9. The ethno-medicinal uses include anti-inflammatory activities, antidiabetic activities, antimicrobial potential, analgesic properties, etc.

Table 2.9: Traditional uses of mahua flowers

Sl. No	Plant parts used	Traditional uses	References
01	Flower juice	Cure skin diseases	Sinha et al., 2017
02	Flowers	Flower decoction is used for the treatment of pitta	Sinha et al., 2017
03	Flowers	Used to cure diarrhea and colitis due to astringent property	Bisht et al., 2018
04	Flower juice	Fresh flower juice is used to arrest bleeding	Sinha et al., 2017
05	Flower juice	Used as nasal drops for the treatment of headache due to sinusitis	Sinha et al., 2017
06	Flower	Analgesic properties	Chandra et al., 2001
07	Bark	Antidiabetic activities, cures rheumatism	Kumar et al., 2011
08	Leaf	Hepatoprotective, Cytotoxicity, Antioxidant and Wound healing activities	Sinha et al., 2017
09	Stem	Antimicrobial activities	Khond et al., 2009
10	Seed cake	Anti-inflammatory, anti-ulcer, and hypoglycemic activity	Das et al., 2022
11	Raw flower	Increases lactation	Mishra and Padhan, 2013
12	Flower	Flowers when mixed with milk cures impotency	Yadav etal., 2024
13	Flower	Flowers roasted with ghee acts as a cooling agent to cure piles	Sinha et al., 2017
14	Flower	Cures eye diseases	Khare et al., 2018
15	Flower	Used as a tonic to cure chronic bronchitis, pharyngitis and bronchitis	Mishra and Padhan, 2013

2.7 Mahua as a food product

- ***Raw intake of*** mahua: Despite the high availability and rich source of nutrition for mahua flowers in the Indian belt, the consumption of mahua flowers as a source of food supplements in rural and urban belts of India is much lower. Only a small portion of the total production of mahua flowers is consumed raw, cooked or fried (Bisht et al., 2018).
- ***Sugar syrup:*** Sugar syrup was prepared from dry mahua flowers because of its sweetness (Benerji et al., 2010). Before the concentrate was prepared, it was decolorized using slacked lime and activated charcoal. Activated charcoal was used at a concentration of 3.5–5.0%, which is considered the best decolorizing agent for the preparation of the desired concentrate (Patel and Naik, 2010). The desired syrup, which is used as a sweetening agent, was used for the preparation of various food products. Different researchers have used alternative methods for the preparation of sugar syrup from mahua flowers. Abhyankar and Narayana, 1942, extracted 90% of sugar during the preparation of syrup with hot water. The syrup was clarified via slacked lime, which was then passed over by activated charcoal. The syrup retained 47.79% of the reducing sugars and 12.96% of the nonreducing sugars. Sutaria and Magar, 1955, extracted sugar syrup from dry mahua flowers with hot/cold distilled water and ethyl alcohol. They have clarified the juice using lime, magnesium salt, activated charcoal, etc. The study revealed that activated charcoal was the best clarifying agent, removing up to 82–85% of the color. Chand & Mahapatra (1983) reported the production of sugar syrup from dried mahua (*M. latifolia*) via a two-step countercurrent extraction procedure. The extracted syrup was clarified by adding lime, and finally, the pH was maintained at 5.5 by adding citric acid solution. After clarification, the syrup was subjected to decolorization by using activated carbon at two stages. First, the syrup was treated with 5% (w/v) activated carbon at 80°C for 20 minutes. The extract was filtered under suction, and the filtrate was again decolorized with 3% (w/v) activated carbon under the same conditions. The decolorized filtrate, having a light yellow color, was concentrated under vacuum to one third of its volume. The concentrated syrup was stored in a glass bottle after adding 50 ppm (w/v) sodium metabisulphite. The authors reported the production of 550 kg of syrup from 1 ton of dried mahua flower.
- ***Jam, jelly, marmalade, and pickle:*** Jam was prepared in addition to citric acid using fully grown mature fruits that are still unripe. Furthermore, the pulp is converted to marmalade, which is also a food product. Similarly, jelly was prepared from mahua flowers and guava

pulp, and the pulp was also used for the preparation of pickles (Mishra et al., 2021). The jam and jelly were also prepared from fresh mahua flowers by Patel, 2008. The overall acceptability (texture, color, flavour, taste, etc.) of the prepared products was tested via a hedonic test.

- ***Confectionary and bakery:*** Due to the sweet taste of the mahua flower concentrate, it is used for the preparation of several products, such as cakes, biscuits and candy (Bisht et al., 2018).
- ***Sauces and Purees:*** After the stamens are removed, fresh flowers are used for the preparation of purees, which are further processed to make sauces (Patel, 2008).
- ***Fermented products:*** Due to their high sugar content, dry mahua flowers act as a rich source of fermented products. Yadav et al., 2009, prepared wine from fresh mahua flowers. Fowler et al., 1920 mentioned several fermented products from mahua flowers, including brandy, ethanol, lactic acid, alcohol, acetone, etc.

2.8 Other uses

Daver et al. (1944) extracted mahua yeast from an alcohol factory and analysed its vitamin contents. According to their study, yeast extracted from mahua samples compared favourably with torula yeast and was better than distillery yeast. Lender, 1922, provided a detailed description of Indian mahua and isolated a new species of yeast, *Zygosaccharomyces mahwae*, from a culture in sterilized must, into which some of the drug was placed. Fowler & Gilbert (Fowler & Gilbert, 1930) reported the production of organic manure by adding mahua flowers to waste organic matter. Daji et al. (1951) reported the role of molasses, mahua flowers and mannitol in soil nitrogen fixation. Hasan & Bhate, 1928, used mahua flowers (produced after fermentation and distillation) as cattle feed. Feeding experiments to determine their food value, their effects on the health of the animals and the quality of milk yielded favourable results overall. Saha & Singh, 1991, developed a new, natural and antibacterial mahua flower agar medium for fungal culture.

2.9 Safety study of dry mahua on an animal model

The effects of a diet containing processed and unprocessed mahua syrup on albino rats were investigated by Kotwal, 2000. Undesirable changes in biochemical and histological parameters were observed in the unprocessed syrup. However, the processed syrup did not result in any changes in the albino rats. The author concluded that the syrup could be efficiently substituted up to 50% of the calorie source without any damaging effects.

The protein quality of Mahua flowers was analysed as described by Jayasree et al. (1998). On the basis of their diet, the animals were divided into three different groups: a protein-free group, a control group and a treatment group (mahua flowers) with casein, ground nut oil and mahua flowers. The results revealed that the weight gain and food intake of the mahua flower were similar to those of the control group. The rats were able to ingest mahua flowers. Protein intake was similar in both groups. Moreover, no adverse effects were detected in either group of animals. On the basis of their results, they assesed that the flower might be recommended as an important food component for rural people.

The effects of feeding dried mahua flowers on the growth and feed conversion efficiency of crossbred gilts were observed by Bora and Singh (1994). The experiment was performed by taking dried mahua flowers as a substitute for maize. The plants were divided into three groups, which were fed 100% maize, 50% mahua flowers + 50% maize or 100% mahua flowers. The results revealed that there was no significant difference in weight gain between the control and treated groups.

2.10 Major findings from the literature review

- Large quantities of flowers are produced every year, but most of them visit the liquor distillation unit because of a lack of suitable processing technologies.
- The composition of flowers is strongly dependent on climatic conditions.
- The flowers are edible and are a rich source of sugar (glucose & fructose), protein, minerals (Ca, P & Fe) and vitamins.
- The protein quality of this flower is superior and comparable to that of groundnut. The overall nutritional quality of flowers is better than that of raisins and banana.
- The flowers have numerous ethno-medicinal and pharmacological uses that can be used for the treatment of several diseases.
- As the literature suggests that flowers have no toxic effects, they could be used for the development of value-added food products.

CHAPTER-III

COMPREHENSIVE TOTAL CARBOHYDRATE AND PHYTOCHEMICAL PROFILING OF FRESH AND DRY MAHUA FLOWERS (*MADHUCA INDICA*)

3. Background

The tree is also known as "**Tree of Life**" as it provides various products which is important for the rural communities. *Madhuca indica* is a multi-purpose tree which fulfills the 3 major F's i.e. Food, Fodder and Fuel (Sarkar et al., 2022). It mainly grows in the Southeast Asia, and the tropical regions of Africa. The tree is well known for its edible flowers, fruits and seeds. Since early ages the tree is important for the tribal and the poor. It is a vital part of their culture, tradition and diet. The rural are dependent on the tree in terms of nutrition and income. As the flower is a rich source of sugar, it is used for the development of various products like country liquor, kheer, pitha etc. (Gupta et al., 2012)

3. 1 Nutritional Outline of Mahua

The nutritional composition of mahua varies depending on the part of the tree being consumed. The flowers, seeds, and fruits are all rich in different nutrients. Understanding the nutritional profile of Mahua is crucial for appreciating its importance in the diet of millions of people. The flowers of mahua are considered to be a rich source of nutrients which have tremendous health benefits. They are the staple food for the poor and the tribal people. The health benefits of mahua flowers are due to the present of various macronutrients, micronutrients and bioactive compounds (Mishra et al., 2023).

3.2 Macronutrients

a. Carbohydrates: Mahua is a rich source of carbohydrates which delivers a rapid and continuous source of energy. They are the major portion of the flower's dry weight. The sugars that are present in the flower are in the simple form and they are easily digestible. By which they can offer a quick energy boost without having any side effects. The simple sugars which are present in the flowers are sucrose, glucose and fructose and these sugars are easily digestible by the body (Jha et al., 2013).

b. Proteins: These are essential for the growth and repair of the tissues, enzymes, cells etc. The protein content of mahua flower is moderate which is essential for the growth and maintenance of the body. When compared to the other tribal food products, the protein content of mahua flower is comparatively higher. The flowers also contain some vital amino acids that is essential for the growth. Although the protein content is less than the animal source and some legumes but still the flower is a good source of protein (Singh et al., 2021).

c. Fats: The fat content that are found in the flower is very minimal but the fat which are present in the flower are comparatively good for health. The flowers contain unsaturated fatty acid rather that saturated fatty acids which is much healthier. These unsaturated fatty acids are good for the heart health as they help in lowering the cholesterol. The fats help to store energy and is helpful for the absorption of fat-soluble vitamins (Sharma et al., 2013).

3.3 Micronutrients

a. Vitamins: Mahua is a rich source of Vitamin-C which helps to heal wound, repairs skin and improves the immune function. It improves the iron absorption capacity and promotes collagen formation which is crucial for bones, skin and blood vessels. It also contains several B-Vitamins like thiamine (Vit-B_1), riboflavin (Vit-B_2), and Niacin (Vit-B_3) (Singh et al., 2021). The major function of riboflavin is, it produces energy and helps in the metabolism of proteins, fats and carbohydrates. It helps in making skin, eyes and nerves healthy. Thiamine helps in the carbohydrate metabolism and also supports the nervous system functions. It is also essential for energy production. The function of niacin is to repair DNA, makes the digestive system healthy. Most importantly it regulates the cholesterol level (Frank, 2015).

b. Minerals: The major groups of minerals that are present in the mahua flowers are iron, calcium, phosphorus, magnesium and potassium. Mahua prevents the most common problems especially in women and children i.e. anemia due to the presence of iron. It is a rich source of iron which is required for the production of hemoglobin. Consuming these flowers boost the iron level which ultimately prevents iron deficiency in the body. For a healthy bone and teeth, calcium is considered as an important mineral. Mahua is a good source calcium which can help in maintaining healthy teeth and bones. It also prevents muscle contraction and supports nerve functions. Researchers have also identified the presence of phosphorous in mahua flowers (Singh et al., 2021). In the conjuction with calcium it makes the teeth and bone healthy. Another important mineral i.e. magnesium found in mahua flowers help in the enzymatic reactions in the body. It also regulates the blood sugar level and prevents high blood pressure. Potassium is considered as an electrolyte which plays a vital role in heart and muscle functions. It maintains the fluid balance. Regulate high blood pressure and prevents muscle cramps.

c. Trace elements: Trace elements like manganese, zinc and copper are found in mahua flowers which is important for immune system, wound healing and antioxidant defense. These trace minerals also support in enzymatic developments that support complete metabolic tasks (Singh et al., 2021).

3.4 Bioactive Compounds

Apart from micronutrients and macronutrients, mahua flowers is also rich in certain bioactive compounds like phenols, flavonoids, saponins etc. These bioactive compounds contributes towards the pharmacological properties.

- ***Flavonoids:*** Flavonoids present in mahua flowers contributes towards the antioxidant properties. These bioactive compounds have various pharmacological importance such as anti-inflammatory, anti-cancer, antibacterial and antiviral properties making the flowers beneficial in combating different chronic health diseases. These compounds also improves the heart health by reducing oxidative stress and lowering the blood pressure levels (Mutha et al., 2021).
- ***Phenols:*** Phenols also contribute towards the antioxidant properties found in mahua flowers. They neutralize the free radicals found in the body by which the cells are protected from damage. Thus, it reduces the oxidative stress, prevents cancer and improves the heart health (Mutha et al., 2021).
- ***Saponins:*** Saponins are one of the compounds found in mahua flowers. Although there are several side effects of saponins but they have anti-inflammatory properties, control the blood sugar level, cholesterol. It helps in weight management and improves the liver health (Sharma et al., 2023).

3.5 Nutritional components and health benefits of mahua flowers

Due to the presence certain nutritional components mahua have wide spread health benefits. These health benefits should be utilized by the people who are suffering from malnutrition, anemia and other diseases. Some of the health benefits are mentioned below as studies by Mishra et al., 2023.

1. ***Energy Boosting:*** As discussed earlier, mahua is a rich source of carbohydrates and natural sugar which provides instant energy. Mahua offers a rapid and constant energy lift, which is predominantly advantageous in areas where people accomplish physically challenging tasks.
2. ***Anemia Prevention***: Iron is crucial for the formation of hemoglobin, which supports in conveying oxygen throughout the body. The iron present in mahua flower, seeds makes it an exceptional food for combating anemia, mostly among women and children.
3. ***Improved Digestion:*** The high fiber content found in mahua supports in endorsing healthy digestion and even bowel movements. It can be particularly supportive in inhibiting constipation and encouraging gut health.
4. ***Heart Health:*** The unsaturated fats found in Mahua seeds contribute to heart health by reducing bad cholesterol levels and maintaining healthy blood pressure. Potassium and magnesium also play a role in regulating heart function and preventing cardiovascular diseases.
5. ***Immune Boosting:*** The vitamin C present in mahua flowers plays a vital part in improving the immune system, thus assisting the body to fight for infections and diseases. Furthermore, antioxidants found in mahua support to diminish oxidative stress, thus dropping the danger of chronic illnesses.
6. ***Bone Health:*** The minerals like calcium and phosphorus found in mahua are significant for retaining strong bones and combating osteoporosis. These minerals upkeep bone mass and help prevent fractures.
7. ***Skin Health:*** In Ayurveda it is used for the treatment of skincare endorsing healthy skin, dropping wrinkles, and increasing wound healing potential.

3.6 Significance of Nutritional Profiling of mahua flowers for Societies (Sreenivasa et al., 2024):

Nutritional profiling of mahua is significant for numerous causes like:

1. ***Can be used as sustainable Food****:* By accepting the nutritional significance of mahua, it can be acknowledged as a sustainable and reasonable source of nutrition for people in rural regions where the use of other nutrient-rich foods is inadequate.
2. ***Improving the public health****:* Nutritional sketching permits governments, health groups, and societies to make knowledgeable conclusions about including mahua into public health nutrition programs, particularly for people that undergo from malnutrition or other deficiencies (such as anemia).
3. ***Cultural Preservation****:* Apart from nutritional importance, it has cultural implication for many native groups. Endorsing its consumption based on its nutritional importance supports to preserve local traditions while refining health consequences.
4. ***Food Security****:* Mahua grows in the leanest season of agriculture and being a hardy and drought-resistant tree, it is an outstanding food during times of shortage. Its nutritional sketching helps to safeguard that it can be used efficiently in food security initiatives.

The nutritional outlining of mahua is crucial for accepting its capacity to recover the health of people, predominantly in rural areas where it is cultivated. By identifying the miscellaneous nutrients in mahua flowers Governments and health administrations can use this information to increase its cultivation, consumption, and trade, contributing to better public health, economic growth, and food security. This plant, with its ironic nutritional outline, serves as a significant apparatus in both fighting malnutrition and supporting livelihoods for rural people. The character of mahua as a dietary supplement highlights the need for further investigation and investment in its potential for refining the lives of billions.

3.7 Materials and Methods

3.7.1 *Preparation of plant extracts*

Fresh flowers of *Madhuca indica* were collected from local sites in Sambalpur and dried in a traydrier. The fresh and dried flowers were extracted via different solvents, such as methanol, hydroethanol (50%), and an aqueous mixture, in a Soxhlet extraction system at 40 °C until the maximum phytoconstituents were leached from the plant samples. The flower extracts were concentrated via evaporation via a rotary evaporator at 40 °C, followed by lyophilization.

3.7.2 *Fourier transform infrared (FT-IR) spectroscopic analysis*

The crude flower extracts of *Madhuca indica* were subjected to FT-IR spectroscopic analysis via a Bruker Alpha-II FT-IR spectroscope to identify the presence of different functional groups (Hota et al. 2024).

3.7.3 *Estimation of total phenolic content (TPC)*

The total phenolic content (TPC) of the crude extracts was estimated via the modified Folin–Ciocaltaeu method (Xue et al. 2017), with some minor modifications. The reaction mixture was incubated in the dark, and the absorbance was measured at 765 nm by using gallic acid as a standard. The experiment was performed in triplicate (Rosyantari et al., 2021).

3.7.4 Estimation of total flavonoid content (TFC)

The total flavonoid content of the crude extracts was estimated following the standard aluminum chloride colorimetric assay (Xue et al. 2017) with slight modifications. The absorbance of the reaction mixture was measured at 420 nm by using quercetin as a standard. The experiment was performed in triplicate (Rosyantari et al., 2021).

3.7.5 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH oxygen radical scavenging activity of the extracts of the mahua flowers was determined as described by Asraoui et al. (2017), with some minor changes. The prepared DPPH solution was added to the different concentrations of extracts, and the reaction mixtures were incubated in the dark for 30 min. Ascorbic acid was used as a positive control. The absorbance was measured at 517 nm (Nurhayati et al., 2024), and the percentage of radical scavenging activity was calculated via the following formula:

$$\text{DPPH radical scavenging activity (\%)} = [(Abs_{Control} - Abs_{Sample})/(Abs_{Control})] \times 100$$

The IC_{50} (concentration of plant sample with 50% DPPH radical scavenging) was calculated via a log dose–inhibition curve of ascorbic acid. The experiment was performed in triplicate.

3.7.6 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic) acid (ABTS) radical scavenging activity

ABTS scavenging activity was determined as described by Asraoui et al. (2017), with slight modifications. The ABTS reagent was prepared by mixing the ABTS solution and potassium per sulfate solution and allowing it to react in the dark for 12 h. The ABTS reagent was mixed at different concentrations and allowed to react for 15 minutes. After the incubation period, the absorbance was measured at 734 nm, and the percentage of ABTS scavenging was calculated. The IC_{50} (concentration of plant sample with 50% ABTS radical scavenging) was calculated via a log dose–inhibition curve of ascorbic acid (Nurhayati et al., 2024). The ABTS reagent without the sample was treated as a control.

$$\text{ABTS scavenging activity (\%)} = [(Abs_{Control} - Abs_{Sample})/(Abs_{Control})] \times 100$$

3.7.7 Ferric reducing antioxidant power **(FRAP) assay:**

The ferric reducing antioxidant power assay was carried out according to the method described by Xiao et al. (2020), with some modifications. This reaction was monitored by measuring the change in absorbance at 593 nm. To determine the FRAP values of the different extracts, gradient concentrations of 5, 10, 15, 20, 30, 50, 70 and 100 µg/ml were mixed with the FRAP reagent and incubated for 10 minutes in the dark. The standard curve was prepared using ascorbic acid. The results were quantified as the FRAP value of the extracts and expressed as the concentration of Fe(II) in µM/g of extract (Nurhayati et al., 2024),. The calculation of the FRAP value is described in the equation below:

$$\text{FRAP value (Fe(II) µM/g of extract)} = c*V*t/m,$$

where c is the Fe(II) concentration in µM in the diluted sample, V is the sample volume in ml, t is the dilution factor, and m is the weight of the extract in grams.

3.7.8 Gas chromatography–mass spectrometry (GC–MS) with RI detector analysis

GCMS analysis was performed on a Shimadzu Nexis GC-2030 coupled with a Shimadzu GCMS-QP2020 NX mass spectrometer (Babu et al. 2024). Separation was carried out on an SH-I-5Sil MS ultrainert capillary column (30 m × 250 µm × 0.25 µm). The chromatographic elution was programmed with an initial temperature of 60 °C and a hold time of 2 minutes and then increased to 230 °C at a rate of 3 °C min^{-1} and a hold time of 5 minutes. The carrier gas was helium with a constant flow rate of 0.8 ml min^{-1}. The sample inlet temperature was set at 210 °C. A total of 0.5 µl of the sample (1000 µg/ml) was injected in split mode with a split ratio of 20:1, and a solvent delay of 3 minutes was used. For MS detection, ions were formed by electron impact at 200 °C via a mass-selective detector. The transfer line was maintained at 280 °C, and masses were scanned in the quadrupole from m/z 35 to 400u. The obtained mass spectra were searched on the basis of their percentage probability, score and reverse score for the identification of compounds in the NIST 2020 database (MS Search; NIST, MSS Ltd. Manchester, England). (Momtaz et al., 2024)

3.7.9 Ultrahigh-performance liquid chromatography high-resolution mass spectrometry (UHPLC-HRMS) coupled with quadrupole time-of-flight (Q-TOF) analysis

The extracts of mahua flowers was prepared and filtered through a 0.22 μm PTFE syringe filter. The filtered sample was taken for UHPLC-HRMS coupled with Q-TOF analysis. The study was accomplished on a Xevo G3 QToF (Waters Corporation, MA, USA) with Acquity UPLC I Class Plus and MassLynx software (Waters Corporation, USA) and processed through Progenesis QI software (Waters Corporation, USA). Chromatographic separation was carried out using an Acquity UPLC HSS T3 column (100 × 2.1 mm, 1.8 μm) (Waters Corporation, USA). The column was maintained at 40 °C, and the sample temperature was maintained at 15 °C during the analysis. The analysis was carried out in ESI mode was from a mass range (*m/z*) of 50–1200 *m/z*, a low collision energy of 6 eV, a high collision energy of 10–40 eV (ramp) and 10–30 eV (ramp) for +ve mode and -ve mode respectively. The flow rate was set at 0.4 ml/min for a gradient program using 0.1% formic acid in water as solvent A and 0.1% formic acid in acetonitrile as Solvent B as mobile phase. The volume ratio of solvent B was changed as follows: 5% B → 0–1 min, 5–25% B → 1–5 min, 25–35% B → 5–8 min, 35–45% B → 8–11 min, 45–55% B → 11–14 min, 55–90% B → 14–20 min, 90–5% B → 20–20.1 min, and 5% B → 20.1–25 min. 5µl of the test solutions were injected for phytochemical screening. The phytochemical compounds present in the *D. volubilis* hydroethanolic extract were identified and confirmed by their respective mass ions, fragmentation patterns, offline and online mass spectral databases, and related literature (Wang et al. 2024).

3.7.10 Total Carbohydrates profiling

The total carbohydrate profile was done through HPLC. The sample was prepared by hydrolyzing the carbohydrate complex into simple sugars using acids and enzymes followed by neutralization and filtration. The mobile phase was prepared using water and acetonitrile or buffer depending on the column used. The sample was injected into the system equipped with a detector using an appropriate column for separation. Then, the carbohydrates were separated based on their properties with peaks which appeared on the chromatogram corresponding to different sugars. Further the carbohydrates were quantified by comparing the area of these peaks with the known standard (Sharma et al., 2024).

3.8 Results

3.8.1 Fourier transform infrared (FT-IR) spectroscopic analysis

The FTIR spectra of fresh mahua flowers extracted with 50% methanol, 100% methanol, and aqueous solvents showed distinct differences due to the varying polarities of the solvents. In the 50% methanol extract, the O-H peak at approximately 3300 cm^{-1} was slightly broad, reflecting the presence of both water and methanol, whereas the 100% methanol extract presented a narrower O-H band due to less hydrogen bonding. Additionally, aromatic C=C stretching near 1600 cm^{-1} would also be more prominent in the 50% and 100% methanolic extracts, which is better at extracting nonpolar compounds such as flavonoids. In the aqueous extract, the O-H stretch dominated, with broader and less intense peaks in other regions, as water primarily extracts polar compounds, leading to weaker C-H, C=O, and C=C stretches. Overall, the solvent polarity significantly influences the extraction of functional groups, with methanol being more efficient for less polar compounds and water favouring the extraction of hydrophilic components (Fig. 7.1 A&B).

In the case of dry mahua flowers, the FTIR spectra of the 50% methanol, 100% methanol, and aqueous extracts presented some notable changes compared with those of fresh flowers due to the loss of water content and possible structural changes in the flower components. For the 50% methanol extract, the O-H stretch (~3300 cm^{-1}) would be less broad than that in fresh flowers, as the drying process would reduce the intrinsic moisture in the sample, resulting in less hydrogen bonding. The aromatic C=C stretch (~1600 cm^{-1}) would also likely be more pronounced, especially for phenolic compounds, as drying can increase the concentration of these nonpolar substances in the dried material. For the 100% methanol extract, similar trends were observed, but with even sharper and more intense peaks. The O-H stretch was narrower than that of the 50% methanolic extract, reflecting the loss of water in the sample. The carbonyl and aromatic stretches are likely more intense than those in the fresh flower spectrum, as methanol is particularly efficient at extracting nonpolar compounds that may become more concentrated in the dried sample. For the aqueous extract, O-H stretching still dominated the spectrum, but it might be narrower than that of fresh flowers because of the reduced moisture content in the dried sample. Additionally, the intensity of the C=O and C=C stretches might decrease, as water is less efficient at extracting these compounds from dried flowers. However, polar compounds that are easily water soluble may still be extracted, but the overall intensity and sharpness of the peaks are lower than those of methanol extracts.

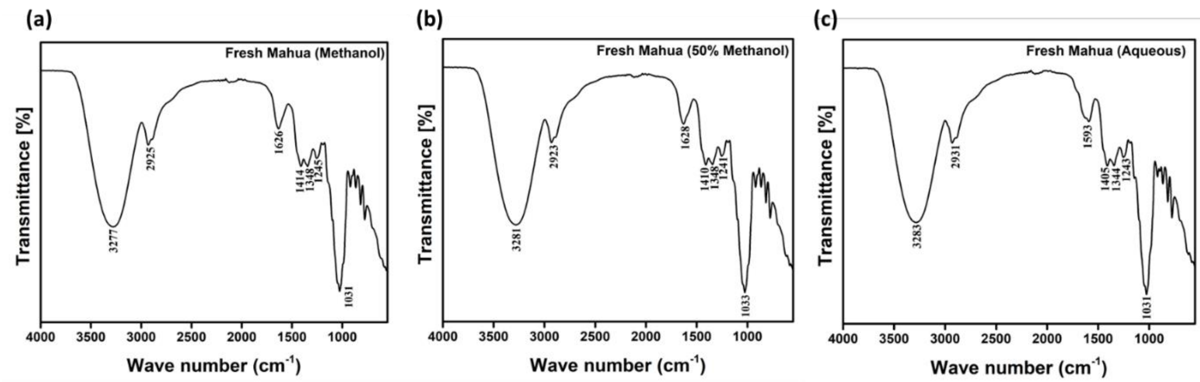


Figure 7.1 FT-IR spectra of fresh flower extract (A), 100% methanolic extract (B), 50% methanolic extract (C), and aqueous extract of *M. indica* in the range of 550--4000 cm^{-1}. The spectrum exhibited several absorption bands corresponding to various functional groups present in the sample.

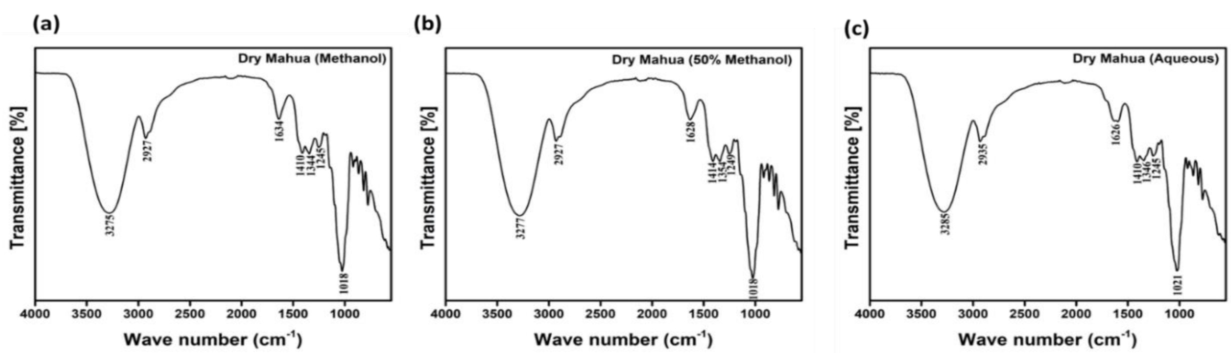


Figure 7.2 FT-IR spectra of dry flower extract, (A) 100% methanolic extract, (B) 50% methanolic extract, and (C) aqueous extract of *M. indica* in the range of 550--4000 cm^{-1}. The spectrum exhibited several absorption bands corresponding to various functional groups present in the sample.

3.8.2 Determination of total phenolic and flavonoid contents

Phenols and flavonoids are considered natural compounds with potential antioxidant activities. These are the second-highest group of compounds found in almost all medicinal plants. The presence of phenols and flavonoids disrupts the chain reaction of reactive oxygen species and prevents damage caused by reactive oxygen species (Patle et al. 2020). These compounds possess neuroprotective, anticancer, and cardioprotective activities (Pandey and Rizvi, 2009). The TPC of the methanolic, hydromethanolic (50%), and aqueous extracts of *M. indica* for both the fresh and dry flower extracts were 2.68 ± 0.18, 1.10 ± 0.15 and 4.90 ± 0.17 mg GAE/g of extract for the fresh flower extracts and 2.34 ± 0.12, 0.52 ± 0.13 and 1.17 ± 0.04 mg GAE/g for the dry flower extracts, respectively, as obtained from the gallic acid standard curve for the fresh and dry flower extracts (y=0.0127x + 0.03260, R^2 = 0.9863, y=0.0127x + 0.0326, R^2 =0.9863). Similarly, the TFCs for fresh and dry flower extracts (methanolic, hydromethanolic (50%), and aqueous extracts) were observed to be 1.45 ± 0.36, 5.35 ± 0.46 and 6.08 ± 0.85 mg QE/g of fresh flower extract and 6.42 ± 0.88, 6.40 ± 0.27 and 5.53 ± 0.38 mg QE/g of dry flower extract, respectively, as obtained from the quercetin standard curve (y= 0.0003x + 0.0254, R^2 = 0.9705, y=0.0005x + 0.0085, R^2 =0.9844) (Fig. 8&9).

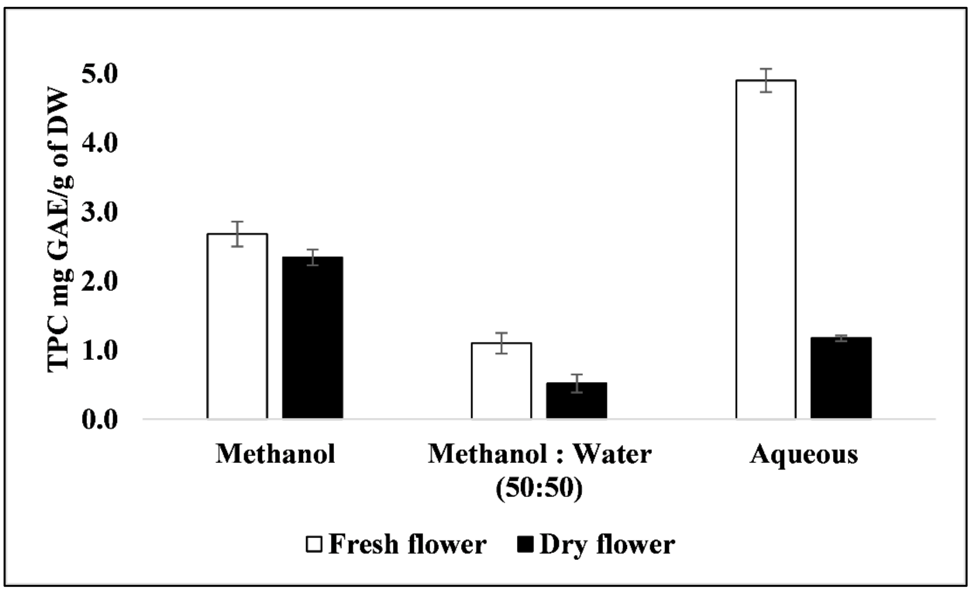


Figure 8 The graph illustrates the Total Phenolic Content (TPC) in the methanolic and aqueous extract of both fresh and dried Mahua flowers. TPC was quantified using the Folin-Ciocalteu method, expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g DW). Data are presented as mean ± standard deviation (SD) of three independent replicates.

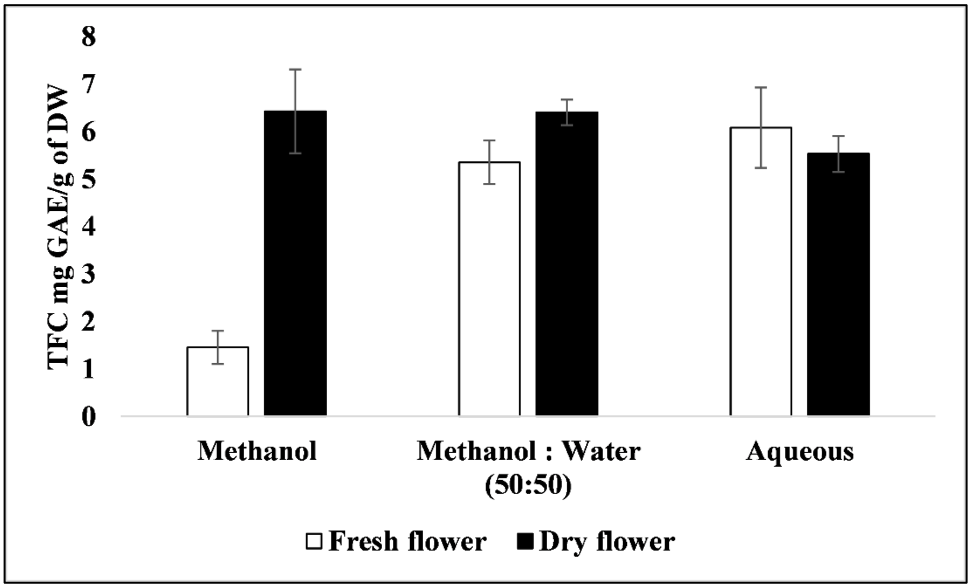


Figure 9 The graph illustrates the Total Flavonoid Content (TFC) in the methanolic and aqueous extract both fresh and dried Mahua flowers. TFC was determined using the aluminum chloride colorimetric method, expressed as milligrams of quercetin equivalents (QE) per gram of dry weight (mg QE/g DW). Data are presented as mean ± standard deviation (SD) of three independent replicates.

3.8.3 Antioxidant activity

Owing to the presence of phenols and flavonoids in flower extracts, there is a reduction in oxidative stress, and these compounds act as free radical scavengers. The present study elaborates the antioxidant efficacy of extracts of fresh and dry mahua flowers via DPPH, ABTS and FRAP. The antioxidant activityactivities of the different extracts of fresh and dry mahua flowers on the basis of their DPPH and ABTS scavenging activities. With increasing concentrations of the plant extracts, the DPPH radical scavenging activity increased. The aqueous extracts of fresh and dry flowers presented DPPH radical scavenging activity, with the lowest IC_{50} values of 150.49 µg/ml and 100.34 µg/ml, respectively. In comparison, the antioxidant agent ascorbic acid has an IC_{50} value of 8.00 µg/ml. In contrast, the aqueous extracts of fresh and dry flowers presented the lowest IC_{50} values of 81.77 µg/ml and 82.85 µg/ml, respectively, while whereas ascorbic acid had an IC_{50} value of 1.41 µg/ml (Fig. 2A). Among the different extracts of fresh and dry flowers, the aqueous extract of fresh flowers and the hydromethanolic extract of dry flowers presented the highest FRAP values of 21.44 Fe(II) µM/g and 21.23 Fe(II) µM/g of extract, respectively (Fig. 2C).

3.8.4 GC–MS analysis

The representative base peak chromatograms of fresh and dry flower (methanolic and hydromethanolic) extracts are depicted in Figure, and the identified phytochemicals are presented in Table. Chromatograms depict the identified phytochemicals on the basis of their characteristic retention time, molecular formula, and fragmentation pattern (Fig. 10.1-10.4), Table 3.1-3.4). According to the literature, the phytochemical constituents that have been identified from the GC–MS of methanolic and hydromethanolic extracts of fresh mahua flowers have numerous pharmacological activities. For example, the compound 3-amino-2-oxazolidinone is used as a potent quorum-sensing inhibitor of *Pseudomonas aeruginosa* and *Chromobacterium violaceum* (Jiang et al. 2020). It also exhibited antihypertensive and monoamine oxidase inhibitory activity (Kaul and Grewal, 1972). Dihydroxyacetone is an antifungal agent against causative agents of dermatomycosis (Stopiglia et al. 2011). 1,3,5-Triazine-2 has numerous pharmacological activities, including antibacterial, antimalarial, antiviral, and anti-inflammatory activities (Singh et al. 2021). 5-Hydroxymethylfurfural has antiquorum-sensing and biofilm potential against gram-positive pathogens and has potential against *K. pneumonia and P. aeruginosa* (Vijayakumar and ramanathan, 2018; Kaushal and Sharma, 2022). Quinic acid is a potent compound that has several biological activities, including anticancer, antimicrobial, antiviral, analgesic, antidiabetic, and antioxidant potential (Benali et al. 2022). The thymine found in the hydromethanolic extract of fresh Mahua flowers plays a key role in the body and converts carbohydrates and fats into energy. It also helps in glucose metabolism and inhibits the effects of glucose and insulin on arterial smooth muscle proliferation (Smith et al. 2021). 2-Propenoic acid is considered a secondary metabolite that has anticoagulant properties. It is used to treat venous thromboembolism, and the compound also has anticancer potential (Sahu et al. 2023). One of the major compounds i.e. 3-deoxy-D-mannoic lactone, found in the hydromethanolic extract of fresh mahua flowers has been shown to have antimicrobial activity with anti-quorum-sensing potential (Wani et al. 2024).

There are certain bioactive compounds that are present in both fresh and dry mahua flowers (methanolic and hydromethanolic extracts), such as dihydroxyacetone, thymine, and 2-propanmine. However, there are also some additional compounds found in dry mahua flowers (methanolic and hydromethanolic extracts) that gain pharmacological importance. 2-Propanamine, a major compound found in dry Mahua flower extracts, is used to treat arterial hypertension, which prevents angina and sinus (Cacabelos and Torrellas, 2016). (S)-5-Hydroxymethyl-2[5H]-furanone possesses antibacterial activity and inhibits quorum sensing, as reported by Gallego et al. (2016). Butanedioic acid has anticancer, antibacterial and antifungal potential (Qasem et al. 2016). 1,2,3-Benzenetriol is a bioactive compound that has antioxidant, antibacterial and antiviral potential (Ehaimir et al. 2023).

Table 3.1: List of compounds identified via GC–MS in the methanolic extract of *M. indica* (fresh flower).

Peak#	Compound Name	Antimicrobial activity	References
2	3-Amino-2-oxazolidinone	Showed antibacterial activity against *P. aeruginosa* and act as Quorum-sensing inhibitor against *C. violaceum*	Jiang et al. 2020
3	Dihydroxyacetone	It is used as an antifungal agent against dermatophytes and *Candida* spp.	Stopiglia et al. 2011
7	1,3,5-Triazine-2,	The compound exhibited antibacterial and antifungal activity. It acts as an inhibitor of *S, aureus* (4 µg/ml), *P. aeruginosa* (64-128 µg/ml) and *E. coli* (16 µg/ml).	Singh et al. 2020
11	4H-Pyran-4-one	The compound exhibited significant antibacterial activity against *S. pneumonia, B. subtilis, P. aeruginosa* and *E. coli* at MIC ranging from 0.007-3.9 µg/ml	Afifi et al. 2017
14	5-Hydroxy methylfurfural	It has a potential to inhibit quorum sensing mediated biofilm formation against *C. violaceum*	Vijayakumar and Ramanathan, 2018
15	1,2,3-Propanetriol	Exhibited antibacterial activities against *S. entteritidis, B. cerus, E. coli, S. aureus*	Saeed et al. 2023
23	1,2,3-Benzenetriol	The compound exhibited antibacterial and inhibit quorum sensing activity against *C. violaceum*	Deryabin and Tolmacheva, 2015
29	Quinic acid	It has numerous pharmacological activities which also includes antibacterial potential and acts as quorum sensing inhibitor against *S. aureus*	Bai et al. 2019

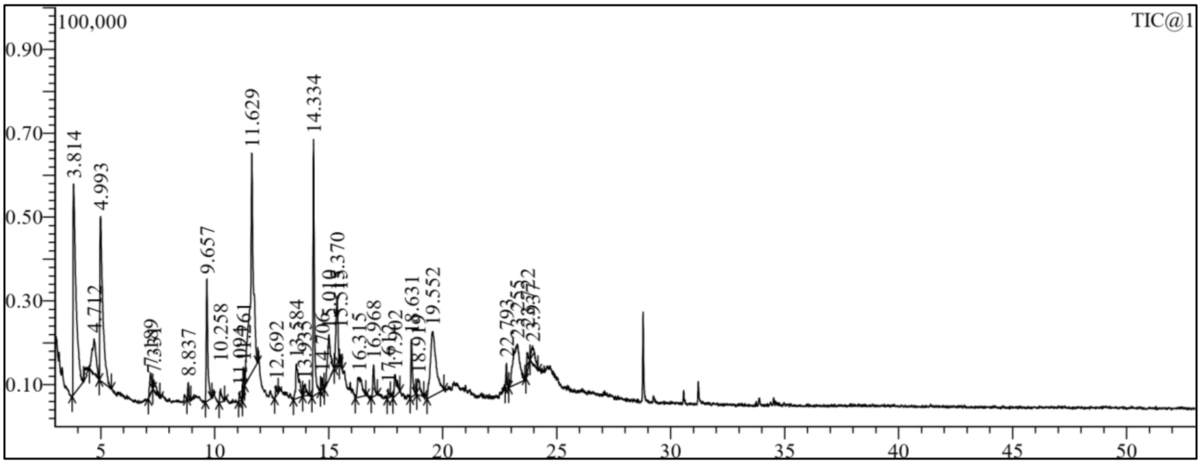


Figure 10.1 The figure shows the GC–MS spectrum of the methanolic extract of *M. indica* (fresh flower). The spectrum exhibits a series of peaks corresponding to different molecular ions, with their intensities representing the relative abundance of each species.

Table 3.2: List of compounds identified via GC–MS in the hydromethanolic extract of *M. indica* (fresh flower).

Peak#	Compound Name	Antimicrobial activity	References
2	3-Amino-2-oxazolidinone	Showed antibacterial activity against *P. aeruginosa* and act as Quorum-sensing inhibitor against *C. violaceum*	Jiang et al. 2020
3	Dihydroxyacetone	It is used as an antifungal agent against dermatophytes and *Candida* spp.	Stopiglia et al. 2011
7	Pentanoic acid	The compound has antibacterial ctivity against *S. aureus* and *E. coli*	Song et al. 2015
11	2(1H)-Pyridinone	The compound and its derivatives exhibited antibacterial activity against *S. aureus, B. subtilis, E. coli* and *P. vulgaris*	Saadon et al. 2022
13	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Reported to have antimicrobial potential	Yu et al. 2012
15	5-Hydroxymethylfurfural	It has a potential to inhibit quorum sensing mediated	Vijayakumar and ramanathan, 2018

		biofilm formation against *C. violaceum*	
16	1,2,3-Propanetriol	Exhibited antibacterial activities against *S. entteritidis, B. cerus, E. coli, S. aureus*	Saeed et al. 2023
22	1,2,3-Benzenetriol	The compound exhibited antibacterial and inhibit quorum sensing activity against *C. violaceum*	Deryabin and Tolmacheva, 2015

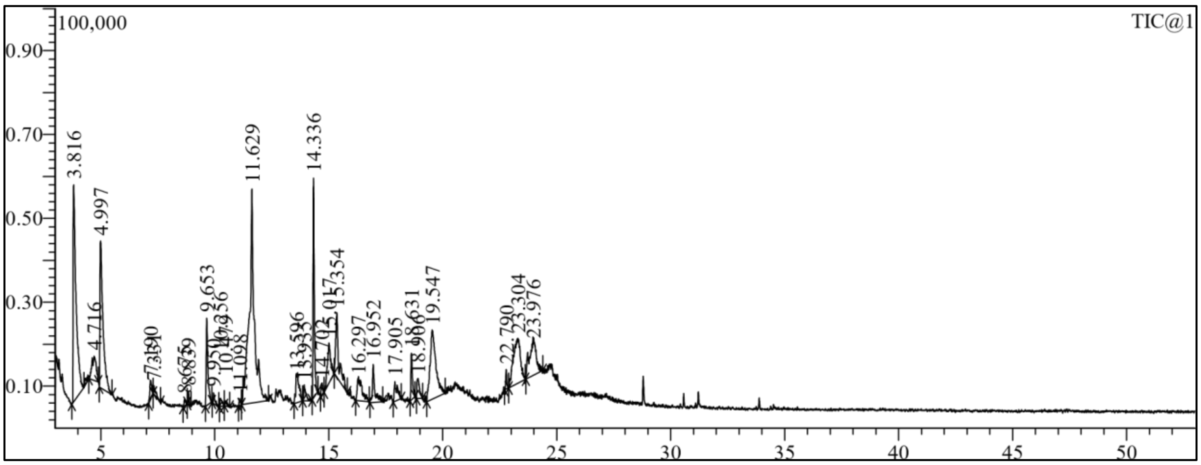


Figure 10.2 The figure shows the GC–MS spectrum of the *M. indica* (fresh flower) hydromethanolic extract. The spectrum exhibits a series of peaks corresponding to different molecular ions, with their intensities representing the relative abundance of each species.

Table 3.3: List of compounds identified via GC–MS in the methanolic extract of *M. indica* (dry flower).

Peak#	Compound Name	Antimicrobial activity	References
2	2,3-Butanediol	2,3-butanediol acts as a neutralizer in the acidic surroundings of the intestinal cells	Yi et al. 2016
5	3-Amino-2-oxazolidinone	Showed antibacterial activity against *P. aeruginosa* and act as Quorum-sensing inhibitor against *C. violaceum*	Jiang et al. 2020
6	Dihydroxyacetone	It is used as an antifungal agent against	Stopiglia et al. 2011

		dermatophytes and *Candida* spp.	
16	2(3H)-Furanone	Exhibits antibacterial and antibiofilm potential against Gram positive bacteria	Sharafutdinov et al. 2019
17	(S)-5-Hydroxymethyl-2[5H]-furanone	Exhibits antibacterial and antibiofilm potential against Gram positive bacteria	Sharafutdinov et al. 2019
21	5-Hydroxymethylfurfural	It has a potential to inhibit quorum sensing mediated biofilm formation against *C. violaceum*	Vijayakumar and ramanathan, 2018
22	1,2,3-Propanetriol	Exhibited antibacterial activities against *S. entteritidis, B. cerus, E. coli, S. aureus*	Saeed et al. 2023
29	1,2,3-Benzenetriol	The compound exhibited antibacterial and inhibit quorum sensing activity against *C. violaceum*	Deryabin and Tolmacheva, 2015
34	3-Deoxy-d-mannoic lactone	Reported to have antibacterial activity	Shobana et al. 2009

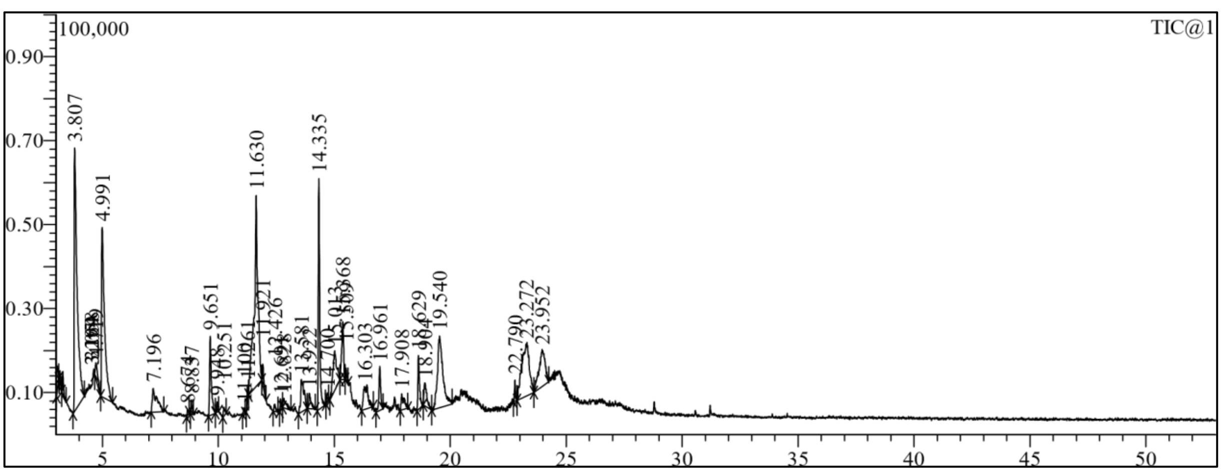


Figure 10.3 The figure shows the GC–MS spectrum of the *M. indica* (dry flower) methanolic extract. The spectrum exhibits a series of peaks corresponding to different molecular ions, with their intensities representing the relative abundance of each species.

Table 3.4: List of compounds identified via GC–MS in the hydromethanolic extract of *M. indica* (dry flower).

Peak#	Compound Name	Antimicrobial activity	Refrences
2	2,3-Butanediol	2,3-butanediol acts as a neutralizer in the acidic surroundings of the intestinal cells	Yi et al. 2016
4	3-Amino-2-oxazolidinone	Showed antibacterial activity against *P. aeruginosa* and act as Quorum-sensing inhibitor against *C. violaceum*	Jiang et al. 2020
5	Dihydroxyacetone	It is used as an antifungal agent against dermatophytes and *Candida* spp.	Stopiglia et al. 2011
15	2(3H)-Furanone	Exhibits antibacterial and antibiofilm potential against Gram-positive bacteria	Sharafutdinov et al. 2019
18	5-Hydroxymethylfurfural	It has a potential to inhibit quorum sensing mediated biofilm formation against *C. violaceum*	Vijayakumar and ramanathan, 2018
25	1,2,3-Benzenetriol	The compound exhibited antibacterial and inhibit quorum sensing activity against *C. violaceum*	Deryabin and Tolmacheva, 2015
31	3-Deoxy-d-mannoic lactone	Reported to have antibacterial activity	Shobana et al. 2009

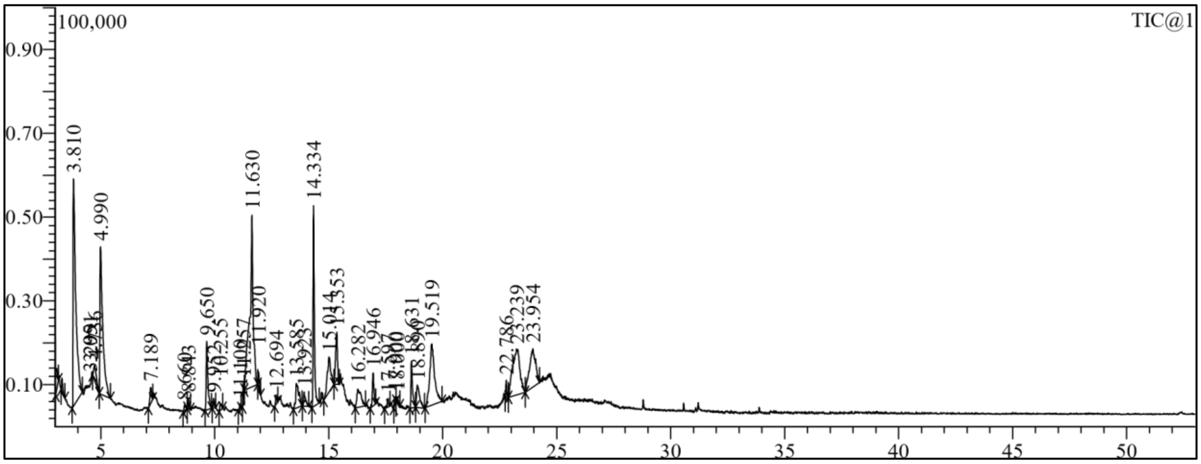


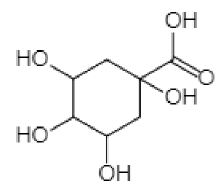
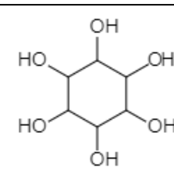
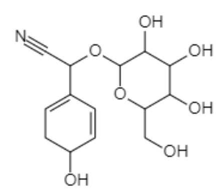
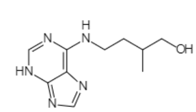
Figure 10.4 GC–MS spectrum of the *M. indica* (dry flower) hydromethanolic extract. The spectrum exhibits a series of peaks corresponding to different molecular ions, with their intensities representing the relative abundance of each species.

