



## Formulation and Process Development of Biofuel and other products from Mahua (*Madhuca latifolia* L.)

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### ABSTRACT

The genus *Madhuca*, belonging to the family of Sapotaceae, is a multifunctional tree and is considered a boon by the tribal's who are forest dwellers and keenly conserve this tree. Mahua flower, seed, fruits and their products are used as a food, fodder and are a non-conventional ingredient in carp feedas well as used as an exchanger in tribal and rural areas. Mahua seeds are rich in edible oil so they have economic importance. Mahua seed oil proved to be cheap and suitable substrate for the production of biofuel. The bioethanol production from Mahua flowers could be increased by developing the traditional methodology, as it is expected to benefit the people of tribal areas in India as well as the bioenergy demand in world in the long run. Flowers have been reported to have various bioactivities and ethno-medicinal uses. Mahua flowers are known to play an immense role in the preparation of valued added food products (biscuit, cake, laddu, candy, bar, jam jelly, sauces etc).

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### 1. Introduction

Mahua (*Madhuca* sp.) trees are widely distributed in the forests of the Australian and Asian continents (Gupta *et al.*, 2012). It is a major tree in the tropical mixed deciduous forests of West Bengal, Bihar, Odisha, Madhya Pradesh, Punjab and Uttar Pradesh as well as the sub-mountainous region of the Himalayas in India (Gupta *et al.*, 2012; Sinha *et al.*, 2017) since it is adapted to arid situations and produced in excess of a million tonne annually (Gupta *et al.*, 2012; Bakhara *et al.*, 2016). In a report, Bakhara *et al.* (2016) reported that Mahua has been widely planted in the Deccan Peninsula and Northern India's plains, and it is usually propagated by seeds (Gupta *et al.*, 2012; Bakhara *et al.*, 2016). Mahua flower is one of the most important Non-timber forest products (NTFP), playing a major role in the tribal economy (Bakhara *et al.*, 2016). Its many parts have been used to make food, fuel, fertiliser, cattle feed and oil (Ramadan *et al.*, 2016; Behera & Ray, 2019).

In many states, cooperative corporations, purchase flowers at a minimum support price to protect collectors from the middleman's exploitation. However, the majority of the flowers degrade/get decomposed in the government go downs because to a lack of adequate post-harvest processing technology (Bakhara *et al.*, 2016). Value added food products from Mahua have been modified or enhanced to have a higher market value and/or a longer shelf life. Different marketable value-added food products like dried flower, candied flower, glazed flower, Mahua bar, Ready-to-Serve beverages (RTS), squash, jam, laddu and cake were prepared and the products were well appreciated by the consumers (Bakhara *et al.*, 2016). In this project, specified marketable products such as Mahua powder, candy/toffee/lollipop, laddu and biscuits/cake, soft drink/cold drinks added ethnomedicinal values (antidiabetic, anticarcinogenic, anti-inflammatory, antimicrobial, etc.) has been prepared for the consumers (Badukale *et al.*, 2021).

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Mahua has helped to improve the socioeconomic conditions and rural livelihoods of these communities (Behera & Ray, 2019). In addition, it has been used to better understand the relationships between various socioeconomic factors (location, wealth status, gender, education, and seasonality) that affect the degree of dependence on Mahua, in various districts and agro ecological zones of India.

## 2. Opportunity

Economic security accompanied by food security can be provided to the tribal by exploring the opportunities of Mahua plant (Nayak & Sahoo, 2020). However, various medicinal and functional properties have been accorded to the various parts of the tree. According to several researches, there is a need for strategy for the harvesting of flowers with appropriate value addition; this is a serious attempt to provide information on the various food products and edible uses of flower, fruit and seed of Mahua tree targeting it to provide prominent raw materials to the industry (Nayak & Sahoo, 2020). Mahua flowers can be seen as sugar replacers and formulated to form various functional foods. Mahua fruit can be minimally processed to be available as a vegetable source. Mahua seed oil provides array of applications as butter replacer or blending oil. There is a need to boost Mahua in our economy as the tree is a boon for the tribal across Indian forests (Behera & Ray, 2019).

## 3. Process Development of Biofuel

The quest for new and inexpensive carbohydrates sources for the development of bioethanol and biodiesel is gaining traction around the world. Mahua flowers and seed oil are an alternative forest biomass to develop bio-refinery production (Banerjee and Samanta, 2018; Bai *et al.*, 2021).

### 3.1. Bioethanol from Mahua Flower

The recent studies have demonstrated that Mahua flowers can serve as a renewable feedstock for commercial bio-ethanol production (Swain *et al.*, 2007; Mohanty *et al.*, 2009; Behera *et al.*, 2010a; 2010b; 2012; Behera *et al.*, 2016). Mahua flowers are a natural, non-food quality, low-cost carbohydrate substrate found in non-agricultural environments or forest ecosystem that can be used to make bioethanol instead of food-grade sugar/starchy crops like maize and sugarcane (Behera *et al.*, 2016). Mohanty *et al.* (2009), *Saccharomyces cerevisiae* used solid state fermentation (SsF) to produce ethanol from Mahula flowers. The maximum ethanol concentration (225.0 g/Kg flower) was obtained after 72 hours of fermentation and found to be optimal at ideal parameters (70% moisture, pH 6.0, and 30°C). Batch fermentation of Mahula flowers was carried out by Behera *et al.* (2010) using immobilised cells (in agar