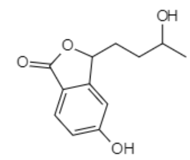
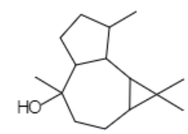
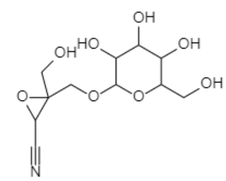
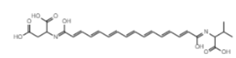
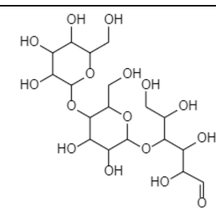
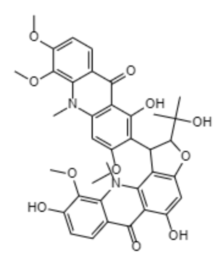
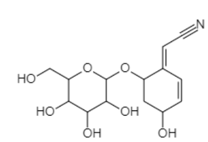
3.8.5 UHPLC-HRMS analysis

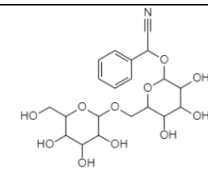
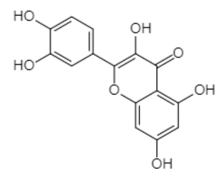
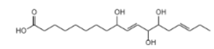
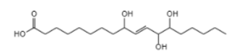
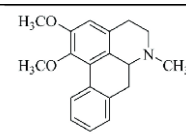
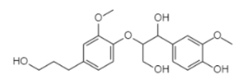
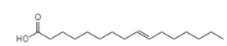
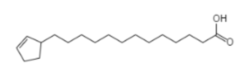
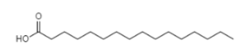
HRMS studies accurately elucidate various pharmacological compounds, highlighting their therapeutic potential. This study reveals the complex molecular structures and diverse mechanisms of action of these compounds, thereby contributing to the increasing frontier of pharmacological research and growth (Fig.11). The table 3.5 shows the list of compounds identified from the methanolic extract of dry Mahua flowers. Quinic acid, which is a natural compound, has significant health effects and clinical value. This compound has numerous pharmacological activities, including antibacterial, antiviral, antioxidant and anti-inflammatory activities (Benali et al., 2022). According to Bai et al. (2019), the compound showed antibacterial activity against *S. aureus* and its reference strain ATCC-29213. It has also been reported that this compound has the potential to inhibit biofilm formation at subMIC concentrations of 1.25 mg/ml. Germacrene D-4-ol is an organic compound that possesses antibacterial and antifungal potential against *S. aureus*, ATCC-25923, *S. sanguinis*, ATCC-49456, *E. coli*, ATCC-25922, *S. tiphymurium*, ATCC-14028, *C. albicans*, ATCC-18804, *A. flavus*, and *F. proliferatum* at MICs ranging from 3.91--125 µg/ml according to dos Santos et al. (2015) and Perez Zamora et al. (2018). The remarkable potential of this compound for antimicrobial and antifungal effects has been described by Abdel-Gawad et al. (2024). Quercetin, a known flavonoid, has shown antibacterial activity along with antibiofilm- and antiquorum-sensing potential. The compound inhibited 95% of the biofilm matrix of *E. faecalis* and 50% of the biofilm production of *S. aureus* (Nguyen and Bhattacharya, 2022).

According to Kannan et al. (2022), this compound has antibacterial and antiquorum-sensing activity against *Serratia marcescens.* The compound fusidic acid also has the potential to inhibit the growth of bacteria, especially gram-positive bacteria, i.e., *S. aureus* (Nigan and Singh, 2014). Palmitoleic acid is a naturally derived compound that is used for the treatment of rough skin and acne. This compound has been studied for its antibacterial activity against *S. aureus* and *Propionibacterium acnes* (Watanbe et al., 2021).

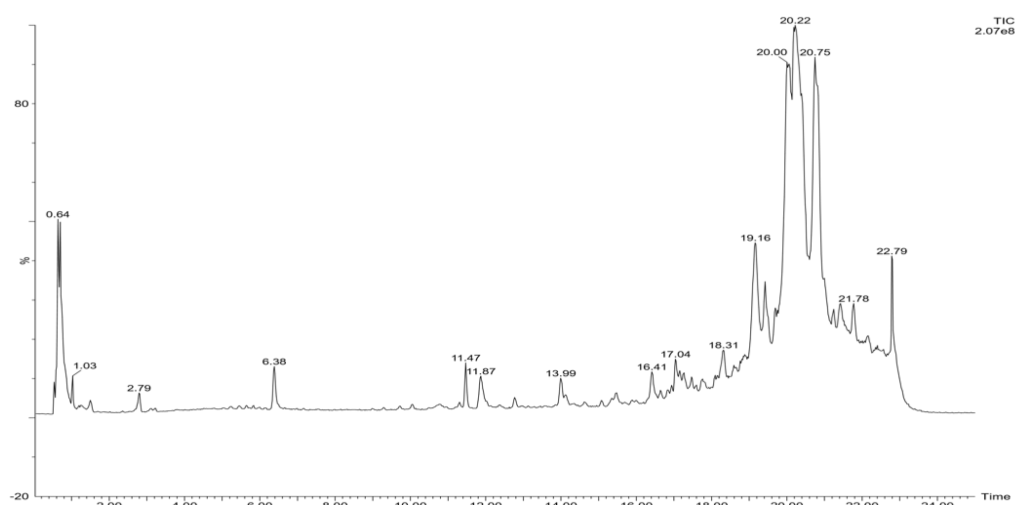
Table 3.5: List of compounds identified from *UHPLC-HRMS analysis*

Description & Formula	**RT (min) & Mode**	**Neutral mass (Da)**	***m/z***	**Fragmentation (*m/z*)**	**Structure**
Quinic acid ($C_7H_{12}O_6$)	0.64 (+ve)	192.063	234.097	213.0866, 188.0917, 170.0811, 163.0601, 145.0496, 130.0498, 116.0706, 112.0393, 102.0549, 98.0600, 84.0444	
D-Fructose ($C_6H_{12}O_6$)	0.87 (+ve)	180.062	203.052	185.042	
2 s)-2-[(4 s)-4-hydroxycyclohexa-1,5-dien-1-yl]-2-{[(2r,3r,4 s,5 s,6r)-3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]oxy}acetonitrile ($C_{14}H_{19}NO_7$)	2.79 (+ve)	313.116	336.105	296.1129, 244.0816, 194.0812, 177.0421, 152.0706, 134.0401, 107.0467	
(2r)-2-methyl-4-(3 h-purin-6-ylamino)butan-1-ol ($C_{10}H_{15}N_5O$)	4.56 (+ve)	221.127	222.134	207.1115	

(3 s)-5-hydroxy-3-[(3r)-3-hydroxybutyl]-3 h-2-benzofuran-1-one (C12H14O4)	11.87 (+ve)	222.089	245.078	214.0391, 177.0547, 149.0234, 121.0284, 93.0335	
Germacrene D-4-ol (C15H26O)	19.26 (+ve)	222.198	223.205	153.1274, 125.0961, 83.0492, 69.0335	
(2 s,3r)-3-(hydroxymethyl)-3-({[(2r,3r,4 s,5 s,6r)-3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-(C11H17NO8)	0.64 (-ve)	291.094	290.087	272.0776, 230.0670, 200.0564, 191.0561, 179.0561, 161.0455, 128.0353, 89.0244, 71.0138	
Kopsidine A (C25H30N2O8)	0.65 (-ve)	486.202	521.171	387.1117, 341.1035, 323.0930, 179.0587, 89.0244	
d-maltotriose (C18H32O16)	0.67 (-ve)	504.167	549.166	503.1617, 409.0987, 341.1089, 323.0983, 221.0666, 197.0561	
Citbismine C (C37H36N2O11)	0.67 (-ve)	684.231	683.224	503.1585, 179.0587	
2-[(1z,4 s,6r)-4-hydroxy-6-{[(2r,3r,4 s,5 s,6r)-3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]oxy}cyclohex-2-en-1-ylidene]acetonitrile (C14H19NO7)	2.82 (-ve)	313.115	358.114	132.0454	

Amygdalin ($C_{20}H_{27}NO_{11}$)	3.25 (-ve)	457.157	456.150	148.0377	
Quercetin ($C_{15}H_{10}O_7$)	7.56 (-ve)	302.042	301.035		
fulgidic acid ($C_{18}H_{32}O_5$)	8.82 (-ve)	328.224	327.217		
pinellic acid ($C_{18}H_{34}O_5$)	8.92 (-ve)	330.240	329.232		
N-Methylnuciferine ($C_{20}H_{24}NO_2^+$)	14.26 (-ve)	310.180	309.173		
(+)-Cnicin ($C_{20}H_{26}O_7$)	14.26 (-ve)	378.168	377.161		
Palmitoleic acid ($C_{16}H_{30}O_2$)	20.19 (-ve)	254.223	253.216		
Chaulmoogric acid ($C_{18}H_{32}O_2$)	20.47 (-ve)	280.239	279.232	253.2173, 241.2173	
Palmitic acid ($C_{16}H_{32}O_2$)	21.13 (-ve)	256.239	255.232		

TOF MS ES+



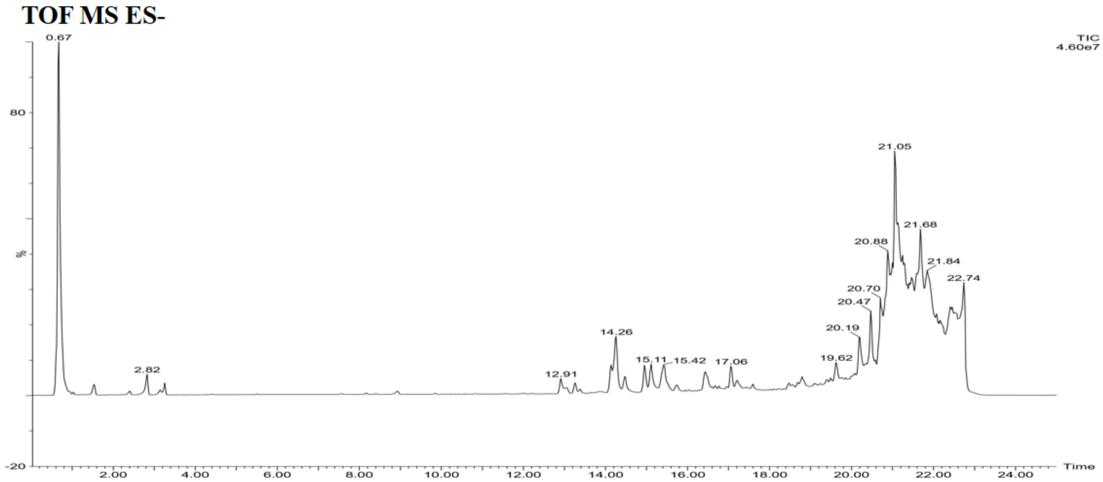


Figure 11 HRMS spectrum of *M. indica* (dry flower methanolic extract) acquired in [positive/negative] ion mode. The spectrum exhibits a series of peaks corresponding to different molecular ions, with their intensities representing the relative abundance of each species.

3.8.6 Total Carbohydrate profiling:

Since traditional times, the mahua flowers are used as a sweetener and used for the preparation of several local products. The sweetness of the mahua flower is due to the presence of several sugar. The chromatograms in Fig. 12.1-12.4 depicts the presence of several sugar. The methanolic extract of fresh mahua flowers contains fructose (15.3%), glucose (14.18%), xylose (0.25%), and sucrose (6.01%). Likewise, the aqueous extract of the fresh mahua flower contains fructose (15.39%), glucose (13.09%), xylose (0.15%), and sucrose (0.57%). The carbohydrate profiling of dry mahua flower was also done where the methanolic extract exhibited fructose (16.51%), glucose (15.54%), xylose (0.29%), and sucrose (2.55%) and the aqeous extract exhibited fructose (8.61%), glucose (13.71%), and sucrose (0.09%).

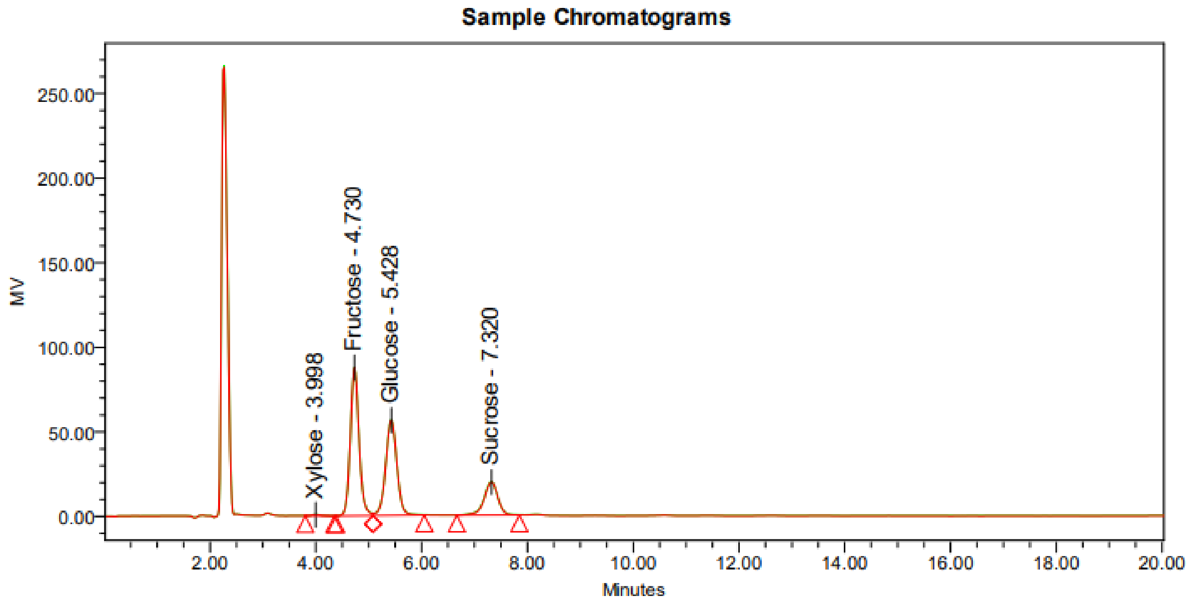


Figure 12.1 The chromatograms depicts the carbohydrate composition of methanolic extract of fresh Mahua analyzed using HPLC with refractive index detection. Peaks represent individual carbohydrates, identified by retention time and compared with standards.

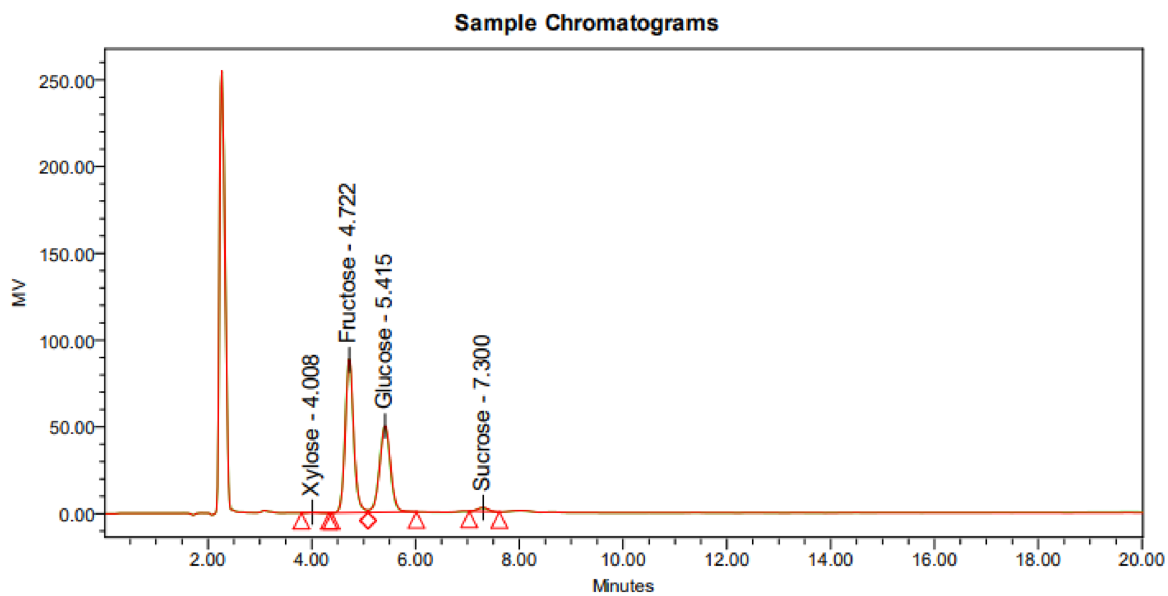


Figure 12.2 The chromatograms depicts the carbohydrate composition of aqueous extract of fresh Mahua analyzed using HPLC with refractive index detection. Peaks represent individual carbohydrates, identified by retention time and compared with standards.

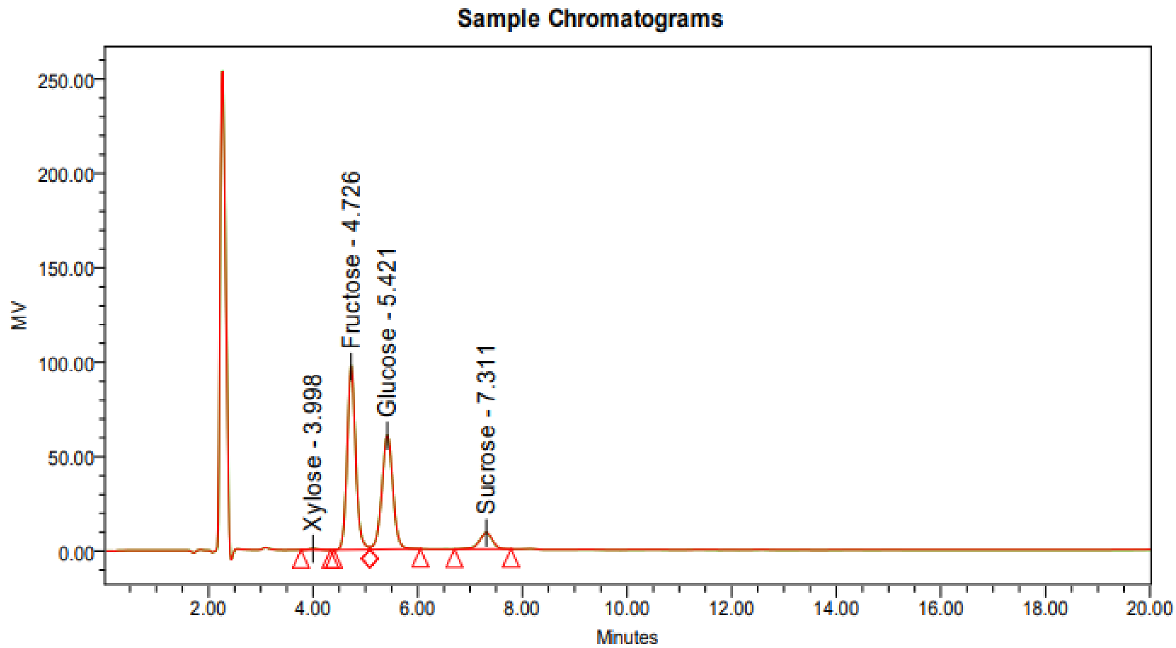


Figure 12.3 The chromatograms depicts the carbohydrate composition of methanolic extract of dry Mahua analyzed using HPLC with refractive index detection. Peaks represent individual carbohydrates, identified by retention time and compared with standards.

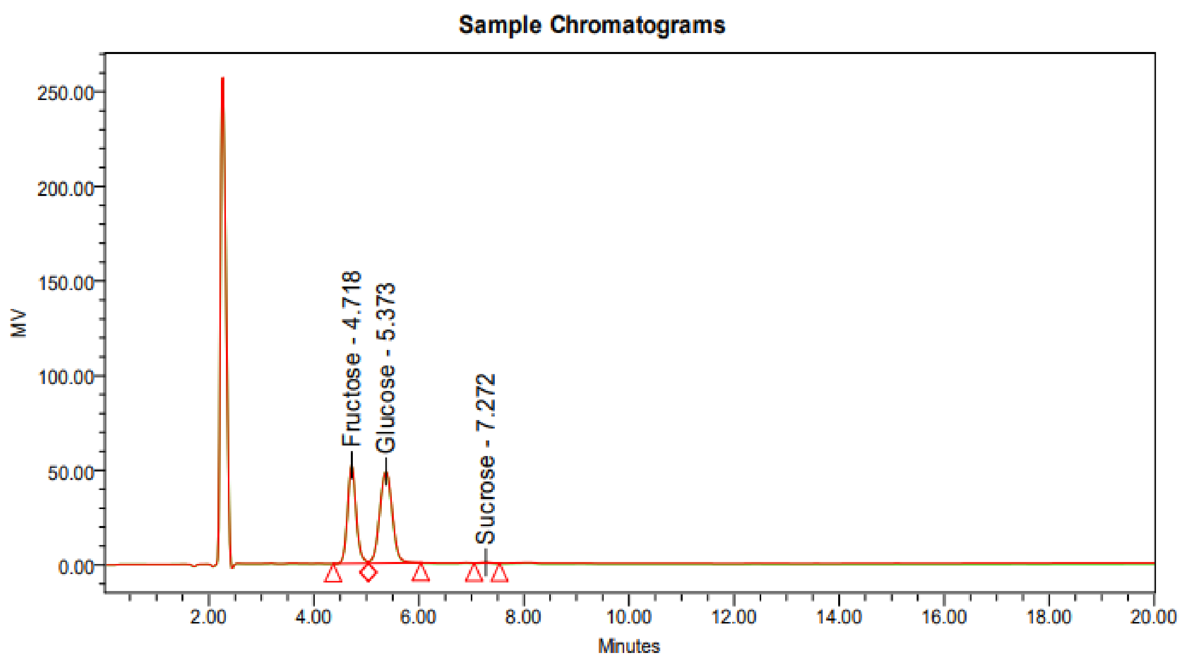


Figure 12.4 The chromatograms depicts the carbohydrate composition of aqueous extract of dry Mahua analyzed using HPLC with refractive index detection. Peaks represent individual carbohydrates, identified by retention time and compared with standards.

3.8.7 Discussion

The different profiling of mahua flowers (fresh and dry) exhibited significant differences between each other, emphasizing their valuable potential and therapeutic benefits. Fresh Mahua flowers are considered by a higher moisture content, which effects in their sugars and phytochemicals (Pinakin et al., 2020). The sugars found in fresh flowers include sucrose, glucose, xylose and fructose, which are vital for inviting pollinators (Endo et al., 2018). The sugars are water-soluble which are sweet in taste and are used for the production of mahua liquor (Sinha et al., 2017). The results of the current study are reliable with those of Sinha et al. (2017), approving the existence of these soluble sugars and their role in the flavor profile of the flowers, which are critical for the beverage's production. As described by the Sethi, 2019, the drying process decreases the water content which leads to the increase in the concentration of sugar. Certain compound may be preserved during the drying process but it may degrade some sugars due to heat (Pinakin et al., 2020). The present study indicated that Mahua flowers are rich in phenolic compounds, flavonoids, and other bioactive constituents. These findings are similar with Singh et al., 2017, who have likewise described the profusion of these bioactive complexes in Mahua, elucidating their potential for therapeutic and antioxidant applications. The results specify that Mahua flowers are ample in phenolic, flavonoids, and other bioactive elements, emphasizing their important prospective for antioxidant and therapeutic properties. Our result depicts that fresh Mahua flowers donates to their raised phenolic content, which in turn improves their antioxidant activity, helping to combat oxidative damage in the body. A similar remark was reported by Arun et al., 2017 who also observed that the phenolic compounds in fresh flowers show a crucial role in boosting antioxidant capacity.The drying process reduces the phenolic content and show variation in the antioxidant activity (Singh et al., 2017). The flavonoid content also varies from fresh and dry mahua flowers. Dry Mahua flowers revealed a higher flavonoid content compared to fresh flowers, which may be due to the loss of water in drying process (Pinakin et al., 2020). This finding is relatable with the results of the present study, which also demonstrates a higher flavonoid concentration in the dry flowers. Still the dried flowers retain the phenols and flavonoid content, which are more stable and less prone to degradation throughout the drying procedure. The DPPH assay was done to measure the free radical scavenging activities of the flower extracts. It revealed that dried flowers exhibited slightly enhanced antioxidant activity due to the concentration of bioactive compounds. The FRAP assay, assesed the reducing power of antioxidants, which confirmed the antioxidant potential of Mahua flowers, suggesting that these flowers can efficiently reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), which can protect the body from oxidative stress. The ABTS assay was done to evaluate the ability of the flowers which can combat oxidative stress.

3.8.8 Conclusion

The complete nutritional and phytochemical analysis of fresh and dried Mahua flowers, *Madhuca indica*, has been established to hold significant value both as a nutrient-rich food item and as a storehouse of bioactive compounds with considerable therapeutic potential. Fresh Mahua flowers are rich in essential macronutrients like carbohydrates, proteins, and dietary fiber, and are rich in micronutrients and vitamins, especially vitamin C. The removal of water during the drying process enhances the concentration of these nutrients, making dried flowers an even richer source of essential nutrients. Dried Mahua flowers show increased mineral, protein, and carbohydrate concentrations, which may provide more health benefits when consumed in this form. Phytochemically, Mahua flowers are a rich source of bioactive compounds, including alkaloids, flavonoids, saponins, tannins, and terpenoids. These compounds have a wide range of biological activities, including antioxidant, anti-inflammatory, antimicrobial, and antidiabetic effects. Flavonoids are specifically known for their strong antioxidant properties, which combat oxidative stress and may reduce the risk of chronic diseases such as heart disease and cancer. Phenolic compounds show antimicrobial and anti-inflammatory activity, making Mahua flowers very valuable for use both in preventive and therapeutic applications. Although the drying process can break down some volatile compounds, it produces a more stable product with longer shelf life, ensuring that beneficial nutrients and phytochemicals in Mahua flowers are preserved in dried form. This makes the dried Mahua flowers an ideal candidate for the production of medicinal extracts, functional foods, and nutraceuticals, extending their usability across different sectors. In conclusion, Mahua flowers, fresh or dried, contain a rich array of nutritional and phytochemical constituents that offer significant health benefits. The diverse bioactive compounds present make them a promising natural resource for a variety of applications, from traditional medicine to modern pharmaceutical and food industries. Future research focused on their pharmacological properties, mechanisms of action, and potential for commercialization will further elucidate the full range of benefits that Mahua flowers can offer, contributing to their recognition as a valuable plant in the pursuit of health and wellness.

CHAPTER-IV

EXPLORING THE ANTIBACTERIAL AND ANTIBIOFILM EFFICACY OF MAHUA (*MADHUCA INDICA*): A PHYTOCHEMICAL AND BIOLOGICAL INVESTIGATION

4. Background

Plant-based medicines are considered the backbone of traditional medicines prescribed in the Ayurvedic era because of their potential pharmacological efficacy. Hence, they are recognized as a major source of new drug development (Najmi et al. 2022). More than 75% of the population of developing countries still have faith in traditional medicines. In the modern era, due to poor hygiene and sanitation, the consumption of contaminated food and water, inadequate vaccination, a weakened immune system, several environmental factors, and severe bacterial infections are increasing (Jubair et al. 2021).

4.1 Introduction

Many of these bacteria are resistant to several common antibiotics. Several factors cause antibacterial resistance in bacteria, including the overuse and misuse of antibiotics, gene mutation, horizontal gene transfer, and, most importantly, a lack of new antibiotics (Padma, 2022). In this context, our research focused on finding noble substitutes for antibiotics to address the growing threat of antibiotic-resistant bacteria (Stachelek et al. 2021). Under these circumstances, there is a need to increase the screening of plants with medicinal value. In this context, the butternut tree scientifically known as *Madhuca indica* (Sapotaceae family) is considered a universal solution in ayurvedic medicine. In religious communities, mahua is considered to have ornamental and decorative value (Pandey and Rakesh, 2023). Traditionally, the flower is used as an analgesic, diuretic, cooling agent, etc. Similarly, it is used to treat helminths, tonsillitis, and bronchitis. Traditionally, flowers are also used as cooling agents (Shrirao et al. 2017). The flowers are fleshy and edible and have high reducing sugar content and nutritional value. Owing to their high sweetening index, these tribes use flowers for the preparation of several food products (Lungade and Karadbhajne, 2022). According to Lungade and Karadbhajne (2022), dried mahua flowers are a rich source of protein (6.67 g/100 g) and carbohydrates (68 g/100 g). Depending on the geographical region, the sugar content of dry mahua flowers varies from 40--70%. According to Singh et al. (2021), flowers can be used as sugar substitutes and have very high vitamin and mineral contents. According to Kendre and Wakte, 2021, flowers have several phytoconstituents, including carotene, ascorbic acid, thaimine, riboflavin, niacine, folic acid, biotene, and inositoole. The aroma of a flower is due to the presence of 2 acetyl-1 pyrroline (Badukale et al. 2021). In addition to being a sugar substitute, flowers are rich in protein and contain certain essential minerals, such as potassium, calcium, iron, and phosphorous (Sreenivasa et al. 2024). Owing to the presence of several bioactive compounds, these plants exhibit several pharmacological activities. The aqueous and alcoholic extracts of mahua flowers exhibited analgesic activity (Shrirao et al. 2017). A detailed study revealed that the plant possesses saponins, flavonoids, glycosides, triterpenoids, and saponins (Pinakin et al. 2020). The plant parts have been used for the treatment of various infections, wounds, arthritis, heart diseases and several disorders. An in vivo study revealed that the plant has antioxidant, antidiabetic, immunomodulatory, and cardioprotective properties (Sreenivasa et al. 2024). The mahua flower juice is used as a tonic for the treatment of tonsillitis, bronchitis, and helminths (Mishra et al. 2023). However, the search for new bioactive compounds from plants remains a potential area of investigation. This can be accomplished by conducting several phytochemical analyses. The present study focused on the phytochemical composition (specifically phenolic and flavonoid contents), phytochemical profile (based on GC–MS and UPLC–Q–TOF–MS), antioxidant activity, antibacterial and antibiofilm activities of Mahua flower extracts.

4.2 Methodology

4.2.1 Preparation of plant extracts

Fresh flowers of *Madhuca indica* were collected from local sites in Sambalpur and dried in a traydrier. The fresh and dried flowers were extracted via different solvents, such as methanol, hydroethanol (50%), and an aqueous mixture, in a Soxhlet extraction system at 40 °C until the maximum phytoconstituents were leached from the plant samples. The flower extracts were concentrated via evaporation via a rotary evaporator at 40 °C, followed by lyophilization.

4.2.2 *Antimicrobial activities of M. indica flower extracts*

4.2.3 Bacterial culture maintenance

To assess the antimicrobial activities of the *M. indica* flower extracts against clinical bacterial strains, the bacterial isolates were collected from Veer Surendra Sai Institute of Medical Sciences and Research (VIMSAR), Burla, Sambalpur, Odisha, India. The bacterial strains used for preliminary screening were *Staphylococcus aureus*, *Enterococcus faecalis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Shigella* sp., *Proteus* sp., *Salmonella typhi, S. Paratyphi A* and *S. Paratyphi B.* The bacterial strains were cultured and maintained in nutrient broth (NB) media.

4.2.4 Preliminary antimicrobial screening

To determine the antibacterial efficacy of the extracts, an initial screening was performed on ten different clinically available strains. The antibacterial screening was carried out via the agar well diffusion method via Mueller–Hinton (MH) agar plates as described by Hota et al. (2024). DMSO was used as a control. The MH agar plates were subjected to incubation overnight at 37 °C. The antibacterial activity was measured by measuring the holo zone around the well.

4.2.5 Determination of the minimum inhibitory concentration (MIC)

The MIC was evaluated by following the CLSI guidelines for the 2-fold serial microdilution method as described by Cheruvanachari et al. (2022). The selected bacterial cultures (0.5 McFarland) were supplemented with serially diluted mahua flower extracts at different concentrations (20, 10, 5, 2.5, 1.25, 0.625, 0.312 and 0.156 mg/ml). The 96-well plates were incubated overnight at 37 °C. A bacterial culture without plant extracts was considered a negative control, whereas a bacterial culture with the antibiotic Imipenem (IC) was considered a positive control. The absorbance of the reaction mixture was measured at 600 nm in a microplate reader. At the MIC, the antibacterial potential of the flower extract and Imipenem was determined via the agar well diffusion method. The results were taken in triplicate. (Hota el al. 2024)

4.2.6 Anti-biofilm efficacy of M. indica flower extracts

4.2.6.1 Tube adhesion assay

The effects of *M. indica* flower extracts and Imipenem (IC) on the formation of biofilm matrices in the selected bacterial strains were evaluated via tube adhesion assays via the crystal violet staining method, light microscopic method, and confocal scanning microscopic assay as described by Cheruvanachari et al. (2023). The evaluation of biofilm inhibition in the presence of the flower extracts was performed via both qualitative and quantitative methods. The tube adhesion assay revealed the ability of bacteria to adhere to different surfaces through biofilm matrix formation when treated with or without plant extracts and IC.

4.2.6.2 Microscopic analysis of bacterial biofilms

For light microscopic analysis, the selected bacterial strain was grown in sterile NB medium supplemented with or without sub-MIC levels of the flower extracts and IC in a 6-well microtiter plate containing sterile coverslips. The 6-microtiter plates were incubated overnight at 37 °C. After incubation, the culture medium was discarded, and the attached biofilm matrices were washed with sterile PBS and further stained with 0.1% crystal violet for 20 min. The stained coverslips were then mounted onto slides and visualized under a light microscope (Quasmo, PZRM-26) (Cheruvanachari et al., 2023). Moreover, for the confocal laser scanning microscopic assay, acridine orange was used to stain the biofilm matrix containing coverslips, which was visualized under a microscope as described by Mhade and Kaushik (2023).

4.2.6.3 Crystal violet staining assay

For the quantitative estimation of biofilm formation after treatment with sub-MIC levels of the flower extracts, the inhibitory concentration (IC) was determined via the crystal violet staining method described by Hota et al. (2024). Briefly, the selected bacterial strains were grown in sterile nutrient broth supplemented with or without plant extracts at 37 °C overnight. After the completion of the incubation period, the culture media was discarded, and the attached biofilm matrices were washed in saline phosphate buffer (PBS) solution (pH 7.0), followed by staining with 0.1% crystal violet for approximately 20 min. The excess strains were removed, 95% ethanol was added to the stained biofilm matrices over the polystyrene surface, and the optical density (OD) was measured at 540 nm.

4.2.6.4 Cell surface hydrophobicity assay

The cell surface hydrophobicity assay was carried out as described by Danchik et al. (2021). The test pathogens were harvested with and without the flower extracts. The supernatant was discarded, and the samples were washed with phosphate buffer. The residue was resuspended in PBS and divided into two equal parts. The optical density of one fraction was measured at 600 nm, and the remaining fraction was added to toluene and allowed to undergo phase separation. The aqueous phase was pipetted out and measured at 600 nm. The hydrophobicity (%) was calculated via the following formula:

Hydrophobicity (%) = (Initial cell density − final cell density)/Initial cell density × 100

4.3 Results

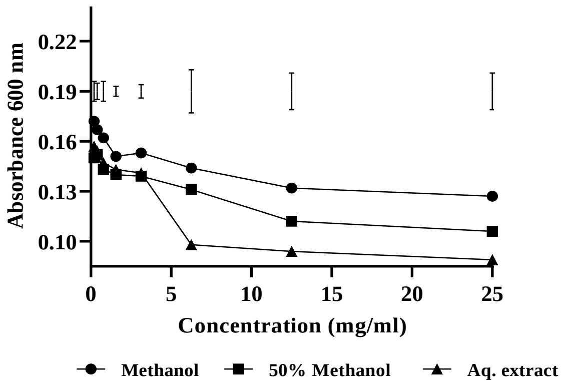
4.3.1 Antibacterial activity

Fresh dry mahua flower extracts (methanol, hydromethanol, and aqueous) were screened against 10 different bacterial cultures. Compared with dry flower extracts (methanol, hydromethanol, and aqueous), fresh mahua flower extracts (methanol, hydromethanol, and aqueous) did not show much antibacterial potential. The dry mahua flower extracts showed activity against *S. aureus, E. faecalis, P. aeruginosa* and *K. pneumoniae*. It also exhibited potential against reference strains of the abovementioned bacteria, i.e., MTCC-740, MTCC-439, MTCC-2488, and MTCC-109. Furthermore, the antibacterial and antibiofilm activities of dry mahua flower extracts (methanol, hydromethanol, and aqueous) were investigated via *S. aureus*, *E. faecalis*, *P. aeruginosa,* and *K. pneumoniae* and the reference strains MTCC-740, MTCC-439, MTCC-2488 and MTCC-109.

4.3.2 Determination of the minimum inhibitory concentrations (MICs)

For the methanolic extract, the dry flower had an MIC of 12.5 mg/ml against *S. aureus*, MTCC-740, *E. faecalis*, MTCC-439, *K. pneumonia*, and MTCC-109 and an MIC of 6.25 mg/ml against *P. aeruginosa*, MTCC-2488. For hydromethanolic (50% methanol), the calculated MIC was 12.5 mg/ml against *S. aureus*, MTCC-740, *P. aeruginosa*, and MTCC-2488 and 6.25 mg/ml against *E. faecalis*, MTCC-439, *K. pneumonia* and MTCC-109. The aqueous extract had the lowest MIC of 3.125 mg/ml against MTCC-740, *E. faecalis*, and MTCC-439, as shown in Fig. 13.1. The pathogens were highly sensitive to treatment with *M.*

indica (dry mahua extracts, i.e., methanol, 50% methanol and aqueous extracts), with the highest zone of inhibition against *E. faecalis,* i.e., 14.52 ± 0.23 mm.



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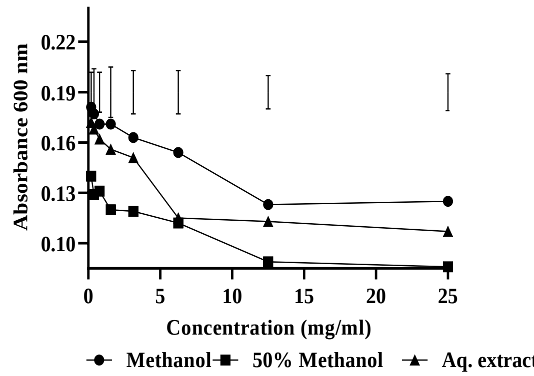

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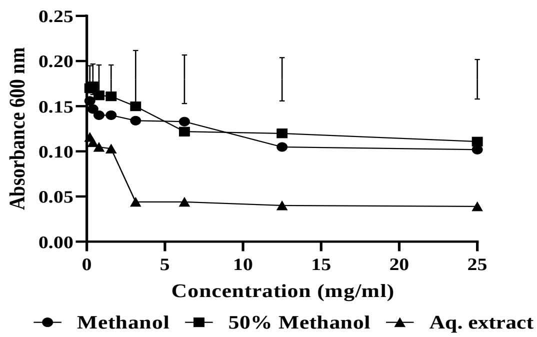

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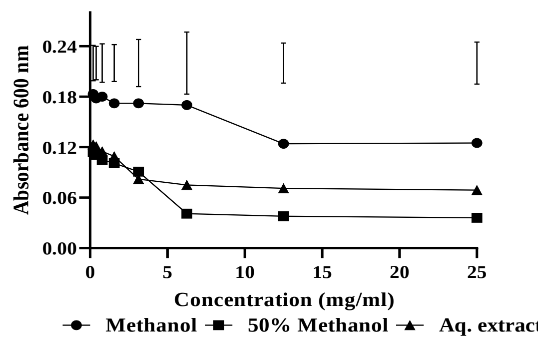

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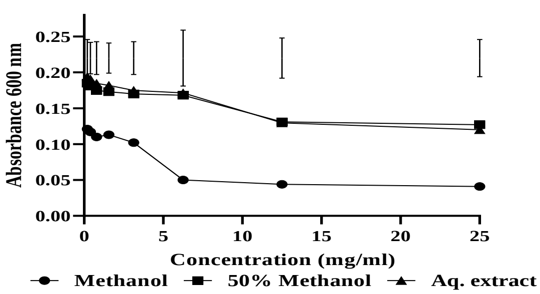

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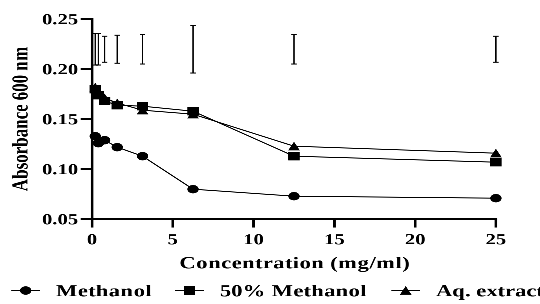

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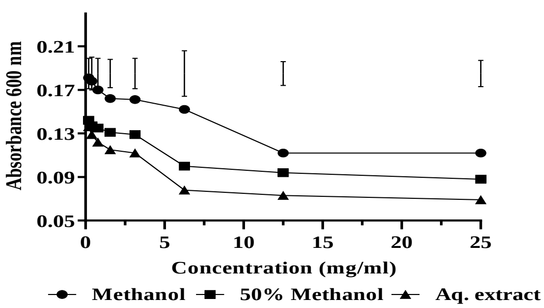

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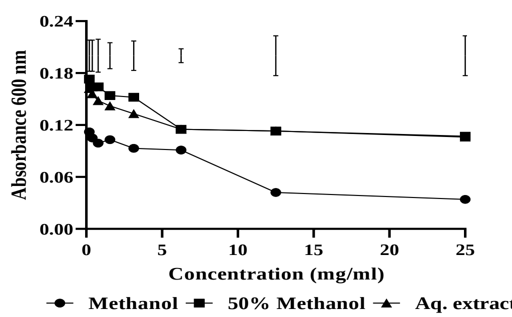


(h)

Figure 13.1 Minimum inhibitory concentrations (mg/ml) of different extracts of *M. indica* (dry mahua flower) against (a) *S. aureus*, (b) MTCC-740, (c) *E. faecalis*, (d) MTCC-439, (e) *P. aeruginosa*, (f) MTCC-2488, (g) *K. pneumonia* and (h) MTCC-109 via the serial dilution method. The graph shows the growth of the bacteria in the presence of increasing concentrations of the extracts. The vertical bar represents the least significant difference (LSD) at $p \leq 0.05$.

4.3.2 Antibiofilm efficacy

(a) Tube adhesion assay

To determine the antibiofilm potential of flower extracts, a tube adhesion assay is commonly used to assess the formation or inhibition of biofilms in test tubes. A quantitative assay is used to assess biofilm formation on the basis of attachment to test tubes. The test tubes were visually observed for any adherent biofilm. As shown in Fig. 13.2, biofilms were present and distributed throughout the control test tubes. In the control tubes, there was a thicker attachment of the biofilm layer in the bottom and sidewall of the tubes, which indicates the strength and extent of biofilm formation. Similarly, the tube adherence assay revealed the presence of minor rings at the liquid–air interface and slight or no attachments at the surface or bottom surface when the test pathogens were supplemented with sub-MICs of different extracts of *M. indica* and Imipenem.

(b) Crystal violet staining method

The graph (Fig. 13.3) displays the absorbance values obtained from the crystal violet staining method measured at 540 nm. The bars represent the mean absorbance for the control, antibiotic and treatment groups. A higher absorbance represents greater biofilm biomass, which indicates that more dye has bound to the biofilm matrix. In contrast, in terms of the absorbance of antibiotics, i.e., Imipenem and treated groups, there was a reduction in the absorbance, which indicates a reduction in biofilms when antibiotics are given at subMICs. The results provide a clear quantitative measure of biofilm formation and the effectiveness of interventions aimed at biofilm reduction, supporting conclusions about the biofilm-forming capacity of various microbial strains or the efficacy of biofilm-disrupting treatments.

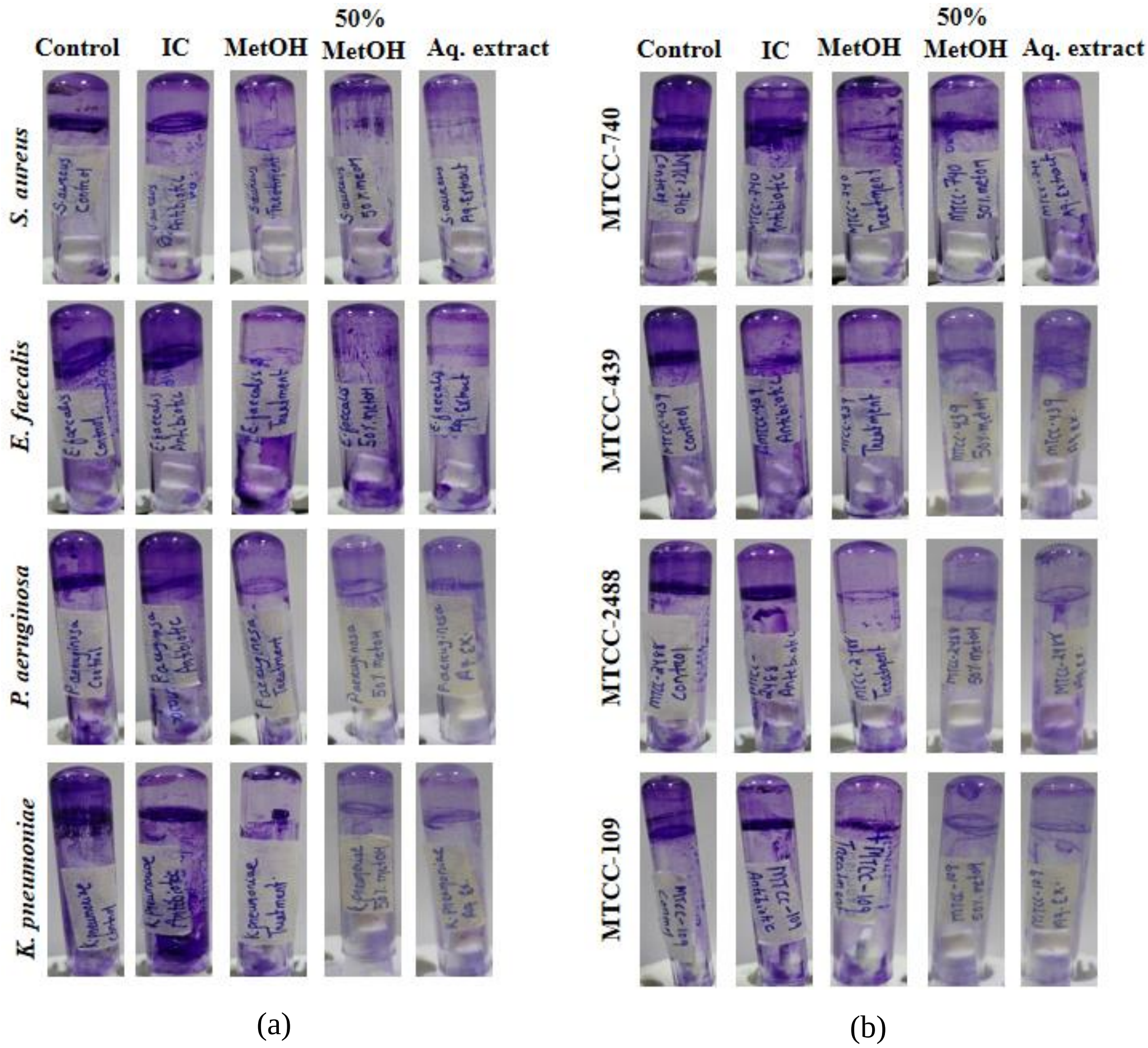
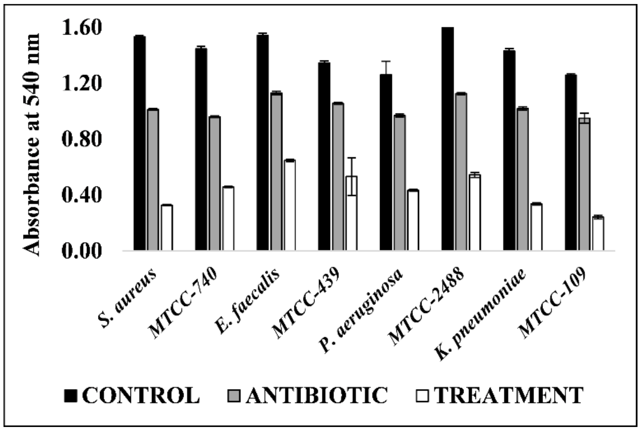


Figure 13.2 The figure qualitatively illustrates biofilm formation and inhibition. The control test tubes (without treatment) show biofilm adherence by visible layers in the walls and bottom of the tubes. The antibiotics and the treated tubes exhibited minimal or very thin layers of biofilms, which indicates the inhibition of biofilm formation.



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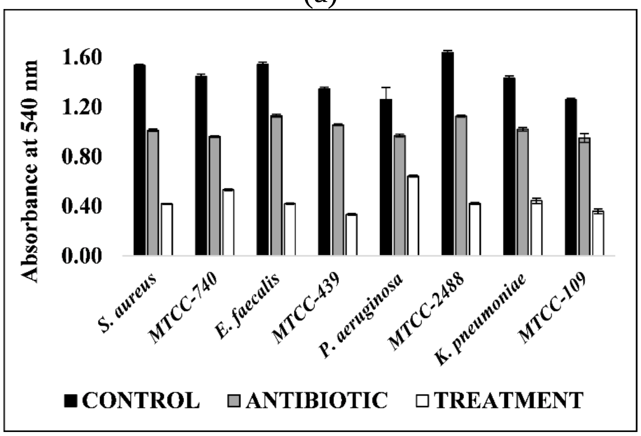

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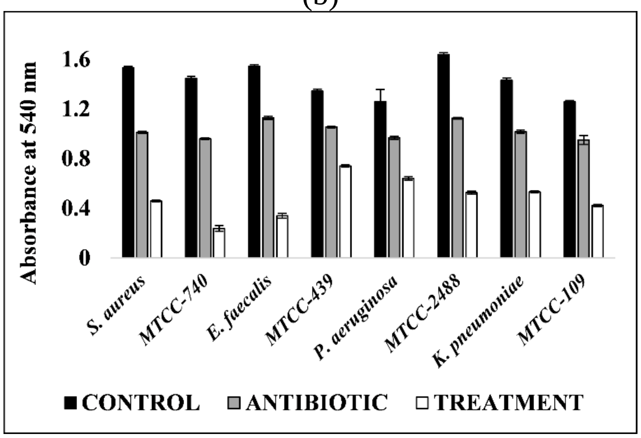
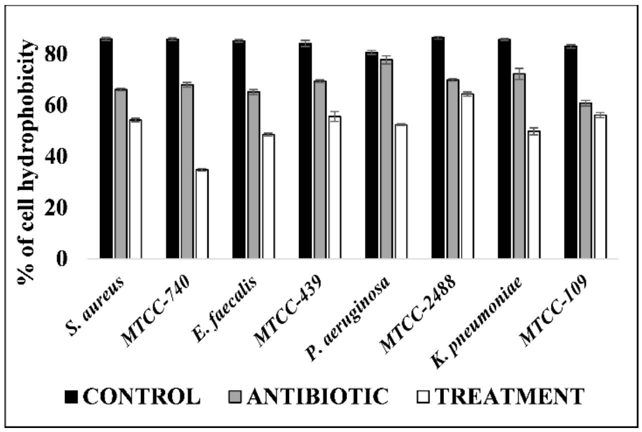


(c)

Figure 13.3 The bars represent the mean absorbance, indicating biofilm formation and inhibition. The higher the absorbance is, the greater the degree of biofilm formation. The bars for the antibiotic-treated groups presented relatively low absorbance values, which demonstrated their effectiveness in reducing biofilm formation in the (a) methanolic extract, (b) hydromethanolic (50% methanol) extract, and (c) aqueous extract. The error bars represent the standard deviations.

(c) Cell surface hydrophobicity assay

The percentage of cell hydrophobicity measured in the biofilm inhibition assay is presented in Figure. The control bar shows that with increasing absorbance, there is an increase in surface hydrophobicity, which leads to increased biofilm formation. The hydrophobic nature of the pathogens enhances the initial adhesion of the microorganisms. Similarly, the bars that represent the antibiotic and treated groups are less hydrophobic, which indicates a lower absorbance value. At subMICs, the bacteria are hydrophilic, which hinders the adhesion of the test pathogens (Fig. 13.4).



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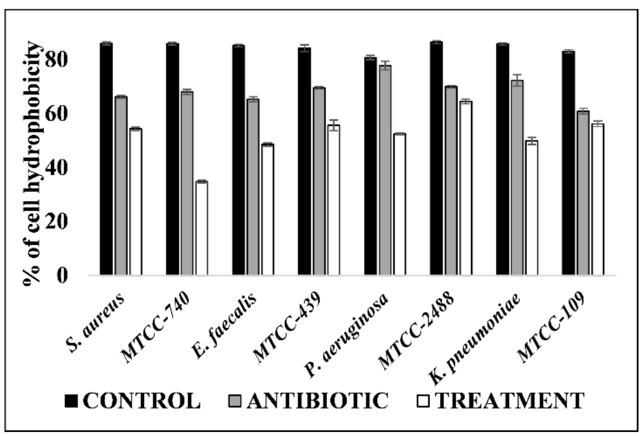

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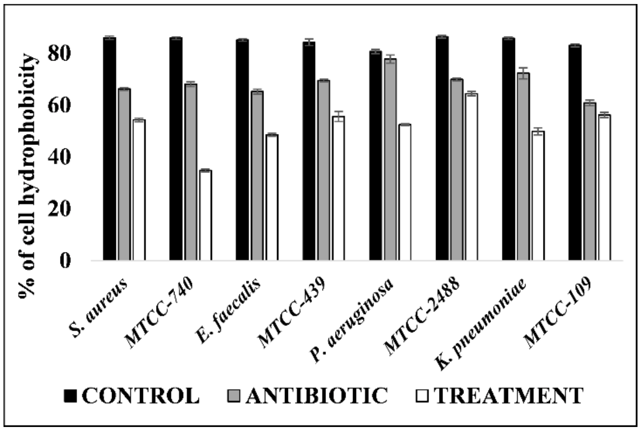
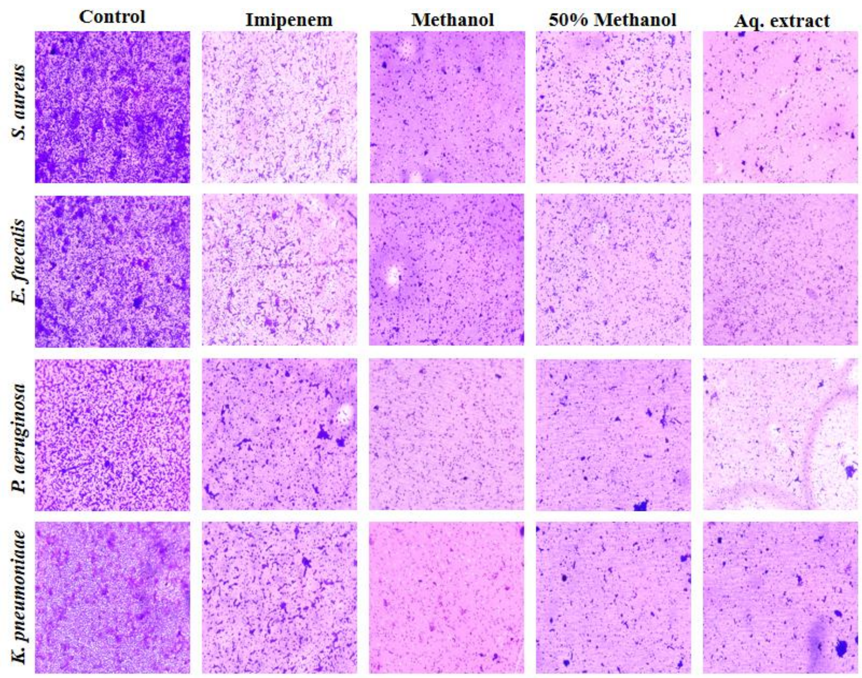


(c)

Figure 13.4 The graph shows the percentage of hydrophobicity measured in a biofilm inhibition assay. The data represent the percentage of hydrophobic cells relative to the total cell population, which was calculated from the absorbance values at 600 nm: (a) methanolic extract, (b) hydromethanolic (50% methanol) extract, and (c) aqueous extract. The error bars represent the standard deviations. The error bars depict the standard deviation.

(d) Light microscopic assay

The light microscopic assay is considered a valuable tool for assessing biofilm inhibition because it provides visual insights into biofilm formation and the effects of treatments. The morphology and density of the biofilms formed under untreated (control) conditions were compared with those of the biofilms formed in the presence of subMICs of antibiotic (Imepenem) and *M. indica* flower extracts. Under a light microscope, the control slides were observed to be healthy and exhibited dense aggregates of cells fixed in extracellular matrix material. In contrast, a reduction in biofilm biomass or decreased feasibility of biofilm cells compared with that of control samples indicates biofilm inhibition. There was a major decline in the biofilm production of the antibiotic (imepenem) and *M. indica* flower extracts when they were treated with sub-MIC concentrations. A light microscopic study revealed that, in the control, the bacterial cells were more clustered than those in the plants treated with the *M. indica* flower extracts (Fig. 13.5).



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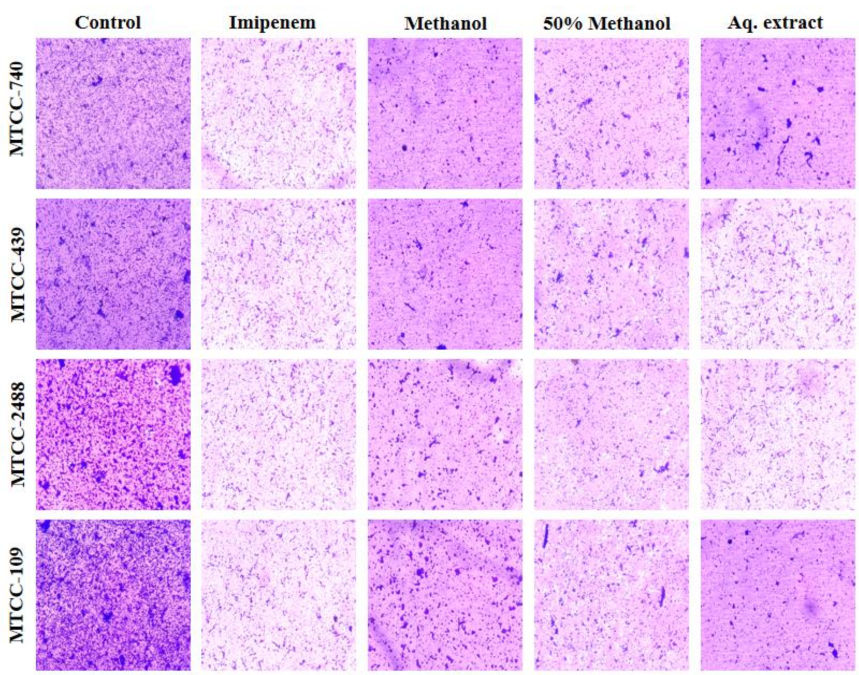
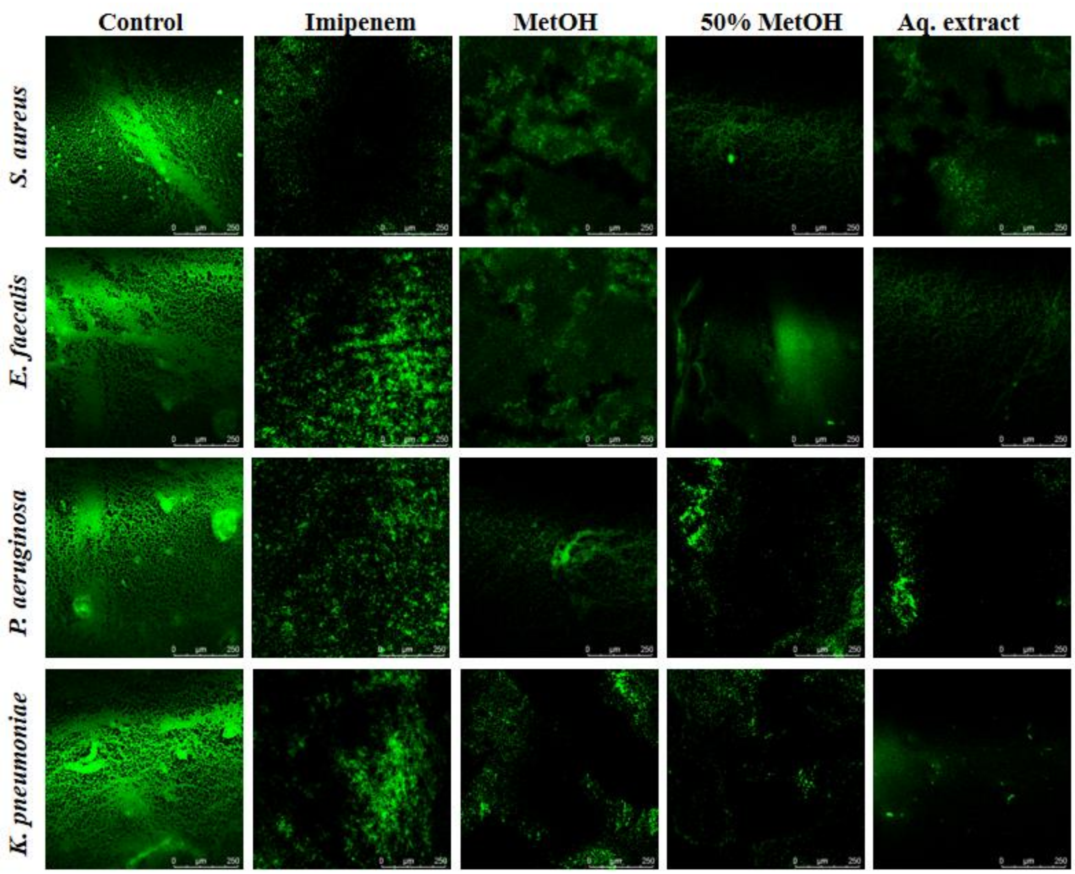


(b)

Figure 13.5 Effects of subMICs of imipenem and flower extracts of *M. indica* on biofilm formation in test pathogens via light microscopy: (a) clinical isolates and (b) MTCC strains. A decrease in biofilm biomass, reformed biofilm morphology, or reduced viability of biofilm cells compared with those of the control samples indicate biofilm inhibition.

(e) Confocal laser scanning microscopic assay

The images (Fig. 13.6) illustrate the 3D structures of biofilms formed by the test pathogens under control conditions versus treatment with imipenem and *M. indica* flower extracts. The control biofilms presented thick accumulation and robust structures, whereas the treated samples presented major alterations in architecture, characterized by reduced biomass and less distinct clustering. Quantitative analysis revealed a marked reduction in biofilm thickness and fluorescence intensity in the treated samples, indicating effective inhibition of biofilm formation. These findings suggest that the imepenem and *M. indica* flower extracts significantly disrupt the ability of cells to adhere to and form mature biofilms, ultimately highlighting their potential as therapeutic agents for managing biofilm-associated infections.



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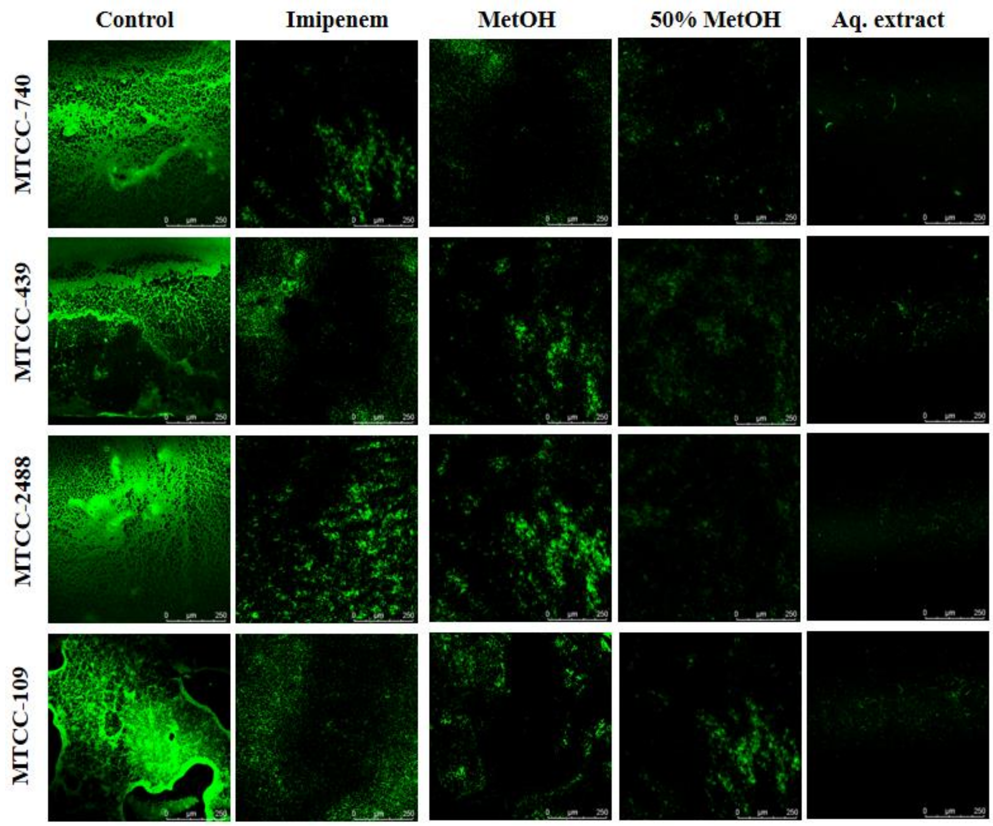


(b)

Figure 13.6 Effects of imipenem and *M. indica* flower extracts at subMICs on biofilm formation by the (a) test pathogens and (b) their reference strains. The control biofilms presented dense, uniform structures with major cell accumulation, whereas the treated biofilms presented compact biomass and less clustering.

4.4 Discussion

The Indian butternut tree, i.e., *M. indica,* is significant for several reasons. The tree provides support for biodiversity. It fulfils the major F's, i.e., food, fodder and fuel (Mishra et al. 2023). The presence of trees prevents soil erosion and improves soil fertility. These findings hold considerable cultural importance. The flowers are edible and are used for making several food products. They are considered to be a source of nutrition for local communities (Yadav et al. 2024). Flowers are traditionally used to treat rheumatism, diabetes, eczema, orchitis, etc. (Mishra et al. 2021). The flower also has anthelminthic activity, antioxidant activity and anticancer activity at a concentration of 200 g/ml. Compared with dry flowers, fresh flowers have high phenolic and flavonoid contents, i.e., 50–100 mg GAE/g fresh weight, 30–70 mg GAE/g dry weight, and 25.3±1.0 GAE/g fresh weight (Singh, 2017;

Singh et al. 2020). The flavonoid content ranges from 20–40 mg QE/g fresh weight, 15–30 mg QE/g dry weight, and 13.1±0.3 QE/g fresh weight (Singh, 2017; Singh et al. 2020). The phenolic content contributes to the antioxidant properties of flowers and is associated with several health benefits and antimicrobial activities. However, there was a decrease in flavonoid and phenolic contents in dry flowers due to heat and light exposure. Similarly, compared with dried mahua flowers, fresh flowers have greater antioxidant value. The FRAP value ranged from 101.6 ±0.8 QE/g fresh weight for the fresh mahua flower extract. The DPPH scavenging activity of the fresh mahua flower extract ranged from 89.4 ±0.1. Similarly, the ABTS activity ranged from 74.1 ± 1.1 mmol Trolox/g FW in fresh mahua flowers. As discussed in the Results section, the compounds identified via GC–MS and UHPLC–HRMS analysis have antibacterial, antibiofilm and pharmacological properties. Compounds such as quinic acid, germacrene D-4-ol, quercetin, fusidic acid, 3-amino-2-oxazolidinone, dihydroxyacetone, 1,3,5-triazine-2,5-hydroxymethylfurfural, quinic acid, 3-deoxy-d-mannoic acid, butanedioic acid and 1,2,3-benzenetriol have antibacterial, antibiofilm, anti-QS, antiviral, antioxidant and anti-inflammatory activities. Both fresh and dry mahua flowers exhibit antibacterial activity (Pinakin et al. 2020; Singh et al. 2020). The fresh and dry flowers exhibited activity against both gram-positive and gram-negative bacteria. Fresh flowers showed activity against *S. aureus* (0.5–1.0 mg/ml), *E. coli* (1.0–1.5 mg/ml), *S. typhi* (1 mg/ml), and *P. aeruginosa* (1.0–2.0 mg/ml). The dry flowers exhibited activity against *S. aureus* (1.0–2.0 mg/ml), *E. coli* (2.0–3.0 mg/ml), *S. typhi* (2.0 mg/ml) and *K. pneumoniae* (2.0–3.0 mg/ml) (Mishra and Poonia, 2019). Flowers possess several phytoconstituents, such as vitamin A and vitamin C (Prajapati et al. 2021). The flower is a rich source of protein (6.67 g/100 g), sugar (54.06 g/100 g), calcium (139.00 mg/100 g), phosphorus (137.00 mg/100 g), thiamine, riboflavin and niacin. The flower also contains amino acids such as leucine, isoleucine, lysine, and methionine (Lungade and Karadbhajne, 2022).

4.5 Conclusion

M. indica has significant potential as a natural remedy for several types of bacterial infection. Overall, the flower is a rich source of bioactive compounds such as phenols, flavonoids, and antioxidants. These compounds increase the antibacterial, antibiofilm, and antioxidant potential, which ultimately contributes to the therapeutic potential of the flower. These findings illustrate that dried Mahua flower extracts have antibacterial and antibiofilm potential against the ESKAPE group of pathogens and their reference strains. Eventually, Mahua (*Madhuca indica*) emerged as a promising aspirant in the kingdom of natural medicine, blending traditional knowledge with scientific review. Its multifaceted bioactive profile not only highlights its role in traditional healing practices but also positions it as a valuable reserve in modern therapeutic uses. As the modern healthcare community is pursuing real and effective replacements to battle antibiotic-resistant bacteria, Mahua could play an essential role in determining future approaches for bacterial infections and biofilm control.

CHAPTER-V

DEVELOPMENT OF VALUE-ADDED FOOD PRODUCTS FROM MAHUA FLOWERS AND THEIR NUTRITIONAL PROFILE

5.1Background

The increasing demand for the development of value-added food products from nontimber forest products (NTFPs) has gained significant attention in recent years, predominantly in terms of food security, the growing demand of consumers and sustainable agriculture (Mondo et al., 2024). This process not only promises to solve the global problem, i.e., food challenges faced by the population by utilizing NTFPs but also contributes to promoting biodiversity and supporting the livelihoods of the people (Zhu and Lo, 2021).

5.2 Introduction

Owing to the sustainability and economic benefits of mahua flowers, they have gained significant advantages in rural communities. It has gained economic potential because of its sustainability, especially in rural regions where people rely on NTFPs for their livelihoods (Talukdar et al., 2021). The Mahua flowers produced in the leanest season of agriculture and support the livelihood of the poorest of the poor. The tree is drought tolerant and does not need any extra care for its growth (Bisht et al., 2018). Deforestration or land degradation is not essential for the harvesting of mahua flowers, which makes it an environmentally viable alternative to many other agricultural practices. The development of value-added food products from mahua flowers could generate income sources for rural farmers. This could eventually create jobs in small-scale industries. Among several bioresources found in nature, Mahua (*Madhuca indica*), a flowering tree found in India, is considered to have extraordinary potential for the development of value-added food products that can fulfil these multiple objectives.

5.2.1 The Mahua Tree: Ecological and Cultural Significance

The tree is found in the Indian subcontinent and survives in the tropical and subtropical climates of India, Sri Lanka, and Nepal. More precisely, it is found in the dry forests of central India, i.e., Odisha, Madhya Pradesh, and Maharashtra (Bisht et al., 2018). Mahua is a hardy tree that plays a vital ecological role in its natural environment, contributing to the balance of soil, the conservation of water, and the maintenance of biodiversity in forested areas (Shah et al., 2024). In addition to their ecological importance, plants also bear certain cultural importance. It is widely worshiped by tribal communities. The flowers and fruits are integral parts of their local customs, food practices and traditional medicines. Unaware of their nutritional significance, locals use flowers for the production of an alcoholic beverage named *"country liquor"* or Mahua liquor (Johar et al., 2020). The flowers are traditionally believed to have certain medicinal benefits, such as anti-inflammatory, antimicrobial, and antioxidant properties.

5.2.2 Traditional Practices and Potential for Mahua-based Food Products

Mahua flowers are an integral part of the life of tribal and rural communities. The flowers are rich in carbohydrates, and owing to their sweet taste, they have been used for the preparation of several food products (Singh et al., 2021). The locals used the flowers in the production of alcoholic beverages. Historically, flowers have been used, especially in the rural areas of India. It is considered a rich source of carbohydrates, so it can be used for the preparation of certain value-added food products (Mishra et al., 2023). Locals employ flowers for the preparation of alcoholic beverages, i.e., *country liquor.* In the present era, flowers are recognized as a source of superfood, and interest in their nutritional and bioactive compounds has increased. In the modern food industry, the potential of mahua flowers is still untapped. This is mainly due to the lack of research on how to incorporate mahua flowers into commercially viable products. In today's era, there has been a rising trend towards the utilization of underutilized NTFPs, which also includes the investigation of Mahua flowers in value-added food production. The incorporation of mahua flowers into food products provides basic nutrition and can eradicate diseases such as malnutrition.

5.2.3 Nutritional and bioactive composition of Mahua flowers

The flowers are not only a rich source of sugar but also contain a wide range of macronutrients and micronutrients, including sugars, animoacids, vitamins, etc. (Sinha et al., 2017), which makes them excellent candidates for the development of value-added food products. Reports also suggest that it contains a good amount of phenols, flavonoids, tannins, and antioxidants, which can prevent several diseases, such as cardiovascular disease, cancer, and diabetes. Vitamins such as vitamin C, B vitamins, and minerals such as iron, magnesium, and potassium are found in mahua flowers (Seenivasa et al., 2024). The presence of these nutrients contributes to the health benefits of mahua-based food products, making them not only energy-dense but also nutritionally rich.

5.2.4 Research gaps and opportunities

Although mahua flowers have gained enough recognition as functional foods or superfoods, there is still a certain research gap related to the processing of mahua flower concentrate, the development of value-added food products and the commercialization of food products. Although some articles have focused on the composition of mahua flowers, the rest of the research still needs to be explored. There is also a need to investigate the optimization techniques of food products. The present study provides an opportunity to explore these gaps and capitalize on the significance of Mahua flowers to create advanced, sustainable, and nutritionally enriched food products. By doing so, this study aims to contribute to the field of food science, provide economic prospects for tribal and rural communities, and address the increasing demand for functional foods internationally.

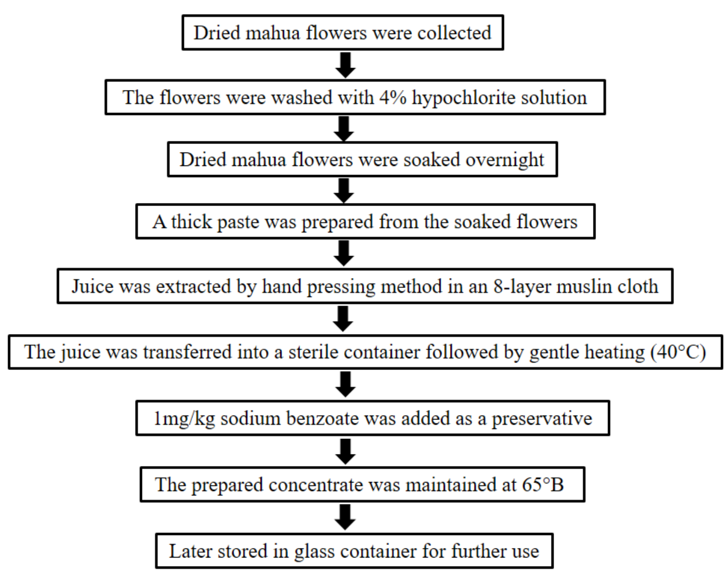
5.3 Materials and methods

5.3.1 Preparation of value added food products from mahua flowers

5.3.1.1 Preparation of Mahua Concentrate

Ingredients:

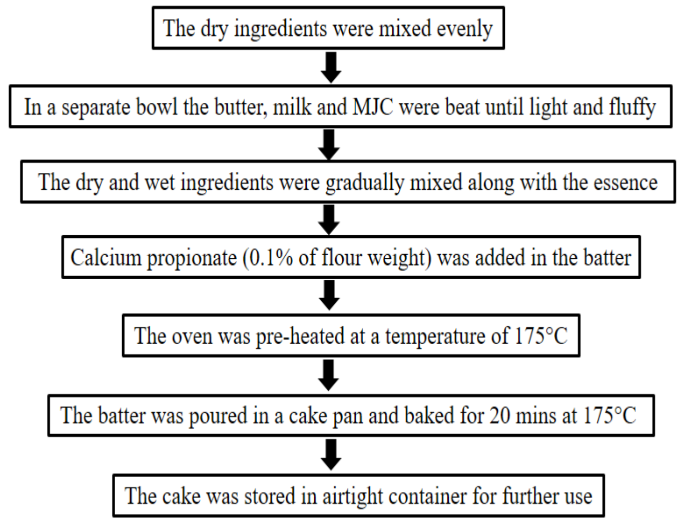
- Dried mahua flowers
- Hypochlorite solution
- Sodium benzoate
- Muslin Cloth



5.3.1.2 Preparation of Mahua Cake

Ingredients:

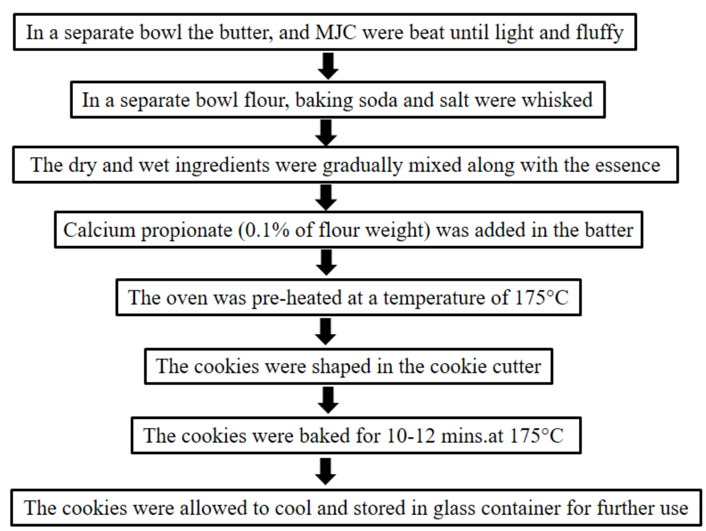
- Mahua juice concentrate (MJC): 100 ml
- All-purpose flour: 200 g
- Butter: 100 g
- Milk: 100 ml
- Baking powder: 1tsp.
- Baking soda: ½ tsp.
- Jaggery: 50 g
- Calcium propionate: 0.1%



5.3.1.3 Preparation of Mahua Cookies

Ingredients:

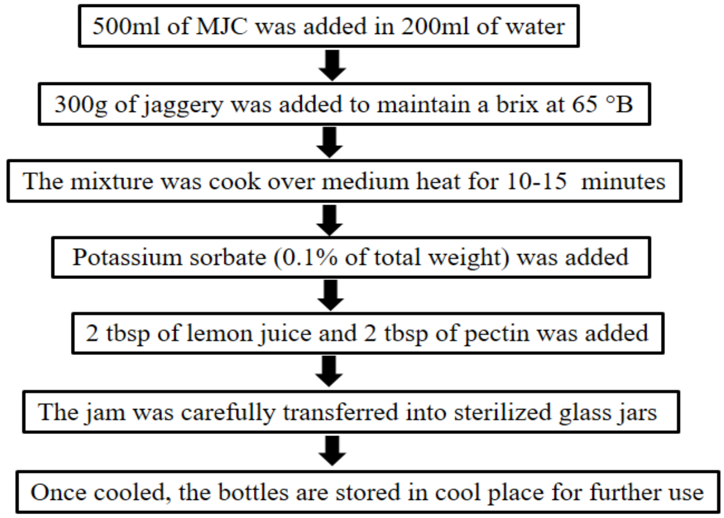
- Mahua juice concentrate (MJC): 50 ml
- All-purpose flour: 150 g
- Butter: 100 g
- Milk: 1--2 tsp.
- Baking powder: 1tsp.
- Baking soda: ½ tsp.
- Jaggery: 50 g
- Sodium benzoate: 0.1%



5.3.1.4 Preparation of Mahua Jam

Ingredients:

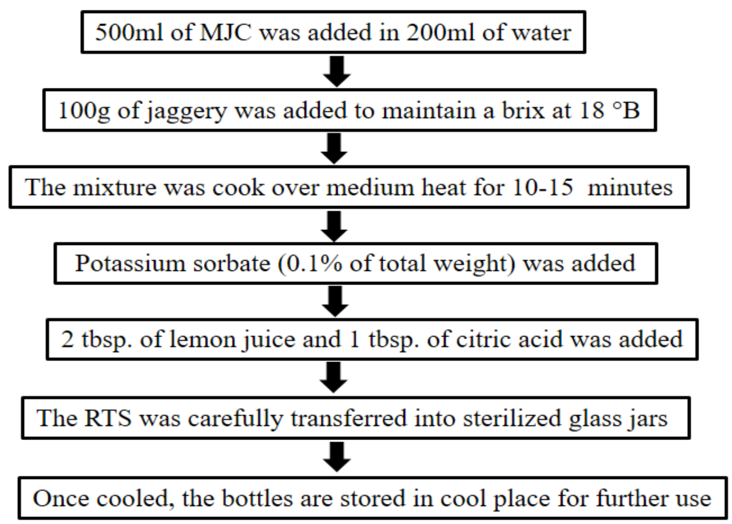
- Mahua juice concentrate: 500 ml
- Jaggery: 300 g
- Lemon juice: 2tbsp.
- Pectin: 2 tbsp.
- Potassium sorbet: 0.1%



5.3.1.5 Preparation of the Mahua Ready-to-Serve Drink

Ingredients:

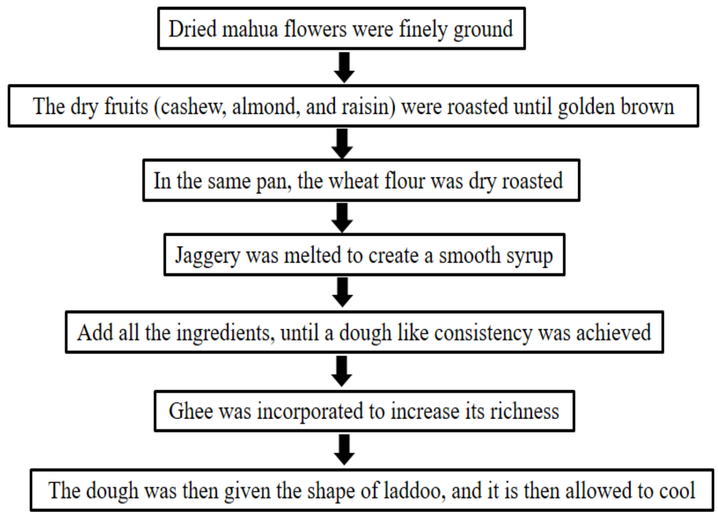
- Mahua juice concentrate: 500 ml
- Jaggery: 100 g
- Lemon juice: 2tbsp.
- Citric acid: 1 tbsp.
- Potassium sorbet: 0.1%



5.3.1.6 Preparation of Mahua Laddoo:

Ingredients:

- Dried mahua flowers: 200 g
- Dry fruits (Cashew, almond, raisins): 50 g each
- Wheat flour: 50 g
- Jaggery: 50 g
- Ghee: 20 ml



5.3.2 Proximate analysis

5.3.2.1 Moisture:

The moisture content of the sample was estimated via the oven drying method. First, a dry container was weighed, a known sample was added, and the initial weight was recorded. The sample was placed in an oven at 105°C for 4–6 hours until it dried. After that, the sample was removed from the oven and cooled in a desiccator, and the final weight was noted (Wang et al., 2022). The moisture content was calculated via the following formula:

Moisture content (%) = (Initial Weight- Final Weight)/Initial Weight X 100

5.3.2.2 pH:

The pH of each sample was estimated using a pH meter. A small proportion of each sample was dissolved in distilled water at a ratio of 1:10. The solution was homogenized properly. The pH meter was calibrated with standard buffer at pH values of 4, 7, and 10 to ensure accurate readings. After the calibration, the electrode node was dipped in the sample solution, and the pH value was noted (Vu et al., 2023).

5.3.2.3 Brix:

The brix of each sample was determined using a hand refractometer. The instrument was calibrated by placing a drop of distilled water into the prism followed by adjustment of the screw until the reading reached 0^0 B. The prism was cleaned before every measurement. The sample was placed onto the prism, the plate was covered evenly, and through the eyepiece, the brix was recorded where the light–dark boundaries appeared on the scale (Jaywant et al., 2022).

5.3.2.4 Sodium and potassium contents:

Sodium (Na) and potassium (K) contents were determined using a flame photometer. Standard solutions of Na and K were prepared in a serial dilution. The flame produced in the instrument is a mixture of air and propane or natural gas, and it is heated to high temperatures. The light emitted by the excited atoms of Na and K was detected by a photodetector. The emission intensity was measured at wavelengths of 589 nm and 766 nm for Na and K, respectively. The graph was plotted for the standards, and the Na and K contents were determined from the standard graph (Asch et al., 2022).

5.3.2.5 Protein estimation:

Protein estimation was performed based on the Lowry method, with bovine serum albumin (BSA) used as a standard. Briefly, the samples, along with the standard, were incubated with alkaline copper solution, and Folin–Ciocalteu reagent was subsequently added. After an incubation period of 30 minutes, the absorbance was measured at 750 nm. The protein content was quantified via a standard curve generated from the standard (Rizwi et al., 2022).

5.3.2.6 Carbohydrate estimation:

The carbohydrate content of the food product was estimated using the anthrone method. The anthrone reagent reacts under acidic conditions to obtain a green color complex, which is measured at 620 nm. The carbohydrate content was calculated by comparing the absorbance of the sample with a standard curve with glucose as the standard (Sarkar et al., 2024).

5.3.2.7 Reducing sugar content:

The reducing sugars present in the food samples were quantified by the dinitrosalicylic acid (DNS) method. In this method, DNS was reduced in an alkaline medium, which subsequently formed an orange–red complex. The intensity of the color was measured spectrophotometrically at 540 nm. The reducing sugars were further quantified by comparison with the calibration curve, in which glucose was used as a standard (Alomari et al., 2024).

5.3.2.8 Fat estimation:

The fats from the sample were extracted by the Soxhlet method using hexane. The solvent comprising the extracted fat was gathered in the flask, and the solvent was evaporated in the rotary evaporator, leaving behind the fat (Chen et al., 2024). The weight of the extracted fat was then estimated using the following formula:

Fat content (%) = (weight of sample (g)/weight of fat extracted (g)) ×100

5.3.2.9 Determination of the calorific value

The calorific value of the prepared food products was estimated using a bomb calorimeter, where a defined mass of the sample was combusted in the calorimeter and the released heat increased the temperature of the surrounding water (Hopper et al., 2023). It is calculated using the following formula:

Calorific value = Q_{total}/m_{food}

where

$Q_{total} = m_w . c_w . \Delta T + C_{bomb} \cdot \Delta T$

- m_w: mass of water (g),
- c_w: specific heat capacity of water (4.18 J/g°C),
- ΔT: temperature change of the water (°C),
- C_{bomb}: heat capacity of the bomb (J/°C),
- $\mathbf{m_{food}}$: mass of the food sample (g).

5.3.3 Nutritional Profiling

5.3.3.1 Total carbohydrate profiling

The total carbohydrate profile was determined via HPLC. The sample was prepared by hydrolysing the carbohydrate complex into simple sugars using acids and enzymes followed by neutralization and filtration. The mobile phase was prepared using water and acetonitrile or buffer depending on the column used. The sample was injected into the system equipped with a detector using an appropriate column for separation. The carbohydrates were subsequently separated on the basis of their properties, with peaks that appeared on the chromatogram corresponding to different sugars. Furthermore, the carbohydrate contents were quantified by comparing the areas of these peaks with those of known standards (Sharma et al., 2024).

5.3.3.2 Total amino acid profiling

The total amino acids found in the samples were quantified via HPLC. The samples were prepared by hydrolysing the proteins and peptides with 6 N hydrochloric acid (HCL) at

110^0 °C for 24 hours, and the samples were neutralized with sodium hydroxide (NaOH). To enhance the detection of amino acids, the samples were derivatised with o-phthaldialdehyde (OPA) or fluorenylmethyloxycarbonyl (FMOC). The mobile phase was prepared using water and acetonitrile or buffer depending on the column used. The samples were injected into an HPLC system equipped with a UV or fluorescence detector. On a reverse-phase column, the amino acids were separated at different retention times. The amino acids were subsequently separated on the basis of their properties, with peaks that appeared on the chromatogram corresponding to different amino acids. Furthermore, the amino acids were quantified by comparing the areas of these peaks with those of known standards (Teo et al., 2024).

5.3.3.3 Total vitamin profiling

The vitamins found in the samples were quantified using HPLC. The samples were prepared by extraction with different solvents, such as methanol, ethanol, hexane, and chloroform, depending on the type of vitamin. The solutions were filtered to remove particulates, if any. The vitamins were quantified via high-performance liquid chromatography (HPLC) with a UV or fluorescence detector. The mobile phase was prepared to be suitable for the analysis of vitamins, such as water, methanol, acetonitrile, or buffer solutions. Reverse-phase columns (C18) were used for fat-soluble vitamins, and anion exchange or normal-phase columns were used for water-soluble vitamins. The samples were injected, and the separation of vitamins was analysed on the basis of their retention time. The chromatographic peaks were analysed to identify the vitamins and further quantified by comparison with standard vitamins (Rashid et al., 2024).

5.3.4 Sensory evaluation

Using the hedonic scale, the sensory evaluation of the food products was carried out, which involved a panel of ten (10) people. The panelists presented the products under controlled conditions to minimize bias. They used a 9-point hedonic scale, where 1 indicated “dislike extremely” and 9 indicated “like extremely” to rate their acceptance of attributes such as taste, texture, color, aroma and appearance. The products were presented randomly to avoid order effects, and the panelists were instructed to clean their palates after every sample. The results were then analysed to determine the average score, indicating the overall acceptability of the food product (Triandini et al., 2024).

5.3.5 Shelf life

The shelf-life of the products was assessed by yeast, mold and bacterial counts according to FSSAI guidelines. The samples were prepared under aseptic conditions to prevent any further contamination. The samples were serially diluted in a sterile diluent. For the yeast and mold counts, the samples were spread onto selective agar, i.e., potato dextrose agar (PDA), and incubated at 25–300°C for 3–5 days. Similarly, for the bacterial count, the samples were spread on nutrient agar (NA) at a temperature of 35–370°C for 24–48 hours. After the incubation period, the colonies were further counted, and the number of colony forming units (CFUs) per gram was calculated (Assocle et al., 2024).

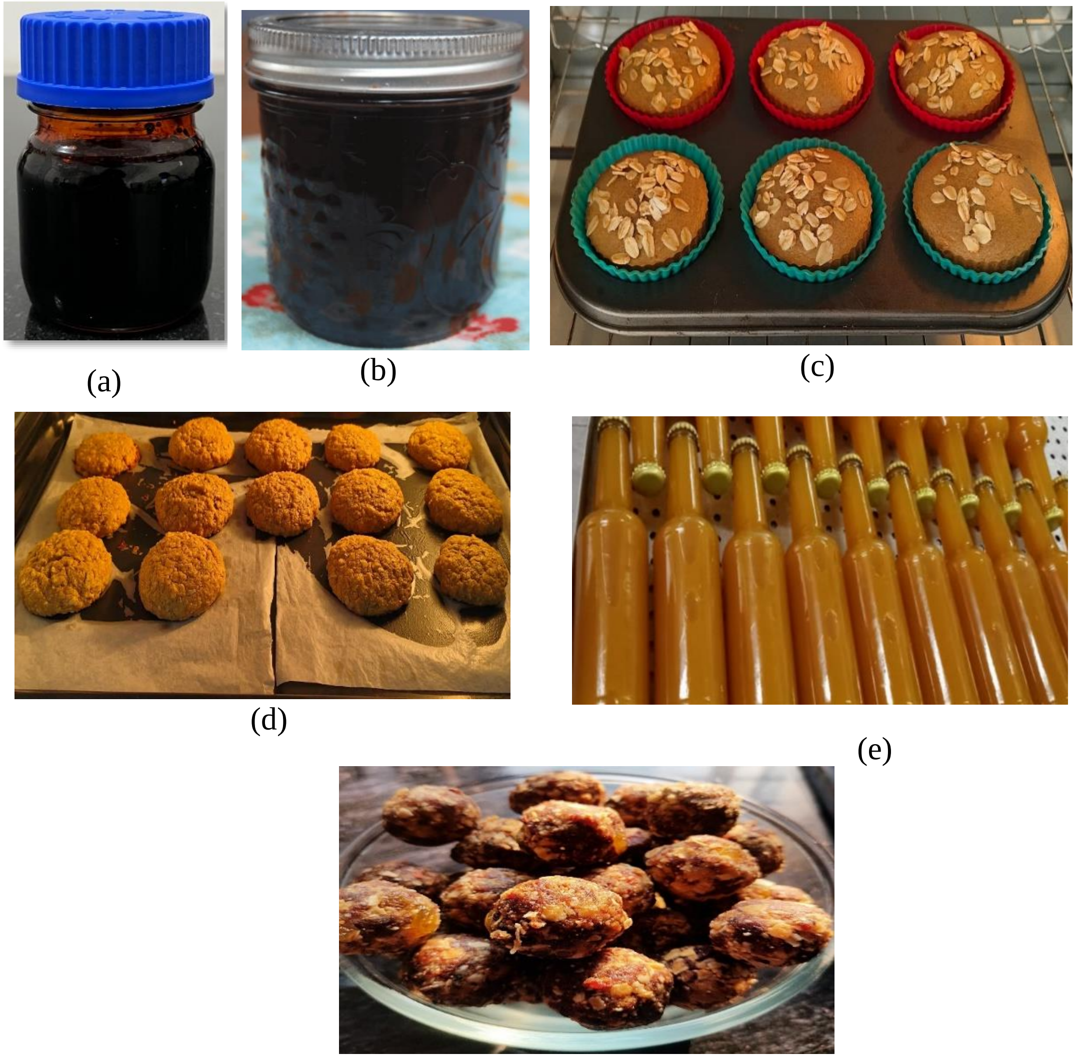
5.3.5.1 Detection of Aflatoxins:

The detection of aflatoxins (B1, B2, G1, and G2) in food samples involves several key steps, including sample preparation, extraction, clean-up, and analytical detection. Initially, the food sample was homogenized (usually 25 g) and is extracted using a mixture of acetonitrile and water. The solution is then centrifuged to distinct the liquid from the solid. The supernatant is passed through a column for clean-up, which removes interfering matrix components and isolates the aflatoxins. After eluting the aflatoxins from the column, the sample was evaporated and re-formed for examination. The aflatoxins are detected using HRMS. Calibration curves are made using standard aflatoxin solutions to calculate the quantity of aflatoxins present in the sample. The detected aflatoxin levels are related to regulatory limits set by authorities like the FDA or WHO to ensure food safety (Wang et al., 2022).

5.4 Results

5.4.1 Proximate analysis:

Several value-added food products have been developed from mahua flowers, including Mahua concentrate, Mahua cake, Mahua cookies, Mahua jam, Mahua RTS and Mahua laddoo (Fig. 14.2). The results revealed significant variations in moisture; pH; brix; protein, carbohydrate, reducing sugar, and fat contents; and caloric value (14.3-14.9). The MJC had a moisture content (%) of 75.24± 1.85 and a pH of 4.63±0.18, indicating mild acidity in nature and a high brix of 64.83±0.24^{0}B, which indicates its sugar content. The protein and carbohydrate contents were 3.84±0.10 g/100 g and 91.14±2.29 g/100 g, respectively, with a reducing sugar content of 43.25±1.80 g/100 g. The amount of fat was relatively low, i.e., 0.95±0.02 g/100 g, contributing to a caloric value of 330±3.25 kcal. In mahua cake, the moisture content was 34.99±1.22% at a pH of 6.3±0.16, and the brix value was 21.67±0.47, which indicated the presence of a certain amount of sugar. The protein content was 2.56±0.26^{0}B, with a carbohydrate content of 77.01±0.38 g/100 g and a reducing sugar content of 34.94±1.71 g/100 g. The fat content was 15.73±0.41 g/100 g, with a caloric value of 280±3.32 kcal. Similarly, in Mahua cookies, the moisture content was 8.2±0.36% at a pH of 6.73±0.16 and a brix of 17.10±0.29 ^{0}B, indicating the sugar concentration. Its protein content was 2.39±0.27 g/100 g, with carbohydrates accounting for 67.30±0.20 g/100 g and reducing sugars accounting for 38.37±1.33 g/100 g, which reflects sweet and energy-dense cookies. The fat content was 20.27±0.72 g/100 g, reflecting a caloric value of 285±3.35 kcal. The mahua jam presented a moisture content of 25.66±1.25% at a low pH of 4.13±0.12 and a relatively high brix of 61.33±0.47 ^{0}B, which indicates that a high sweetness index is required for preservation. The protein and carbohydrate contents were 3.13±0.36 g/100 g and 81.98±1.39 g/100 g, respectively, with 45.68±1.69 g/100 g of reducing sugars. The fat content was minimal at 3.31±0.26 g/100 g, and the caloric value was 215±2.29 kcal. The ready-to-use beverage prepared from mahua has a high moisture content of 87.17±0.6%, a pH of 4.33±0.18 and a brix of 17.83±0.62 ^{0}B, which indicates that it is a moderately sweet drink. The protein content was 3.56±0.43 g/100 g, with 70.56±0.65 g/100 g carbohydrates and 34.17±1.28 g/100 g reducing sugars. It is considered a large fat drink, with a fat amount of 1.41±0.17 g/100 g and a caloric value of 120±3.54 kcal. The nutritious mahua laddoo has a moisture content of 15.66±0.37%, a pH of 5.5±0.24 and a brix of 11.33±0.47 ^{0}B. The protein content was 3.92±0.28 g/100 g, the carbohydrate content was 76.33±0.74 g/100 g, and the reducing sugar content was 40.09±4.05 g/100 g, indicating a highly energy-dense composition. The addition of ghee increased the fat content to 23.35±0.25 g/100 g at a caloric value of 350±3.85 kcal. These results elucidate the various nutritional shapes of these products, which can be consumed in everyday life.



(a) (b) (c)

(d) (e)

(f)

Figure 14.1 Different value-added food products derived from mahua flowers (A) Mahua concentrate, (B) Mahua jam, (C) Mahua cake, (D) Mahua cookies, (E) Mahua RTS, (F) Mahua laddoo

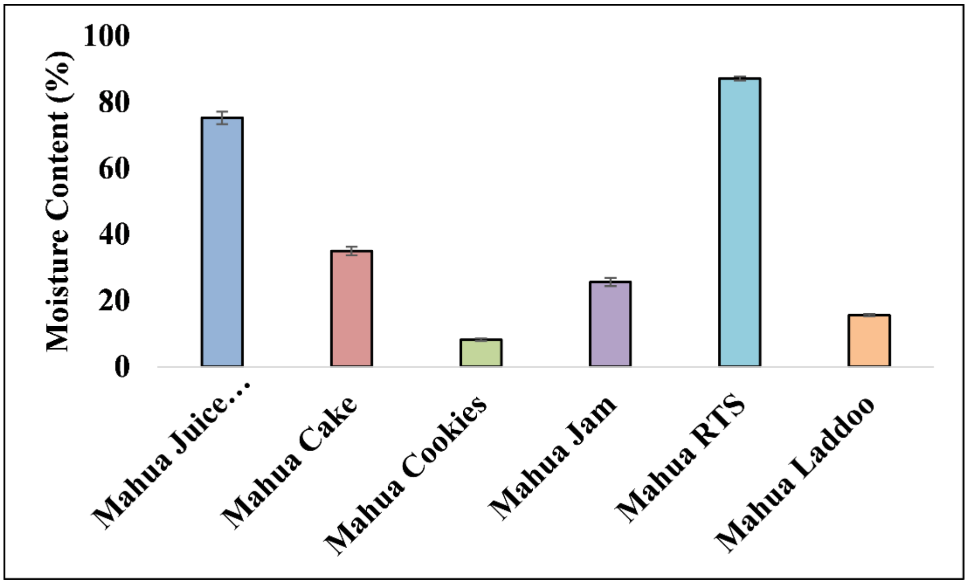


Figure 14.2 This figure illustrates the moisture content of various food samples determined through standard drying methods. The moisture levels are depicted as a percentage of the total weight of each sample.

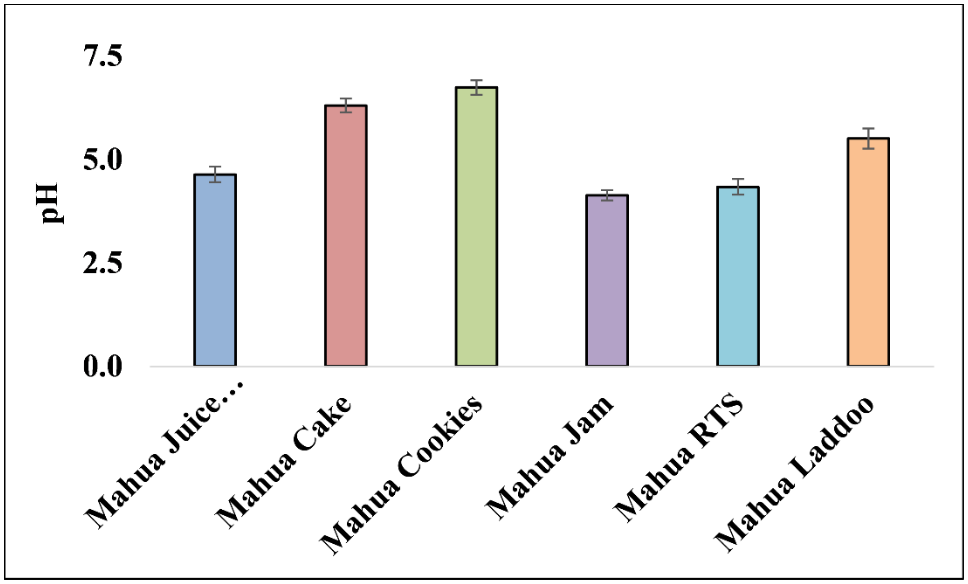


Figure 14.3 The pH values of the food samples were measured via a pH meter. These values reflect the varying levels of acidity and alkalinity present in the food products.

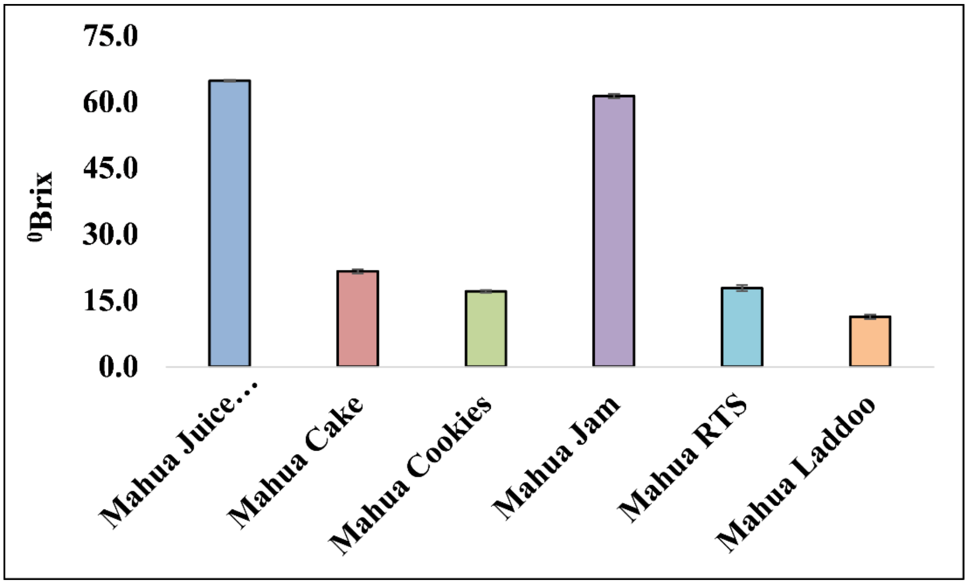


Figure 14.4 This figure illustrates the brix content of various food samples. The Brix values, representing the total soluble solids content (mainly sugars), were measured using a refractometer.

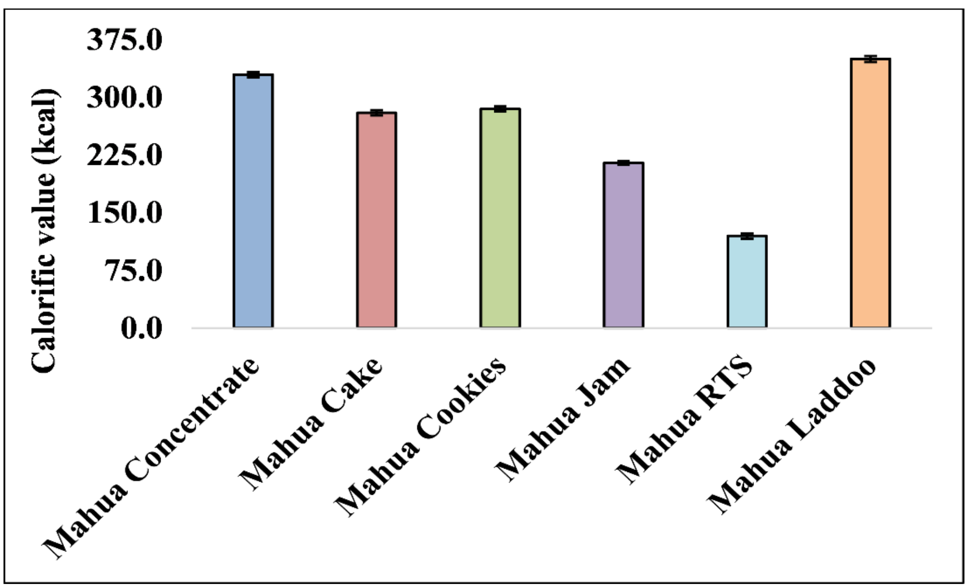


Figure 14.5 This figure illustrates the calorific value of various food samples. The figure depicts that, mahua laddoo exhibited highest calorific value.

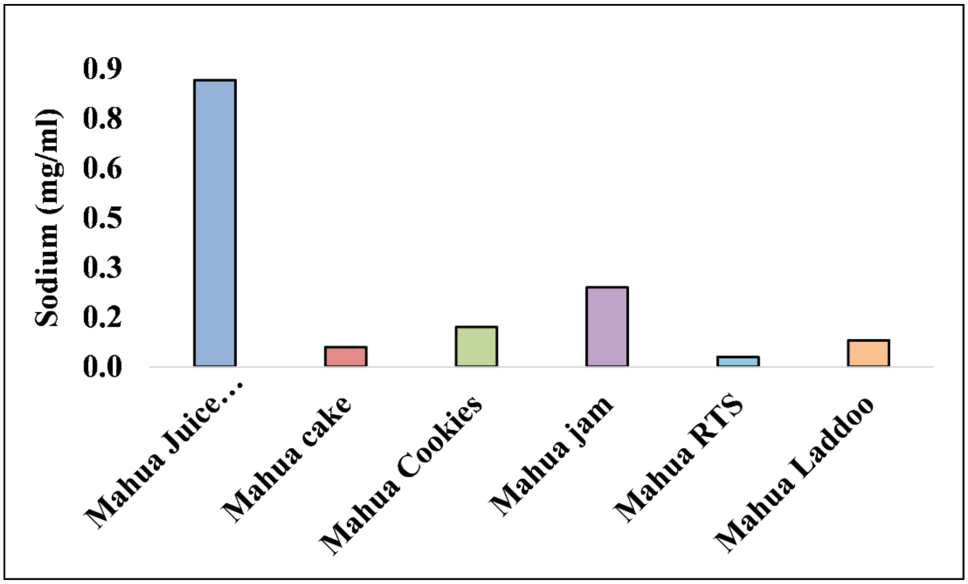


Figure 14.6 This figure illustrates the Sodium content of various food samples. The figure depicts that, mahua concentrate exhibited highest sodium value.

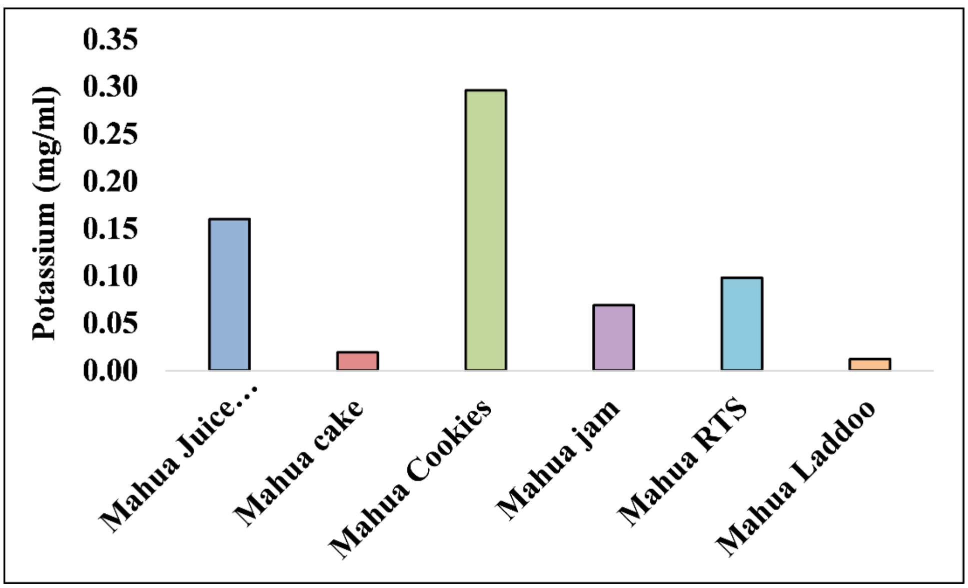


Figure 14.7 This figure illustrates the Potassium content of various food samples. The figure depicts that, mahua cookies exhibited highest sodium value.

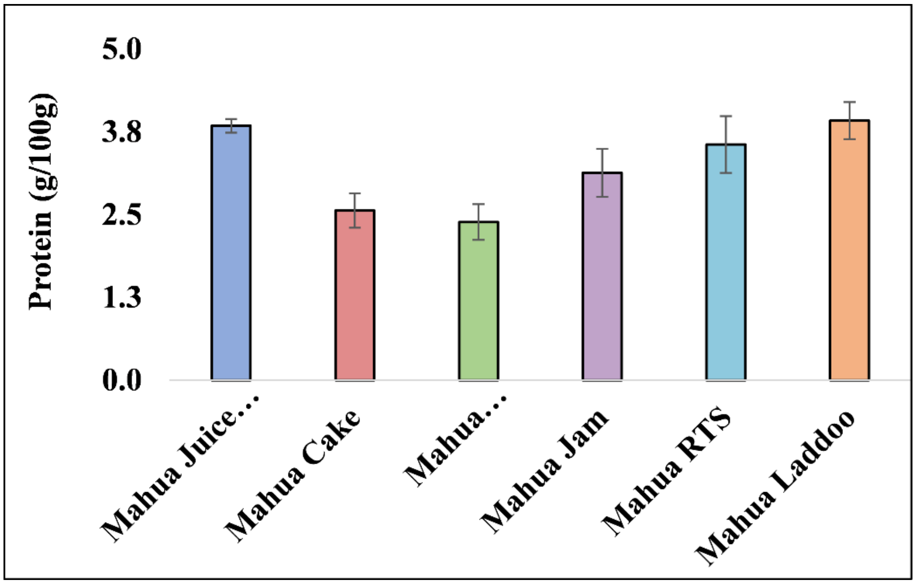


Figure 14.8 This figure illustrates the protein content of various food samples. The figure depicts that, mahua laddoo exhibited highest protein content.

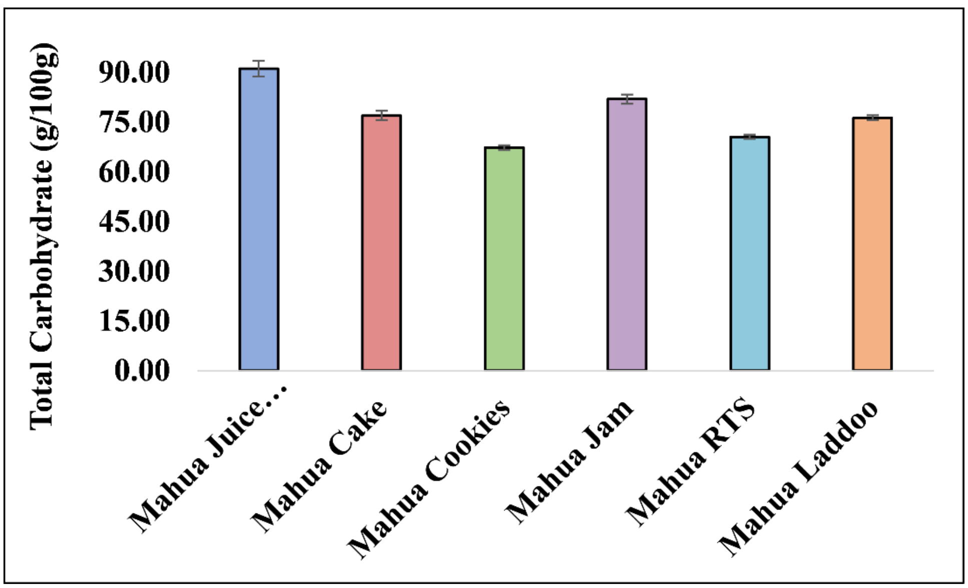


Figure 14.9 This figure illustrates the total carbohydrate content of various food samples. The figure shows that the Mahua juice concentrate presented the highest carbohydrate content.

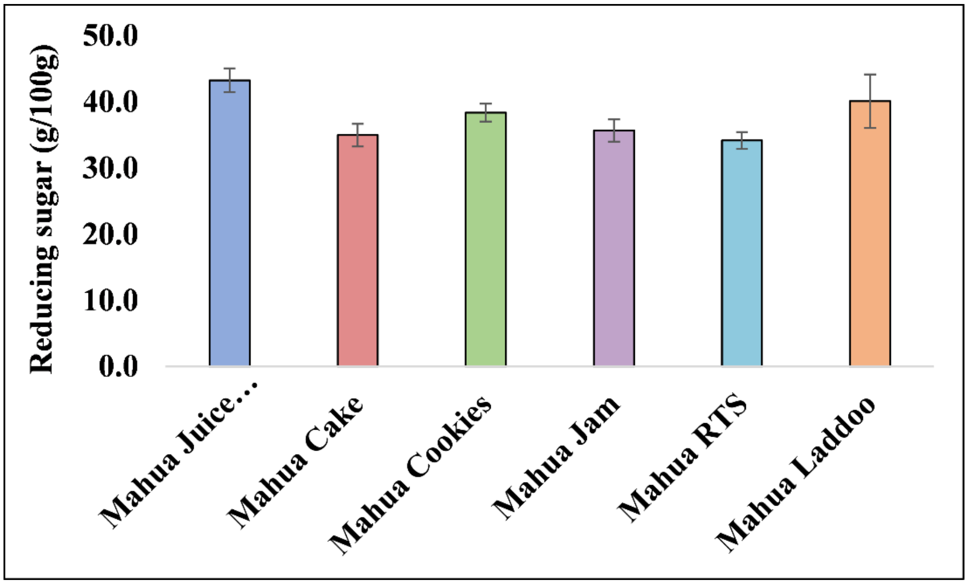


Figure 14.10 This figure illustrates the reducing sugar content of various food samples. The figure shows that the mahua juice concentrate presented the highest reducing sugar content.

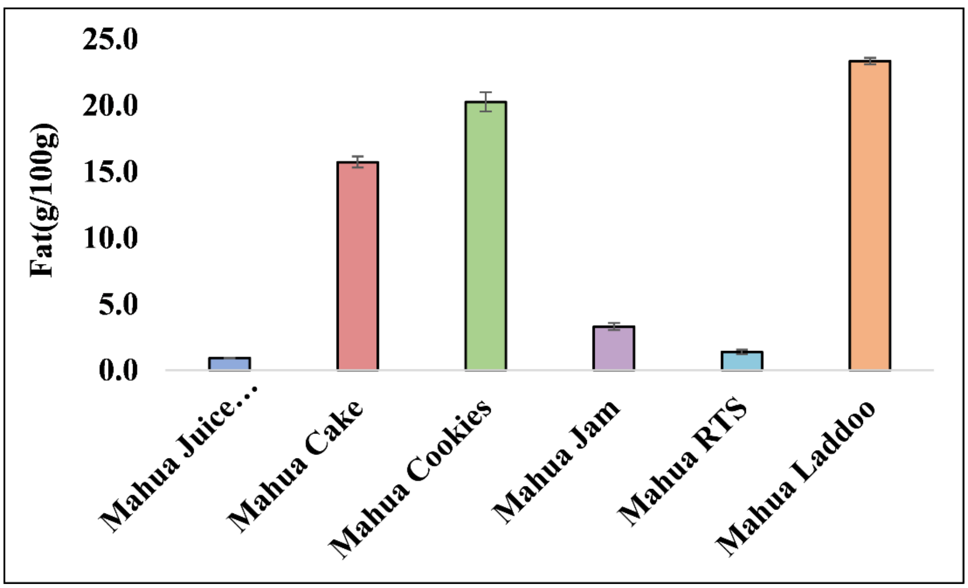


Figure 14.11 This figure illustrates the total fat content of various food samples. The figure shows that the Mahua juice concentrate presented the lowest fat content.

5.4.2 Nutritional Profiling

5.4.2.1 Total carbohydrate content:

The total carbohydrate contents, such as those of glucose, fructose, sucrose, and xylose, were quantified using HPLC. Chromatographic analysis revealed distinct peaks for each sugar content in various food samples (Fig. 15.1-15.10). The sugar contents of various food products are depicted in Table. Fructose (%) and glucose (%) were found to be highest in the Mahua juice concentrate (MJC), and xylose (%) was found to be highest in the Mahua cake. Mahua Jam presented the greatest percentage of sucrose (%), and Mahua cake and cookies presented lactose due to the addition of milk during the preparation of the products (Table 4.1).

Table 4.1: The table depicts the sugar content (fructose, glucose, xylose, sucrose and lactose) of the developed food products

Srl. No.	Sample Name	Fructose (%)	Glucose (%)	Xylose (%)	Sucrose (%)	Lactose (%)
1	MJC	23.16	20.84	0	8.14	0
2	Mahua Cake	2.79	2.58	0.17	9.08	6.21
3	Mahua Cookies	3.12	2.31	0.08	8.92	5.65
4	Mahua Jam	15.38	18.06	0	86.78	0
5	Mahua RTS	10.69	10.76	0	8.9	0
6	Mahua Laddoo	14.47	11.83	0.14	69.07	0

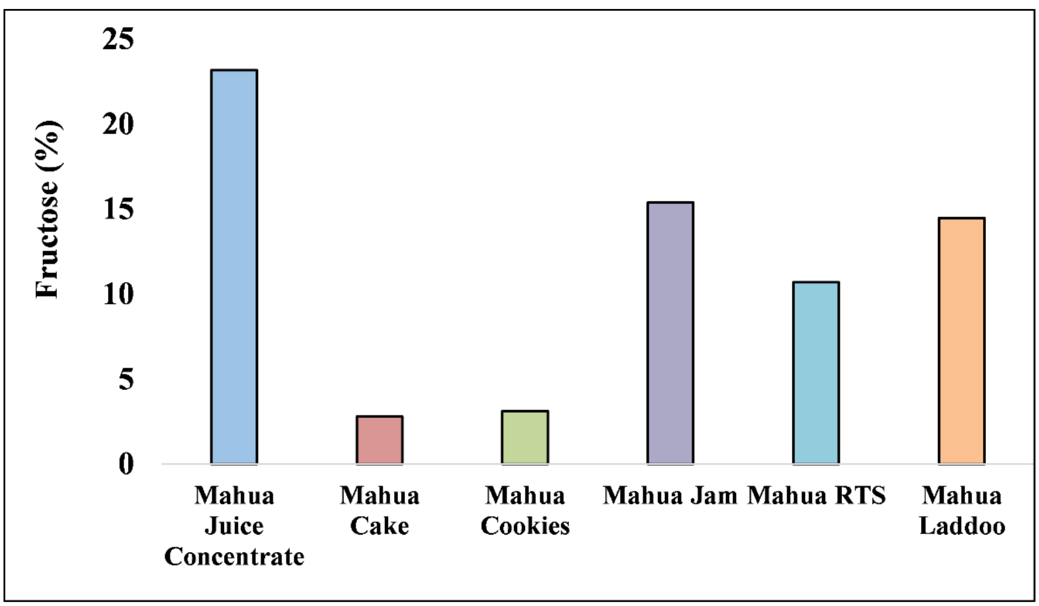


Figure 14.12 Graph depicts the percentage of fructose in the food products. The x-axis corresponds to different food categories, while the y-axis indicates the percentage of fructose content.

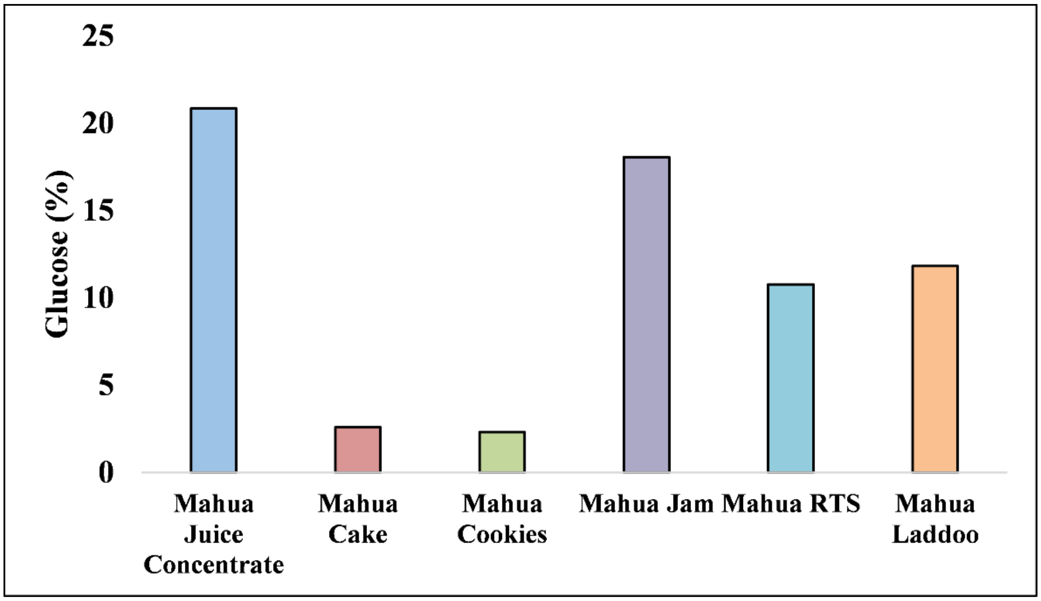


Figure 14.13 Graph depicts the percentage of glucose in the food products. The x-axis corresponds to different food categories, while the y-axis indicates the percentage of fructose content.

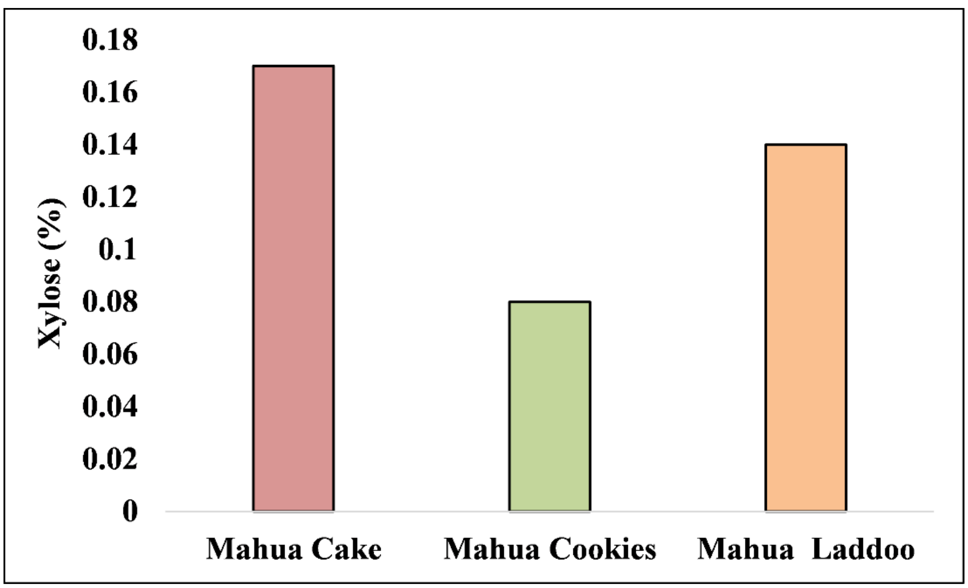


Figure 14.14 Graph depicts the percentage of xylose in the food products. The x-axis corresponds to different food categories, while the y-axis indicates the percentage of fructose content.

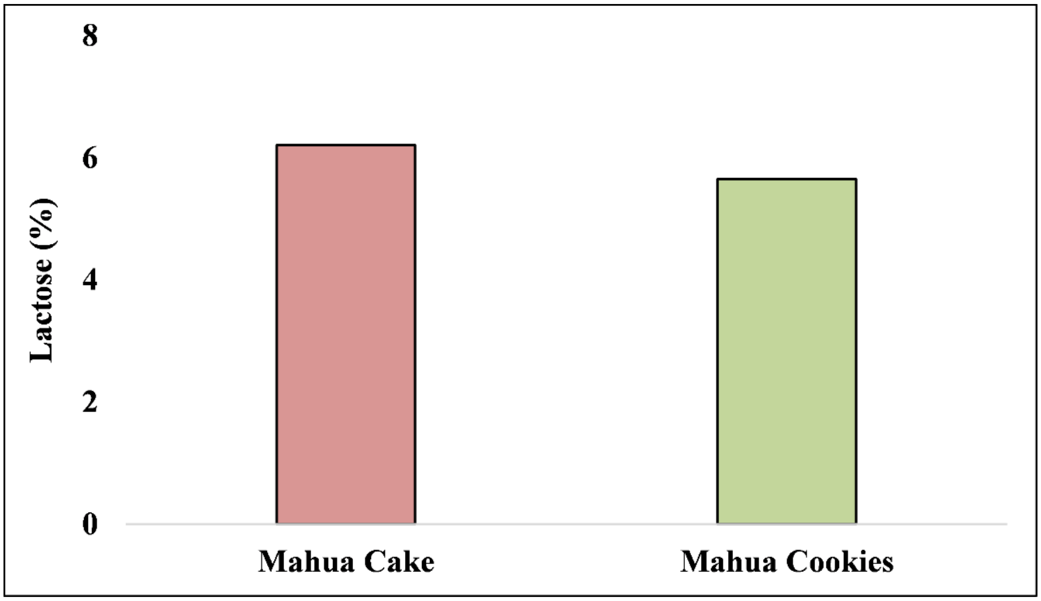


Figure 14.15 Graph depicts the percentage of lactose in the food products. The x-axis corresponds to different food categories, while the y-axis indicates the percentage of fructose content.

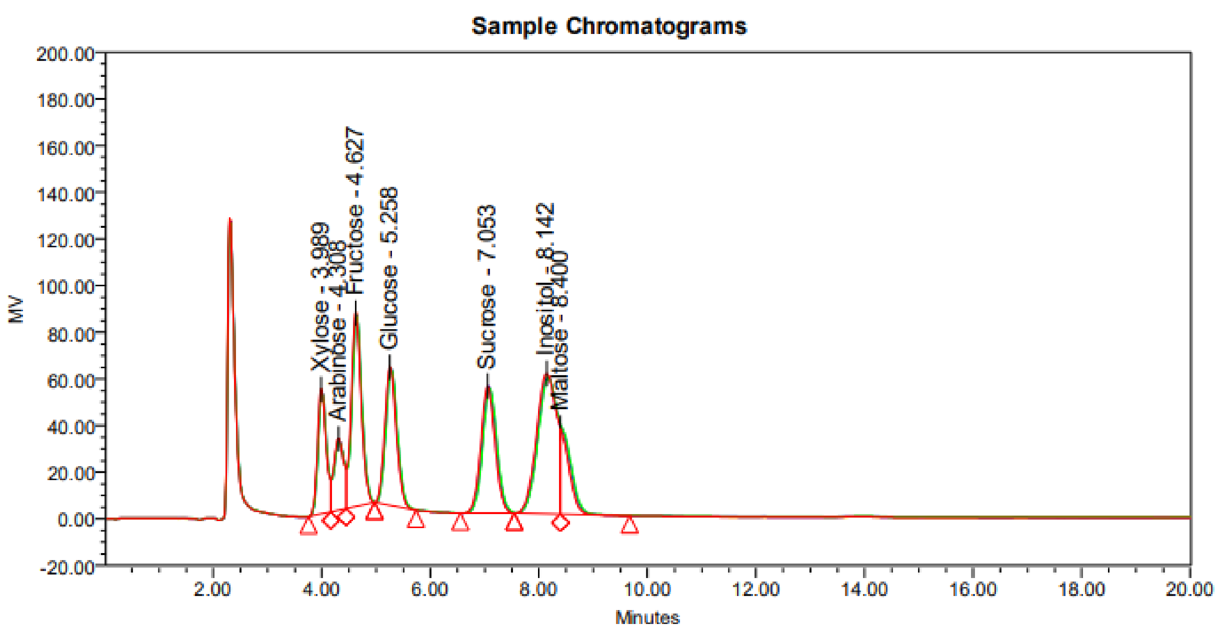


Figure 15.1 Chromatograms depict the carbohydrate composition of the prepared standard analysed using HPLC with refractive index detection. The peaks represent individual carbohydrates identified by retention time and compared with standards.

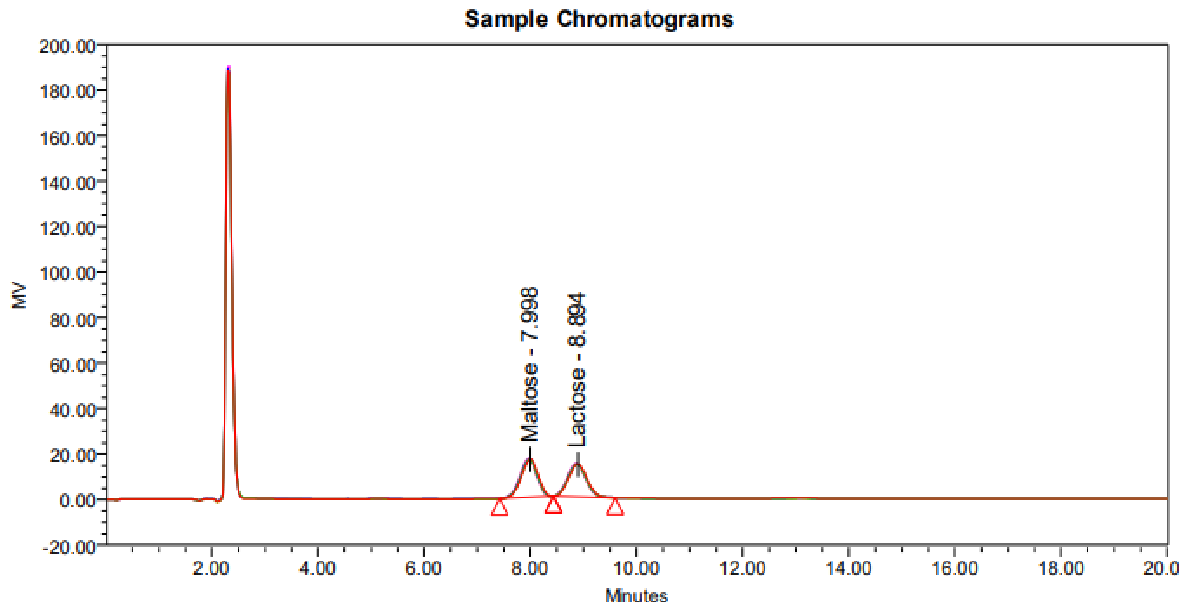


Figure 15.2 Chromatograms depict the carbohydrate composition (malose and lactose) of the prepared standard analysed using high-performance liquid chromatography (HPLC) with refractive index detection. The peaks represent individual carbohydrates identified by retention time and compared with standards.

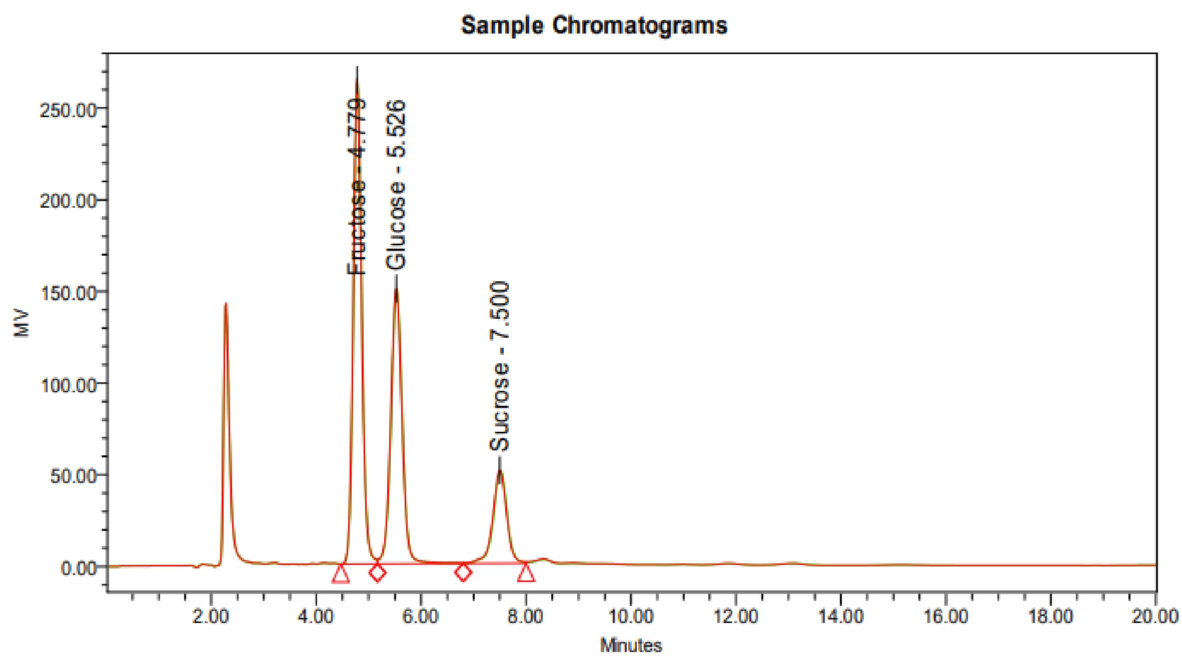


Figure 15.3 Chromatograms showing the carbohydrate composition of the Mahua concentrate analysed using HPLC with refractive index detection. The peaks represent individual carbohydrates identified by retention time and compared with standards.

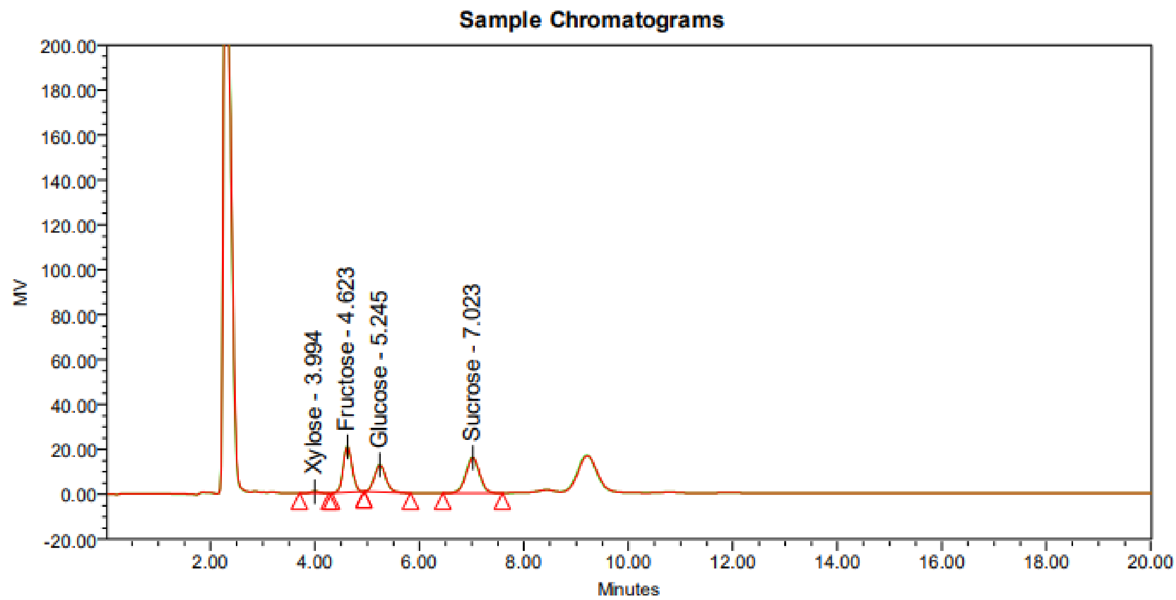


Figure 15.4 Chromatograms showing the carbohydrate composition of Mahua cake analysed using HPLC with refractive index detection. The peaks represent individual carbohydrates identified by retention time and compared with standards.

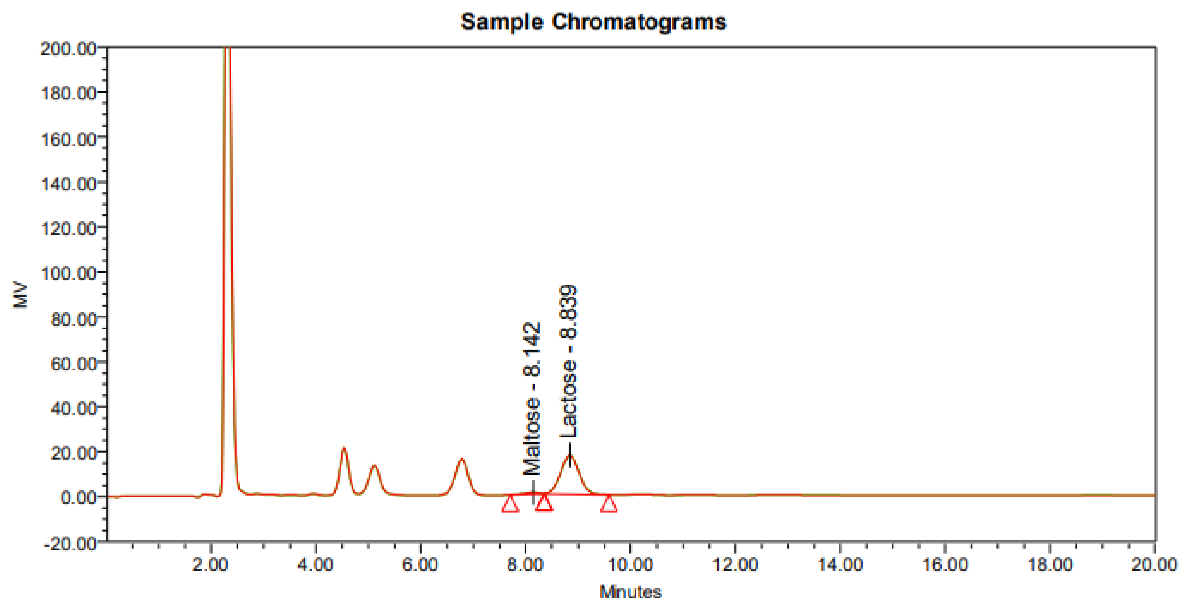


Figure 15.5 Chromatograms showing the carbohydrate composition of Mahua cake (Maltose+Lactose) analysed using HPLC with refractive index detection. The peaks represent individual carbohydrates identified by retention time and compared with standards.

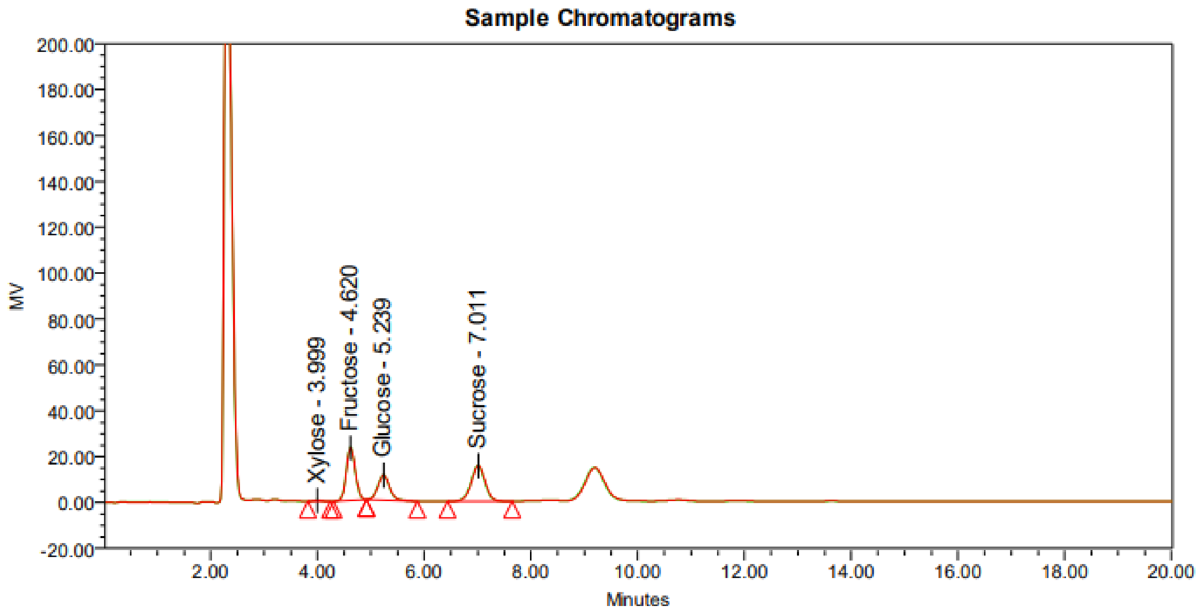


Figure 15.6 Chromatograms showing the carbohydrate composition of Mahua cookies analysed using HPLC with refractive index detection. The peaks represent individual carbohydrates identified by retention time and compared with standards.

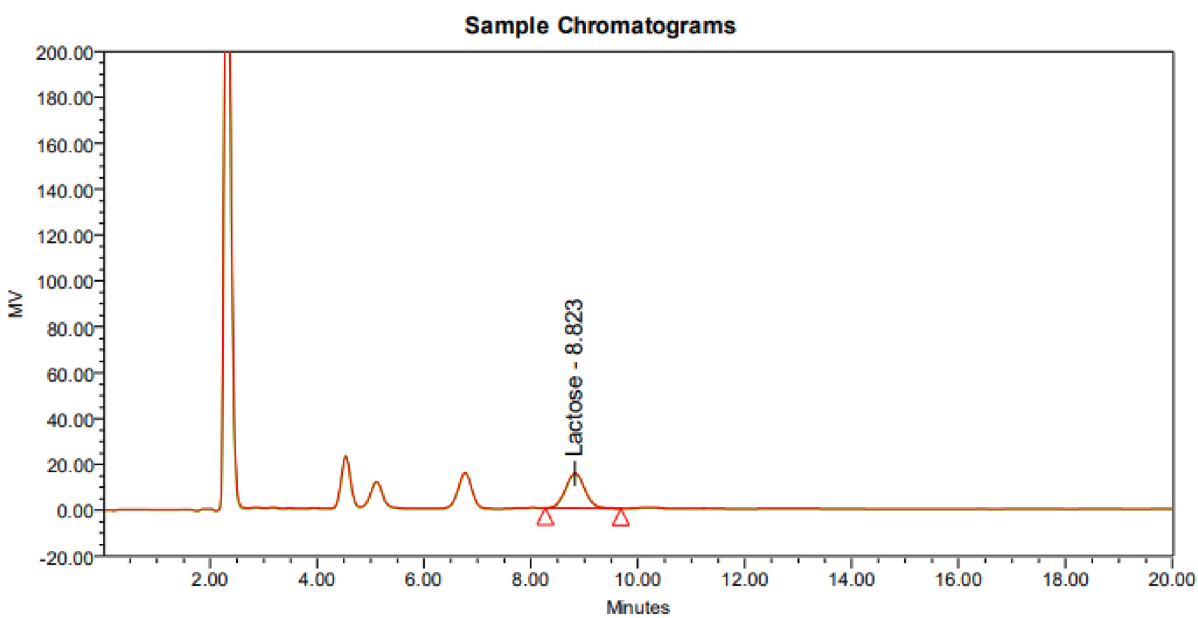


Figure 15.7 Chromatograms showing the carbohydrate composition of Mahua cookies (Maltose+Lactose) analysed via high-performance liquid chromatography (HPLC) with refractive index detection. The peaks represent individual carbohydrates identified by retention time and compared with standards.

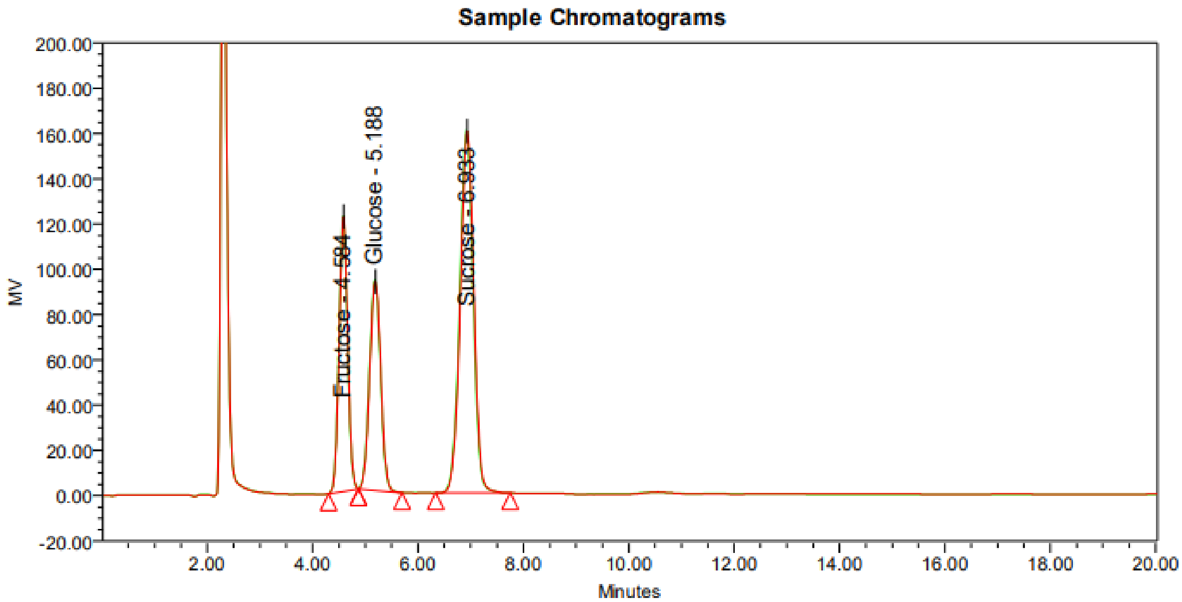


Figure 15.8 Chromatograms depicting the carbohydrate composition of Mahua jam analysed using HPLC with refractive index detection. The peaks represent individual carbohydrates identified by retention time and compared with standards.

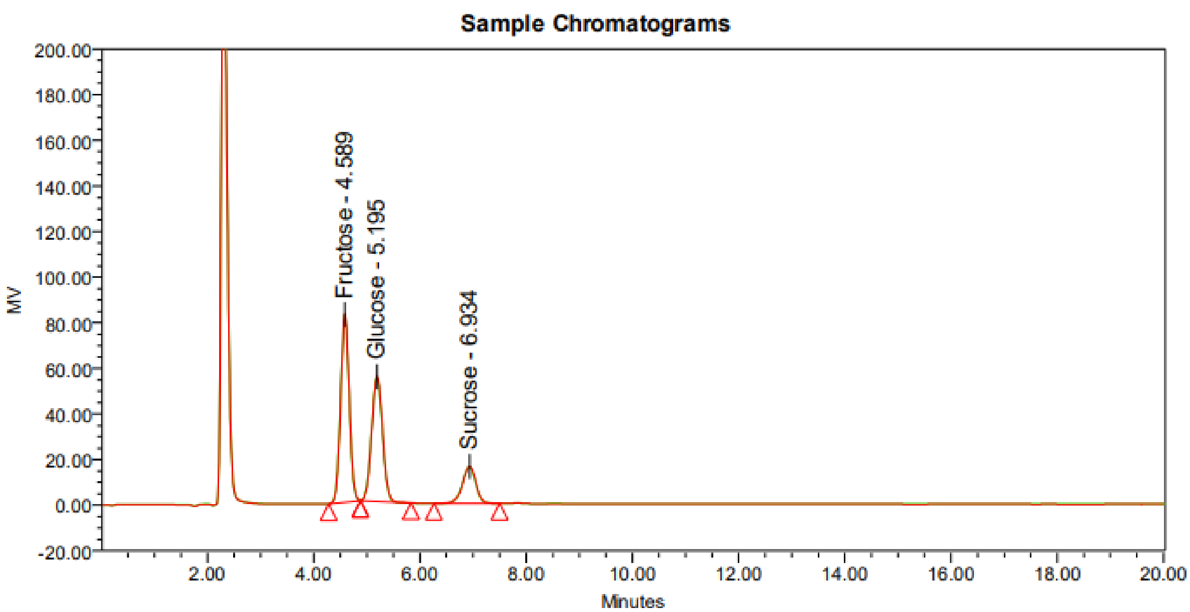


Figure 15.9 Chromatograms depicting the carbohydrate composition of Mahua RTS analysed using HPLC with refractive index detection. The peaks represent individual carbohydrates identified by retention time and compared with standards.

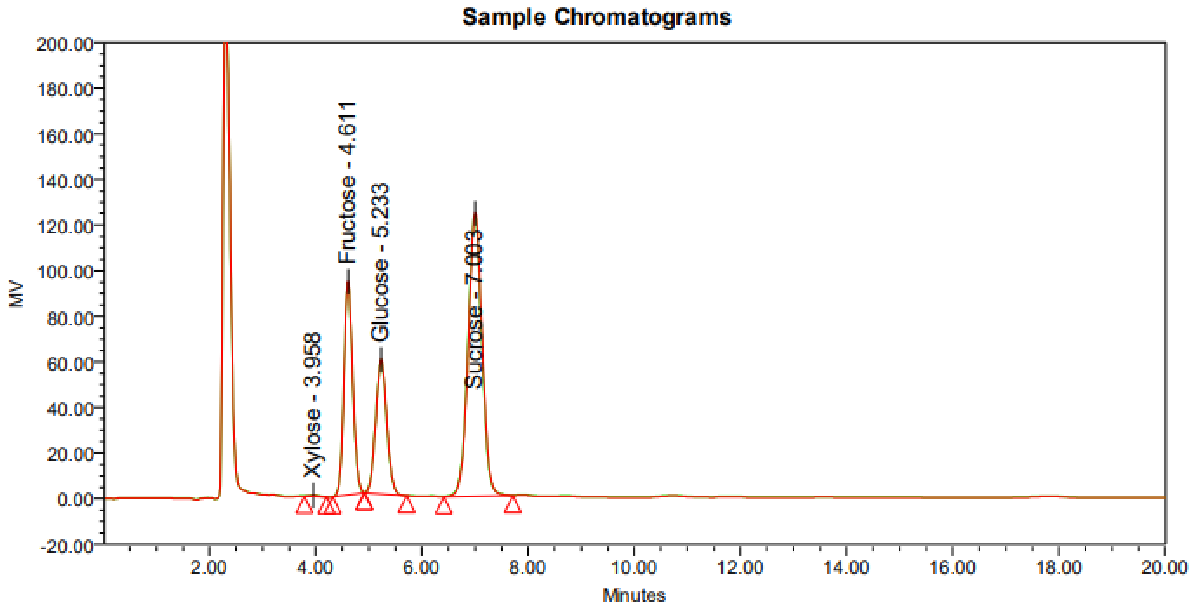


Figure 15.10 Chromatograms depict the carbohydrate composition of Mahua laddoo analysed via high-performance liquid chromatography (HPLC) with refractive index detection. The peaks represent individual carbohydrates identified by retention time and compared with standards.

5.4.2.2 Total amino acid profiling:

The total amino acid contents, such as those of histidine, serine, arginine, glycine, and aspartic acid, were quantified using HPLC. Chromatographic analysis revealed distinct peaks for each amino acid in various food samples (Fig. 16.1-16.13). The sugar contents of various food products are depicted in Table. Fructose (%) and glucose (%) were found to be highest in the Mahua juice concentrate (MJC), and xylose (%) was found to be highest in the Mahua cake. Mahua Jam presented the greatest percentage of sucrose (%), and Mahua cake and cookies presented lactose due to the addition of milk during the preparation of the products (Table 4.2).

Table 4.2: This table depicts the presence of essential and nonessential amino acids in the value-added food products derived from Mahua flower concentrate.

Sr. No.	Name of Amino acids	Mahua Juice Concentrate (g/100 g)	Mahua cake (g/100 g)	Mahua cookies (g/100 g)	Mahua Jam (g/100 g)	Mahua RTS (g/100 g)	Mahua Laddoo (g/100 g)
1	Histidine	0.144	0.184	0.189	0.055	0.042	0.467
2	Serine	0.053	0.171	0.188	0.015	0.012	0.143

3	Arginine	0.193	0.841	1.024	0.053	0.053	0.615
4	Glycine	0.044	0.084	0.098	0.035	0.027	0.129
5	Aspartic Acid	0.020	0.096	0.102	0.009	0.004	0.073
6	Glutamic Acid	0.271	0.500	0.584	0.179	0.190	0.607
7	Threonine	0.045	0.086	0.082	0.032	0.011	0.171
8	Alanine	0.028	0.103	0.108	0.008	0.006	0.067
9	Proline	0.075	0.118	0.129	0.028	0.027	0.155
10	Cystine	0.032	0.151	0.176	0.013	0.012	0.074
11	Lysine	0.028	0.098	0.102	0.007	0.006	0.074
12	Tyrosine	0.075	0.253	0.270	0.021	0.018	0.194
13	Methionine	0.007	0.051	0.060	0.001	-	0.028
14	Valine	0.023	0.074	0.052	0.008	0.008	0.068
15	Isoleucine	0.039	0.142	0.152	0.010	0.009	0.102
16	Leucine	0.064	0.267	0.289	0.018	0.015	0.183
17	Phenylalanine	0.040	0.163	0.182	0.011	0.008	

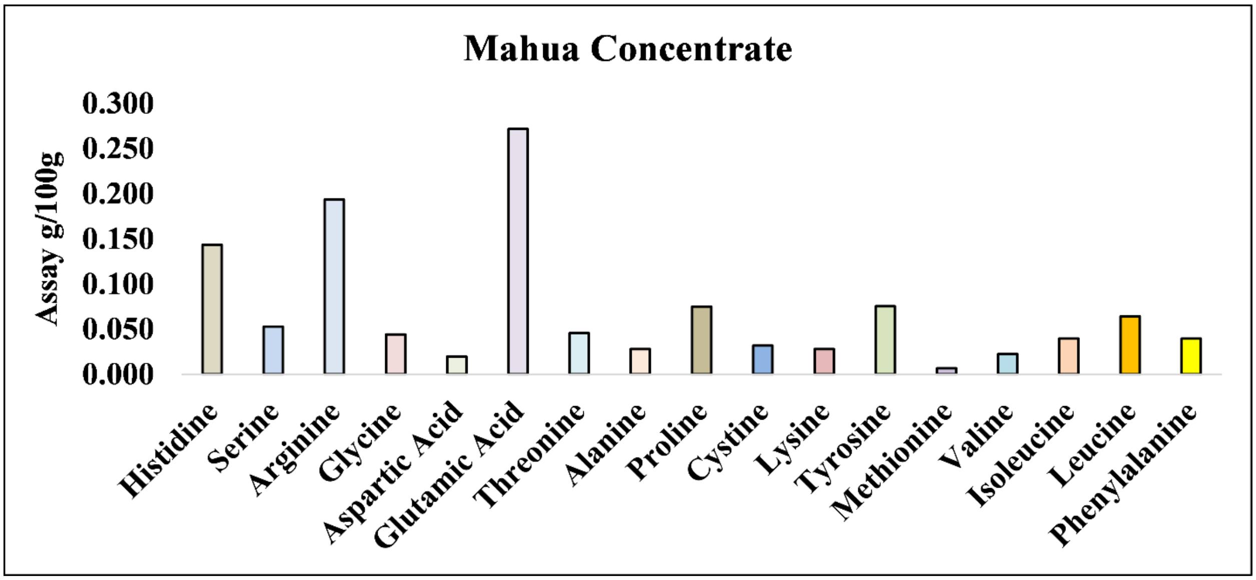


Figure 16.1 The graph depicts the total amino acid composition of the Mahua concentrate analysed via high-performance liquid chromatography (HPLC), with data expressed in g/100 g of sample.

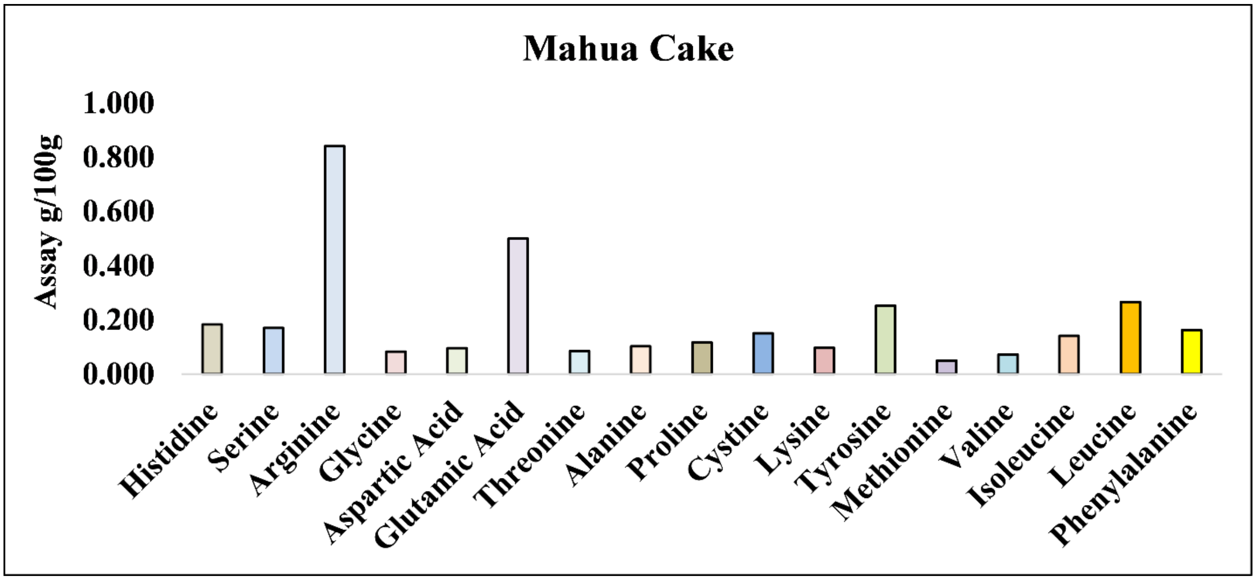


Figure 16.2 The graph depicts the total amino acid composition of mahua cake analysed using high-performance liquid chromatography (HPLC), with data expressed in g/100 g of sample.

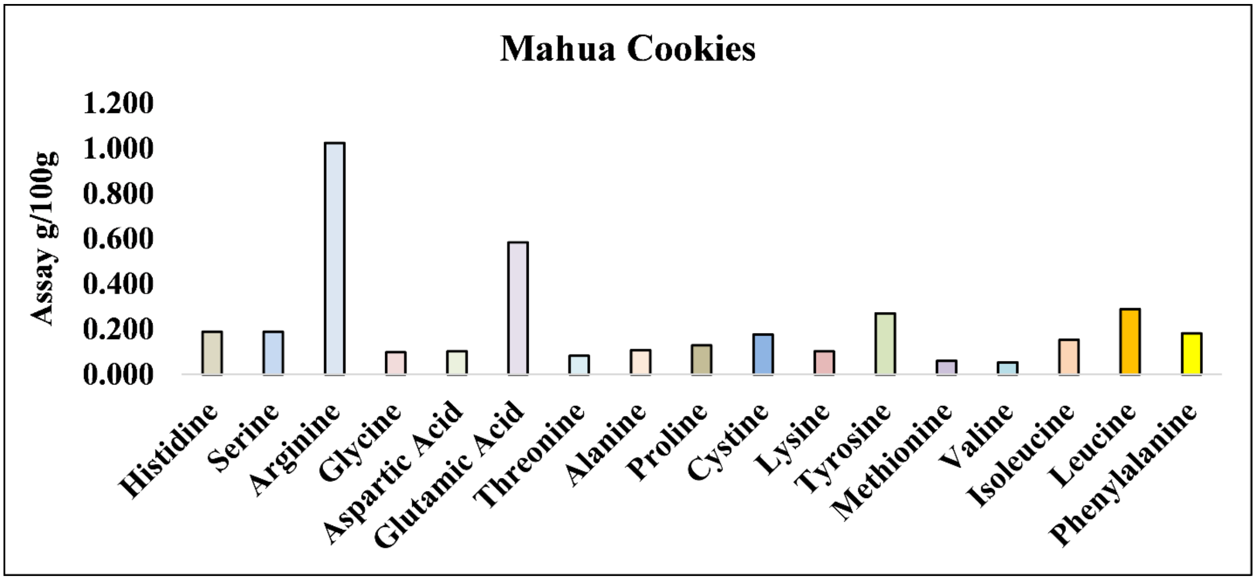


Figure 16.3 The graph depicts the total amino acid composition of Mahua Cookies analysed using high-performance liquid chromatography (HPLC), with data expressed in g/100 g of sample.

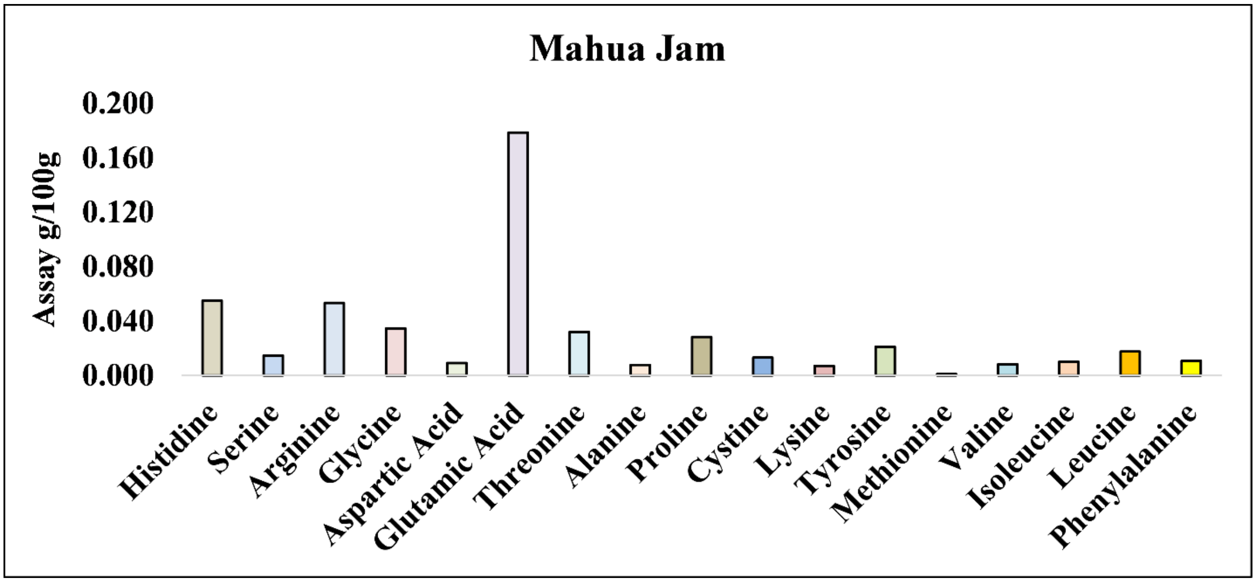


Figure 16.4 The graph depicts the total amino acid composition of Mahua Jam analysed using high-performance liquid chromatography (HPLC), with data expressed in g/100 g of sample.

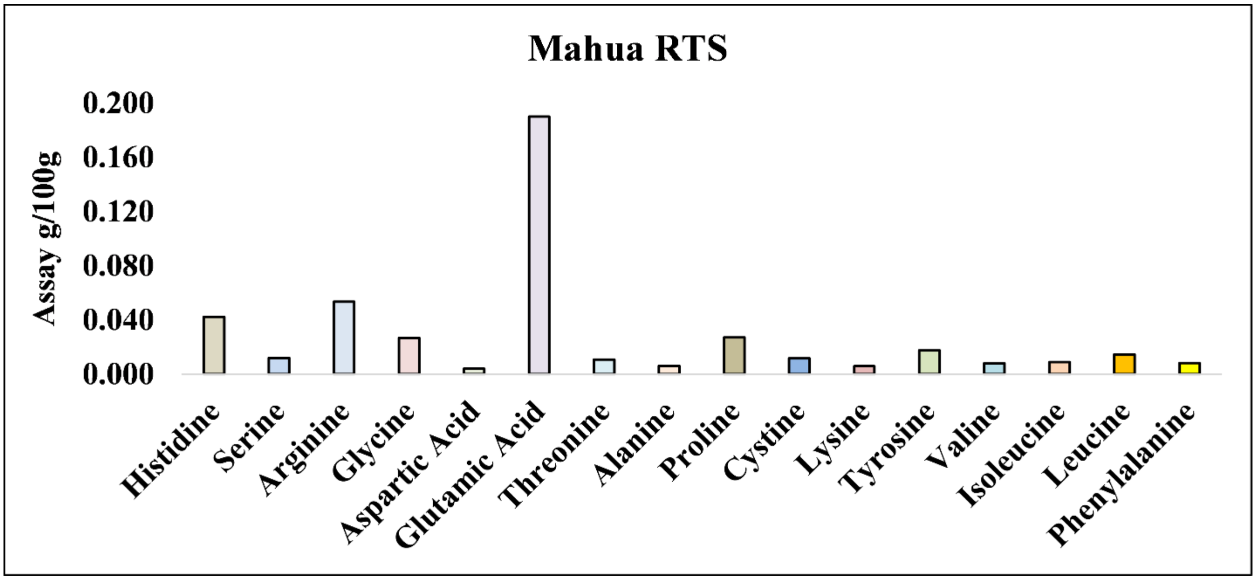


Figure 16.5 The graph depicts the total amino acid composition of Mahua RTS analysed using high-performance liquid chromatography (HPLC), with data expressed in g/100 g of sample.

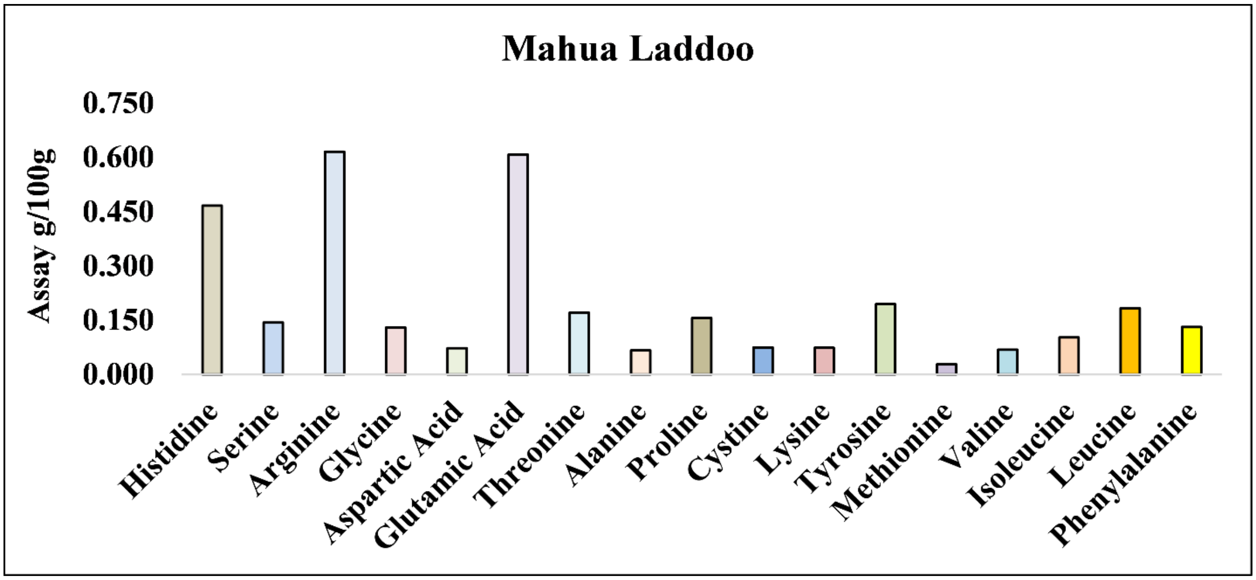


Figure 16.6 The graph depicts the total amino acid composition of Mahua Laddoo analysed using high-performance liquid chromatography (HPLC), with data expressed in g/100 g of sample.

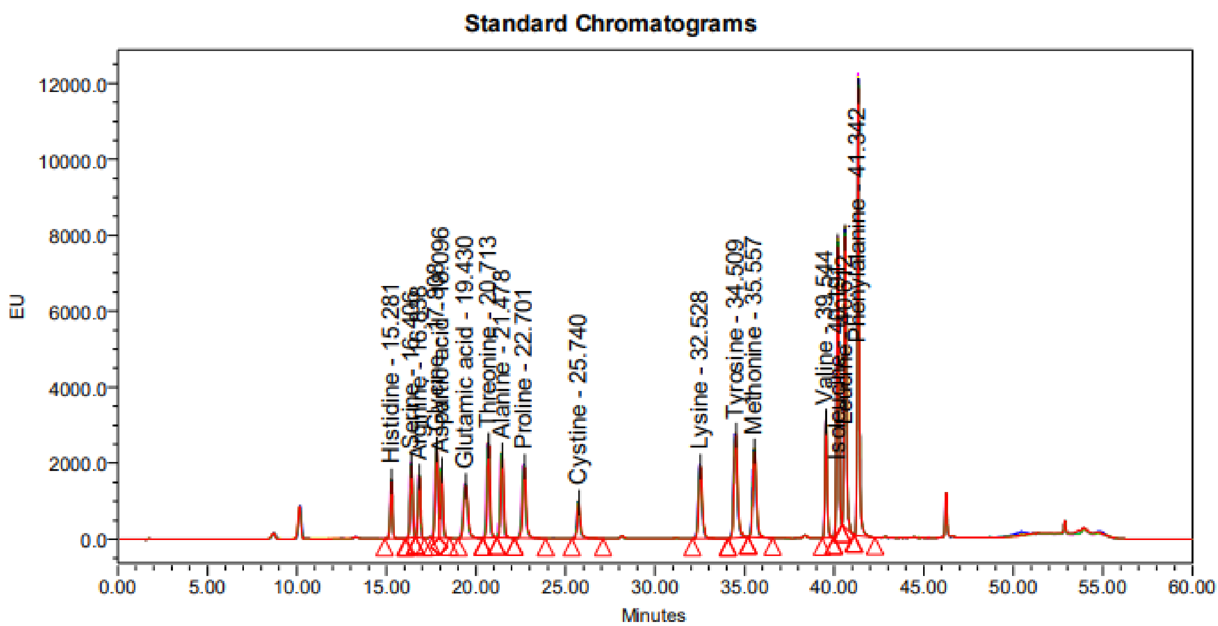


Figure 16.7 Chromatograms depicting the amino acid (essential and nonessential) composition of the prepared standards analysed via HPLC with refractive index detection. The peaks represent individual carbohydrates identified by retention time and compared with standards.

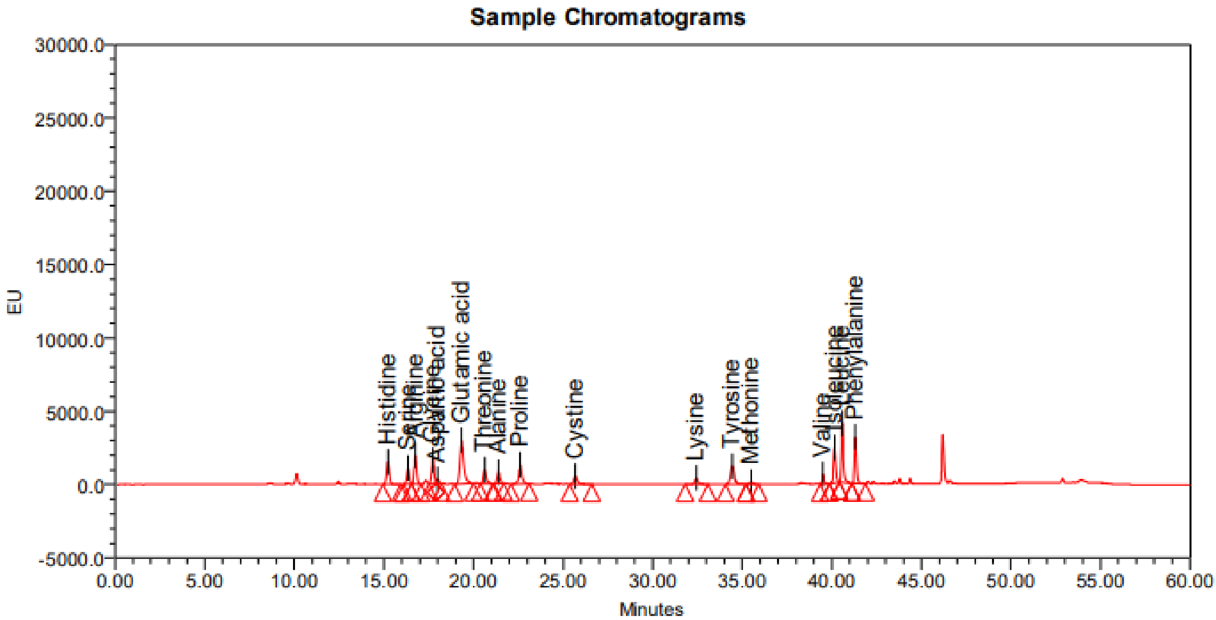


Figure 16.8 Chromatograms depicting the amino acid (essential and nonessential) composition of the Mahua concentrate analysed via HPLC with refractive index detection. The peaks represent individual carbohydrates identified by retention time and compared with standards.

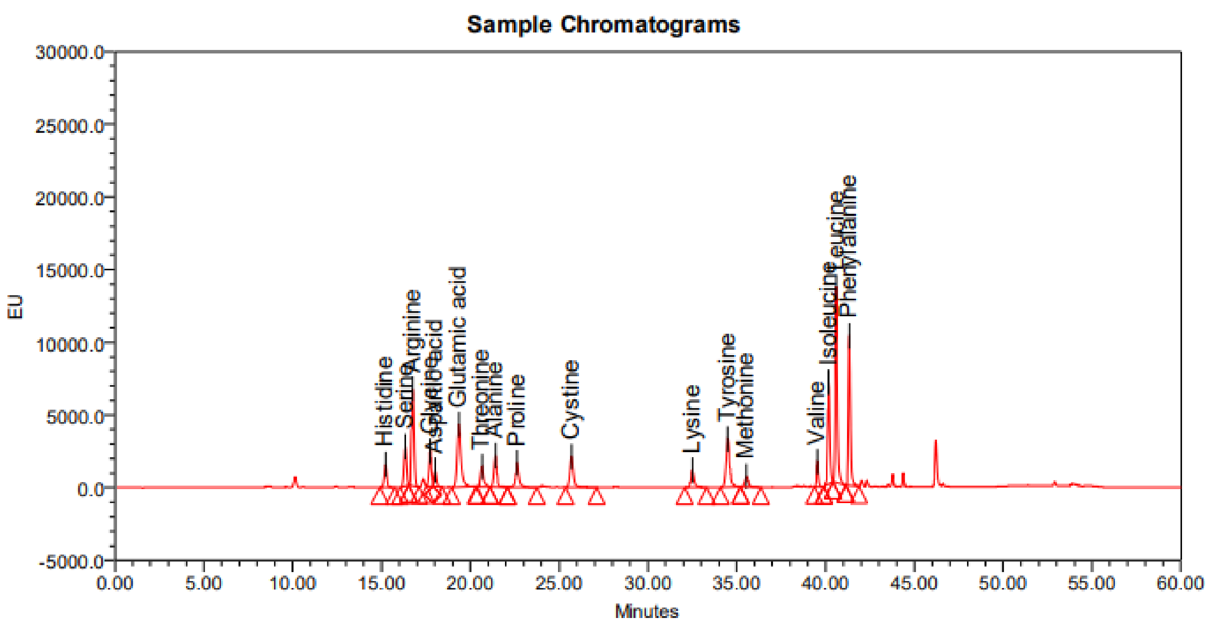


Figure 16.9 Chromatograms depicting the amino acid (essential and nonessential) composition of mahua cakes analysed via HPLC with refractive index detection. The peaks represent individual carbohydrates identified by retention time and compared with standards.

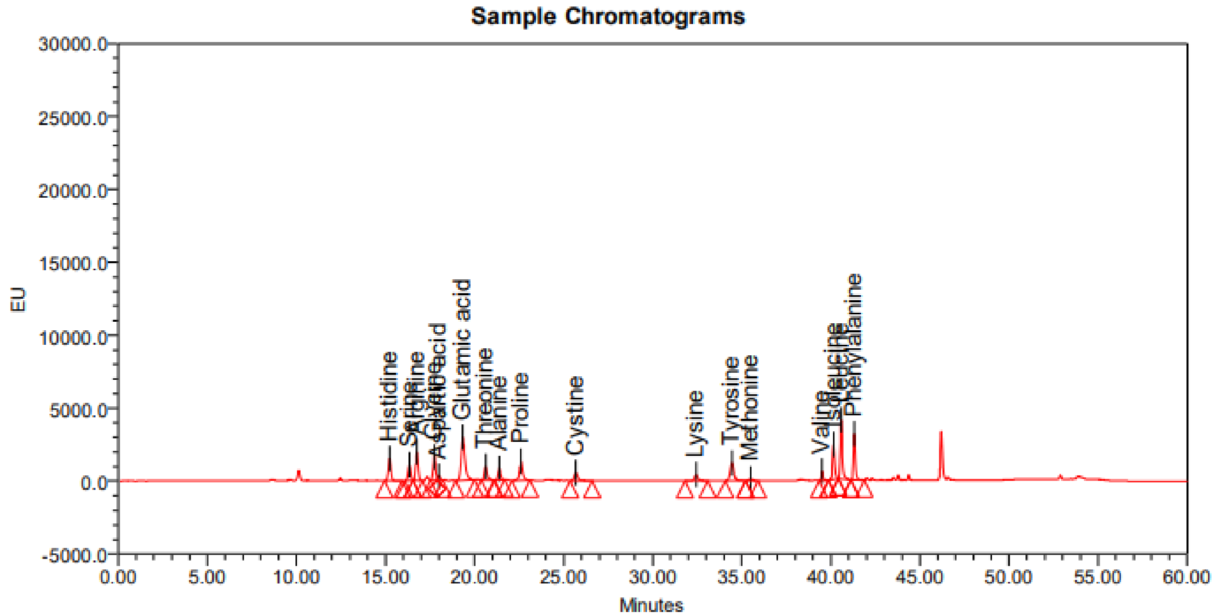


Figure 16.10 Chromatograms depicting the amino acid (essential and nonessential) composition of the Mahua cookies analysed via HPLC with refractive index detection. The peaks represent individual carbohydrates identified by retention time and compared with standards.

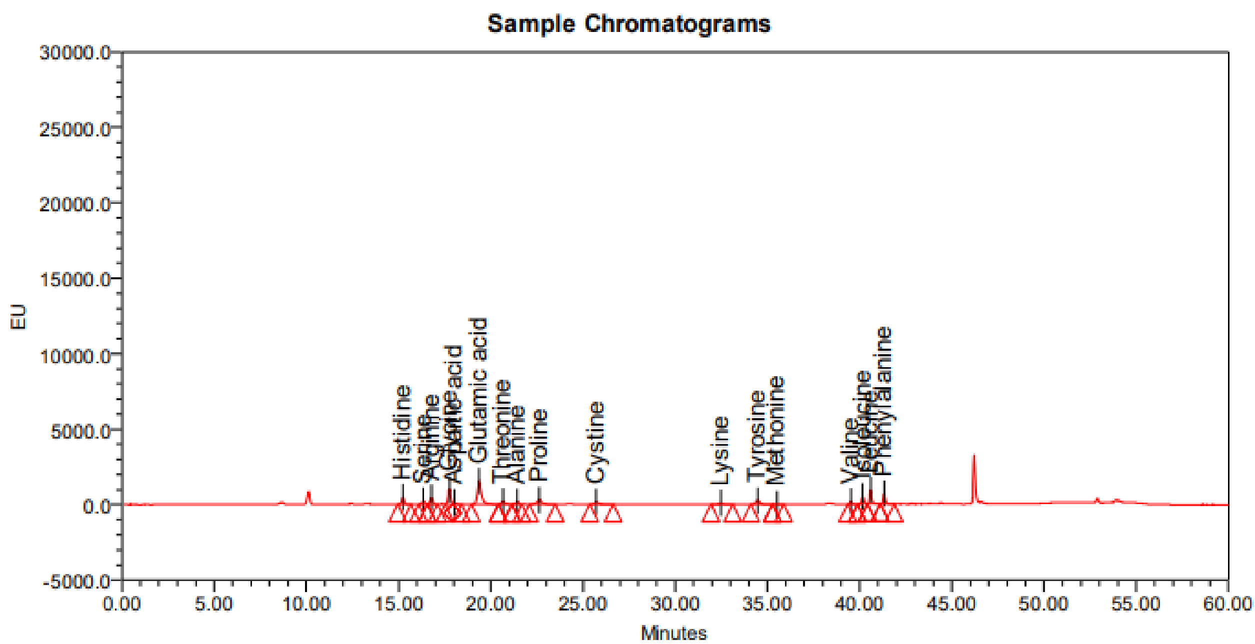


Figure 16.11 Chromatograms depicting the amino acid (essential and nonessential) composition of Mahua jam analysed via high-performance liquid chromatography (HPLC) with refractive index detection. The peaks represent individual carbohydrates identified by retention time and compared with standards.

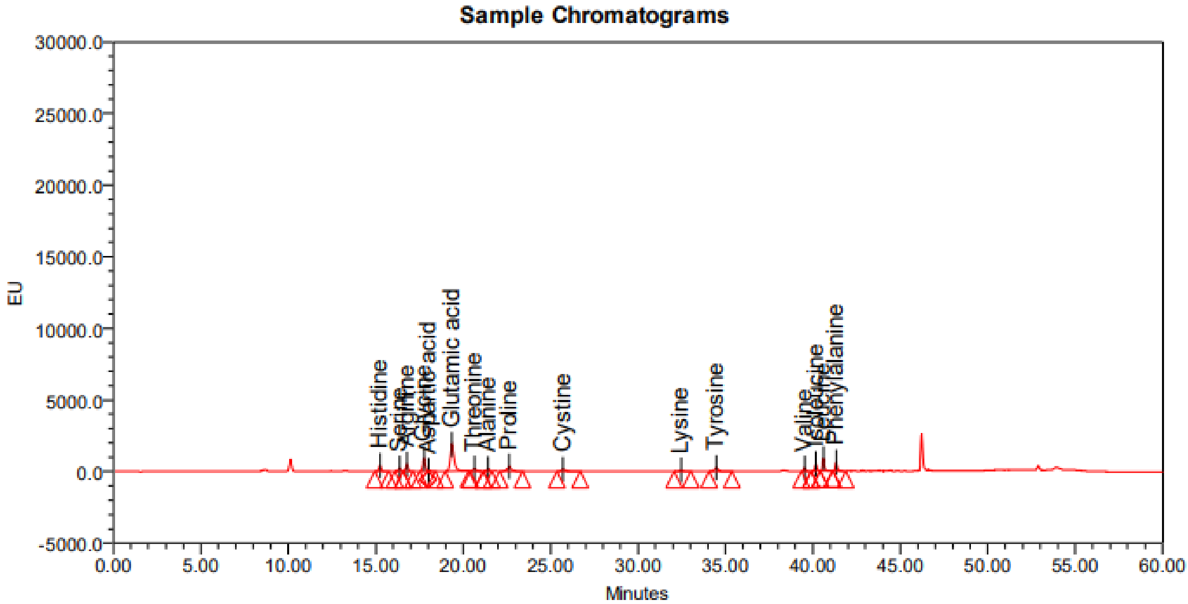


Figure 16.12 Chromatograms depicting the amino acid (essential and nonessential) composition of Mahua RTS analysed via HPLC with refractive index detection. The peaks represent individual carbohydrates identified by retention time and compared with standards.

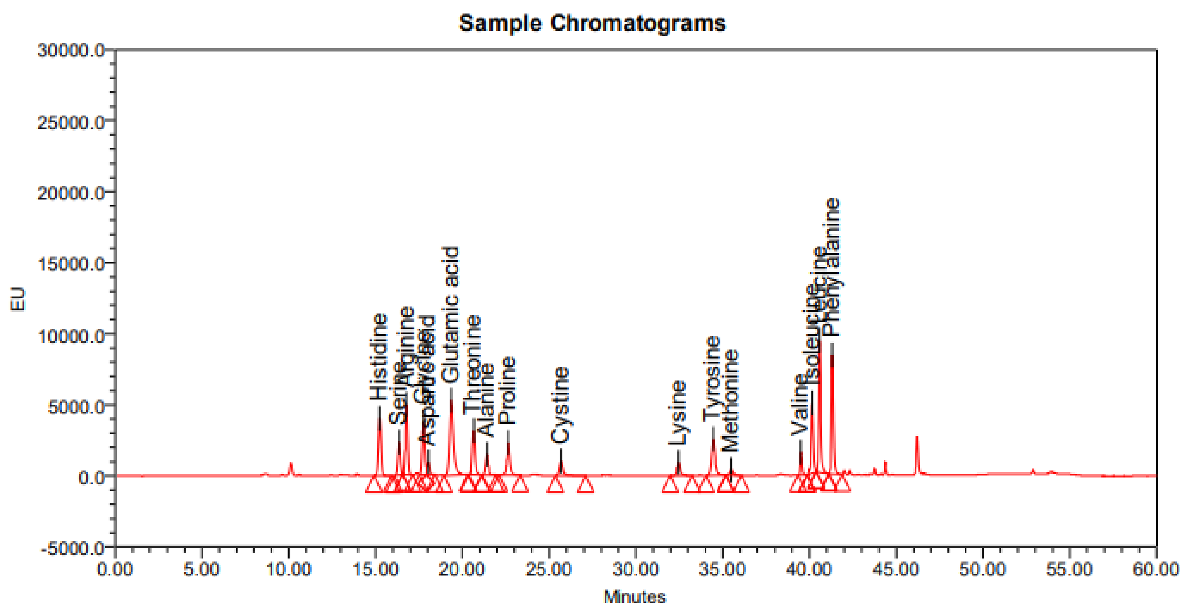


Figure 16.13 Chromatograms depicting the amino acid (essential and nonessential) composition of Mahua laddoo analysed via HPLC with refractive index detection. The peaks represent individual carbohydrates identified by retention time and compared with standards.

5.4.2.3 Vitamin profiling:

The presence of vitamins in food products plays a crucial role in maintaining health status. Vitamin B5 plays a major role in energy production, cellular function and various metabolic processes. The presence of riboflavin increases energy metabolism and maintains healthy eyes and skin. Folic acid is essential for DNA synthesis and the division of cells. It is also essential for pregnant women to prevent neural tube defects. The metabolic processes of carbohydrates, fats and proteins are carried out in the presence of coenzyme A, which is synthesized by vitamin B5. These vitamins are essential in the diet, specifically through fortified or value-added food products.

The vitamin profiling of the prepared value-added food products is carried out via high-performance liquid chromatography (HPLC), which quantifies the levels of riboflavin, folic acid, and vitamin B5 (16.14-16.27). The amount of riboflavin used was different, with several products showing increased levels due to fortification, which makes them good sources of vital nutrients. Riboflavin was mainly found in Mahua concentrate (0.03%), Mahua cake (0.02%), Mahua cookies (0.01%), Mahua jam (0.01%), and Mahua RTS (0.04%). The presence of riboflavin (vitamin B2) increases energy production, as it supports the translation of carbohydrates, fats, and proteins into working energy. It also acts as an antioxidant to protect cells from damage. A good amount of riboflavin consumption is important for avoiding deficiencies that can lead to indications such as sore throat, skin cracks, and fatigue. Folic acid was also present in considerable amounts, especially in products such as Mahua concentrate (0.01%), Mahua cake (0.02%), Mahua cookies (0.02%), Mahua jam (0.01%), Mahua RTS (0.02%) and Mahua laddoo (0.03%). The presence of folic acid (vitamin B9) is important for DNA synthesis and cell division, which makes it more valuable for pregnant women. These findings support the prevention of neural tube flaws in developing embryos, which supports healthy fetal growth. Moreover, it helps in the formation of red blood cells and reduces the risk of anaemia. The presence of vitamin B5 was also noted in the prepared food products from mahua flowers. Nicotinic acid was found mainly in the mahua flower concentrate at a concentration of 0.02%. It has also been shown to be present in products such as Mahua cookies (0.02%), Mahua jam (0.05%), Mahua RTS (0.02%) and Mahua laddoo (0.05%). It plays a major role in converting food into energy by metabolizing carbohydrates, fats, and proteins. Furthermore, it helps maintain cholesterol levels by encouraging the production of good HDL cholesterol. Thaimine was detected in products such as Mahua concentrate (0.05%), Mahua cookies (0.03%), Mahua cookies (0.02%), Mahua jam (0.01%), Mahua RTS (0.04%), and Mahua laddoo (0.03%). It is important for converting carbohydrates into energy and supports the proper brain and nervous system. It helps to maintain healthy muscle function and endorses cardiovascular health. Acceptable thiamine consumption is vital for avoiding conditions such as beriberi, which can cause nerve and heart damage. Pyridoxine was also identified in the Mahua concentrate (0.01%). Similarly, cyanocobalmine was found in Mahua cake at a concentration of 0.02%. The presence of pyridoxine facilitates the metabolism of amino acids and helps to produce neurotransmitters. Additionally, it helps in the production of RBCs and supports brain and immune functions. Cyanocobalamin (vitamin B12) is important for DNA synthesis and nerve health and helps in the formation of red blood cells. It also prevents anaemia and supports neurological functions. As these products contain a good amount of vitamins, they can be incorporated into the daily diet of all age groups to prevent several diseases.

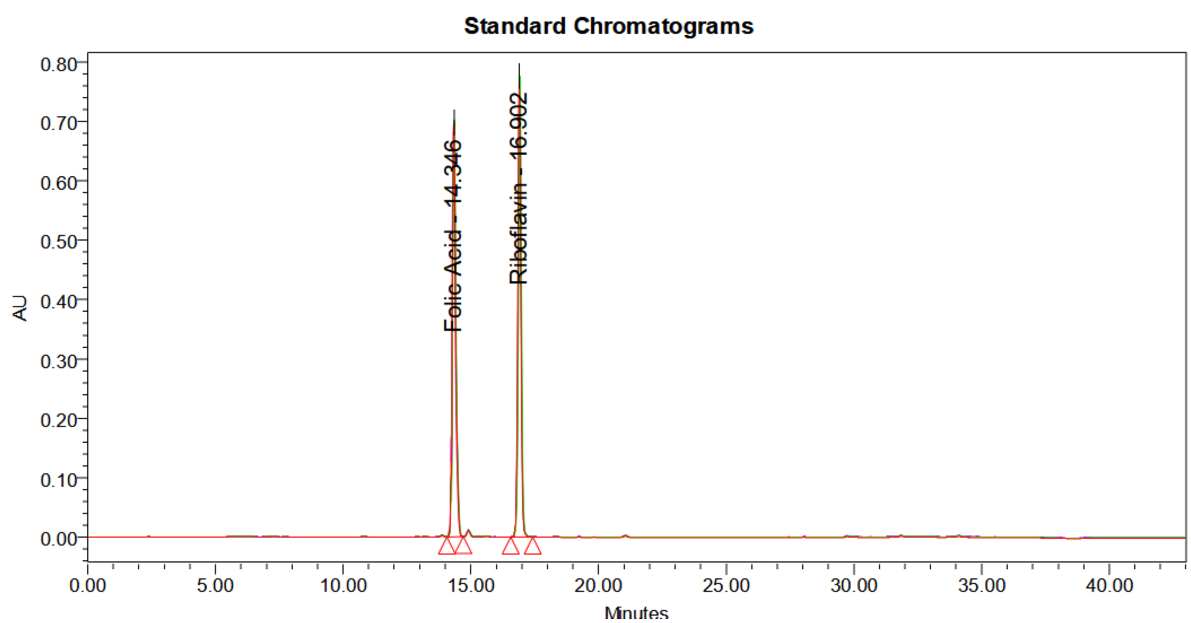


Figure 16.14 Chromatogram showing the analysis of riboflavin and folic acid standards via HPLC. The chromatogram identified distinct peaks for riboflavin and folic acid. The sharp peaks indicate the successful resolution and identification of riboflavin and folic acid.

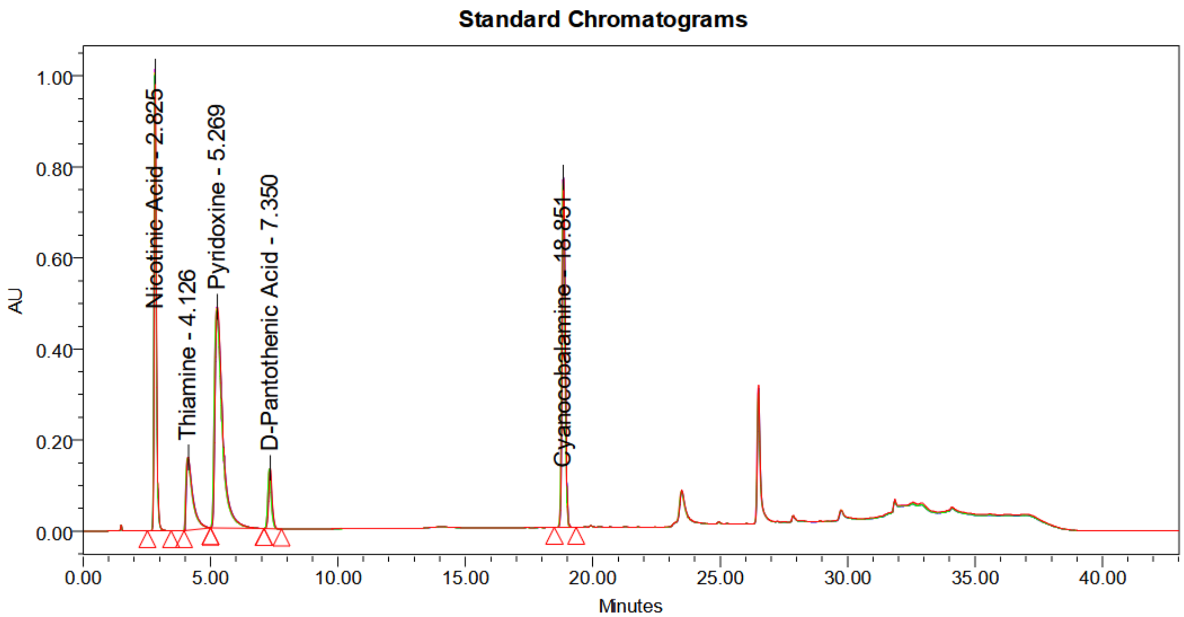


Figure 16.15 Chromatogram showing the analysis of nicotinic acid, thiamine, pyridoxine, D-pantothenic acid, and cyanocobalmine standards via HPLC. The chromatogram shows the distinct peak for each vitamin. The sharp peaks indicate the successful resolution and identification of vitamin B5.

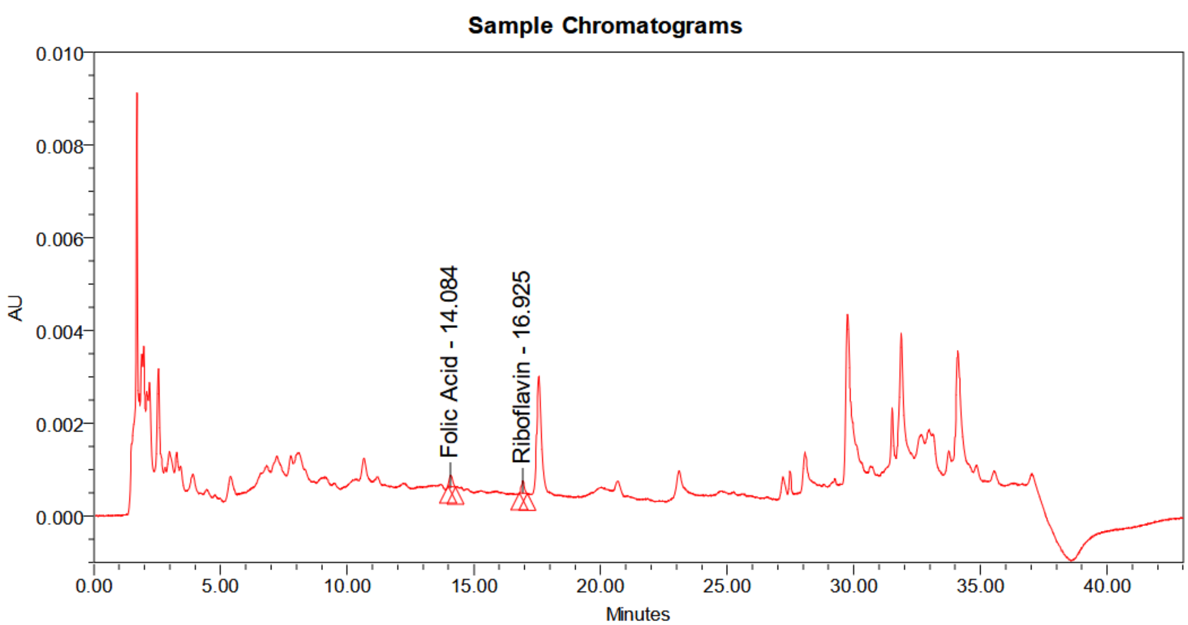


Figure 16.16 Chromatograms showing the separation of folic acid and riboflavin in the Mahua concentrate. Each peak corresponds to a specific vitamin. The X-axis represents the retention time, whereas the Y-axis represents the peak intensity. The vitamins were quantified via comparison with standards.

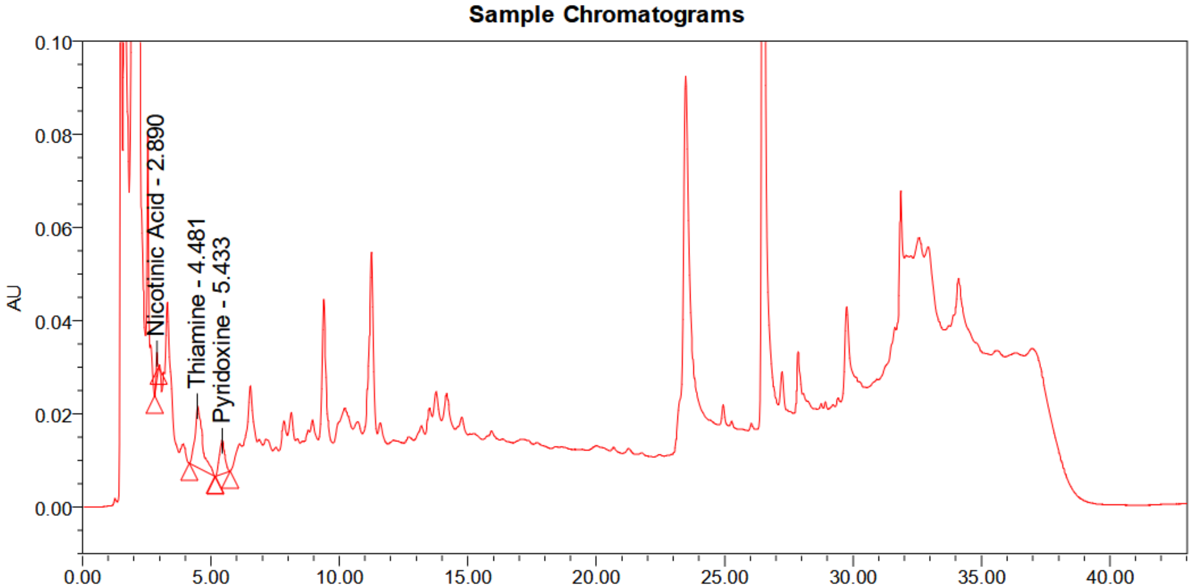


Figure 16.17 Chromatograms showing the separation of vitamin B5 in the Mahua concentrate. Each peak corresponds to a specific vitamin. The X-axis represents the retention time, whereas the Y-axis represents the peak intensity. The vitamins were quantified via comparison with standards.

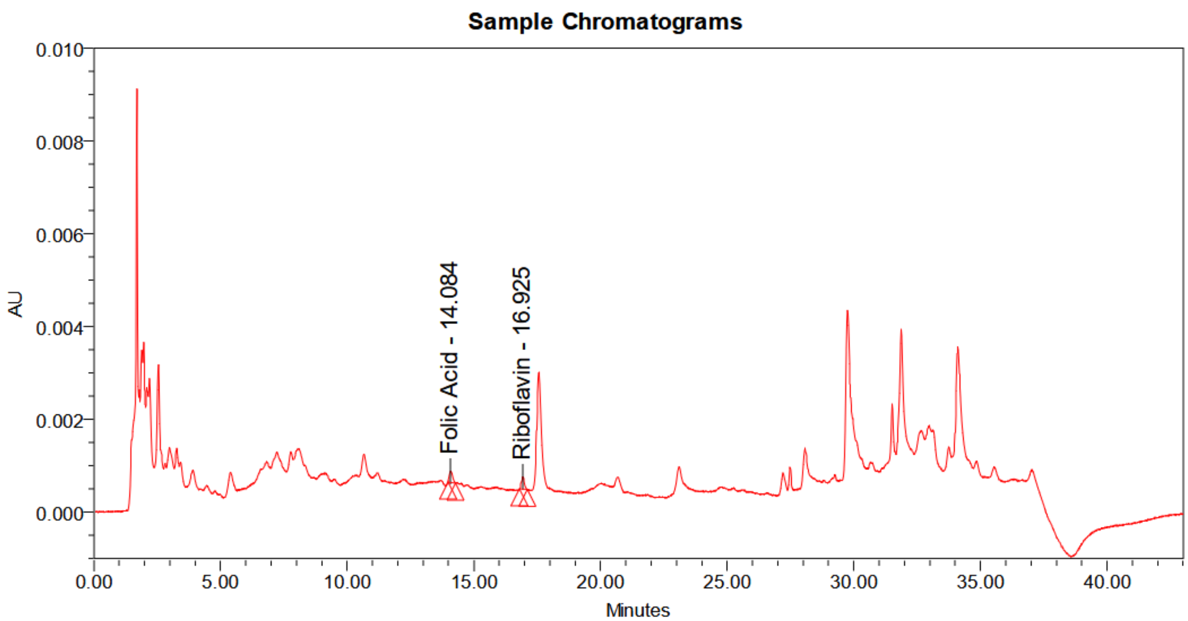


Figure 16.18 Chromatograms showing the separation of folic acid and riboflavin in Mahua cake. Each peak corresponds to a specific vitamin. The X-axis represents the retention time, whereas the Y-axis represents the peak intensity. The vitamins were quantified via comparison with standards.

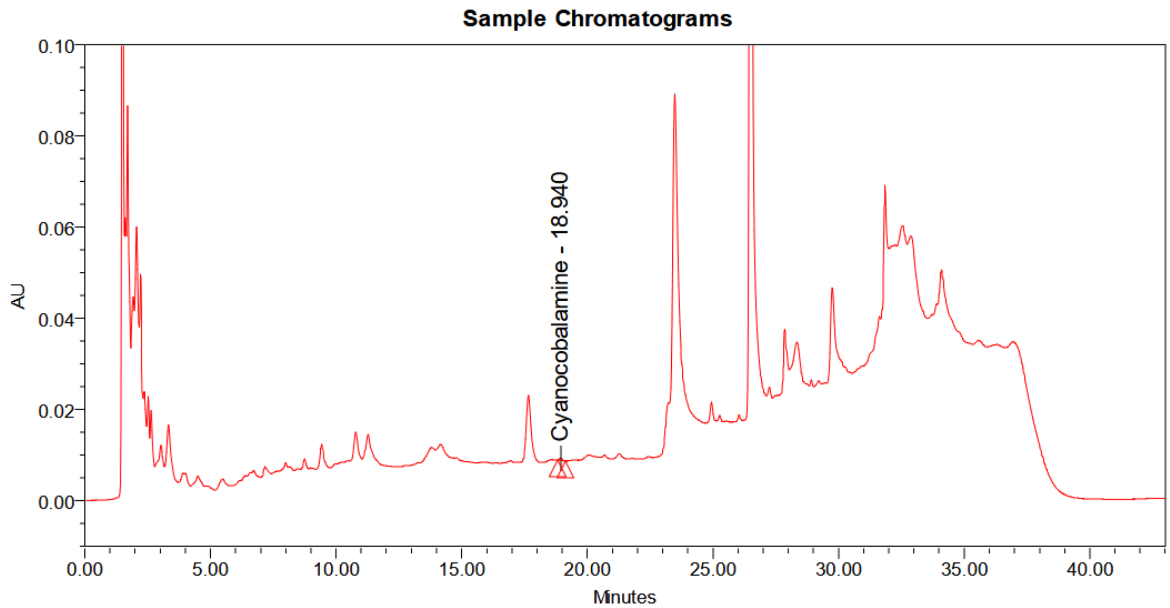


Figure 16.19 Chromatograms showing the separation of cyanocobalmine in Mahua cake. Each peak corresponds to a specific vitamin. The X-axis represents the retention time, whereas the Y-axis represents the peak intensity. The vitamins were quantified via comparison with standards.

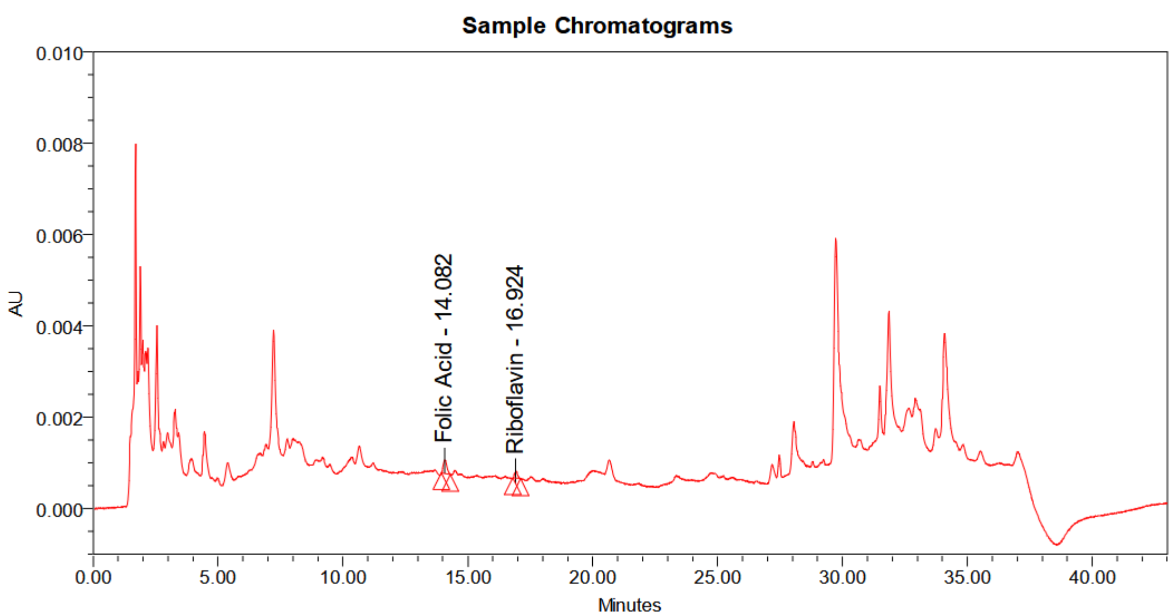


Figure 16.20 Chromatograms showing the separation of folic acid and riboflavin in Mahua cookies. Each peak corresponds to a specific vitamin. The X-axis represents the retention time, whereas the Y-axis represents the peak intensity. The vitamins were quantified via comparison with standards.

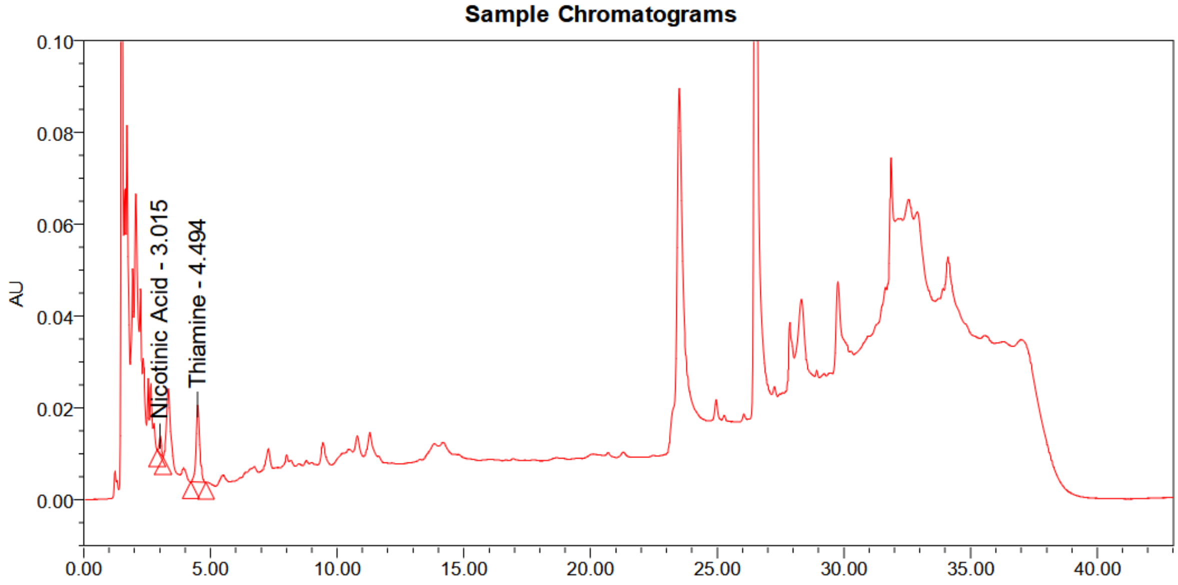


Figure 16.21 Chromatograms showing the separation of nicotinic acid and thaimine in Mahua cookies. Each peak corresponds to a specific vitamin. The X-axis represents the retention time, whereas the Y-axis represents the peak intensity. The vitamins were quantified via comparison with standards.

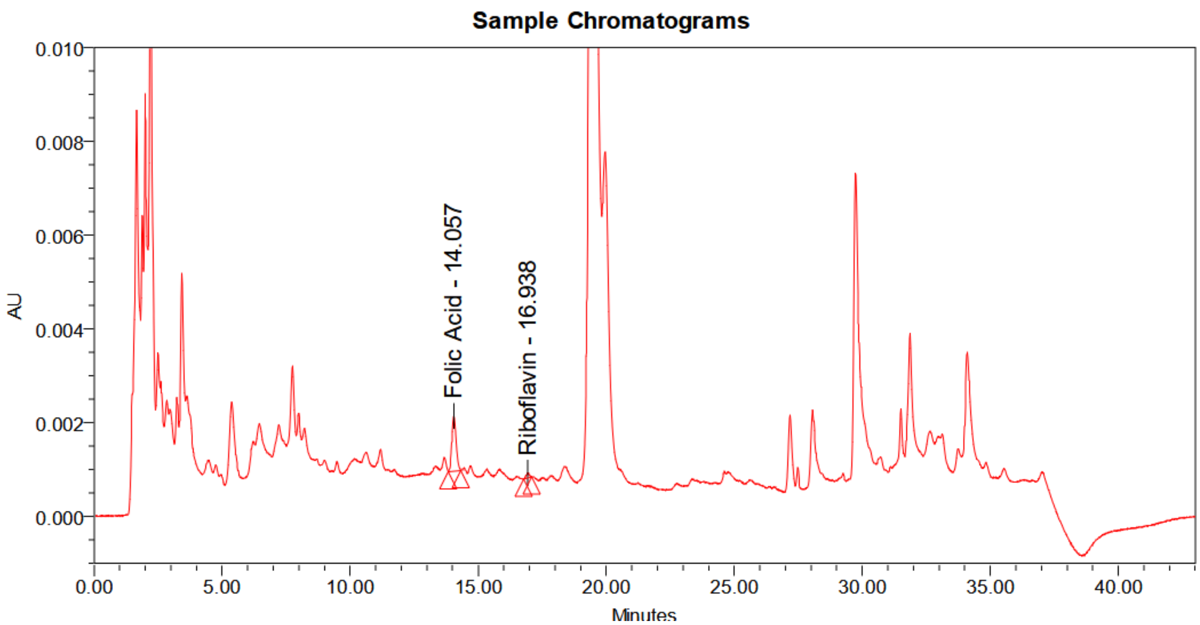


Figure 16.22 Chromatograms showing the separation of folic acid and riboflavin in Mahua jam. Each peak corresponds to a specific vitamin. The X-axis represents the retention time, whereas the Y-axis represents the peak intensity. The vitamins were quantified via comparison with standards.

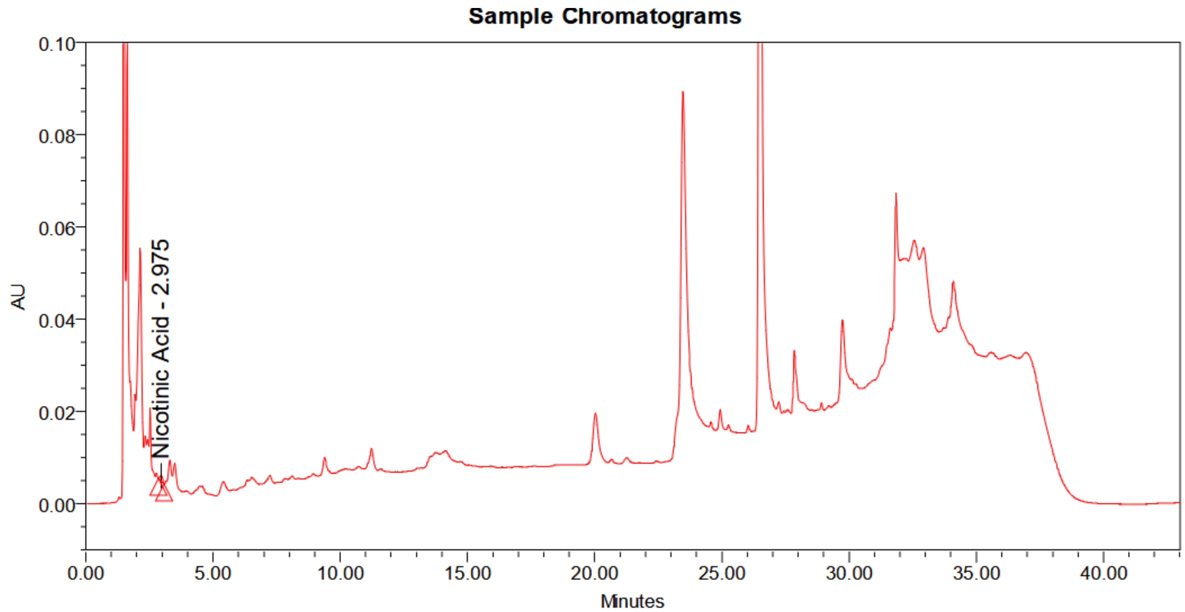


Figure 16.23 Chromatograms showing the separation of nicotinic acid in Mahua jam. Each peak corresponds to a specific vitamin. The X-axis represents the retention time, whereas the Y-axis represents the peak intensity. The vitamins were quantified via comparison with standards.

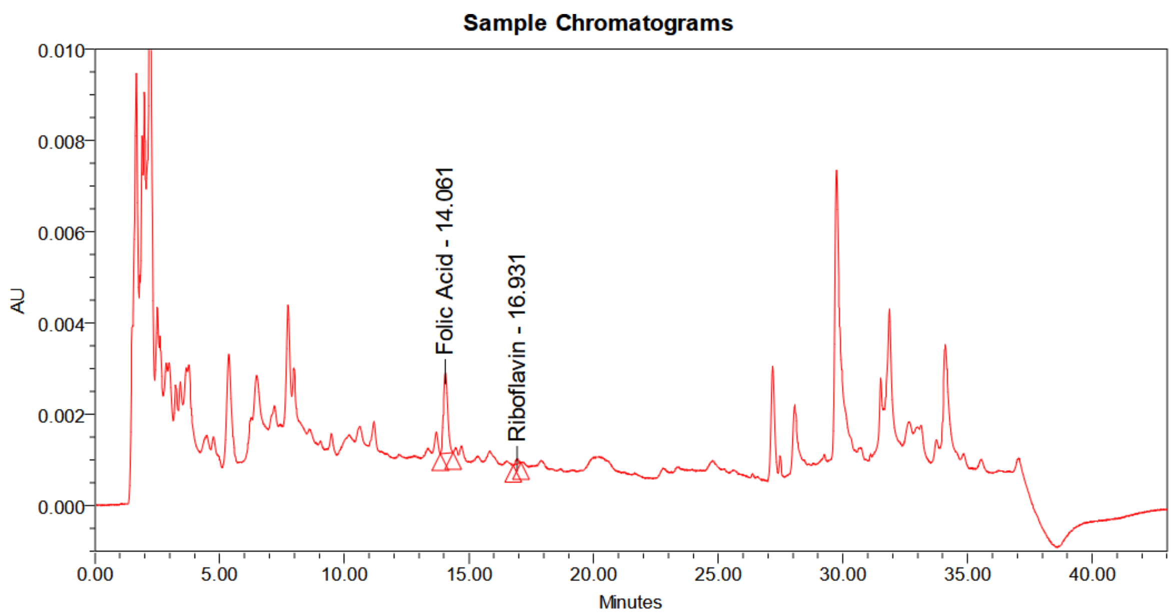


Figure 16.24 Chromatograms showing the separation of folic acid and riboflavin in Mahua RTS. Each peak corresponds to a specific vitamin. The X-axis represents the retention time, whereas the Y-axis represents the peak intensity. The vitamins were quantified via comparison with standards.

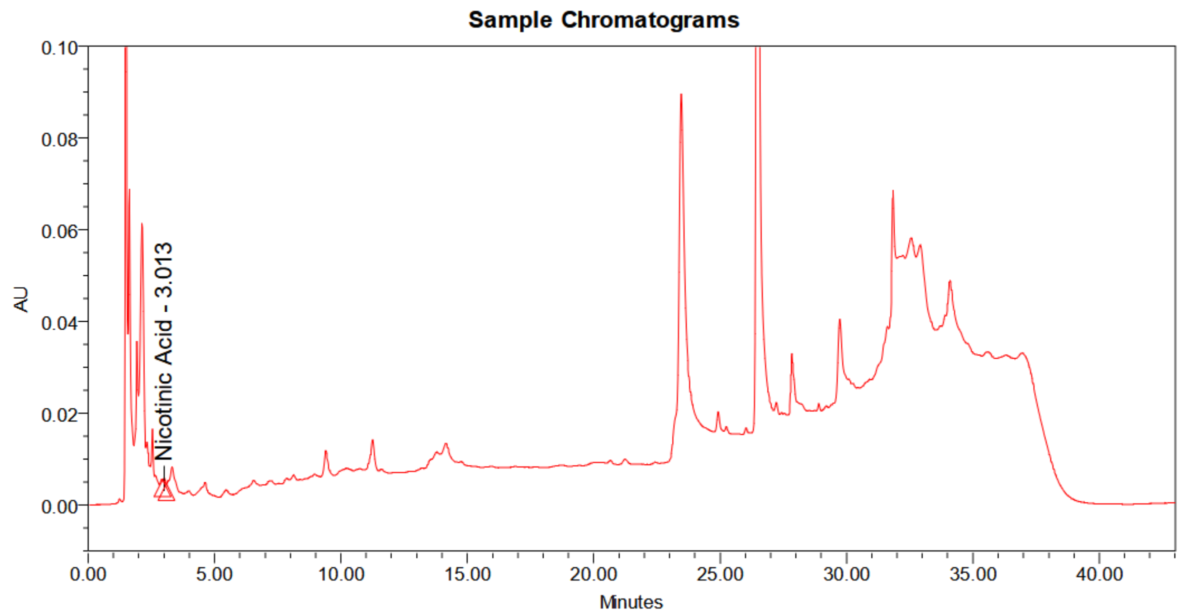


Figure 16.25 Chromatograms showing the separation of nicotinic acid in Mahua RTS. Each peak corresponds to a specific vitamin. The X-axis represents the retention time, whereas the Y-axis represents the peak intensity. The vitamins were quantified via comparison with standards.

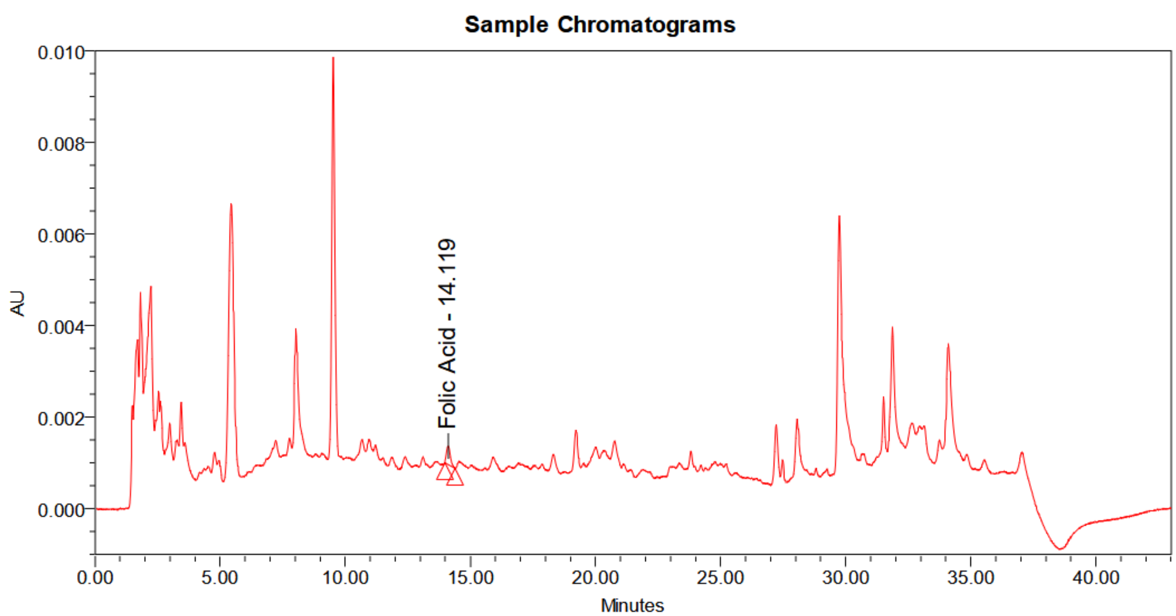


Figure 16.26 Chromatograms showing the separation of folic acid from Mahua laddoo. Each peak corresponds to a specific vitamin. The X-axis represents the retention time, whereas the Y-axis represents the peak intensity. The vitamins were quantified via comparison with standards.

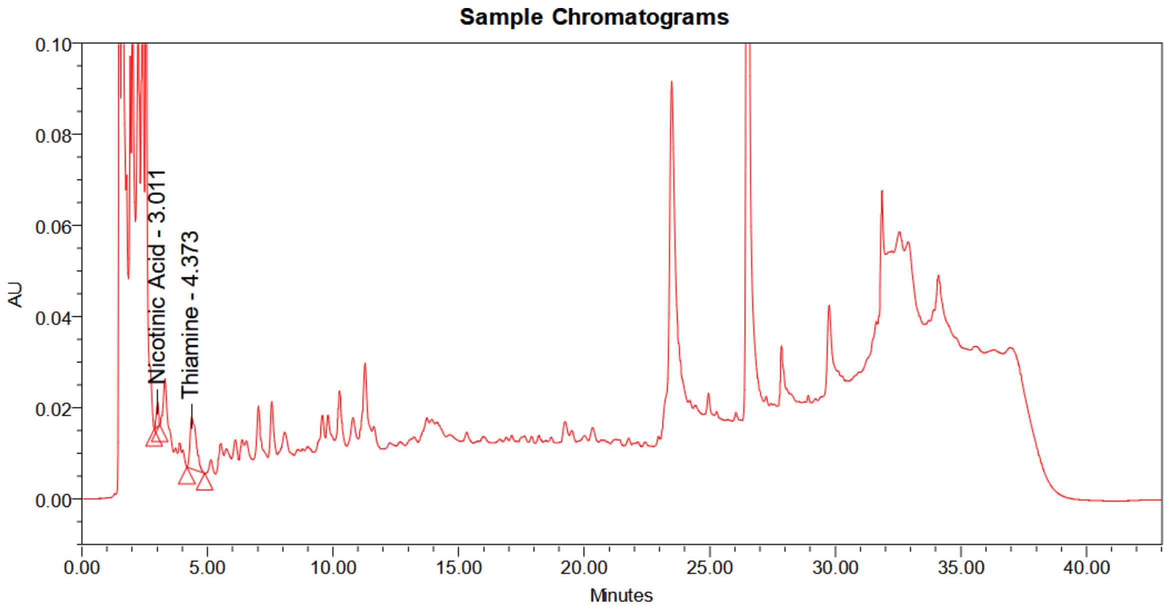


Figure 16.27 Chromatograms showing the separation of nicotinic acid and thaimine in Mahua laddoo. Each peak corresponds to a specific vitamin. The X-axis represents the retention time, whereas the Y-axis represents the peak intensity. The vitamins were quantified via comparison with standards.

5.4.3 Sensory evaluation:

The sensory evaluation of different value added food products like mahua concentrate, mahua cake, mahua cookies, mahua jam, mahua RTS and mahua laddoo were evaluated by taking randomly 10 panelists. The evaluation of different products were done based on their colour, odour, texture, taste, mouth feel and appearance. Mahua cake and cookies were appreciated for their colour, texture, and aroma. The cake offers a soft texture while the cookies offers a crispy edge and chewy center. Mahua jam were appreciated for their colour, spreadable texture and aroma. The colour of RTS was highly accepted. The drink was said to be fresh, juicy having a sweet aroma. Laddoos were appreciated for their soft, roasted aroma, firm texture while the concentrates were visually consistent, with rich colours and a smooth, viscous texture. Overall, the products exhibited outstanding sensory potentials, contributing to their market demand and consumer recognition (Fig. 17.1-17.6).

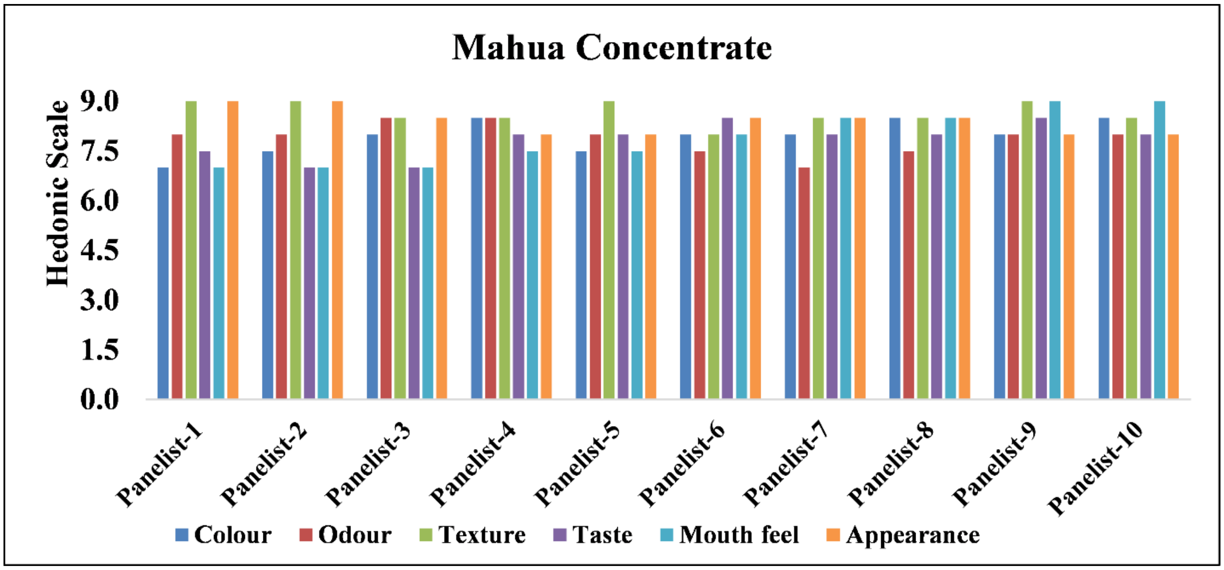


Figure 17.1 The graph depicts the sensory result of 10 panelists of mahua concentrate based on colour, odour, texture, taste, mouth feel and appearance. The rating were assessed on 9-point hedonic scale (Y-axis), where 1 indicated extremely dislike and 9 indicates extremely like.

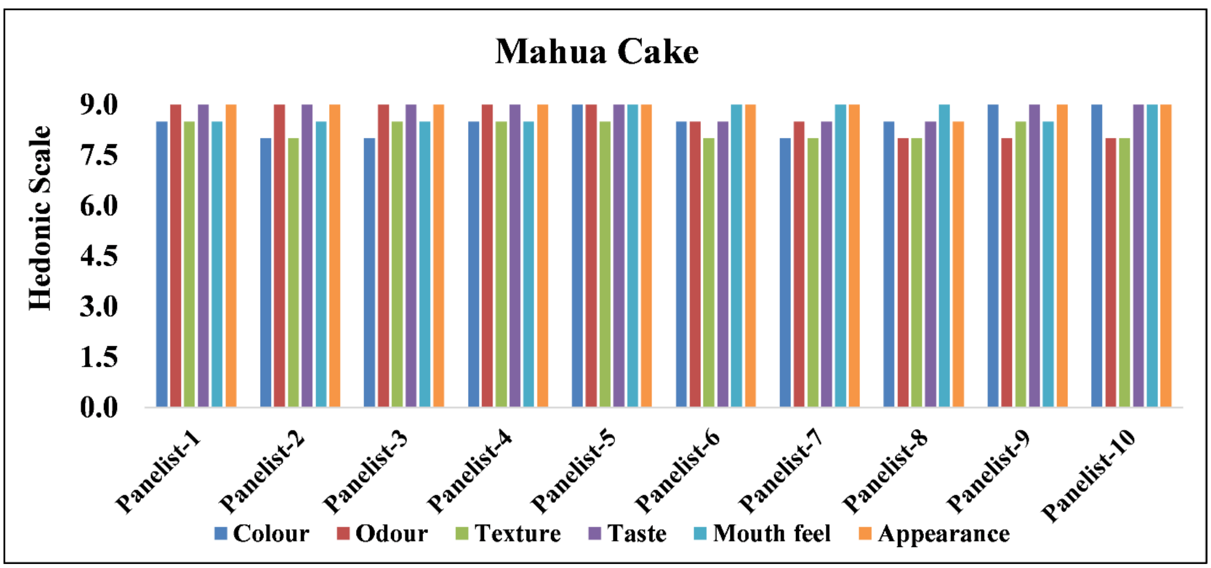


Figure 17.2 The graph depicts the sensory result of 10 panelists of mahua cake based on colour, odour, texture, taste, mouth feel and appearance. The rating were assessed on 9-point hedonic scale (Y-axis), where 1 indicated extremely dislike and 9 indicates extremely like.

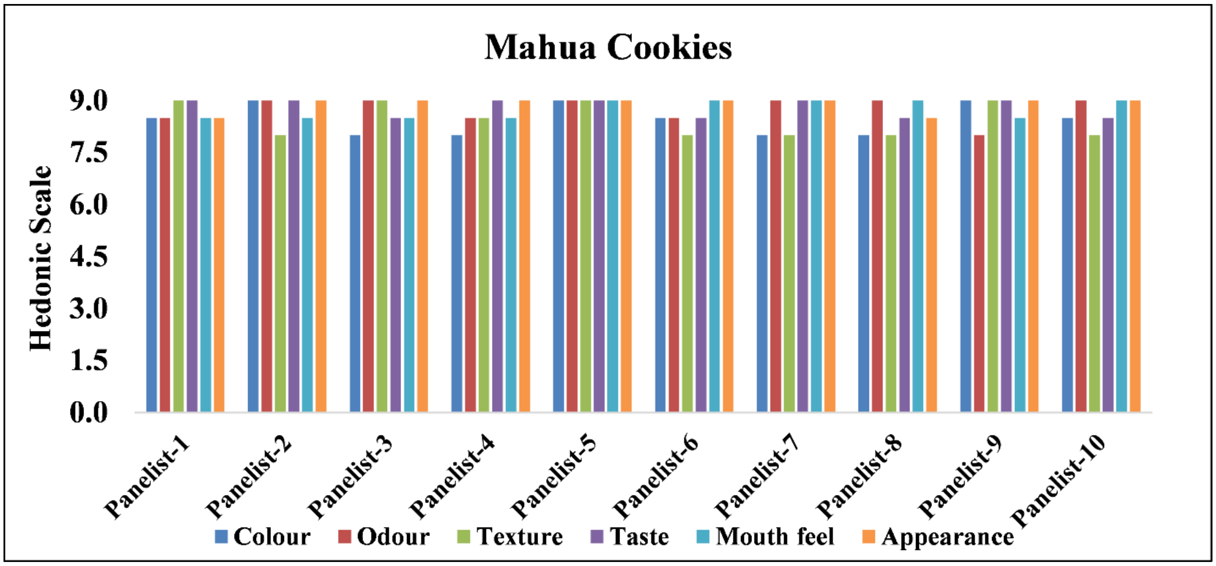


Figure 17.3 The graph depicts the sensory result of 10 panelists of mahua cookies based on colour, odour, texture, taste, mouth feel and appearance. The rating were assessed on 9-point hedonic scale (Y-axis), where 1 indicated extremely dislike and 9 indicates extremely like.

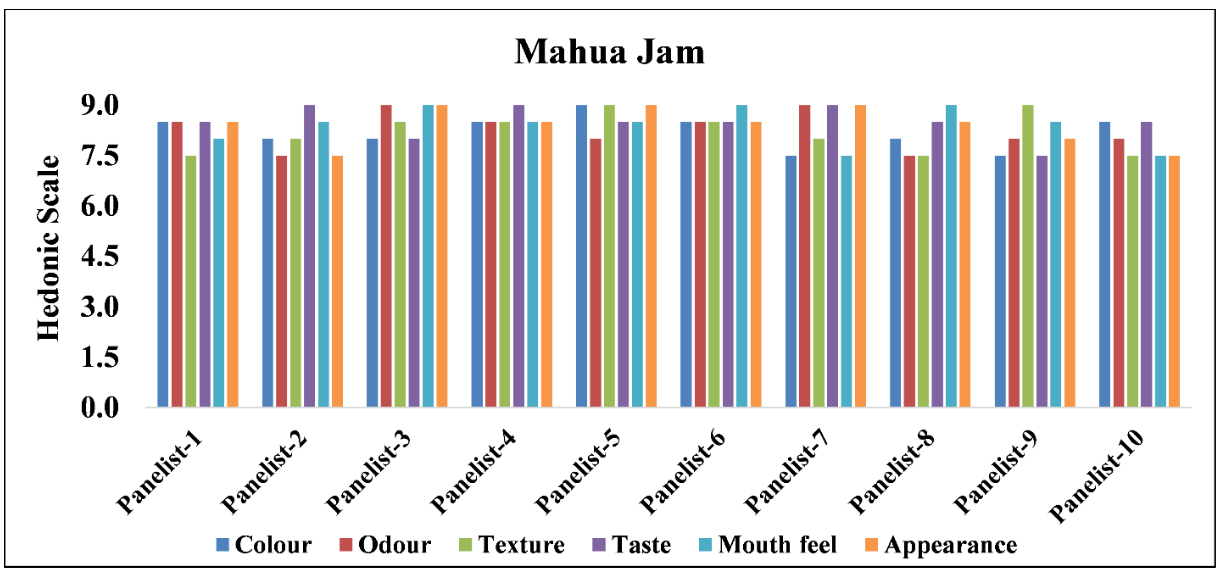


Figure 17.4 The graph depicts the sensory result of 10 panelists of mahua jam based on colour, odour, texture, taste, mouth feel and appearance. The rating were assessed on 9-point hedonic scale (Y-axis), where 1 indicated extremely dislike and 9 indicates extremely like.

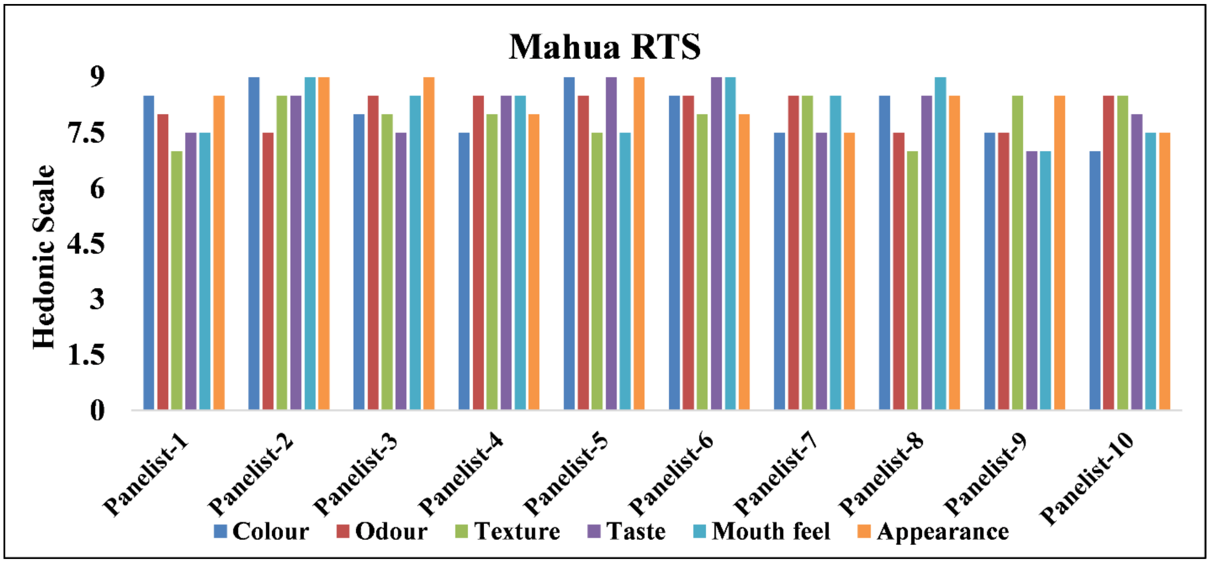


Figure 17.5 The graph depicts the sensory result of 10 panelists of mahua RTS based on colour, odour, texture, taste, mouth feel and appearance. The rating were assessed on 9-point hedonic scale (Y-axis), where 1 indicated extremely dislike and 9 indicates extremely like.

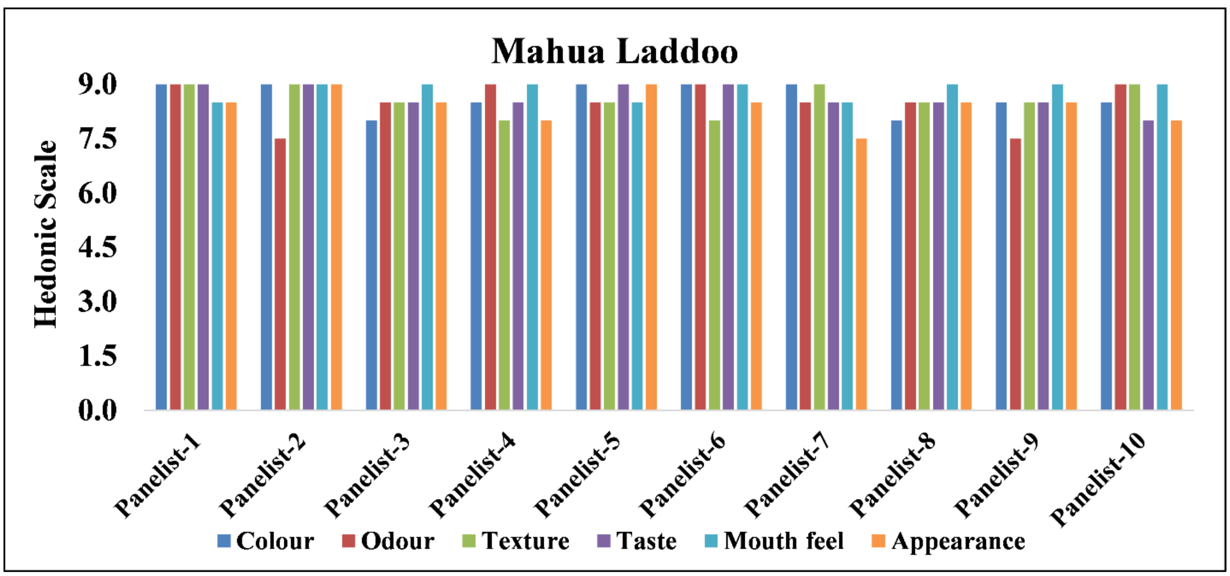


Figure 17.6 The graph depicts the sensory result of 10 panelists of mahua laddoo based on colour, odour, texture, taste, mouth feel and appearance. The rating were assessed on 9-point hedonic scale (Y-axis), where 1 indicated extremely dislike and 9 indicates extremely like.

5.4.4 Shelf life of food products

The shelf-life of the products depends on the storage conditions. The shelf life of perishable products such as dairy and meat is shorter, ranging from days to weeks, but processed products such as packaged foods and canned foods have longer shelf lives, ranging from several months to years, due to the use of preservatives and packaging, which reduces the exposure to air and moisture. The shelf-life of products can be influenced by several external factors, such as temperature, humidity and light exposure. Proper storage conditions, such as keeping the products in a cool and dry environment and airtight packaging, can increase the shelf-life of the products. In addition, some foods have an optimal shelf life beyond which their quality, flavour, texture, and appearance may deteriorate. Monitoring products on a regular basis and proper handling plays a significant role in maintaining the quality of food products.

The experimental data revealed that after a period of three months, the products that were initially considered safe for consumption began to contain yeast, mould and bacteria (Table 4.3). In the earlier stages, the products did not contain any visible contaminants, making them suitable for consumption as per the FSSAI guidelines. However, on passing days, there is visible growth of yeast, mold and bacteria. Within three months, the products were in safe and acceptable condition and did not carry any health risks. The changes after this time underscore the natural spoilage process of the products, even if they are initially preserved under optimal conditions. The visible growth of yeast, mold and bacteria elucidates that despite early stability, the products have now closed the window for safe consumption, highlighting the importance of strict adherence to storage recommendations, expiry dates, and regular monitoring of food products. This finding confirms that food safety can change over time and strengthens the need to consume food products within their optional periods to avoid possible health threats from microbial infection.

Over the storage period, the nutritional aspects of the products gradually deteriorated, with key components such as proteins, carbohydrates, and reducing sugars showing a slow but constant decrease (Fig. 17.7-17.9). Over time, the stability of these nutrients begins to decline due to several factors, such as enzymatic reactions, oxidation and breakdown of several Phyto compounds within the products. Proteins, crucial nutrients that are required for tissue repair and helpful for several significant functions within the body, slightly degrade over a period of three months, leading to a reduction in their bioavailability. Similarly, carbohydrates, which are a primary source of energy, lose some of their potential over a period of three months, which results in a slight decrease in their effectiveness as quick energy sources. Reducing sugars, which play a dynamic role in various metabolic processes, also gradually decrease as they are broken down during extended storage. Since the loss of these nutrients is not extreme within the initial days, it becomes more distinct as the food products are maintained for prolonged periods. The overall decrease in nutritional quality highlights the importance of consuming food products within their optimum shelf life to safeguard them from their projected health benefits, as extended storage can lead to increased nutrient loss, which may disturb their overall efficacy.

Table 4.3: The shelf-life of the value-added food products derived from Mahua flowers with added preservatives. Bacterial load and Yeast/Mold Count within the safe limit as per FSSAI *Bacterial load: 1×10^2/g to 1×10^3/g *Yeast/Mold load: 50/g to 1×10^2/g

Food Products	**Aerobic plate count/g**	**Yeast and mold count/g**
	0^{th} day	
Mahua Juice Concentrate	0	0
Mahua cake	0	0
Mahua cookies	0	0
Mahua jam	0	0
Mahua RTS	0	0
Mahua laddoo	0	0
	15^{th} day	
Mahua Juice Concentrate	0.1×10^1	0.12×10^1
Mahua cake	0	0
Mahua cookies	0	0
Mahua jam	0	0
Mahua RTS	0.12×10^1	0.14×10^1
Mahua laddoo	0	0
	30^{th} day	
Mahua Juice Concentrate	0.1×10^1	0.12×10^1

Mahua cake	0	0
Mahua cookies	0	0
Mahua jam	0	0
Mahua RTS	0.12×10^1	0.14×10^1
Mahua laddoo	0	0
45th day		
Mahua Juice Concentrate	0.14×10^1	0.12×10^1
Mahua cake	0.1×10^1	0
Mahua cookies	0	0
Mahua jam	0.1×10^1	0.1×10^2
Mahua RTS	0.14×10^1	0.14×10^1
Mahua laddoo	0	0
60th day		
Mahua Juice Concentrate	0.2×10^1	0.12×10^1
Mahua cake	0.15×10^1	0.5×10^1
Mahua cookies	0.9×10^1	0.8×10^1
Mahua jam	0.1×10^1	0.1×10^2
Mahua RTS	0.22×10^1	0.14×10^1
Mahua laddoo	0.9×10^1	0.1×10^1
75th day		
Mahua Juice Concentrate	1.0×10^1	2×10^1
Mahua cake	2×10^1	1×10^1
Mahua cookies	1.9×10^1	1×10^1
Mahua jam	2.5×10^1	1.9×10^2
Mahua RTS	2.8×10^1	2.1×10^1
Mahua laddoo	1.5×10^1	0.1×10^1
90th day		
Mahua Juice Concentrate	5×10^1	4×10^1
Mahua cake	3.5×10^1	3.3×10^1
Mahua cookies	2.8×10^1	2.9×10^1
Mahua jam	4.9×10^1	5.6×10^2

Mahua RTS	7.4 x 10^1	5.5 x 10^1
Mahua laddoo	2.9 x 10^1	3.2 x 10^1
105th day		
Mahua Juice Concentrate	3x 10^2	2.2x 10^3
Mahua cake	2.4x 10^2	1.9x 10^3
Mahua cookies	2.2x 10^2	3.9x 10^3
Mahua jam	6.9x 10^2	3.3x 10^4
Mahua RTS	7.8x 10^2	6.6x 10^4
Mahua laddoo	4.5x 10^2	3.7x 10^3

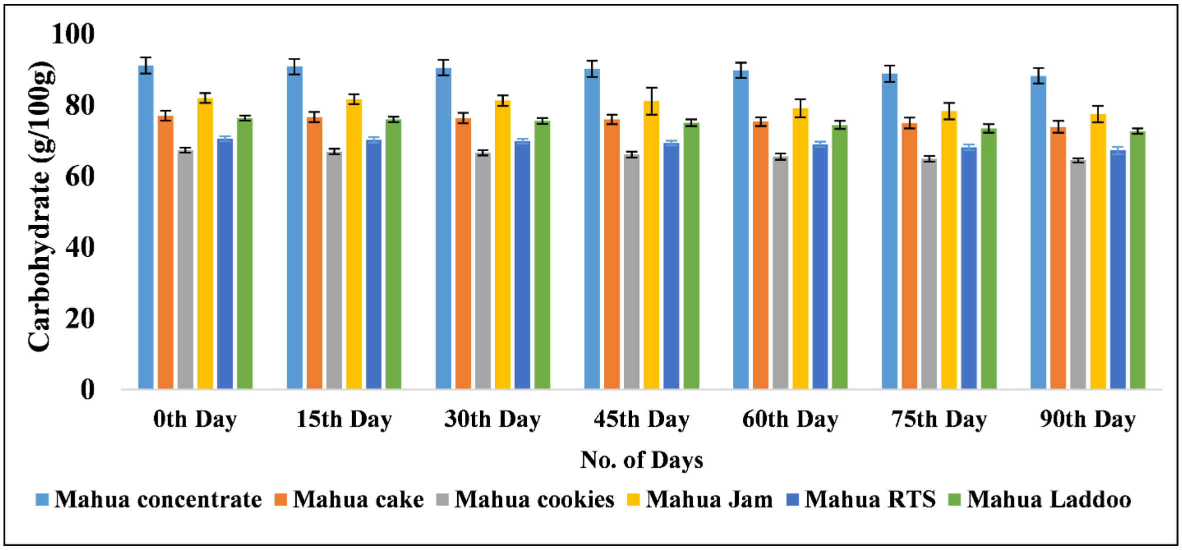


Figure 17.7 The graph shows the decrease in carbohydrate content of food products from days ^{0} to 90. During the storage phase, the carbohydrate content decreases due to the breakdown of starches and sugars through enzymatic activity and microbial fermentation.

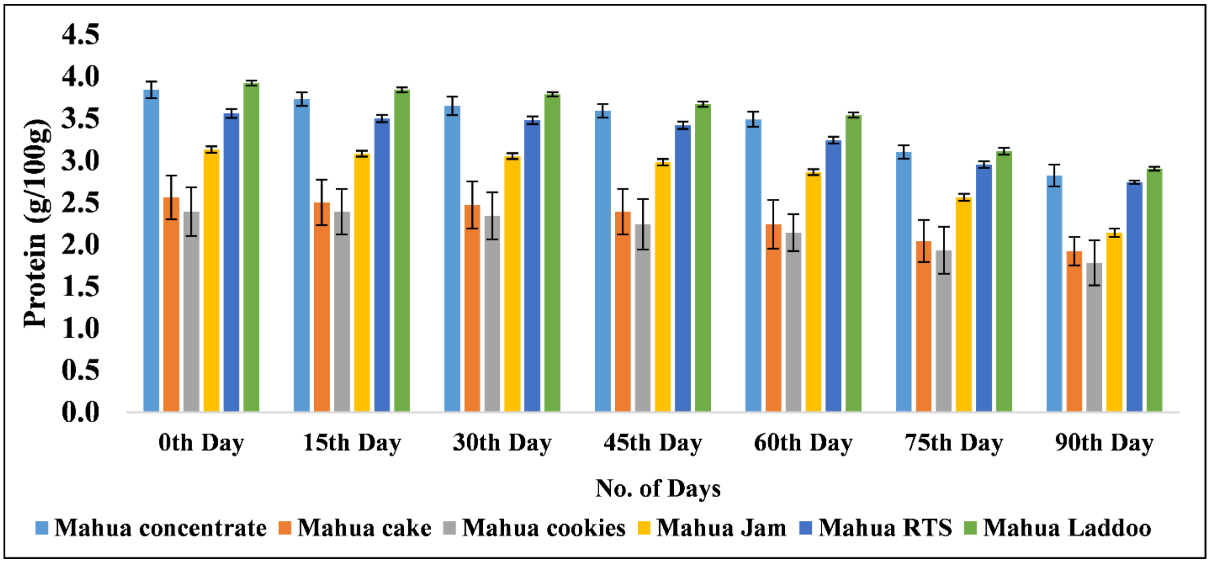


Figure 17.8 The graph shows the decrease in protein content of food products from the 0th day to the 90th day. During the storage period, protein levels decrease due to protein breakdown, which may be due to microbial activity and enzymatic processes, such as proteolysis.

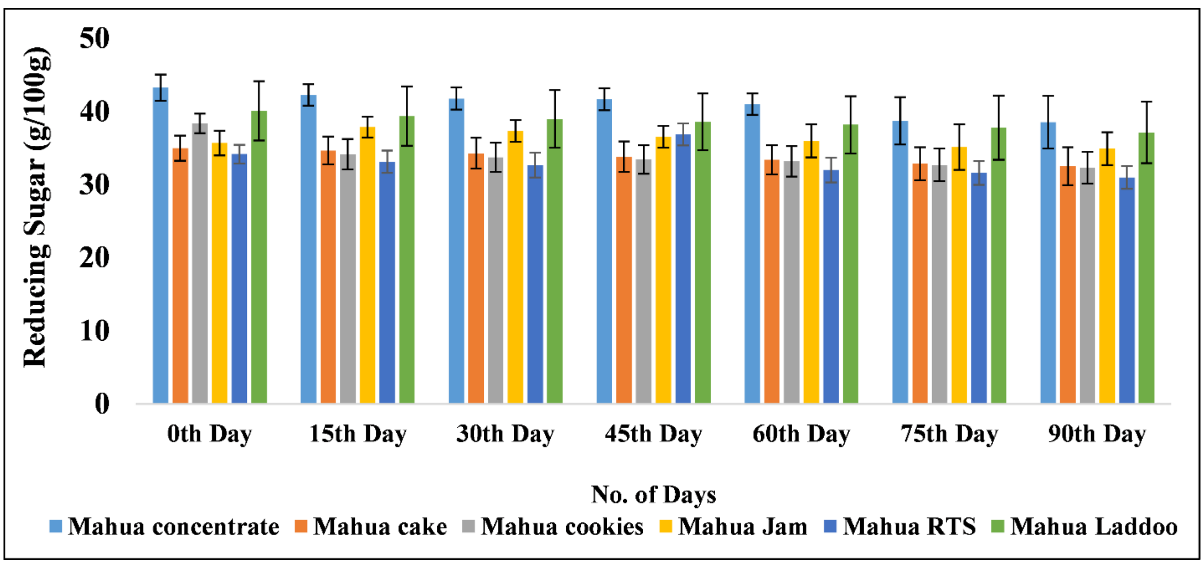


Figure 17.9 The graph shows the decrease in the reducing sugar content of the food products from the 0th to the 90th day. This decline is due to the change of reducing sugars into other compounds through enzymatic activities and microbial fermentation.

5.4.4.1 Detection of aflatoxins

The major aflatoxins, i.e., B1, B2, G1 and G2, which are produced mainly by *Aspergillus* species, are the most toxic mycotoxins. B1 is a strong carcinogen found in contaminated food, whereas B2, G1 and G2 are less toxic than B1 is. These mycotoxins are key to food security because of their ability to contaminate food products. The analysis was accomplished via standard aflatoxin solutions of B1, B2, G1, and G2 at concentrations of 5, 10, 25, 50, 75, and 100 ppb. These standards were used to standardize the chromatographic system and ensure accurate identification and quantification of the mycotoxins present in the food samples. The standards (Fig. 17.10) allowed for exact comparison and confirmed that no peaks corresponding to aflatoxins were present in the tested food products. The analysis of standard aflatoxins (B1, B2, G1, and G2) in food samples at concentrations of 5, 10, 25, 50, 75, and 100 ppb revealed no significant presence of these mycotoxins. Despite testing a broad range of concentrations, the chromatographic results revealed no major peaks corresponding to aflatoxins B1, B2, G1, or G2 in the food samples (Fig. 17.11-17.16). This suggests that the food products analysed did not contain noticeable levels of aflatoxins within the method's limits of quantification. The absence of aflatoxins in these samples indicates that they are free from contamination by these mycotoxins at the tested concentrations, confirming their safety in terms of aflatoxin contamination.

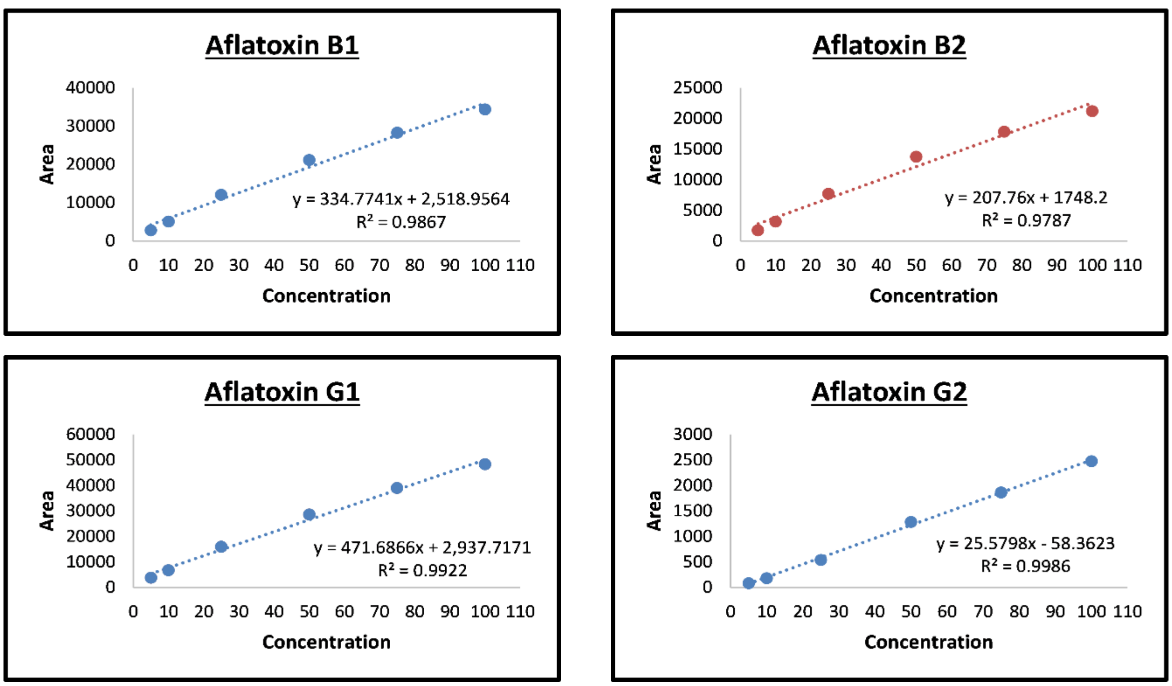


Figure 17.10 Calibration curve showing the relationships between the concentrations of aflatoxins B1, B2, G1, and G2 (ranging from 5 to 100 ppb) and the peak areas determined via HPLC analysis. The x-axis represents the aflatoxin concentration (ppb), and the y-axis represents the measured peak area.

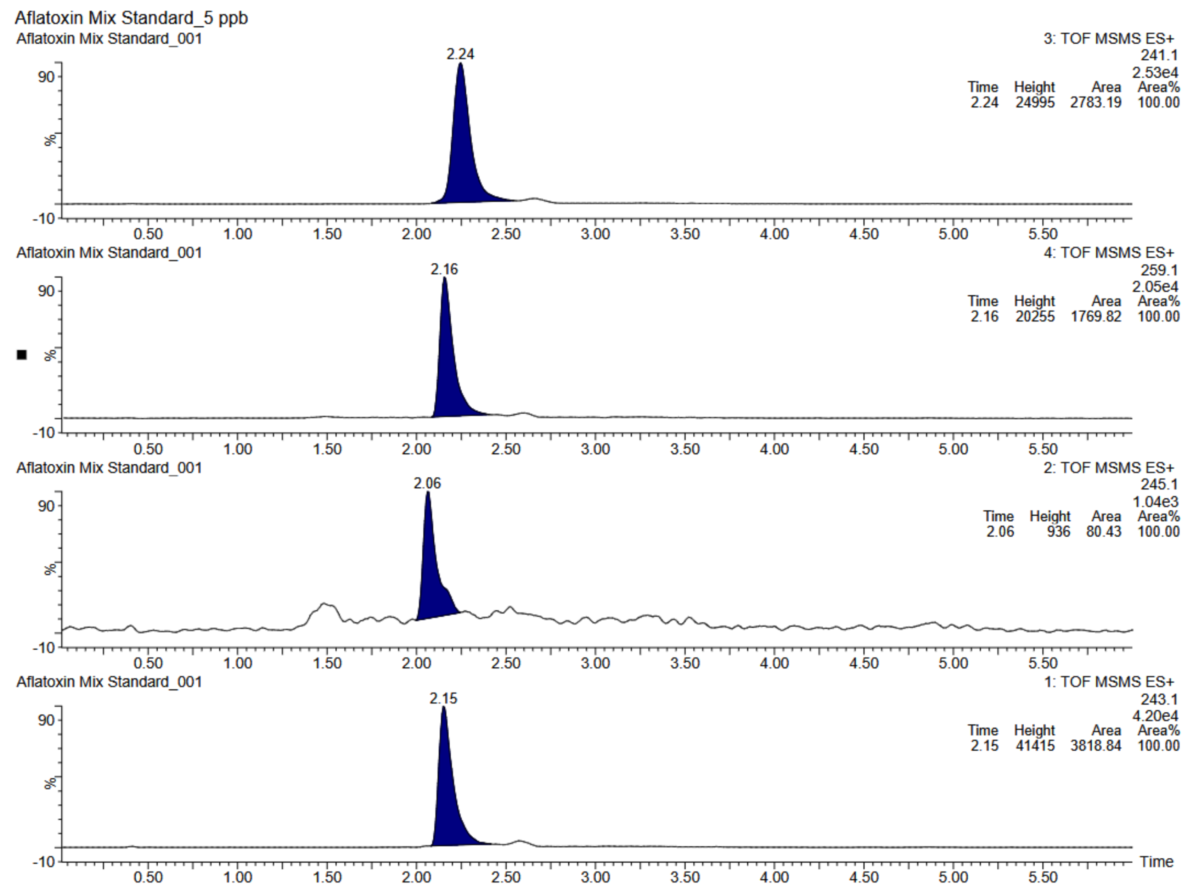


Figure 17.11 This figure elucidates the HPLC chromatograms for aflatoxins B1, B2, G1, and G2 at a concentration of 5 ppb. The x-axis represents the retention time (minutes), while the y-axis shows the peak area.

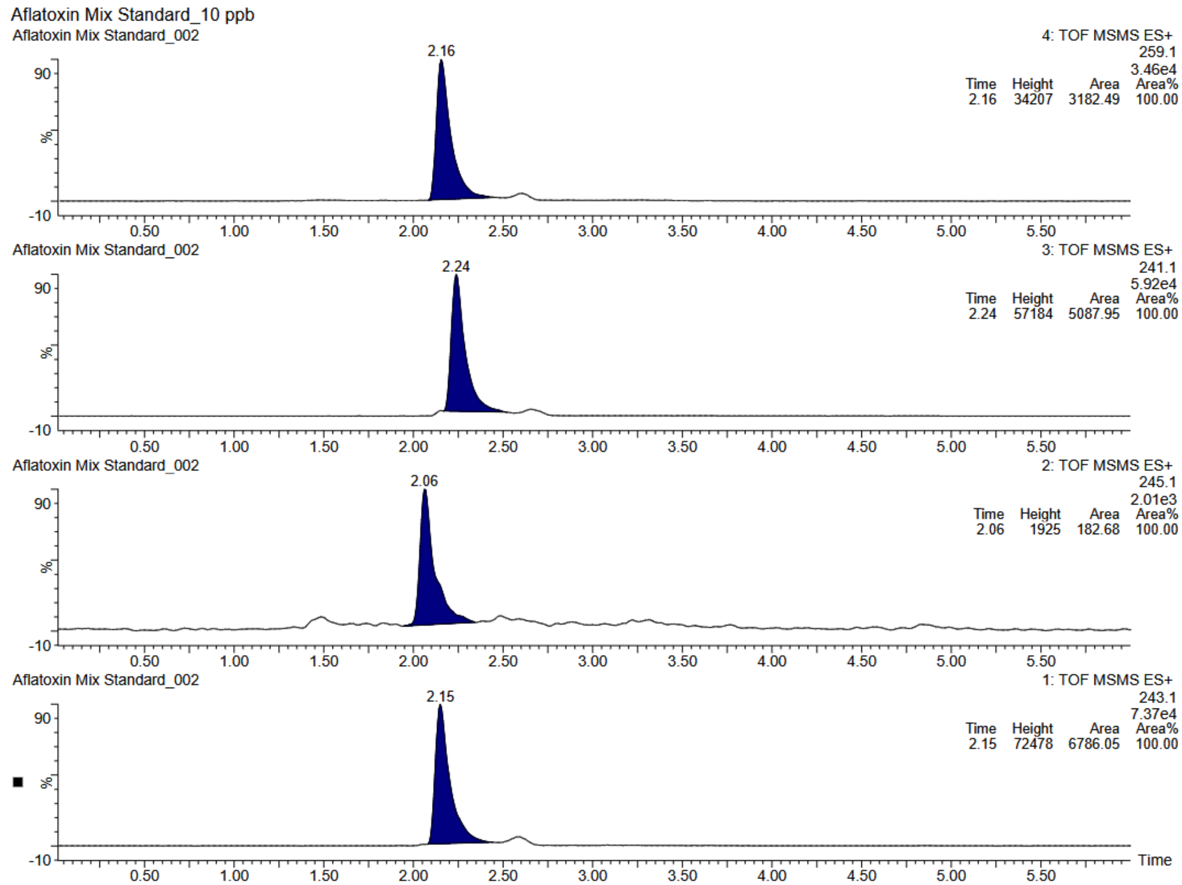


Figure 17.12 This figure elucidates the HPLC chromatograms for aflatoxins B1, B2, G1, and G2 at a concentration of 10 ppb. The x-axis represents the retention time (minutes), while the y-axis shows the peak area.

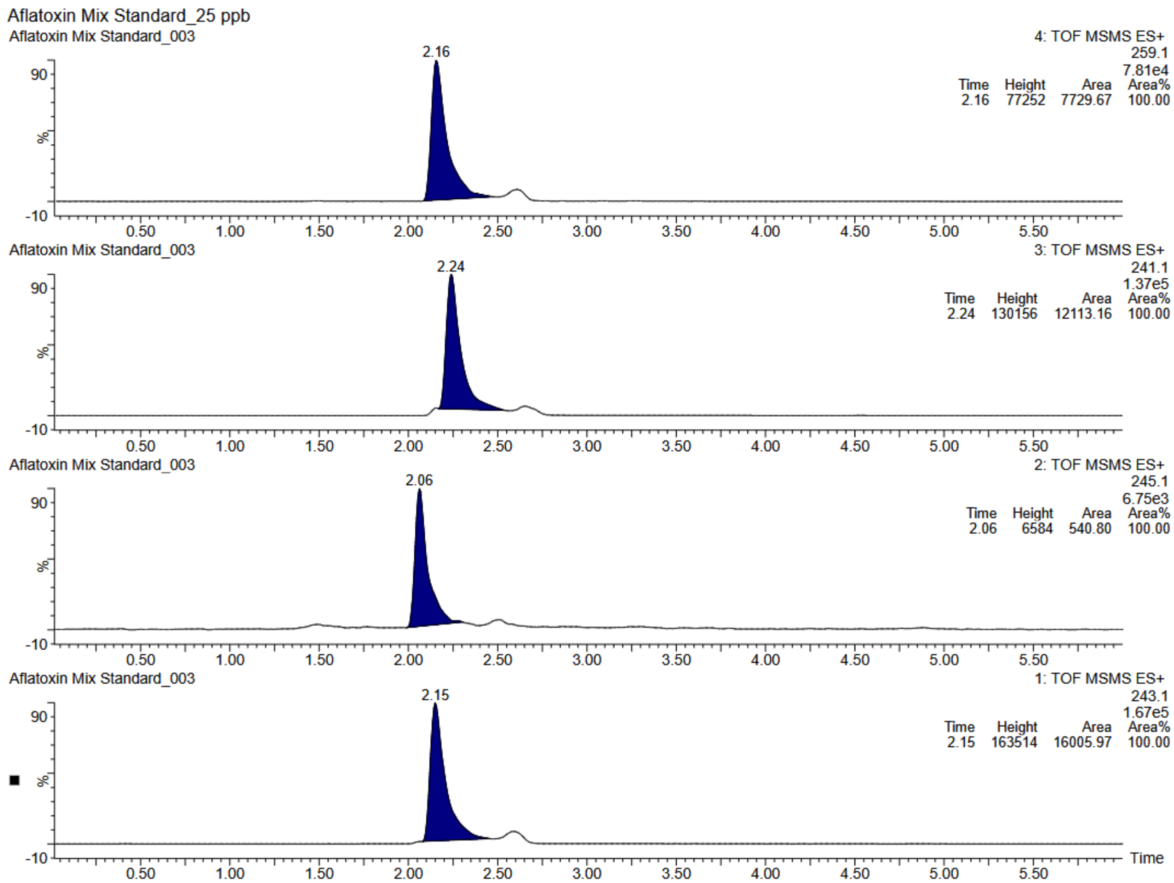


Figure 17.13 This figure elucidates the HPLC chromatograms for aflatoxins B1, B2, G1, and G2 at a concentration of 25 ppb. The x-axis represents the retention time (minutes), while the y-axis shows the peak area.

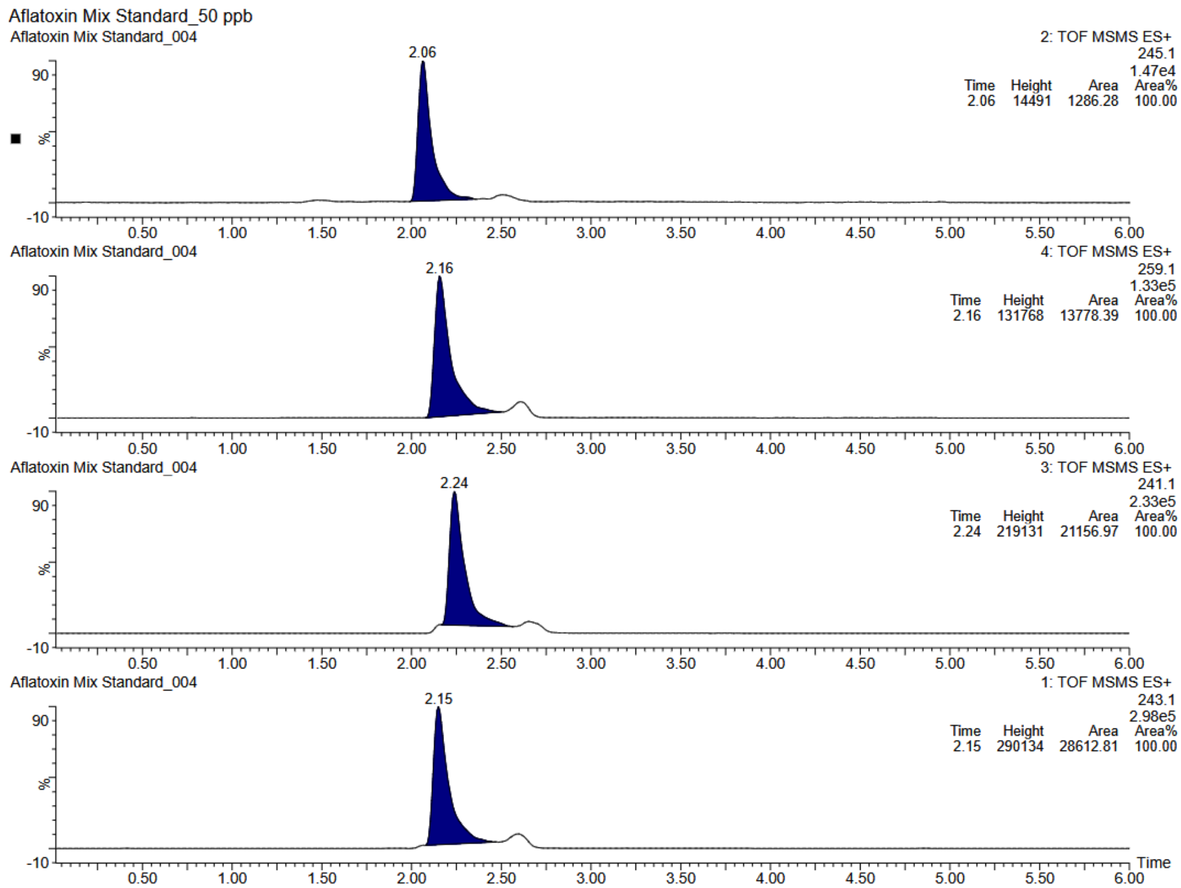


Figure 17.14 This figure elucidates the HPLC chromatograms for aflatoxins B1, B2, G1, and G2 at a concentration of 50 ppb. The x-axis represents the retention time (minutes), while the y-axis shows the peak area.

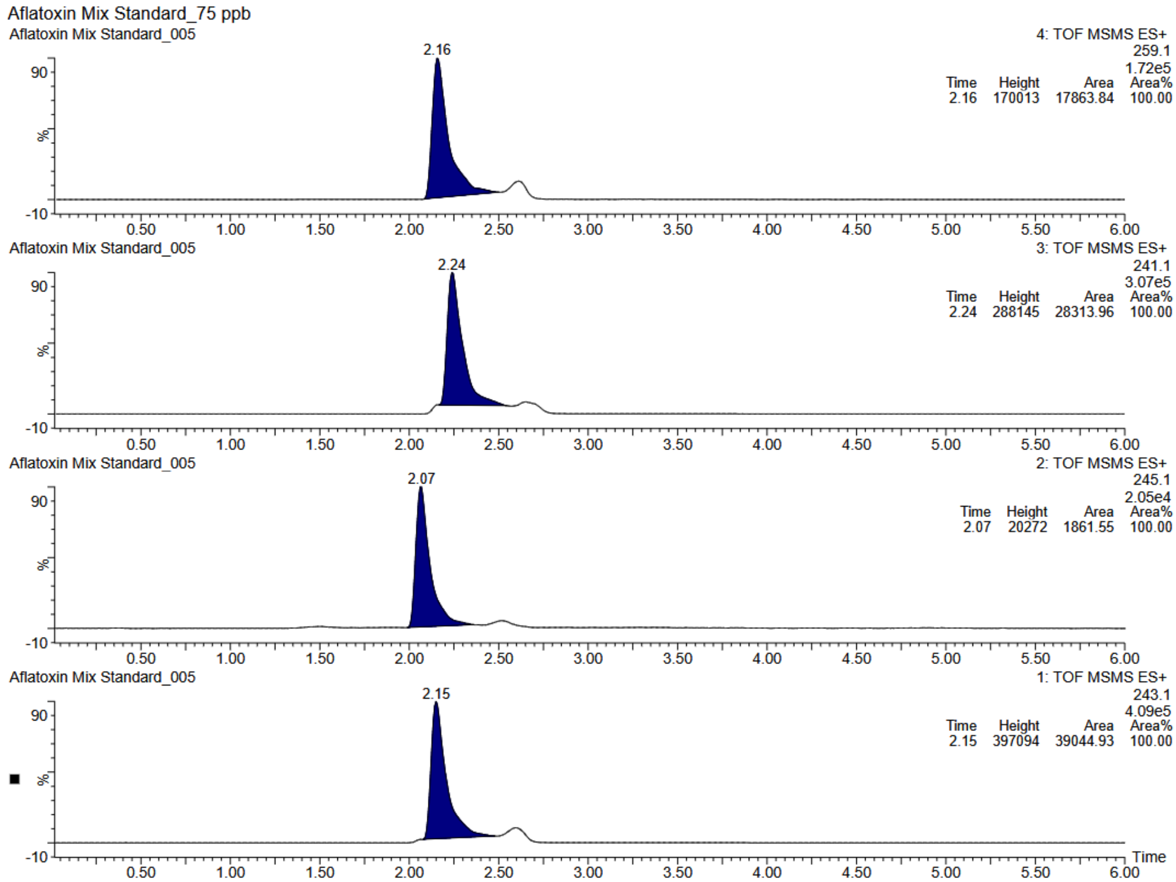


Figure 17.15 This figure elucidates the HPLC chromatograms for aflatoxins B1, B2, G1, and G2 at a concentration of 75 ppb. The x-axis represents the retention time (minutes), while the y-axis shows the peak area.

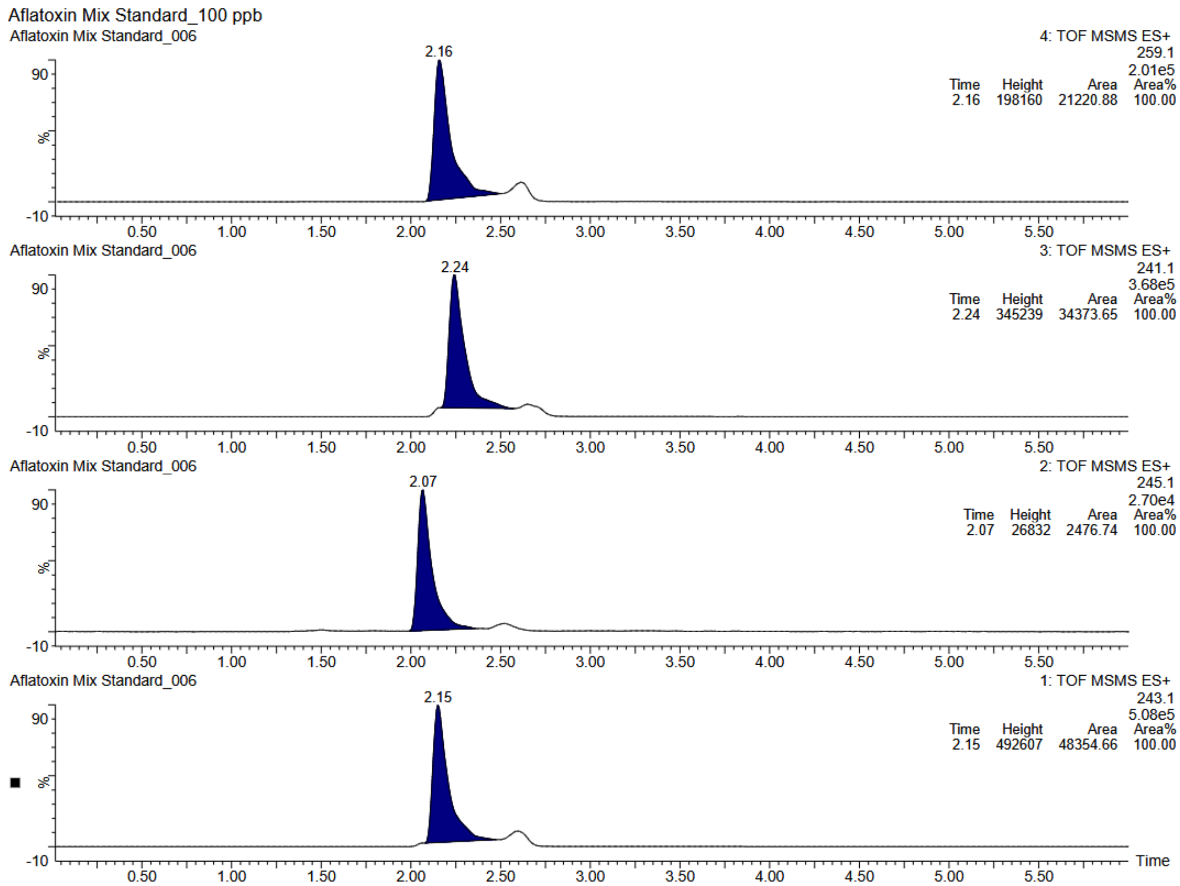


Figure 17.15 This figure elucidates the HPLC chromatograms for aflatoxins B1, B2, G1, and G2 at a concentration of 100 ppb. The x-axis represents the retention time (minutes), while the y-axis shows the peak area.

5.5 Discussion

The products that have been prepared from mahua flowers have gained importance as sources of nutrition, particularly in places where mahua trees are abundant. The flowers are used for the preparation of alcoholic beverages, i.e., “Country liquor” (Jamir, 2024), but their potential in creating nutritionally enriched food products is highly recognized. The proximate information of mahua cakes, mahua cookies, mahua jams, mahua RTS and mahua laddoos provides valuable insights into their nutritional configuration and energy value. Like mahua cake and cookies, cakes and cookies that are prepared with other flours characteristically have high carbohydrate and fat contents (Owheruo et al., 2024). The cakes are high in moisture, and the cookies are firmer because of their lower moisture content. Jam has a relatively high sugar content but a low fat content because its relatively high water content makes it energy dense.

Laddoos are high in carbohydrates and fats because of the incorporation of ghee and their protein content, especially when made with legumes or nuts (Nayak et al., 2024). Studies have shown that cakes and cookies made with whole grains tend to have better nutritional outlines, with higher protein contents than those made with refined ingredients (Ahmed et al., 2024). Jam are rich in sugar but low in fat, while laddoos are considered to be calorie-dense and can offer more nutritional assistance when enriched with protein and fibre (Joshi et al., 2024). The nutritional profile of these developed products is dependent on the ingredients used during preparation.

Sugar profiling: Sugar profiling of Mahua flower products revealed a mix of reducing sugars and nonreducing sugars, which contributes to both their sweetness and useful role in the human figure (Dwivedi et al., 2022). The reducing sugars are important for providing quick-release energy. Like other flower-based food products, such as those made from hibiscus or neem flowers, mahua flowers have relatively high concentrations of simple sugars, including glucose and fructose, which facilitate quick energy production. A study by Sharma et al. (2018) revealed that the sugar profile of Mahua flower products is helpful in balancing energy levels, particularly in diets that need quick energy release. This is vital for persons involved in intense physical action or those with greater caloric needs.

Amino acid profiling: Amino acid profiling is another critical feature of the nutritional excellence of Mahua flower-based food products. Mahua flowers contain a variety of essential amino acids, including leucine, lysine, and valine, which are important for muscle repair and immune function (Irine and Neha, 2023). The amino acid profile of mahua flowers and their products is similar to that of other protein-rich flowers, such as the moringa flower, which is known for its greater amino acid composition (Singh et al., 2022). Studies have suggested that despite the protein content in mahua flowers, it still delivers a balanced amino acid outline, making it a useful supplement in protein malnutrition, predominantly in areas where protein deficiencies are common. A study by Ritika et al. (2024) verified the high availability of these amino acids when processed into foodstuffs, increasing their potential as valuable protein sources.

Sensory evaluation: Sensory evaluation of Mahua flower-based food products plays a critical role in their acceptance by customers. Mahua flowers are known for their sweet flavour, which can be exceptional in value-added products. Sensory studies conducted by

Mishra et al. (2023) indicated that products such as mahua flower syrup, jams, and sweets are well accepted for their pleasant aroma, taste, and texture. The slight floral sweetness and minor bitterness of mahua flowers make it suitable for use in both sweet and salty dishes (Kolkar et al., 2024). This miscellaneous sensory profile provides a broad variety of prospects for product development, especially in the food industry, with the aim of consumers looking for exceptional flavours. When linked to other floral food products, such as rose or jasmine, mahua flowers have a gentler flavour, making them more flexible for a variety of cooking uses (Kumar, 2021).

Shelf life and microbial growth: Shelf life is one of the most important factors in defining the commercial feasibility of any food product (Yadav et al., 2022). In the case of Mahua flower-based products, the shelf-life is affected by various factors, such as nutritional content and storage conditions. Research has shown that these products are prone to microbial growth after a period of three months. Singh et al. (2022) reported that after three months of storage, Mahua flower-based food products exhibited visible growth of yeast, mold, and bacterial contamination, indicating a substantial decline in both safety and quality. This finding matches the results in other food products developed from natural floral bases, where microbial growth becomes an alarm beyond a certain shelf time. The presence of these microorganisms after three months highlights the need for better preservation techniques to increase shelf-life and prevent spoilage (Karanth et al., 2023). The microbial load also highlights the status of proper hygiene during processing and handling to mitigate contamination.

Loss of nutrients over time: Over a three-month storage phase, there was significant nutritional loss in Mahua flower-based food products, mostly in terms of protein, carbohydrates, and reducing sugars. While these losses were not extreme, they were sufficient to influence the overall nutritional profile of the product. Protein deprivation was found to be negligible but noticeable, which was similar to the findings of studies on other plant-based products, such as moringa and hibiscus (Khare et al., 2022). These studies suggest that enzymatic and microbial activities promote the steady collapse of nutrients over time, with the most significant losses occurring in the last few weeks of storage (Rehman et al., 2024). In contrast, other researchers, such as Reddy et al. (2015), reported similar nutrient losses in plant-based food products, highlighting that extended storage often primes to a gradual degradation of both macro- and micronutrients (Haghighi et al., 2024). The loss of reducing sugars, although minimal, can impact the taste and sweetness of a product, potentially decreasing its consumer appeal.

5.6 Conclusion

The products that were developed from mahua flowers, such as mahua concentrate, mahua cake, mahua cookies, mahua jam, mahua RTS and mahua laddoo, have shown potential in offering nutritious, sustainable, and traditionally appropriate alternatives to conventional food products. The proximate study of these products revealed a promising nutritional shape, with acceptable levels of proteins, fats, carbohydrates, etc. Total sugar profiling revealed that these food products are good sources of both reducing sugars and complex carbohydrates, making them valuable energy sources. Furthermore, the amino acid profile highlights the existence of essential amino acids, which are vital for human health, predominantly for growth and tissue repair. Sensory evaluation of the products validated their acceptability, with a stable taste, texture, and aroma, contributing to their potential addition to normal diets. During storage for three months, there are several significant deliberations regarding the shelf-life of food products. There was an observation of microbial growth, such as visible growth of yeast, mold and bacterial load, which indicated that there was a decrease in product safety after the 3^{rd} month of storage, which made the product unfit for consumption. While the products were primarily safe for consumption and retained their nutritional profile, the presence of microbial adulteration after the 3^{rd} month highlights the need for better preservation methods to extend the shelf-life. In addition, there was a slight decrease in nutritional integrity over the three months, particularly with respect to proteins, carbohydrates, and reducing sugars. These reductions, although slight, highlight the importance of timely consumption to safeguard extreme nutritional advantages and the need for storage under optimum conditions to reduce nutrient loss. The complete findings from the development of mahua flower-based value-added food products point to their feasibility as a nutritious and sustainable food source, although reflections regarding preservation, packaging, and shelf life are serious for their fruitful commercialization. Additional examination into enhancing shelf stability, as well as discovering advanced ways to preserve or even recover their nutritional content over time, will be vital for safeguarding these products not only from being safe but also from providing substantial health assistance to consumers in the long term.

CHAPTER- VI

ANTIANEMIC ACTIVITY OF MAHUA FLOWER CONCENTRATE AND MAHUA LADDOO AGAINST 2,4-DINITROPHENYLHYDRAZINE-INDUCED ANEMIA IN A RAT MODEL

6.1 Background

In Greek, the term anemia means “without blood”. The WHO has recognized anemia as a global problem in the developing countries like India where the percentage vary from 10-20 based on the recent survey (Chauhan et al., 2022). In the anemic condition, there is a reduction of haemoglobin concentrations and red blood cells (Cappellini and Motta, 2015). It is not considered as a disease but an underlying condition which is based upon biological mechanisms (Chaparro and Suchdev, 2019). Iron deficiency anemia is the most widespread nutritional deficiency syndrome and the important cause of anemia in children. The prolonged effect of iron deficiency anemia leads to neurodevelopmental and mental disorders, which may not be fully reversible even with the treatment of the iron deficiency anemia (De Falco et al., 2014). In the present days, the risk of anemia has comparatively reduced due to availability of food supplements fortified with iron still iron deficiency anemia is prevalent across the world. For the formation of RBC, several minerals are required which includes iron, magnesium, cobalt, vitamin A and B group vitamins. But among these iron plays a key role in the delivery of oxygen by Hb in RBC (Kumar et al., 2022). Due to this growing health problem, iron supplements or iron rich diet are being preferred by the nutritionist for the management of anemic patients. The long term use of iron supplements have certain disadvantages. The oral therapy leads to the insufficient absorption of iron and over use of iron supplements leads to several health issues like cancer, neurogenic disorders etc.(Seth et al., 2021). The above mentioned facts clearly denotes that there is a need to find a safe and effective substitute for the controlling of anemia.

6.2 Introduction

Nature has gifted us with varieties of plants which has numerous medicinal and pharmacological properties which can overcome several diseases including anemia. It is necessary to maintain a healthy lifestyle for both the pregnant and lactating women as they require additional nutrients as compared to other women (Ramdas et al., 2011). The daily required dose of iron for those women is 35 mg/day (ICMR, 2009). One of the essential plant is *Madhuca indica* (Mahua), which has all the essential nutrients required for pregnant and lactating women. The plant is widely present all over the country. The flower of the plant has a significant amount of calcium, phosphorus, iron and vitamins which is responsible to overcome several diseases. According to Ahirwar et al., 2020 the dried mahua flowers contain 1412.3 ppm iron which is an essential for haemoglobin. Based on MICR, 2009 standard limits mahua flowers are the best source of iron ions. So based on the above data it can be said that we can overcome from anemia by the consumption of dry mahua flowers. Further several food products can be made from dry mahua flowers like Mahua concentrate, Mahua Laddoo which could be a boon for the pregnant and lactating women. If the flowers can be used for the preparation of several value added food products it can definitely prevent several unnecessary diseases like anemia, calcium deficiency, vitamin deficiency etc.

6.3 Materials and Methods

6.3.1 Preparation of Mahua Juice Concentrate

The juice concentrate was prepared by soaking (overnight) the dried mahua flower in drinking water. The following day, the flowers were ground to make a thick paste and the juice was subsequently extracted by hand pressing method in an 8-layer muslin cloth. The extracted juice was transferred to a sterile container followed by gentle heating at a controlled temperature (40^0 C) until a thick consistency was achieved. The mahua juice concentrate was maintained at a brix of 65^0 B. the prepared juice extract was stored in a glass container for further uses.

6.3.2 Preparation of Mahua Laddoo

To prepare a base for the mahua laddoo, dried mahua flowers (about 200g) were finely ground. The dry fruits (cashew, almonds, raisins) each 50g were roasted until golden brown. At the same pan, wheat flour were dry roasted to release the nutty aroma. Jiggery was used as a binding agent, which was melted at a low temperature creating a smooth syrup. In a large bowl all the above prepared ingredients were mixed till a dough like consistency is achieved. Lastly, ghee is incorporated to increase its richness. The dough is then given the shapes of laddoo and they are then allowed to cool. Further they are stored in an air tight container for future use.

6.4 Proximate analysis

6.4.1 Protein estimation:

For accurate protein quantification, lowry method was used by taking Bovine Serum Albumin (BSA) as a standard. The samples along with the standard were incubated with alkaline copper solution subsequently adding the Folin-Ciocalteu reagent. After an incubation period of 30 minutes, absorbance was taken at 750nm. The protein content was quantified using a standard curve which will be generated from the standard (Rizvi et al., 2022).

6.4.2 Carbohydrate estimation:

The carbohydrate content of the food product is estimated using the anthrone method. There is a reaction of anthrone reagent under the acidic conditions to obtain a green colour complex and measured at 620 nm. The carbohydrate content is calculated by comparing the absorbance of the sample with the standard curve taking glucose as the standard (Sarkar et al., 2024).

6.4.3 Reducing sugar content:

The reducing sugar present in the food samples were quantified by DNS (Dinitrosalicylic acid) method. In this method, the DNS is reduced in an alkaline medium which subsequently form a orange-red complex. The intensity of the colour is measured spectrophotometrically at 540 nm. The reducing sugar is further quantified by comparing with the standard cure where glucose is taken as a standard (Khatri et al., 2020).

6.4.4. Fat estimation:

The fat percentage of the samples is estimated through soxhlet method. The products are placed in the thimble subsequently hexane is heated, evaporated and condensed which passed through the sample repeatedly which dissolves the fat. The solvent comprising the extracted fat is gathered in the flask, and the solvent is evaporated in the rotary evaporator leaving behind the fat (Watkins, 2023). The weight of the extracted fat is then estimated using the formula:

Fat content (%) = (Weight of sample (g)/Weight of fat extracted (g)) ×100

6.4.5 Determination of the calorific value:

The calorific value of the prepared food products was estimated using a bomb calorimeter, were a defined mass of the sample is combusted in the calorimeter and the released heat raises the temperature of the surrounding water (Hopper et al., 2023). It is calculated using the formula:

6.4.6 Calorific value = Q_{total}/m_{food}

Where;

$Q_{total} = m_w . c_w . \Delta T + C_{bomb} \cdot \Delta T$

- m_w: mass of water (g),
- c_w: specific heat capacity of water (4.18 J/g°C),
- ΔT: temperature change of the water (°C),
- C_{bomb}: heat capacity of the bomb (J/°C),
- **m_{food}**: mass of the food sample (g).

6.5 In-vivo study

Wistar albino rats (180-200 g) of either sex supplied by Sambalpur University, Odisha, India, were used for the study. Animals were acclimatized for seven days under standard housing conditions in an animal house approved by the Committee for Control and Supervision of Experiments on Animals. They were housed in polypropylene cages and maintained at 25 ± 2°C, relative humidity 50 ± 10% under 12 h light/dark cycles.

6.5.1 Chemical

The tablet Livogen-XT (P&G Health India) was procured form Merck Ltd., India for research purpose and 2,4-Dinitrophenylhydrazine was procured from SRL Ltd., India for research purpose

6.5.2 Selection of dose

The doses were calculated from the human dose using a conversion factor based on body surface area (Ghosh et al., 1984). The dose of WBG is 54mg/kg daily; therefore, a 1ml per day was selected for the study.

6.5.3 Acute Toxicity

Single dose of different concentrations such as 250, 500, 1000, 2000 and 5000 mg/kg of the of mahua concentrate and mahua laddoo (15-18 gm) were administered orally to six groups with six animals in each group (n=6). They were placed under observation for 14 days after which the number of dead rats was recorded (OECD Test No. 425, 2022). The results showed no signs of toxicity or death in the animals even at highest concentration of 5000 mg/kg.

6.5.4 Subacute Toxicity

Twenty wistar rats were randomly divided into two groups with ten animals in each group (n=10). Mahua concentrate and mahua laddoo (15-18 gm) were administrated orally at a daily dose of 500 mg/kg body weight for 28 days following the repeated oral toxicity test (OECD, 2008). The animals were observed daily during the experiment to detect death or abnormal clinical signs. The body weight, water intake and food intake were recorded. At the end of the day number 29th animal were sacrificed and collected the blood by cardiac heart puncture, collected the all vital organ for the toxicological studies.

6.5.5 Induction of anemia

The anemic model was developed by administrating 2, 4-diphenylhydrazine to the rats via Oral Gavage at a concentration of 40mg/kg for 2 consecutive days. When the haemoglobin is reduced to 30%, the animals were considered to be anaemic (Seth et al., 2021).

6.5.6 Experimental design

Rats were divided into five groups containing six rats in each group (n = 6). Group-I rats were treated as normal control. The rats of Group-II were treated as anemic control induced with 2, 4-diphenylhydrazine consider as a negative group. Group-III was treated as positive control where Livogen XT was given orally. Group-IV & V were the treatment groups where Mahua concentrate and Mahua laddoo were administered to the animals by oral cavage and Chewing method.

6.5.7 Hematological analysis

For the acute and sub-acute toxicity, the animals were anaesthetised by mild anaesthesia (isoflurane) followed by cervical dislocation and blood was collected by cardiac puncture. Blood cell parameters were analysed using CBC analyser. The collected blood was centrifuged at 5000 rpm for 10 mins and for the anemic the blood samples were collected from the retro-orbital on days 0-before the induction of anaemia and on 3rd, 7th, 10th and 15th day after the induction of anaemia. Blood samples were examined for red blood cells (RBCs), hemoglobin (Hb), and hematocrit (HCT) levels (Diallo et al., 2008).

6.5.8 Serum Biochemistry Analysis

For the biochemistry part of the in vivo study 1 ml of blood was taken from the rat and was subjected to centrifugation for 10 min at 10000 rpm so that the cells would accumulate in the palette and the supernatant was collected which contained the serum. This serum was taken in a biochemistry analysis to identify and quantify different types of biomolecules such as glucose(GLU), albumin(AB), Urea, creatinine(CREA), Cholesterol(CHOL), Triglycerides(TGL), Alanine transaminase(ALT), Aspartate aminotransferase(AST), total protein(TP), magnesium(MG), phosphorus(PHOS), calcium(CA), direct bilirubin (DBIL), total bilirubin(TBIL), high-density lipoprotein(HDL), gamma glutamine transpeptidase (GGT), alkaline phosphatase(ALP), low-density lipoprotein(LDL).

6.5.9 Histopathology

For acute and sub-acute toxicity, animals were sacrificed to isolate the liver, lung, kidney, heart, spleen, and femur bone for bone marrow examination. The collected tissues were processed, stained by Hematoxylin and Eosin staining methods, and examined under the light microscope for histological changes. For the anemic model, on the last day i.e. 23rd day, the animals were sacrificed to segregate the femur bone for bone marrow investigation. The organs were preserved in formalin and the tissues were further processed, stained by Hematoxylin and Eosin and studied under the microscope for any histological changes.

6.4 Results

6.4.1Proximate analysis

The proximate analysis of mahua concentrate and mahua laddoo was analyzed which disclose variations in the amount of fat, protein, carbohydrate, reducing sugar contents and calorific value. The proximate composition of the food samples was analyzed, revealing variations in protein, fat, carbohydrate, and reducing sugar contents. Both the food products exhibited a similar protein amount of 3.84 ± 0.10 g/100g and 3.92 ± 0.28 g/100g in mahua concentrate and mahua laddoo. There is no certain variation in the protein content among the food products. These values suggest a significant difference in protein levels among the samples, consistent with the nature of the food types analyzed. The fat content of laddoo exhibited highest fat content i.e. 9.48 % due to the presence of clarified butter and dry fruits but the mahua concentrate showed less fat content i.e. 0.10 % respectively. Carbohydrate was the most ample macronutrient, which is found in mahua concentrate and mahua laddoo. The concentrate exhibited highest carbohydrate content i.e. 91.17 ± 2.29 g/100g as compared with mahua laddoo i.e. 76.33 ± 0.74 g/100g. These variances may be credited due to the changing levels of starches and fibers present in each sample. Reducing sugar levels also varied significantly, with mahua concentrate having 43.25 ± 1.80 g/100g showing the highest reducing sugar content at compared to 40.09 ± 4.05 g/100g in mahua laddoo. The difference in reducing sugars probably imitates the existence of simply digestible carbohydrates, which may affect the sweetness and nutritional shape of samples. These results specify prominent alterations in the macronutrient configuration of the food samples, which may have inferences for their nutritional worth and possible health welfares.

6.4.2 In vivo study

The rats were treated with the food samples at a single dose of different concentrations, such as 250, 500, 1000, 2000 and 5000 mg/kg body weight (acute toxicity study), or daily doses of 50 mg/kg, 200 mg/kg and 500 mg/kg body weight for 28 days (sub-acute toxicity study), as per the OECD guidelines. No signs of mortality were observed. The estimated LD_{50} value of the extract was estimated to be 5000 mg/kg body weight. There was no significant difference in body weight, food intake, or water intake between the untreated and treated groups of animals for the entire duration of the experiment (Table 5.1). At the end of the experiments, all the rats exhibited normal behavior and were healthy. After 28 days of treatment, the rats were anaesthetized and sacrificed, after which blood was collected for biochemical analysis, and vital organs were collected for histopathology study. The blood biochemistry data and complete blood counts (Table 5.2 and Table 5.3) revealed no significant differences ($p>0.05$) between the control and treated rats. The vital organs, such as the kidney, liver, heart, spleen and brain of the animals treated with the extract did not differ from those of the untreated control group (Fig. 18.1). The in vivo study confirmed that the plant extract has no side effects or toxicity and can be considered a safe medicinal plant.

Table 5.1: Evaluation of body weight, food intake, and water intake among control and treated animals. The control and treated group experimental were monitored for body weight, food intake, and water intake at regular intervals over the entire duration of the experiment.

No. of Days	Normal Control	Mahua concentrate	Mahua Laddoo
Body Weight (g)			
01	175.5 ± 6.28	176.3 ± 5.19	180.1 ± 9.65
07	186.4 ± 5.98	186.1 ± 4.32	188.4 ± 3.60
14	201.2 ± 6.20	203.7 ± 5.60	203.9 ± 7.46
21	207.6 ± 11.64	220.2 ± 6.77	219 ± 6.84
28	223.6 ± 10.92	229 ± 12.71	230.7 ± 11.52
Food Intake (g)			
01	17.13 ± 2.33	13.7 ± 1.61	14.58 ± 3.06
07	17.18 ± 2.31	13.82 ± 1.56	15.38 ± 3.15
14	17.07 ± 2.50	13.68 ± 1.59	16.09 ± 3.09
21	17.42 ± 1.97	14.86 ± 1.67	16.96 ± 3.11
28	17.72 ± 1.43	16.05 ± 2.27	17.82 ± 3.21
Water Intake (ml)			
01	17.53 ± 3.53	16.79 ± 3.37	18.54 ± 4.38
07	18.4 ± 3.33	17.97 ± 2.48	18.86 ± 3.81
14	18.76 ± 3.97	19.84 ± 3.87	21.8 ± 1.91
21	20.18 ± 4.62	20.83 ± 4.35	20.19 ± 3.98
28	20.65 ± 5.01	21.7 ± 5.50	20.19 ± 3.91

Table 5.2: Blood biochemistry parameters of rats treated with food samples at a daily dose of 200 mg/kg body weight for 28 days. Organ functions show undistinguishable profiles among the control and treated groups. No significant differences could be detected in the liver function test, kidney function test, lipid profile, serum glucose, and different salts between the treated and untreated groups.

Parameter	Control	Mahua concentrate	Mahua Laddoo
Glucose(GLU)	122.83 ± 4.94	110.11 ± 2.28	103.58 ± 4.29
Albumin(ALB)	2.67 ± 0.21	2.92 ± 0.36	2.56 ± 0.32
Urea(UREA)	32.09 ± 2.32	28.33 ± 4.83	29.42 ± 7.62
Creatinine(CREA)	1.4 ± 0.32	1.15 ± 0.24	1.17 ± 0.16
Cholestrol(CHOL)	144.98 ± 11.32	153.08 ± 10.85	143.51 ± 8.61
Triglycerides(TG)	102.66 ± 7.08	127.33 ± 13.18	91.26 ± 23.64

Alanine Transaminase(ALT)	85.99 ± 2.22	102.4 ± 11.13	65.77 ± 29.31
Aspartate Aminotransferase(AST)	34.22 ± 4.6	31.96 ± 0.83	20.67 ± 8.92
Total Protein(TP)	6.92 ± 0.2	6.2 ± 0.4	4.65 ± 1.42
Magnesium(MG)	2.08 ± 0.29	1.78 ± 0.14	1.45 ± 0.31
Phosphorus(PHOS)	4.8 ± 0.65	4.57 ± 0.7	4.34 ± 0.86
Calcium(CA)	9.26 ± 0.26	9.3 ± 0.68	7.58 ± 0.68
Direct Bilirubin(DBIL)	0.37 ± 0.14	0.33 ± 0.14	0.33 ± 0.05
Total Bilirubin(TBIL)	0.37 ± 0.16	0.17 ± 0.12	0.44 ± 0.24
High-density Lipoprotein(HDL)	56.51 ± 4.9	54.51 ± 4.71	61.48 ± 6.68
Gamma-glutamyl Transferase(GGT)	4.41 ± 0.9	4.35 ± 1.56	4.46 ± 1.83
Alkaline Phosphatase(ALP)	77.73 ± 19.26	95.34 ± 13.07	103.67 ± 14.97

Table 5.3: Blood parameters of rats treated with food samples at a daily dose of 500 mg/kg body weight for 28 days. No significant differences could be detected in the WBC count (WBC), monocytes (MON), eosinophils (EOS), neutrophils (Neu) lymphocytes (Lym), Basophiles (Bas), RBC count (RBC), haemoglobin concentration (HB),Hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count between treated and untreated groups.

Parameter	**Control**	**Mahua concentrate**	**Mahua Laddoo**
white blood cell count (WBC (10^3/L))	5.21 ± 0.98	5.1 ± 1.22	8.01 ± 1.03
Neutrophils (Neu# (10^3/L))	4.83 ± 1.36	4.4 ± 1.1	5.69 ± 0.53
Lymphocytes (Lym# (10^3/L))	2.93 ± 0.42	3.01 ± 0.95	3.12 ± 0.9
Monocytes (Mon# (10^3/L))	0.38 ± 0.07	0.51 ± 0.05	0.48 ± 0.12
Eosinophils (Eos# (10^3/L))	0.28 ± 0.05	0.25 ± 0.18	0.23 ± 0.05
Basophil (Bas# (10^3/L))	0.32 ± 0.37	0.4 ± 0.3	0.07 ± 0.03
NLR	1.5 ± 0.23	1.5 ± 0.15	1.89 ± 0.3
PLR	0.01 ± 0	0.02 ± 0	0.04 ± 0.06
red blood cell count (RBC (10^12/L))	4.93 ± 0.5	5.43 ± 0.32	5.28 ± 0.41

Hemoglobin (HGB (g/dL))	13.98 ± 0.5	13.19 ± 0.5	14.65 ± 0.52
HCT	42.91 ± 1.58	41.92 ± 2.55	46.17 ± 1.51
MCV (fL)	82.63 ± 3.23	82.9 ± 4.69	84.68 ± 4.62
MCH (pg)	30.18 ± 1.2	29.58 ± 1.89	30.56 ± 1.19
MCHC (g/L)	32.87 ± 0.49	32.8 ± 1.72	34.05 ± 0.91
RDW-CV	13.15 ± 0.48	12.99 ± 0.64	13.82 ± 1.02
RDW-SD (fL)	40.4 ± 2.43	43.26 ± 1.76	46.31 ± 4.24
platelet count (PLT (10^3/L))	237.66 ± 40.86	264.83 ± 62.53	227.83 ± 34.75
PCT (mL/L)	0.2 ± 0.04	0.25 ± 0.07	0.3 ± 0.07

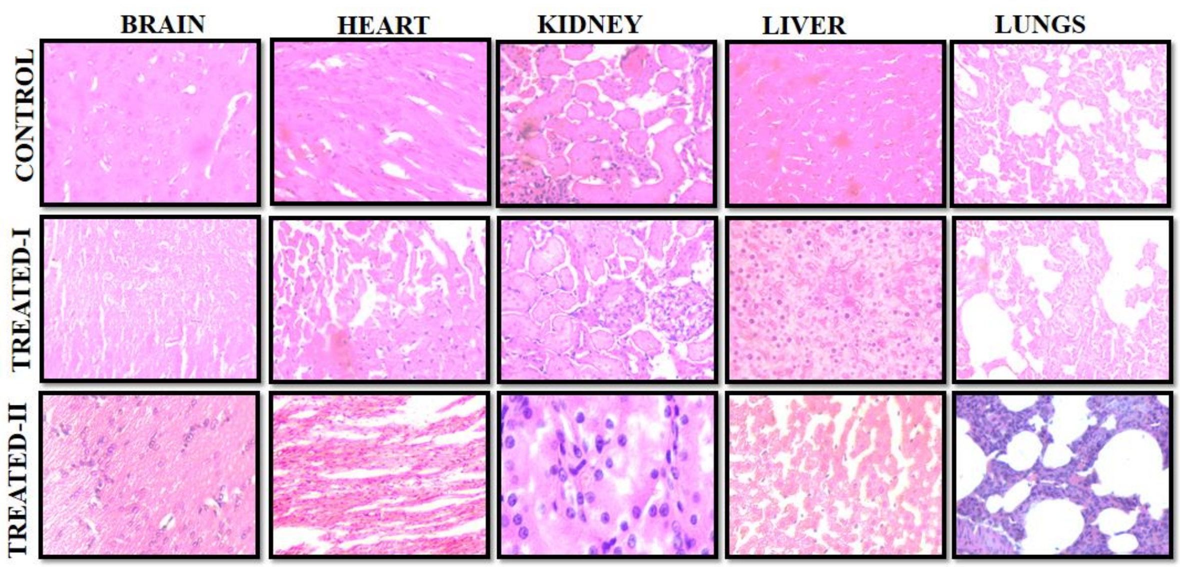


Figure 18.1 Photomicrographs of different vital organs, i.e., the brain, lungs, heart, liver and kidney of control and treated rats at a dose of 200 mg/kg body weight subjected to subacute toxicity for 28 days. There was no significant difference between the control and treated groups of animals with respect to cellular architecture. Where treated-I indicates mahua concentrate and treated-II indicates mahua laddoo.

Blood biochemistry parameters of rats treated with Mahua Concentrate and Mahua Laddoo at a daily dose of 200 mg/kg body weight for 28 days in anemia induced rat model was investigated. Organ functions show undistinguishable profiles among the control and treated groups. No significant differences could be detected in the liver function test, kidney function test, lipid profile, serum glucose, and different salts between the treated and untreated groups (Table 5.2). There was no significant difference in body weight, food intake, or water intake between the untreated and treated groups of animals for the entire duration of the experiment (Fig. 18.2).

After the administration of phenylhydrazine, there is a significant decrease in the RBCs count in the animals (*$p<0.001$*) which is mentioned in Table 5.4 There is an increase in the RBCs count by the treatment of livogen XT, mahua concentrate and mahua laddoo. The food products were found to be more effective than positive control i.e. livogen XT. Likewise, after the induction of phenylhydrazine, the hemoglobin content decreased significantly (*$p<0.001$*). There is an increase in the hemoglobin by the treatment of livogen XT, mahua concentrate and mahua laddoo. The food products were found to be more effective than positive control i.e. livogen XT. Similarly, there is a decrease in the hematocrit (HCT) count (*$p<0.001$*) due to the induction of phenylhydrazine in the rats compared with the normal rats. The decrease in the HCT count was reversed by the treatment of the food products as well as livogen XT. The food products were found to be more effective than positive control i.e. livogen XT. The presence of minimal grade of central foci which contains adipose tissue was observed in anemia induced rats which revealed the depletion of bone marrow cellular population. But with the treatment of the food products (mahua concentrate and mahua laddoo) and livogen XT prevented the histological changes and there was an increase in the bone marrow cellular population (Fig. 18.3).

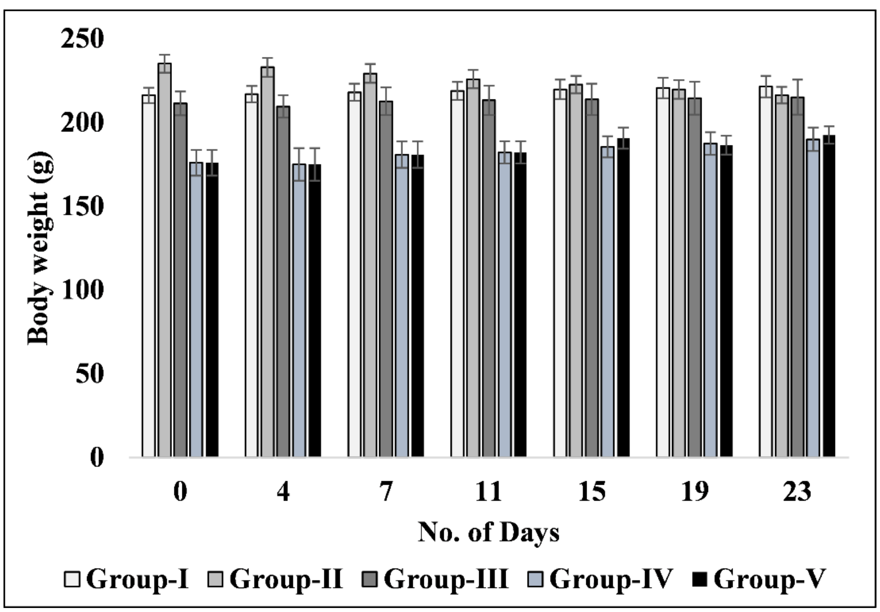


Figure 18.2 Evaluation of body weight among control and treated animals. The control and treated group were monitored for body weight at regular intervals over the entire duration of

the experiment. (Where Group-I: Control Group, Group-II: Negative Control, Group-III: Livogen XT, Group-IV: Mahua Concentrate, Group-V: Mahua Laddoo)

Table 5.4: Blood biochemistry parameters of rats treated with Mahua Concentrate and Mahua Laddoo at a daily dose of 200 mg/kg body weight for 28 days in anemia induced rat model. Organ functions show undistinguishable profiles among the control and treated groups. No significant differences could be detected in the liver function test, kidney function test, lipid profile, and different salts between the treated and untreated groups. Here day 0 is considered for before induction, where all parameters are normal and from day 1 to 23 is considered as an anemic model.

Days	Normal Control	Negative Control	Positive Control	Mahua Concentrate	Mahua Laddoo
Alanine Transaminase (U/L), (Range -10.00 - 125.00)					
Before induction	93.7±27.75	107.9±4.67	117.15±1.89	111.09±4.43	110.59±4.07
4	105.28±5.62	125.98±0.97	107.96±0.82	107.96±0.82	107.92±0.95
7	106.79±7.32	126.26±0.78	113.45±4.98	108.45±0.88	109.62±3.04
11	105.72±8.09	126.46±0.94	115.55±4.2	108.38±1.18	275.97±374.65
15	105.72±6.5	126.83±1.11	112.72±5.07	107.05±1.79	110.05±2.42
19	108.05±5.76	126.98±0.37	114.72±4.45	107.05±1.76	109.38±2.03
23	112.31±7.76	128.65±0.62	113.65±4.64	110.35±3.79	110.35±3.79
Aspartate Aminotransferase (U/L), (Range- 0 - 50.0)					
Before induction	26.65±4.39	30.88±2.22	33.81±3.2	34.43±4.35	34.43±4.35
4	28.11±4	54.45±0.73	41.35±4.25	34.45±0.73	34.45±0.73
7	32.45±4.36	55.46±1.39	36.18±5.5	35.38±1.48	35.38±1.48
11	33.73±4	55.4±1.05	36.6±3.01	42.06±5.16	36.71±2.94
15	32.35±4.47	55.16±0.31	34.93±4.74	36.5±3.05	34.84±4.84
19	34.06±2.05	54.98±0.88	41.16±1.85	35.68±3.7	35.68±3.7
23	35.25±0.69	55.25±0.69	39.38±3.15	38.58±4.76	39.4±3.05
Alkaline Phosphatase (U/L), (Range- 0.1 - 212.0)					
Before induction	152.5±7.01	153±4.43	161±30.67	157.5±23.36	3.79±0.47
4	167.66±9.12	254.33±19.05	157.5±21.78	148.33±11.27	4.07±0.63
7	148.83±11.83	255.5±19.09	155.5±19.09	156±18.93	4.31±0.71
11	161±7.48	266±24.58	166±24.58	159.83±24.43	4.72±0.41
15	149±9.09	244±21.62	144.16±21.56	149.83±15.89	3.89±0.55
19	151±8.2	202.58±91.92	144.16±21.37	142.5±8.24	3.95±0.78
23	149±8.92	255.83±13.95	155.66±13.97	156.16±13.22	4.46±0.88
Total Bilirubin (mg/dl), (Range- 0 - 0.90)					
Before induction	0.44±0.02	0.38±0.08	0.52±0.18	0.45±0.03	0.45±0.03
4	0.52±0.02	0.04±0.02	0.53±0.02	0.53±0.02	0.53±0.02

7	0.59±0.18	0.05±0.02	0.54±0.02	0.54±0.02	0.54±0.02
11	0.4±0.01	0.16±0.18	0.65±0.21	0.43±0.03	0.43±0.03
15	0.53±0.02	0.25±0.31	0.49±0.06	0.52±0.02	0.52±0.02
19	0.49±0.04	0.03±0.01	0.43±0.15	0.5±0.04	0.5±0.08
23	0.5±0.08	0.03±0.01	0.52±0.02	0.53±0.02	0.51±0.02
High-density Lipoprotein (mg/dl), (Range- 35.0 - 88.0)					
Before induction	58.11±5.26	49.85±6.66	61.03±7.26	63.66±5.16	63.66±5.16
4	49.61±6.64	29.53±2.74	55.58±5.56	55.21±5.38	55.21±5.38
7	52.35±5.81	29.86±2.95	60.05±3.73	57.51±5.24	57.51±5.24
11	52.6±6.18	30.48±3.04	63.73±5.2	57.08±4.26	57.08±4.26
15	51.48±1.79	31.11±3.62	70.53±1.55	60.11±4.56	55.13±3.78
19	60.28±2.46	30.13±2.3	70.55±2.09	57.25±5.44	58.68±3.18
23	61.1±7.23	32.35±2.51	67.75±2.97	55.25±3.57	59.61±8.16
Phosphorus (mg/dl), (Range- 3.00-6.20)					
Before induction	4.3±1.21	3.78±0.67	4.33±0.36	3.79±0.47	157.5±23.36
4	4.65±1.09	2.22±0.52	4.03±0.75	4.07±0.63	148.33±11.27
7	4.15±0.56	2.43±0.13	4.72±0.28	4.31±0.71	156±18.93
11	3.57±1.6	1.87±0.34	4.85±0.54	4.49±0.66	159.83±24.43
15	4.41±0.67	1.65±0.56	4.66±0.57	4.72±0.41	145.66±9.56
19	3.89±0.55	2.15±0.47	4.99±1.08	3.41±0.65	142±6.5
23	4.17±0.85	1.86±0.5	4.41±0.7	4.39±0.88	154.5±14.34

Table 5: Effect of Mahua Concentrate and Mahua Laddoo on blood levels of RBCs, Hemoglobin (Hb) and hemocrait (HCT) in anemia induced rat model

Days	Normal Control	Negative Control	Positive Control	Mahua Concentrate	Mahua Laddoo
RBC (10^12/L), (Range- 4.20-6.0)					
Before induction	4.41±0.26	4.28±0.07	4.26±0.03	4.51±0.24	4.51±0.24
4	4.66±0.38	4.13±0.07	4.49±0.06	4.76±0.19	4.76±0.19
7	4.91±0.37	4.01±0.08	4.66±0.11	4.95±0.19	4.95±0.19
11	5.21±0.35	3.91±0.1	5.02±0.2	5.12±0.19	5.12±0.19
15	5.44±0.28	3.78±0.09	5.23±0.23	5.25±0.17	5.25±0.2
19	5.69±0.15	3.68±0.09	5.46±0.24	5.45±0.19	5.57±0.17
23	5.88±0.12	3.45±0.17	5.67±0.26	5.61±0.2	5.78±0.18
HGB (g/dL), (Range- 13-17)					
Before induction	13.31±0.43	13.36±0.34	13.36±0.22	12.8±0.58	12.8±0.58
4	13.55±0.45	12.06±0.46	13.71±0.15	12.25±0.64	12.25±0.64
7	13.8±0.38	11.3±0.53	14.13±0.12	12.16±0.84	12.16±0.84
11	14.18±0.34	10.3±0.45	14.46±0.26	12.58±0.73	12.58±0.73
15	14.41±0.34	9.4±0.46	14.78±0.29	13.06±0.69	13.06±0.69

19	14.6±0.34	8.58±0.43	15.05±0.29	13.51±0.66	13.72±0.93
23	14.78±0.28	7.53±0.22	15.35±0.31	13.93±0.54	14.23±0.62
HCT count (%), (Range- 39-52)					
Before induction	41.5±1.7	41.5±0.95	41.5±1.38	40±0.75	40±0.75
4	43.16±1.21	39.5±0.95	43.33±1.69	38±0.75	38±0.75
7	45.16±0.89	37.66±1.1	45.16±1.21	37.83±1.45	37.83±1.45
11	813.5±1714.4	35.5±1.25	46.5±1.38	39.5±1.38	39.5±1.38
15	41.66±15.95	33.5±2.06	47.83±1.34	42±1.19	42±1.19
19	50±1.52	31.83±2.03	49.33±1.24	43.66±1.57	48.83±2.58
23	51.16±1.46	30.33±2.28	51.33±0.94	45.33±1.38	50±2.67

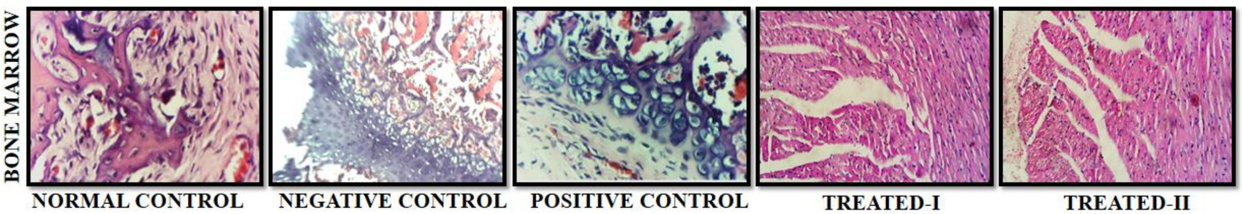


Figure 18.3 Representative images of bone marrow sections from animals of (A) normal control showing presence of adequate number of bone marrow cells; (B) anemic control indicating presence of certain foci of minimal grade in bone marrow containing adipose tissue; (c) Livogen XT and (D) Treated-I: Mahua Concentrate (E) Treated-II: Mahua Laddoo. *(H and E X40).*

6.5 Discussion

Anemia is a major threat to the global health which affects almost about 24.8% of world's total population, 58.5% in Indian population specifically in women and even higher in Odisha i.e. 60.2% of total women population and 71.3% of children are affected (Bhat et al., 2023). The anemic conditions refers to the decrease in the red blood cells or hemoglobin which in turns decreases the oxygen carrying capacity. This is caused due to the deficiency of iron, folic acid and certain vitamins (Wiciński et al., 2020). However, there are certain conventional medications for the treatment of anemia like iron supplements etc. But the interest lies on the natural remedies for the management of the anemia. Mahua tree is abundantly found in India and its flower is well known for its sweetness index (Bisht et al., 2018), hence, it is used for the preparation of several value added food products. The flowers is well known for its nutritional content especially iron which can be used to combat anemia among the women and children. The presence of iron increases the red blood cells formations and also prevents birth defects (Patel and Naik, 2010). “Mahua concentrate and Mahua laddoo” prepared from dried mahua flowers have been explored for the anti-anemic potential. The iron content found in mahua flower is 35 mg/100g. The high iron content enhances the nutritional aspects of mahua, specifically for women and children who are susceptible to iron deficiency (Singh et al., 2021). Although similar studies related to the anti-anemic properties of mahua concentrate and mahua laddoo has not been carried out but the nutritional composition may be the reason for its anti-anemic activity. The proximate analysis of mahua flowers (Singh et al., 2021), mahua concentrate and mahua laddoo have shown that they have several essential nutrients like carbohydrates, proteins, fats, minerals, vitamins which is important for combating anemia. The minerals found in mahua flowers such as iron, copper, zinc could be responsible for the production and maturation of RBCs (Singh et al., 2020). Proteins could also act as an oxygen carrier in the blood stream. Even though the studies suggest encouraging potential, it requires further work in order to gain full information about mechanisms related to its antianemic activity and ensure its safety in human subjects. The currently available data indicate, however, that mahua concentrate and mahua laddoo may certainly signify a valuable traditional food of major importance for treatment with potential advantageous properties in relation to combating anemia.

6.6 Conclusion

In conclusion, the mahua flower concentrate and mahua laddoo exhibited anti-anemic activity in a rat model induced with 2,4-dinitrophenylhydrazine. This study also highlights the therapeutic potential of value-added food products. Both the products display substantial effects in ameliorating anaemia, as demonstrated by enhancements in red blood cell count, haemoglobin levels, and overall haematological parameters. These findings indicate that mahua-based preparations may possess valuable properties, which could contribute to their efficacy in treating anaemia. Further studies are necessary to discover the mechanisms of action, optimal dosages, and potential for clinical application in human populations. However, this investigation offers insights into the use of mahua flowers as a natural medicine for the management of anaemia.

CONCLUSION

In conclusion, mahua flowers (*Madhuca indica*) hold important cultural, nutritional, and medicinal value, with a rich history of use in various regions, especially in India. These flowers not only are home to essential nutrients, such as carbohydrates but alsoalso contain a wide range of phytochemicals with potential health benefits. The complete analysis of both fresh and dry Mahua flowers revealed notable differences in carbohydrate content and phytochemical profile, indicating that drying may improve certain properties while altering other properties. These studies provide critical insights into the nutritional potential of Mahua flowers and their utilization in food, medicine, and traditional practices. The occurrence of bioactive compounds such as flavonoids, alkaloids, and terpenoids further highlights their therapeutic potential, indicating that Mahua flowers can promote health and nutrition, particularly in rural areas. Additional research into the exact pharmacological properties of these compounds could open new doors for the use of Mahua flowers in modern healthcare and industry. Hence, Mahua flowers represent a valuable natural reserve that syndicates cultural inheritance with modern scientific openings. The examination of the antibacterial and antibiofilm efficacy of Mahua has emphasized its important potential as a natural therapeutic agent. A complete phytochemical and biological investigation revealed that Mahua has a rich collection of bioactive compounds, such as flavonoids, tannins, and saponins, which contributes to its antimicrobial efficacy. These results suggest that Mahua extracts not only hinder the development of various bacterial strains but also display promising antibiofilm activity, which is vital for preventing the formation of biofilms that cause chronic infections and antibiotic resistance. These results underscore the potential of Mahua as a substitute treatment in the fight against bacterial contamination, predominantly those resistant to conventional antibiotics. Owing to its traditional use in numerous cultures and its confirmed antibacterial efficacy, Mahua could serve as a valuable contender for further investigation and development in the pharmaceutical and healthcare industries. The development of value-added food products from mahua flowers, such as mahua concentrate, mahua cake, mahua cookies, mahua jam, mahua RTS and mahua laddoo, represents a promising method to improve the nutritional profile of various food items and provides a supportable source of nutrition. Mahua flowers, which are conventionally used for their cultural, medicinal, and nutritional benefits, are increasingly recognized for their potential as functional elements in the food industry. The thorough nutritional sketching of Mahua flowers reveals a rich configuration that comprises essential sugars, vitamins, and amino acids, making them a valuable addition to the human diet. The sugars present in flowers are glucose, fructose, xylose, arabinose, etc.,, which are important energy sources, making them useful for individuals in need of quick energy. In addition to sugars, it comprises numerous vitamins that affect overall immune health, energy metabolism, and skin health. The presence of these vitamins plays a critical role in defending the body from oxidative stress, supporting the immune system, and maintaining healthy skin and connective tissues. In addition, they are rich sources of essential amino acids, which are the building blocks of proteins in the body. These amino acids are important for numerous physiological functions. The equilibrium of amino acids found in Mahua flowers makes them important contributors to protein nutrition, particularly in regions where the ability to access animal-based protein sources is partial. The value-added food products significantly improve the use of this underutilized resource. By including mahua flowers in such products, they can be made more available to a broader population. The value-added food products can help minimize nutritional deficiencies, predominantly in rural groups where protein, vitamins, and energy-dense foods are in short supply. Moreover, these foodstuffs provide economic chances for local groups by enabling them to make jobs in Mahua-based food items. While the potential of Mahua flowers in food product development is clear, further investigation into their processing methods and their ability to hold nutrients is essential. This ensures that the extreme nutritional value is conserved when Mahua flowers are converted into different food products. Moreover, identifying functional properties, such as their antioxidant, antimicrobial, and anti-inflammatory effects, can further enhance the health benefits of Mahua-based food products. The value-added food products represent a highly promising resource for the development of nutritious, value-added food products. Owing to their exclusive mixture of sugars, vitamins, and amino acids, they constitute an outstanding addition to the global food supply, especially in regions that face nutritional challenges. By increasing the use of mahua flowers in food product development, we can advance public health and support local economies. Finally, the antianemic activity of the Mahua flower concentrate and Mahua laddoo against 2,4-dinitrophenylhydrazine-induced anaemia in a rat model provides convincing evidence for the therapeutic potential of Mahua flowers in fighting anaemia. Both the Mahua flower concentrate and Mahua laddoo confirmed substantial enhancements in hematological parameters, such as increased red blood cell count, hemoglobin levels, and hematocrit values, which are indicative of their potential to ease anemia. The bioactive compounds present in Mahua flowers, including vitamins, minerals, and flavonoids, likely contribute to their ability to increase red blood cell production. The results suggest that Mahua flower-based products could aid in natural and operative dietary enhancement for individuals suffering from iron deficiency anaemia or related conditions. Furthermore, these findings highlight the wider potential of Mahua flowers as useful food ingredients, providing therapeutic assistance beyond their traditional practices. Additional research, mostly clinical studies in people, would be vital to determine the effectiveness and safety of Mahua flower products in treating anaemia, flagging the way for their combination into nutritional involvements and health-promoting food products. Overall, Mahua flowers represent a sustainable, nutrient-rich, and bioactive reserve with complex applications in food, medicine, and health. As research continues to discover their full potential, Mahua flowers may play a vital role in addressing both nutritional and health anxieties, contributing a natural answer to some of the most persistent health contests. With further examination and development, Mahua flowers could become an essential part of current nutrition and pharmacology, helping individuals and societies worldwide.

FUTURE PRESPECTIVES

The future perspectives of value-added food products derived from Mahua flowers seem highly promising, predominantly as consumer demand for natural, functional, and sustainable food ingredients endures to rise. Mahua flowers are rich in bioactive compounds, including flavonoids, saponins, phenolic acids, and triterpenoids, which exhibit antioxidant, anti-inflammatory, antimicrobial, and potential anticancer properties. These functional properties position Mahua flowers as a valuable source for developing nutraceutical and health-oriented food products. Mahua flowers could be utilized in the production of functional beverages, energy bars, natural sweeteners, and fortified foods, capitalizing on their nutritional content, which includes carbohydrates, proteins, vitamins (especially Vitamin C), and essential minerals such as calcium and iron. Moreover, the natural sugars present in Mahua flowers, such as glucose, fructose, and sucrose, offer the potential to be incorporated as a healthier alternative to refined sugars, especially in low-glycemic food products. Additionally, Mahua flowers possess prebiotic potential, which could benefit gut health, making them suitable for incorporation into probiotic or digestive health formulations. The sustainable harvesting of Mahua, a native and drought-resistant plant, aligns with growing trends in environmental consciousness, especially in the context of sourcing plant-based ingredients with minimal ecological impact. With advancements in food processing technologies, such as freeze-drying, extraction, and encapsulation, the bioactive compounds in Mahua flowers could be more efficiently preserved, thus enhancing the functional properties and shelf life of end products. As a result, Mahua flower-based ingredients hold significant potential not only for traditional food sectors but also for the emerging markets focused on functional, plant-based, and clean-label products, making it an attractive area for future food innovation.

REFERENCES

1. Abdel-Gawad, DR, Ibrahim, MA., El-Banna, HA., Hassan, WH., & El-Ela, FIA. (2024). Evaluating the Therapeutic Potential of Amygdalin: Cytotoxic and Antimicrobial Properties. *Tissue and Cell*, 102443.
2. Abhyankar, VS, & Narayana, N. (1942) A preliminary note on the preparation of syrup from mahua flowers. Poona Agriculture College Magazine, 33, 168172.
3. Afifi TH, Okasha RM, Ahmed HE, Ilaš J, Saleh T, Abd-El-Aziz A.S. (2017) Structure-activity relationships and molecular docking studies of chromene and chromene based azo chromophores: A novel series of potent antimicrobial and anticancer agents. EXCLI J. 16:868–902.
4. Ahirwar MK, Kirar N., Gupta GS. (2020) Micro Nutrient Values in Mahua Flower Found Naturally, as a Nutritional Potential. *Food & Nutrition Journal,* 5:219.
5. Ahmed WE, Ragab I, Gadallah MG, Alhomaid RM, Almujaydil MS. (2024) Effect of sprouting whole wheat grain on the sensory quality, physicochemical properties, and antioxidant activity of cupcakes. Applied Food Research. 4(1),100412.
6. Alomari KB, Ansari N, Jabeen F, Rahman QI. (2024) Spectrophotometric estimation of reducing sugar in food product. Science and innovation. 45, 346-51.
7. Annalakshmi, R, Mahalakshmi, S, Charles, A, & Sahayam, C. S. (2013). GCMS and HPTLC analysis of leaf extract of Madhuca longifolia (Koenig) Linn. Drug Invention Today, 5, 7680.
8. Anonymous. (1962). Wealth of India, Raw Materials. CSIR.
9. Arun S, Ajay S, Vadivel V. (2017) Effect of extraction conditions on the total phenolic yield of Madhuca longifolia leaves and evaluation of its physico-chemical and antioxidant properties. Journal of Pharmaceutical Sciences and Research 9(7):1188.
10. Asch J, Johnson K, Mondal S, Asch F. (2022) Comprehensive assessment of extraction methods for plant tissue samples for determining sodium and potassium via flame photometer and chloride via automated flow analysis#. Journal of Plant Nutrition and Soil Science. 2, 308-16.

11. Assocle MS, Okidi L, Ongeng D. (2024) The effect of rosemary, ginger, or garlic on microbial shelf life and sensory acceptability of nutritionally enriched cassava-based pancake (Kabalagala). Applied Food Research. 4(1), 100384.
12. Bai, JR, Wu, YP, Elena, G, Zhong, K, & Gao, H. (2019). Insight into the effect of quinic acid on biofilm formed by *Staphylococcus aureus*. *RSC advances*, *9*(7), 3938-3945.
13. Bakhara, CK, Bal, LM, Pal, US, Sahoo, NR, & Panda, MK. (2016). Post-harvest practices and value addition of Mahua (Madhuca longifolia) flower in Odisha. Agricultural Engineering Today, 40, 2228.
14. Banerji, R, & Mitra. (1996). Mahua (Madhuca species): Uses and potential in India. Applied Botany Abstract, 16, 260277.
15. Behera, S, & Swain, MR. (2013). A survey of post-harvest spoilage of mahula (Madhuca latifolia L.) flowers. Interanational Journal of Agricultural Technology, 9, 227235.
16. Belavady, B, & Balasubramanian, SC. (1959). Nutritive value of some Indian fruits and vegetables. Indian Journal of Agricultural Science, 29, 151163.
17. Benali, T, Bakrim, S, Ghchime, R, Benkhaira, N, El Omari, N, Balahbib, A, ... & Bouyahya, A. (2022). Pharmacological insights into the multifaceted biological properties of quinic acid. *Biotechnology and Genetic Engineering Reviews*, 1-30.
18. Bhat BA, & Bhat SA. (2023) Do Increasing Number of Antenatal Care Visits Improve Quality of ANC Services in India.
19. Birk, Y. (1969). In: Toxic Constituents of Plant Foodstuffs. Liener, I.E. (Ed). Academic Press, New York.
20. Bisht V, Neeraj VK, Dalal N. (2018) Mahua an important Indian species: A review. Journal of Pharmacognosy and Phytochemistry. 7(2):3414-8.
21. Bisht, V, Neeraj, S, Solanki, VK, & Dalal N. (2018). Mahua an important Indian species: A review. Journal of Pharmacognosy and Phytochemistry, 7(2), 34143418.
22. Bora, JR, & Singh, VP. (1994). Effect of feeding dried Mahua (Madhuca indica) flowers on growth and feed conversion efficiency of crossbred gilts. Indian Veterinary Journal, 71(10), 988990.
23. Cacabelos, R, & Torrellas, C. (2016). Pharmacoepigenomics. In *Medical Epigenetics* Academic Press.585-617
24. Cappellini MD, Motta I. (2015) Anemia in Clinical Practice-Definition and Classification: Does Hemoglobin Change With Aging? Semin. Hematol. 2015; 52: 261–269.

25. Chand, S, & Mahaptra, SN. (1983). Production of sugar syrup from Mahua (Madhuca latifolia) flower. Research and Industry, 28(1), 2931.

26. Chandel, PK., Prajapati, RK., & Dhruwe, RK. (2018). Documentation of post-harvest methods and value addition of different NTFPs in Dhamtari forest area. Journal of Pharmacognosy and Phytochemistry, 7(1), 15181523.

27. Chaparro CM, Suchdev PS. (2019) Anemia epidemiology, pathophysiology, and etiology in low- and middle-income countries. Ann. N. Y. Acad. Sci. 1450: 15–31.

28. Chauhan S, Kumar P, Marbaniang S.P, Srivastava S, Patel R. (2022) Prevalence and predictors of anaemia among adolescents in Bihar and Uttar Pradesh, India. Sci. Rep.12: 8197.

29. Chen B, Ellefson WC. Fat Analysis. (2024) Cham: Springer International Publishing.InNielsen's Food Analysis 273-286.

30. Cheruvanachari, P, Mishra, M, Pattnaik, S, & Naik, PK. (2023). Determination of antibacterial and anti-biofilm potential of Kewda essential oil against *Staphylococcus aureus* and *Klebsiella pneumoniae*. *Biologia*, *78*(8), 2253-2260.

31. Cheruvanachari, P, Pattnaik, S, Mishra, M, Pragyandipta, P, & Naik, PK. (2022). Terpinen-4-ol, an active constituent of kewda essential oil, mitigates biofilm forming ability of multidrug resistant *Staphylococcus aureus* and *Klebsiella pneumoniae*. *Journal of Biologically Active Products from Nature*, *12*(5), 406-420.

32. Daji, JA., Patel, MK., & Kibe, MM. (1951). Proceedings - Indian Academy of Sciences, Section A, 33B, 322-326.

33. Das U, Ray RC, Hansdah B. (2022) Ethnomedicinal and biological activities of tropical mahua (Madhuca species)–A comprehensive review. Annals of Tropical Research. 44(2):67-88.

34. Das, BK., Choudhury, BK., & Kar, M. (2010). Quantitative estimation of changes in biochemical constituents of mahua (*Madhuca indica* syn. *Bassia latifolia*) flowers during postharvest storage. Journal of Food Processing and Preservation.

35. Daver, MB., & Ahmed, SS. (1944). Mahwa yeast from an alcohol factory and its vitamin content. Indian Medical Gazette, 79(3), 110111.

36. De Falco L, Silvestri L, Kannengiesser C, et al. (2014) Functional and clinical impact of novel TMPRSS6 variants in iron-refractory iron deficiency anemia patients and genotype-phenotype studies. Hum Mutat 35(11).

37. Deryabin, DG, & Tolmacheva, AA. (2015). Antibacterial and anti-quorum sensing molecular composition derived from Quercus cortex (Oak bark) extract. *Molecules*, *20*(9), 17093-17108.
38. Diallo A, Gbeassor M, Vovor A, Eklu-Gadegbeku K, Aklikokou K, Agbonon A, et al. (2008) Effect of Tectona grandis on phenylhydrazine-induced anaemia in rats. Fitoterapia 79:332e6.
39. dos Santos, RC, de Melo Filho, AA, Chagas, EA, Takahashi, JA, Ferraz, VP, Ribeiro, PRE., & Holanda, LC. (2015). Chemical composition, antimicrobial and anti-acetylcholinesterase activities of essential oil from Lantana camara (Verbenaceae) flowers. *Journal of Medicinal Plants Research*, *9*(35), 922-928.
40. Douli, AK., Kharya, MD., & Srivastava, MP. (1968a). Mahua fat in suppository bases Part-1. Indian Journal of Pharmaceutical Sciences, 50, 3335.
41. Dushing, PM, & Surve, VD. (2019). Production of wine from Mahua (*Madhuca indica* L.) flower extract and Pomegranate (*Punica granatum* L.) fruit juice. International Journal of Chemical Studies, 7(1).
42. Dwivedi A, Priyadarshini A, Induar S. (2022) Mahua (*Madhuca longifolia*) flower and its application in food industry: A review. International Journal of Chemical Studies. 10(1):80-4.
43. Ehaimir, SY, Ibrahim, MA, Abdalhalim, AM., Ibrahim, SO, Alzain, AA, Alameen, AA, ... & Abdalrahman, MA. (2023). Antibacterial, Antioxidant Activities, GC–MS Analysis and Docking Studies of Guiera senigalensis (L.) Ethanol Leaves Extract. *Tropical Journal of Natural Product Research*, *7*(1).
44. Elworthy. (1887). Analysis of Mahua flower. Journal on Systemics, Cybernetics and Informatics.
45. Endo A, Maeno S, Tanizawa Y, Kneifel W, Arita M, Dicks L, Salminen S. (2018) Fructophilic lactic acid bacteria, a unique group of fructose-fermenting microbes. Applied and environmental microbiology. 84(19):e01290-18.
46. Fowler, & Gilber, J (1930). Recent experiments on the preparation of organic manure. Agriculture Journal of India, 25, 365383.
47. Fowler, GJ., Behram, JDE, Bhate, SR, Hassan, HK, Mahdihassan, S, & Inuganti, NN. (1920). Biochemistry of Mahua flower. Journal of Indian Institute of Science, 3, 81118.

48. Frank LL. (2015) Thiamin in clinical practice. Journal of Parenteral and Enteral Nutrition. 39(5):503-20.

49. Gaikwad, RD, Ahmed, ML, Khalid, MS, & Swamy, P. (2009). Anti-inflammatory activity of Madhuca longifolia seed saponin mixture. Pharmaceutical Biology, 592597.

50. Gallego, A, Giordano, W, Rosero, E, & Echeverri, F. (2016). Effect of furans and a Pyran on Several Quorum Sensing Factors in Ralstonia solanacearum. *J. Microb. Biochem. Technol*, *8*, 1-5.

51. Ghadge, SV., & Raheman, H. (2006). Process optimization for biodiesel production from mahua (Madhuca indica) oil using response surface methodology. Bioresource Technology, 97(3), 379384.

52. Goodhart, RS., & Sahil, ME. (1980). Modern Nutrition in Health and Disease, 6th Edition. Lea & Febiger, PA, United States.

53. Gopalan, C, Rama Sastri, BV, & Balasubramanian, SC. (2004). Nutritive Value of Indian Foods. National Institute of Nutrition, ICMR, Hyderabad, India.

54. Goud, VV, Patrwardhan, AV, & Pradhan, NC. (2006). Studies on the epoxidation of mahua oil (Madhumica indica) by hydrogen peroxide. Bioresource Technology, 97(12), 13651371.

55. Gupta A, Chaudhary R, Sharma S. (2012) Potential applications of mahua (Madhuca indica) biomass. Waste and Biomass Valorization.175-89.

56. Haghighi ST, Alizadeh M, Salehian R. (2024) Scientific Vanguard: Chronicling the Emerging Innovations Reshaping Our Future. Nobel Sciences;

57. Hasan, K, Habib, & Bhate, SR. (1928). Mahua waste as food for milch cattle. Proceedings 15th Indian Science Congress.

58. Hopper Z, Desbrow B, Roberts S, & Irwin C. (2023) Preparation procedures of food and beverage samples for oxygen bomb calorimetry: A scoping review and reporting checklist. *Journal of Food and Drug Analysis*, 31(2): 232.

59. Hopper Z, Desbrow B, Roberts S, Irwin CG. (2024) Beverage sample preparation and procedures for bomb calorimetry: establishing equivalency in methods. Journal of Food Composition and Analysis. 128:106033.

60. Hota, SS, Sahoo, DR, Pattnaik, S, Mishra, M, Mishra, T, Sahu, T, ... & Naik, PK. (2024). Antioxidant, antibacterial and antibiofilm activities of *Pittosporum napaulense* (DC.)

against drug-resistant *Staphylococcus aureus* and *Shigella* sp. *Natural Product Research*, 1-9.

61. Hsu PK, Tsai YT, Lin YC, & Kuan CM. (2022). Assessment of the acute and subacute toxicity of the ethanolic extract of the aerial parts of Crassocephalum rabens (Asteraceae) in rats. *Toxicology reports*, *9*, 58-63.
62. ICMR. (2009) Nutrient requirements and recommended dietary allowances for Indians. Indian Council of Medical Research report, Hyderabad.
63. Irine Shibu Chacko (2023). Unlocking the Power of Mahua: A Comprehensive Guide. IRJPS. 14: 14.
64. Jamir R. (2024) More than a Country Liquor: Madhuca Longifolia as a Facilitator of SDGs. InTransformative Practices in Archaeology: Empowering Communities and Shaping Sustainable Futures Singapore: Springer Nature Singapore 219-228.
65. Jatoth R, Dhanabal SP, Senthil V, Ganesh T, Selvaraj J, & Venkatesan PS. (2025) Acute oral toxicity study of novel polyherbal formulations by using wistar rats and Swiss albino mice as per OECD 425 TG. *Phytomedicine Plus*, *5*(1): 100672.
66. Jayasree, B., Harishankar, N., & Rukmini, C. (1998). Chemical composition and biological evaluation of Mahua flowers. Journal of Oil Technologist Association of India, 30, 170172.
67. Jaywant SA, Singh H, Arif KM. (2022) Sensors and instruments for brix measurement: A review. Sensors. 22(6):2290.
68. Jha S, Vaibhav V, Suneetha V. (2013) A Culinary Mahua (Madhuca indica) flower from Bihar, India–A potential in Production of Jam, Alcohol for Pharmacological benefits with Fertilizer value. International Journal of Drug Development and Research. 2:362-7.
69. Jha, D, & Mazumder, PM. (2018). Biological, chemical and pharmacological aspects of Madhuca longifolia. Asian Pacific Journal of Tropical Medicine, 11(1), 914.
70. Jiang, K, Yan, X, Yu, J, Xiao, Z, Wu, H, Zhao, M, ... & Lin, F. (2020). Design, synthesis, and biological evaluation of 3-amino-2-oxazolidinone derivatives as potent quorum-sensing inhibitors of *Pseudomonas aeruginosa* PAO1. *European journal of medicinal chemistry*, *194*, 112252.
71. Johar V, Kumar R. (2020) Mahua: A versatile Indian tree species. Journal of Pharmacognosy and Phytochemistry. 9(6):1926-31.

72. Joshi A, Gupta AK, Jha AK, Naik B, Kumar V, Rustagi S. (2024) Nutraceutical Potential of Staple Food Crops. Herbal Nutraceuticals: Products and Processes. 1:329-45.
73. Kannan, S, Balakrishnan, J, Govindasamy, A, & Arunagiri, R. (2022). New insights into the antibacterial mode of action of quercetin against uropathogen *Serratia marcescens* in vivo and in vitro. *Scientific Reports*, *12*(1), 21912.
74. Kapilan, N, & Reddy, RP. (2008). Evaluation of methyl esters of mahua oil (Madhuca indica) as diesel fuel. Journal of the American Oil Chemists' Society, 85, 185188.
75. Karanth S, Feng S, Patra D, Pradhan AK. (2023) Linking microbial contamination to food spoilage and food waste: The role of smart packaging, spoilage risk assessments, and date labeling. Frontiers in Microbiology. 14:1198124.
76. Kaul, CL, & Grewal, RS. (1972). Antihypertensive and monoamine oxidase inhibitory activity of 3-amino-2-oxazolidinone (3AO) and its condensation product with 2-substituted-3-formyl-4-oxo-(4H) pyrido (l, 2-a) pyrimidines. *Biochemical Pharmacology*, *21*(3), 303-314.
77. Kaushal, S, & Sharma, P. (2022). Antimicrobial and antioxidant potential of banana (Musa sapientum) peel extracts of variety Grand naine, major compound 5-hydroxymethylfurfural in methanolic peel extract and its derivatives. *Agricultural Research Journal*, *59*(5).
78. Khare A, Samudre S, Arora A. (2022) Sneak-peek into iron deficiency anemia in India: The need for food-based interventions and enhancing iron bioavailability. Food Research International. 162:111927.
79. Khare P, Kishore K, Sharma DK. (2018) Medicinal uses, Phytochemistry and Pharmacological profile of Madhuca longifolia. Asian Journal of Pharmacy and Pharmacology. 4(5):570-81.
80. Khatri D, & Chhetri SBB. (2020) Reducing sugars, total phenolic content, and antioxidant potential of neighboring plants. *BioMed Research International*, 1: 7296859.
81. Kolkar KP, Malabadi RB, Sadiya MR, Chalannavar RK. (2024) Updates on some medicinal and ornamental plants-Ayurvedic medicines. World Journal of Advanced Research and Reviews. 23(1):111-47.
82. Kotwal, D. (2000). Mahua Nutritional aspects, Manohar publication, Nagpur

83. Kumar S, Arnipalli SR, Mehta P, Carrau S, Ziouzenkova O. (2022) Iron Deficiency Anemia: Efficacy and Limitations of Nutritional and Comprehensive Mitigation Strategies. Nutrients 14: 2976.

84. Kumar SV. A Study on the Valuable Source and Medicinal Use of selected Edible Flowers in the New Normal. Food, Tourism and Hospitality Perspectives.:248.

85. Kumar, S, & Meena, GL. (2018). Procurement and marketing of non-timber forest products. Indian Forester, 144(3), 252259.

86. Kumari, K, Sinha, R, Krishna, G, Kumar, S, Singh, S, & Srivastav, AK. (2018). Sensory and nutritional evaluation of value added products prepared from Mahua flower. International Journal of Current Microbiology and Applied Sciences, 7, 10641070.

87. Lender, A. (1992). Indian Mahwa. Schweizerische Apotheker-Zeitung, 60, 713719.

88. Mahajan, UN., Mahapatra, DK, Mahajan, NM, Kazi, FS, & Baghel, N. (2017). Exploring the role of Mahua oil as potent emulsifier in cream formulations. International Journal of Herbal Medicine, 5(3), 9397.

89. Maithani, G. P. (1994). Management Perspectives of Minor Forest Produce. MFP News.

90. Mandal, S, Datta, B. K, & Bhattacharyya, G. N. (1993). Biochemical analysis of the allergenic pollen of Madhuca indica Gmel. and Spathodea campanulata Beauv. Aerobiologia, 9, 197-200.

91. Mehta N, Singh N, Shirin F. Flower morphometry of *Madhuca indica*: inter and intra population variations in.

92. Mishra A, Nandini V, Joglekar A. (2021) Development of new product by mahua (*Madhuca longifolia*) flowers. Kala Sarovar. 24(1):9-12.

93. Mishra M, Pattnaik S, Singh H, Naik PK. (2023) Exploring the role of Mahua as a functional food and its future perspectives. InRecent Frontiers of Phytochemicals Elsevier 109-121.

94. Mishra, A, & Poonia, A. (2019). Mahua (Madhuca longifolia) flowers: review on processing and biological properties. *Nutrition & Food Science*, *49*(6), 1153-1163.

95. Mishra, AB., & Usha, T. (2019). A review on pharmacological approach of the therapeutic property of Madhuca longifolia (J. Koenig ex L.) J. f. Macbr. flower. Journal of Research in Siddha Medicine, 2(2), 6168.

96. Mishra, S, & Pradhan, S. (2013). Madhuca lonigfolia (Sapotaceae): a review of Its traditional uses and nutritional properties. International Journal of Humanities and Social Science Invention, 2(5), 3036.
97. Misra, PS., & Mitra, CR. (1968). Nitrification of defatted and processed seed meals. Journal of Agricultural and Food Chemistry, 16(4), 701703.
98. Mitra, CR., & Misra, PS. (1967). Amino acids of processed seed meal proteins. Journal of Agricultural and Food Chemistry, 15(4), 697700.
99. Mohod, SM., & Bodhankar, SL. (2013). Antiulcer activity of aqueous extract of leaves of Madhuca indica J. F. Gmel against naproxen induced gastric mucosal injury in rats. Journal of Acute Disease, 2(2), 127133.
100. Momtaz M, Khan MS. (2024) Analysis of chlorpyrifos pesticide residue in locally grown cauliflower, cabbage, and eggplant using gas chromatography–mass spectrometry (GC-MS) technique: a Bangladesh perspective. Foods. 13(11):1780.
101. Mondo JM, Chuma GB, Muke MB, Fadhili BB, Kihye JB, Matiti HM, Sibomana CI, Kazamwali LM, Kajunju NB, Mushagalusa GN, Karume K. (2024) Utilization of non-timber forest products as alternative sources of food and income in the highland regions of the Kahuzi-Biega National Park, eastern Democratic Republic of Congo. Trees, Forests and People. 16:100547.
102. Mulky, MJ, & Gandhi, VM. (1977). Mowrah (Madhuca latifolia) seed saponin toxicological studies. Journal of Applied Chemistry and Biotechnology, 27(6), 708713.
103. Nayak P, Mishra AA, Shukla RN, Biswas R. (2024) Traditional Foods Based on Pseudocereals. InPseudocereals CRC Press 248-263.
104. Nayak, S, & Sahoo, UK. (2020). Contribution of Mahua (Madhuca latifolia Macb.) to household economy of Odisha. Environment and Ecology, 38(2), 20242207.
105. Nguyen, TLA., & Bhattacharya, D. (2022). Antimicrobial activity of quercetin: an approach to its mechanistic principle. *Molecules*, *27*(8), 2494.
106. Nurhayati R, Shoviantari F, Munandar TE, Yuwono M. (2024) Butterfly pea (Clitoria ternatea L.) flower water and ethanol extract: Phytochemical screening, FTIR analysis, and antioxidant activity estimation using comparison of ABTS, DPPH, and FRAP assays. Research Journal of Pharmacy and Technology. 17(5):1973-82.

107. Owheruo JO, Edo GI, Makia RS, Gaaz TS, Okolie MC, Nwaogu MU, Faturoti AO, Akpoghelie PO, Yousif E, Isoje EF, Igbuku UA. (2024) Nutritional qualities of cookies made from wheat/cashew nut composite flour. Food and Humanity. 3:100452.

108. Pandey, AK, & Rakesh, S. (2023). Madhuca longifolia var. latifolia (Roxb.) A. Chev: A plant with medicinal boon. *International Journal of Ayurvedic Medicine*, *14*(3), 593-605.

109. Pandey, KB., & Rizvi, SI. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative medicine and cellular longevity*, *2*(5), 270-278.

110. Patel M, & Naik SN. (2010) Flowers of Madhuca indica JF Gmel.: Present status and future perspectives.

111. Patel M, Naik SN. Flowers of Madhuca indica JF Gmel.: Present status and future perspectives.

112. Patel, M, Jaiswal, S, Naik, B, Naik, M, Saxena, D, & Naik, SN. (2016). Development of antioxidant rich beverage from Mahua (*Madhuca indica*) and Amla (*Emblica officinalis*). Journal of Scientific and Industrial Research, 75(1), 3539.

113. Patel, MK, & Kibe, MM. (1951). Proceedings - Indian Academy of Sciences, 33, 322326.

114. Patel, PK, Janghel, V, Chandel, SS, & Sahu, J. (2019). Madhuca indica (Mahua)-pharmaceutical, nutraceutical and economical importance for tribal people of Chhattisgarh state. International Journal of Pharmaceutical and Phytopharmacological Research, 9(3), 1628.

115. Patel, PK, Prajapati, NK, & Dubey, BK. (2012). Madhuca indica: A review of its medicinal property. International Journal of Pharmaceutical Sciences and Research, 3(5), 12851293.

116. Patle, TK, Shrivas, K, Kurrey, R, Upadhyay, S, Jangde, R, & Chauhan, R. (2020). Phytochemical screening and determination of phenolics and flavonoids in Dillenia pentagyna using UV–vis and FTIR spectroscopy. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *242*, 118717.

117. Perez Zamora, CM, Torres, CA, & Nuñez, MB. (2018). Antimicrobial activity and chemical composition of essential oils from Verbenaceae species growing in South America. *Molecules*, *23*(3), 544.

118. Pinakin, DJ, Kumar, V, Kumar, A, Gat, Y, Suri, S, & Sharma, K. (2018). Mahua: A boon for pharmacy and food industry. Current Research in Nutrition and Food Science, 6(2), 371381.

119. Prajapati, M, Yamgar, D, & Fegade, B. (2021). Comprehensive Profile of Madhuca Longifolia in Pharma and Food Industry. *Current Nutraceuticals*, *2*(1).

120. Puhan, S, Vedaraman, N, Ram, BV., Sankarnarayanan, G, & Jeychandran, K. (2005). Mahua oil (*Madhuca Indica* seed oil) methyl ester as biodiesel-preparation and emission characterstics. Biomass and Bioenergy, 28(1), 8793.

121. Qasem, W, Mohamed, EA, Hamed, AA., El-Sayed, AEKB., & Salah El Din, RA. (2016). Antimicrobial and anticancer activity of some microalgal species. *Egyptian Journal of Phycology*, *17*(1), 33-49.

122. Raheman, H, & Ghadge, SV. (2007). Performance of compression ignition engine with mahua (Madhuca indica) biodiesel. Fuel, 86(16), 25682573.

123. Rajesh, Y, Khan, N. M, Shaikh, AR, Mane, VS, Daware, G, & Dabhade, G. (2023). Investigation of geranium oil extraction performance by using Soxhlet extraction. *Materials Today: Proceedings*, *72*, 2610-2617.

124. Rajgor, N, Gujral, S, Gopaldas, T, et al. (1986). Preliminary trials to investigate the feasibility of using Mahuda flowers (*Mahuda indica* or *Bassia latifolia*) as energy source in an experimental model. Indian Journal of Nutrition and Dietics, 23(1).

125. Ramadan, MF., Mohdaly, AAA, Assiri, A, Tadrod, M, & Niemeyer, B. (2016). Functional characteristics, nutritional value and industrial applications of Madhuca longifolia seeds: an overview. Journal of Food Science and Technology, 53, 21492157.

126. Ramadas V, Geetha C, & Begum K. (2011) Food management and child care, Government of Tamilnadu, 1-293.

127. Ranjana, K, Amit, P, & Kumar, SA. (2018). GC-MS analysis of methanol extract from bark, flower, leaf and seed of Madhuca indica JF gmel. Journal of Pharmacognosy and Phytochemistry, 7(2), 32593266.

128. Rashid MM, Islam S, Uddin MN, Al Mamun MZ, Abedin MJ, Bhuiyan MH, Miah MA. (2024) HPLC-DAD analysis of water-soluble vitamins (B1, B2, B3, B5, B6, C and Biotin) and fat-soluble vitamins (A, D, E, K1 and β-carotene) in commonly consumed pulses in Bangladesh. Applied Food Research. 4(1):100424.

129. Ravat, P, & Dixit, A. (2017). Development and evaluation of gluten free biscuits by incorporation of Mahua powder as natural sugar. International Conference on Recent Trends in Agriculture, Food Science, Forestry, Horticulture, Aquaculture, Animal

Sciences, biodiversity, Ecological Sciences and Climate Change. Journal of Scientific and Industrial Research.

130. Rehman H, Rehman Z, Das TK, Rehman M, Khan BA, Nandi S, Ahmad K, Mohanty SK, Naeem R, Bajaj M, Tuka MB. (2024) Toxicity evaluation and degradation of cypermethrin-contaminated soil using biochar and Bacillus cereus amendments. Scientific Reports. 14(1):1-5.
131. Ritika, Mansi, Rizwana, Kumar H, Bora B, Rather MA, Naik B, Kumar V, Ranjan R, Gupta AK. (2024) Traditional and Underutilized Fruits and Vegetables for Attaining Zero Hunger. InFood Production, Diversity, and Safety Under Climate Change Cham: Springer Nature Switzerland 89-111.
132. Rizvi NB, Aleem S, Khan MR, Ashraf S, & Busquets R. (2022) Quantitative estimation of protein in sprouts of vigna radiate (Mung beans), lens culinaris (Lentils), and cicer arietinum (Chickpeas) via the Kjeldahl and Lowry methods. *Molecules*, 27(3): 814.
133. Rosyantari A, Prasedya ES, Ilhami BT, Martyasari NW, Padmi H, Abidin AS, Ambana Y, Kirana IA, Sunarwidhi AL. (2021) Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and antioxidants activity of marine sponge stylissa flabelliformis ethanol extract. InIOP Conference Series: Earth and Environmental Science 012109.
134. Roy, JK, & Rao, RK. (1959). Mahua spirit and chemical composition of the raw material (Mahua flowers). Proceedings of the Institution of Chemists (India), 31, 6465.
135. Rukmini, C. (1990). Reproductive toxicology and nutritional studies on mahua oil (Madhuca latifolia). Food and Chemical Toxicology, 28(9), 601605.
136. Saadon, KE, Taha, NM, Mahmoud, NA, Elhagali, GA, & Ragab, A. (2022). Synthesis, characterization, and in vitro antibacterial activity of some new pyridinone and pyrazole derivatives with some in silico ADME and molecular modeling study. *Journal of the Iranian Chemical Society*, *19*(9), 3899-3917.
137. Saeed, HM, Ferdosi, MF, Khan, IH, Javaid, A, & Sultan, MW. (2023). Antibacterial activity and GC-MS analysis of white flowers extract of Nerium oleander L. *International Journal of Biology and Biotechnology*, *20*(1), 163-168.
138. Saha, NK, & Singh, BK. (1991). Mahua flower agar medium: a new, natural and anti-bacterial culture medium for fungi. National Academy of Science Letter, 14, 359361.
139. Sahu, MK, Suthakaran, S, Ghosh, SC, Singh, D, Das, A, & Jha, H. (2023). Anticancer activity of secondary metabolite isolated from the rhizospheric fungus *Fusarium*

oxysporum isolate-ABRF1,2-propenoic acid, pentadecyl ester. *Asian Journal of Natural Product Biochemistry*, *21*(2).

140. Sarkar M, Sah A, Agarwal P, Joshi S, & Hait S. (2024) Quantification tests for carbohydrate detection. In *Practical Biochemistry* Bentham Science Publishers 32-54
141. Sarkar M, Sah A, Agarwal P, Joshi S, Hait S. (2024) Quantification Tests for Carbohydrate Detection. InPractical Biochemistry Bentham Science Publishers 32-54.
142. Sarkar PK, Sinha A, Das B, Dhakar MK, Shinde R, Chakrabarti A, Yadav VK, Bhatt BP. (2022) Kusum (*Schleichera oleosa* (Lour.) Oken): A potential multipurpose tree species, it's future perspective and the way forward. Acta Ecologica Sinica. 42(6):565-71.
143. Sarkar, N, & Chaterjee, BP. (1984). Structural studies on a polysaccharide of mahua (Madhuca indica) flowers. Carbohydrate Research, 127, 283295.
144. Sen Gupta, S. (1980). The pattern of tree worship and its significance. Folklore, 21(4), 77.
145. Seth PA, Pawar AT, Mote CS, More C. (2021) Antianemic activity of polyherbal formulation, Raktavardhak Kadha, against phenylhydrazine-induced anemia in rats. *Journal of Ayurveda and Integrative Medicine*, 2021;12: 340-345.
146. Sethi c. *Studies on drying characteristics and storage of mahua flower* (doctoral dissertation, orissa university of agriculture and technology, bhubaneswar).
147. Shah S, Ajaz-ul-Islam M. (2024) Mahua (*Madhuca longifolia* L.) Tree-A lifeline for Tribes of Madhya Pradesh. Publish Popular articles, Newsletters, Success stories, Open Access, AgroScience Today, Magazine, e-ISSN: 2583-0147, 5(11):0975-82.
148. Sharafutdinov, IS, Pavlova, AS, Akhatova, FS, Khabibrakhmanova, AM, Rozhina, EV, Romanova, YJ, ... & Kayumov, AR. (2019). Unraveling the molecular mechanism of selective antimicrobial activity of 2 (5 H)-furanone derivative against Staphylococcus aureus. *International Journal of Molecular Sciences*, *20*(3), 694.
149. Sharma K, Kaur R, Kumar S, Saini RK, Sharma S, Pawde SV, Kumar V. (2023) Saponins: A concise review on food related aspects, applications and health implications. Food Chemistry Advances. 2:100191.
150. Sharma N, Kaur M, Gupta Phutela U, Bhatia S. (2024) Characterization of halotolerant microalga isolated from waterlogged habitats: Deciphering the biochemical profiling and unraveling the molecular identity. Journal of Basic Microbiology. 4:2300496.
151. Sharma, NP. (1992). Managing the World Forest: Looking for Balance Between Conservation and Development. Kendall Hunt Publishing Company.

152. Shrirao, AV., Kochar, NL, Chandewar, AV. (2017). Madhuca longifolia (Sapotaceae): A Review of its Traditional Uses and Phyto-Pharmacological Profile. *Research Chronicle in Health Sciences*, 3(4), 45-50.
153. Shrivastava, RK, Sawarkar, SK, & Bhutey, PG. (1970). Decolorization and deodorization studies on mahua extract. Research and Industry, 15, 114117.
154. Singh K, Sharma D, Mishra A. (2021) Mahua flowers (*Madhuca* sp.) utilization as a carbon-rich natural substrate for the cost-effective bench-scale production of fumaric acid. SN Applied Sciences. 1:89.
155. Singh V, Singh J, Kushwaha R, Singh M, Kumar S, & Rai A.K. (2021) Assessment of antioxidant activity, minerals and chemical constituents of edible mahua (*Madhuca longifolia*) flower and fruit of using principal component analysis. *Nutrition & Food Science*, 51(2):387-411.
156. Singh V, Singh J, Kushwaha R, Singh M, Kumar S, & Rai AK. (2020) Assessment of antioxidant activity, minerals and chemical constituents of edible mahua.
157. Singh, A, Sao, P, Tiwari, S. & Patel, Y.K., (2022). Development of Nutri-Cereals Based Mahua (*Madhuca longifolia*) Laddoo.
158. Singh, R, Mishra, BK, Shukla, KB, Jain, NK, Sharna, KC, Kumar, S, Kant, K, & Ranjan, JK. (2013). Fermentation process for alcoholic beverage production from mahua (*Madhuca indica* JF Mel.) flowers. African Journal of Biotechnology, 12(39), 57715777.
159. Singh, S, Mandal, MK, Masih, A, Saha, A, Ghosh, SK, Bhat, HR, & Singh, UP. (2021). 1, 3, 5-Triazine: A versatile pharmacophore with diverse biological activities. *Archiv der Pharmazie*, *354*(6), 2000363.
160. Singh, V, Kumar, S, Singh, J, & Rai, AK. (2018). Fuzzy logic sensory evaluation of cupcakes developed from the mahua flower (*Madhuca longifolia*). Journal of Emerging Technologies and Innovative Research, 5(1), 411421.
161. Singh, V, Singh, J, Kushwaha, R, Singh, M, Kumar, S, & Rai, AK. (2020). Assessment of antioxidant activity, minerals and chemical constituents of edible mahua.
162. Singh, V. (2017). Phenolic content and antioxidant activity of solvent extracts of mahua (*Madhuca longifolia*) flowers and fruit. *International Journal on Nutraceuticals, Functional Foods and Novel Foods*.
163. Sinha J, Singh V, Singh J, Rai AK. (2017) Phytochemistry, ethnomedical uses and future prospects of Mahua (*Madhuca longifolia*) as a food: a review. J Nutr Food Sci. 7(573):2.

164. Smith, TJ, Johnson, CR, Koshy, R, Hess, SY, Qureshi, UA, Mynak, ML, & Fischer, PR. (2021). Thiamine deficiency disorders: a clinical perspective. *Annals of the New York Academy of Sciences*, *1498*(1), 9-28.
165. Song, MX, Deng, XQ, Wei, ZY, Zheng, CJ, Wu, Y, An, CS, & Piao, HR. (2015). Synthesis and Antibacterial Evaluation of (S, Z)-4-methyl-2-(4-oxo-5-((5-substituted phenylfuran-2-yl) methylene)-2-thioxothiazolidin-3-yl) Pentanoic Acids. *Iranian Journal of Pharmaceutical Research: IJPR*, *14*(1), 89.
166. Soni, S, & Dey, G. (2013). Studies on value-added fermentation of madhucalatifolia flower and its potential as a nutrabeverage. International Journal of Biotechnology and Bioengineering Research, 4(3), 215226.
167. Sreenivasa Rao J, Patil PB, Goudar G, Jaleel A, Qadri SS. (2024) Mahua (*Madhuca indica*): indigenous flower as nutrient and phenolic rich food to combat anaemia and associated disorders. Phytochemistry Reviews. 1-21.
168. Stachelek, M, Zalewska, M, Kawecka-Grochocka, E, Sakowski, T, & Bagnicka, E. (2021). Overcoming bacterial resistance to antibiotics: the urgent need–a review. *Annals of Animal Science*, *21*(1), 63-87.
169. Stopiglia, CDO., Vieira, FJ., Mondadori, AG, Oppe, TP, & Scroferneker, ML. (2011). In vitro antifungal activity of dihydroxyacetone against causative agents of dermatomycosis. *Mycopathologia*, *171*, 267-271.
170. Suhane, A, Rehman, A, & Khaira, HK. (2013). Tribological investigation of mahua oil based lubricant for maintenance applications. International Journal of Engineering Research and Applications, 3(4), 23672371.
171. Sutaria, BP., & Magar, NG. (1955a). Chemical constituents of mowrah flowers (B. latifolia). Part I. Proximate composition. Journal of the Indian Chemical Society, 18, 4349.
172. Sutaria, BP., & Magar, NG. (1955b). Chemical constituent of mowrah flowers (B. latifolia) Part II. Vitamins, enzymes and miscellaneous analysis and effect of storage. Journal of the Indian Chemical Society, 18, 5964.
173. Sutaria, BP., & Magar, NG. (1955c). Chemical constituent of mowrah flowers (B. latifolia) Part III. Preparation of syrup and analysis of flowers from various districts. Journal of the Indian Chemical Society, 18, 7680.

174. Talukdar NR, Choudhury P, Barbhuiya RA, Singh B. (2021) Importance of non-timber forest products (NTFPs) in rural livelihood: A study in Patharia Hills Reserve Forest, northeast India. Trees, Forests and People. 3:100042.
175. Teo M, Khoo LW, Chew W. (2024) A simplified small-scale workflow for determination of complete protein-bound amino acids using pre-column derivatization HPLC method. Journal of Food Composition and Analysis. 135:106571.
176. Triandini IG, Wiranto W, Wangiyana IG. (2024) Sensory evaluation of cookies formulated with rhizopora propagules flour using 9-point hedonic scale. Journal of Agritechnology and Food Processing. 4(2):60-74.
177. Verma, N, Jha, K K, Kumar, U, Deepak, K, Singh, NK, Singh, AK, & Sharma, R. (2014). Biological properties, phytochemistry and traditional uses of Mahua (Madhuca longifolia): A review. International Journal of Advance Research and Innovation, 2(3), 630638.
178. Verma, P, Shirin, F, & Verma, RK. (2013). Research article rot disease of Madhuca indica. International Journal of Current Research, 7(6), 1675116754.
179. Vijayakumar, K, & Ramanathan, T. (2018). Antiquorum sensing and biofilm potential of 5-Hydroxymethylfurfural against gram-positive pathogens. *Microbial pathogenesis*, *125*, 48-50.
180. Vimal, OP., & Tyagi, PD. (1984). Agricole publishing academy. In Energy from Biomass, Vol. I, 119120. (pp. 102 and 165).
181. Vu ND, Nguyen VM, Tran TT. (2023) Effects of pH, total soluble solids, and pectin concentration on color, texture, vitamin C, and sensory quality of mango fruit bar. International Journal of Food Science. 1:6618300.
182. Wang B, Dong Y, Fang Y, Gao W, Kang X, Liu P, Yan S, Cui B, Abd El-Aty AM. (2022) Effects of different moisture contents on the structure and properties of corn starch during extrusion. Food Chemistry. 368:130804.
183. Wang X, Zhao Y, Qi X, Zhao T, Wang X, Ma F, Zhang L, Zhang Q, Li P. (2022) Quantitative analysis of metabolites in the aflatoxin biosynthesis pathway for early warning of aflatoxin contamination by UHPLC-HRMS combined with QAMS. Journal of Hazardous Materials. 431:128531.
184. Wang Z, Chen L, Yang F, Wang X, Hu Y, Wang T, Lu X, Lu J, Hu C, Tu H, Xu G. (2024) High-sensitivity profiling of dipeptides in sauce-flavor Baijiu Daqu by chemical

derivatization and ultrahigh-performance liquid chromatography coupled with high-resolution mass spectrometry. Food Chemistry: X. 21:101097.

185. Wani, PA, Omobolanle, LA, Hamid, B, Fayokemi, RA, Perveen, K, Bukhari, NA, ... & Mastinu, A. (2024). Evaluation of destruction of bacterial membrane structure associated with anti-quorum sensing and ant-diabetic activity of Cyperus esculentus extract. *Heliyon*, *10*(14).
186. Watanabe, T, Yano, S, Kawai, T, Jinbo, Y, & Nonomura, Y. (2021). Selective antibacterial activity of palmitoleic acid in emulsions and other formulations. *Journal of Surfactants and Detergents*, *24*(6), 973-979.
187. Watkins, P. (2023) The use of chloroform to petroleum ether for Soxhlet extraction of fat in meat was compared. *Animal Production Science*, *63*(14): 1445-1449.
188. Wiciński M, Liczner G, Cadelski K, Kołnierzak T, Nowaczewska M, & Malinowski B. (2020) Anemia of chronic diseases: wider diagnostics—better treatment?. *Nutrients*, *12*(6: 1784.
189. Xue Q, Fan H, Li K, Yang L, Sun L, Liu Y. (2017). Comparative evaluations on phenolic antioxidants of nine adulterants and anti-inflammation of four alternatives with their original herb *Erycibe schmidtii*†. RSC Advances. 7: 51151-51161.
190. Yadav AK, Rawat D, Dubey K. Mahua's Medicinal Marvels: (2024) Bridging Traditional Knowledge and Ethnomedicine. Journal of Advancement in Pharmacognosy. 4(1).
191. Yadav, A, Kumar, N, Upadhyay, A, Sethi, S, & Singh, A. (2022). Edible coating as postharvest management strategy for shelf-life extension of fresh tomato (Solanum lycopersicum L.): An overview. *Journal of Food Science*, *87*(6), 2256-2290.
192. Yadav, AK, Rawat D, & Dubey, K. (2024). Mahua's Medicinal Marvels: Bridging Traditional Knowledge and Ethnomedicine. *Journal of Advancement in Pharmacognosy*, *4*(1).
193. Yadav, P, Garage, N, & Diwedi, DH. (2009). Standardization of pre-treatment conditions for mahua wine preparation. Journal of Ecofriendly Agriculture, 4(1), 8892.
194. Yadav, P, Singh, D, Mallik, A, & Nayak, S. (2012). Madhuca longifolia (Sapotaceae), a review of its traditional uses, phytochemistry and pharmacology. International Journal of Biomedical Research, 3(7), 291305.

195. Yadav, S, Suneja, P, Hussain, Z, Abraham, Z, & Mishra, SK. (2011). Prospects and potential of Madhuca longifolia (Koenig) J.F. Macbride for nutritional and industrial purpose. Biomass and Bioenergy, 35(4), 15391544.
196. Yi, HS, Ahn, YR, Song, GC, Ghim, SY, Lee, S, Lee, G, & Ryu, CM. (2016). Impact of a bacterial volatile 2, 3-butanediol on Bacillus subtilis rhizosphere robustness. *Frontiers in microbiology*, *7*, 993.
197. Yu, X, Zhao, M, Liu, F, Zeng, S, & Hu, J. (2013). Identification of 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one as a strong antioxidant in glucose–histidine Maillard reaction products. *Food research international*, *51*(1), 397-403.
198. Zhu L, Lo K. (2021) Non-timber forest products as livelihood restoration in forest conservation: A restorative justice approach. Trees, Forests and People. 6:100130.

LIST OF PUBLICATIONS

1. **Mishra M** and Naik PK. Antianemic activity of mahua flower concentrate and mahua laddoo against 2,4-dinitrophenylhydrazine-induced anemia in a rat model. Int J Pharm Sci & Res 2025, 16(7):1000-10.
2. **Mishra M**, Pattnaik S, Singh H, Naik PK. Exploring the role of Mahua as a functional food and its future perspectives. InRecent Frontiers of Phytochemicals 2023 Jan 1 (pp. 109-121).
3. **Mishra M**, Pattnaik S, Naik PK. Aquatic Biofilms and Their Role in Biogeochemical Cycling. InAquatic Ecosystems and Microbial Biofilms (pp. 133-154).
4. Hota SS, Sahoo DR, Pattnaik S, **Mishra M**, Mishra T, Sahu T, Naik BB, Kalyankumarraju M, Dixit AK, Lamba D, Kumar YR S. Antioxidant, antibacterial and antibiofilm activities of Pittosporum napaulense (DC.) against drug-resistant Staphylococcus aureus and Shigella sp. Natural Product Research. 2024 Sep 27:1-9.
5. Cheruvanachari P, Pattnaik S, **Mishra M**, Pragyandipta P, Pattnaik A, Naik PK. Deciphering the antibiofilm potential of 2-Phenylethyl methyl ether (PEME), a bioactive compound of Kewda essential oil against Staphylococcus aureus. Microbial Pathogenesis. 2023 Jun 1;179:106093.
6. Cheruvanachari P, **Mishra M**, Pattnaik S, Naik PK. Determination of antibacterial and anti-biofilm potential of Kewda essential oil against Staphylococcus aureus and Klebsiella pneumoniae. Biologia. 2023 Aug;78(8):2253-60.
7. Cheruvanachari P, **Mishra M**, Pattnaik S, Naik PK. Determination of antibacterial and anti-biofilm potential of Kewda essential oil against Staphylococcus aureus and Klebsiella pneumoniae. Biologia. 2023 Aug;78(8):2253-60.
8. Pattnaik S, **Mishra M**, Naik PK. Computational Approaches for the Inhibition of ESKAPE Pathogens. InESKAPE Pathogens: Detection, Mechanisms and Treatment Strategies 2024 Mar 30 (pp. 503-544). Singapore: Springer Nature Singapore.
9. Pattnaik S, **Mishra M**, Naik PK. Phytochemicals as Potential Antibacterial Agents Against ESKAPE Pathogens. InESKAPE Pathogens: Detection, Mechanisms and

Treatment Strategies 2024 Mar 30 (pp. 379-419). Singapore: Springer Nature Singapore.

10. Pattnaik S, **Mishra M**, Naik PK. Alternative Strategies for Combating Antibiotic Resistance in Microorganisms. InAntimicrobial Photodynamic Therapy 2024 (pp. 65-109). CRC Press.
11. Pattnaik S, **Mishra M**, Singh H, Naik PK. Novel perspectives on phytochemicals-based approaches for mitigation of biofilms in ESKAPE pathogens: recent trends and future avenues. Recent Frontiers of Phytochemicals. 2023 Jan 1:433-54.

LIST OF CONFERENCES

- **Mishra, M**., & Naik, P.K. Development of value added food products from Mahua Flower Concentrate in Odisha Reasearch Conclave (ORC-2022).
- **Mishra, M**., & Naik, P.K. Anti-anemia effect of standardized extract of Mahua Juice Concentrate on 2,4-phenylhydrazine induced anemia in wistar rats in Odisha Reasearch Conclave (ORC-2023).
- **Mishra, M**., & Naik, P.K. Exploring the Antibacterial and Antibiofilm Efficacy of Mahua (*Madhuca Indica*): A Phytochemical and Biological Investigation in Emerging Trends in Biotechnolog & Bioinformatics (ETBB-2024).
- **Mishra, M**., & Naik, P.K. Comprehensive Nutritional and Phytochemical Profiling of Fresh and Dry Mahua Flowers (*Madhuca indica*) in Odisha Reasearch Conclave (ORC-2024).
- **Mishra, M**., & Naik, P.K. Exploring the Therapeutic Potential of Mahua (Madhuca Indica) Flowers: Antibacterial, Antibiofilm, and Nutritional Insights from Fresh and Dry Forms in Prospects in Biotechnology (PBB-2025).