agar and calcium alginate) and free cells of *S. cerevisiae*. Using immobilised (in agar agar and calcium alginate) and free cells, the ethanol yields were found 151.2, 154.5, and 149.1 g/Kg flowers, respectively. Furthermore, the immobilised cells remained physiologically active for at least three ethanol fermentation cycles without reducing productivity. In another study, Behera *et al.* (2010a) reported the bioethanol production from mahula flowers using immobilised *S. cerevisiae* and *Z. mobilis* in calcium alginate beads. Using immobilised (calcium alginate) *Z. mobilis* and *S. cerevisiae* cells the ethanol yields were reported to be 154.5 and 134.55 g/Kg flowers, respectively. Gedela *et al.* (2016) described a fermentation process using *S. cerevisiae* to produce bioethanol from Mahua flowers. The presence of bioethanol and the percentage of bioethanol were verified using a spectrophotometer (204–240 nm) and an alcohol metre, respectively. The results revealed that 1000 mL of acidic fermented media (pH-4, 5 and 5.7) contained 170.03, 142.3, and 27.7 mL of bioethanol in test-1 (*S. cerevisiae* + Mahua flowers + media), test -2 (Mahua flowers + media), and control (media) respectively. The development of first generation biofuels (bioethanol) from Mahua flowers enriched with high amounts of fermentable sugars was reported by Banerjee and Samanta (2018). For the development of bioethanol from Mahua flower extract, the fermentation method (batch and fed-batch) used yeast strain *S. cerevisiae*-3078 culture. From fresh and 6-month-stored Mahua flowers, the maximum yields of ethanol (33 °C and pH 5.7) after 14 days were found to be 18 and 15% (using batch) and 22 and 16% (using fed-batch fermentation), respectively. Agrawal *et al.* (2019) used a newly isolated strain of *Pichia kudriavzevii* from milk whey to produce bioethanol from Mahua flowers. Using Mahua flowers as a substrate, the isolated yeast was tested for fermentation capacity using a carbohydrate fermentation test. Optimizing operational parameters (fermentation time of 48 hours, temperature of 25°C, and pH of 5.0) resulted in a maximum ethanol yield of 371 g/Kg from the flowers.

### 3.2. Biodiesel from Mahua seed Oil

Biodiesel (fatty acid alkyl ester) has the following advantages to meet the future energy demands; it is biodegradable, environmentally safe, non-toxic, and sustainable (Puhan *et al.*, 2005; Kumar *et al.*, 2018). Biodiesel is made from animal fats, vegetable oils, recycled cooking greases, and waste plastics all over the world. Globally, edible oils such as sunflower, peanut, canola, palm, coconut, and other oils account for 95% of biodiesel output. Owing to the impending scarcity of food crops, these biodiesel productions are not advantageous from edible oils (Atabani *et al.*, 2012; Ansari *et al.*, 2022). It should be obvious to

researchers that they look for advantageous biodiesel sources and concentrate on feedstocks that do not include food crops. Mahua oil is used to keep the cost of vehicle fuel low and stable. As a result, non-edible Mahua oil was chosen as one of the key alternatives to diesel oil (Karmakar *et al.*, 2010; Singh and Singh, 2010; Ansari *et al.*, 2022).

Transesterification can be used to make biodiesel from any Mahua seed oil. Ghadge and Raheman (2005) developed a method for producing biodiesel from Mahua oil (*M. indica*), which contains a high amount of free fatty acids (FFA). The pre-treatment process was reduced the high FFA content of Mahua oil with a methanol-to-oil ratio (0.30-0.35 v/v) in the presence of H<sub>2</sub>SO<sub>4</sub> (1%, v/v) as an acid catalyst. Mahua biodiesel fuel properties were found to be similar to those of diesel, and it met both American and European standards. Kumar *et al.* (2018) reported that the Mahua seed oil was trans-esterified with methanol using acid and alkaline catalyst process to obtain Mahua methyl ester (MME). MME was examined for its chemical composition and physical properties. The performance, combustion, and emission of a computerised single cylinder CI engine fuelled with diesel and B20 (20% vol. of MME biodiesel + 80% vol. diesel) were investigated in experimental tests. The B20 fuel out performed the baseline diesel in terms of efficiency at lower partial loads, combustion, and emissions. The combination of B20 with smaller orifice NHD has shown appreciable results in performance, combustion and emissions. But, the only drawback was NO<sub>x</sub> is found to be increased.

Since Mahua oil contains a high amount of FFA, it is difficult to turn it into biodiesel using a chemical catalyst. Kumari *et al.* (2007) used a commercial preparation of lipase from *Pseudomonas cepacia* as a catalyst for transforming Mahua oil into ethyl esters. Biocatalyst formulations such as cross-linked enzyme aggregates (CLEAs) and protein-coated micro catalysts (PCMCs) produced the best performance. Using 50 mg of lipase, free enzyme powder converted to 98% in 6 hours, CLEAs converted to 92% in 2.5 hours, and PCMCs converted to 99% in 2.5 hours after process optimization. It was also reported that upon further optimization with PCMCs, a more economical and efficient process design would be possible.

Use of non-edible Mahua oil (MO) as fuel in agricultural diesel engine will improve rural economy, sustainability and increase the environmental benefits (Kapilan *et al.*, 2009). They reported that the flash point, density and viscosity of the MO are higher than the diesel, but it has lower calorific value. Mixing diesel with the MO reduces the viscosity of the blends of MO and diesel. From the analysis, it was claimed that the MO can be partially

substituted for diesel oil in the diesel engine, without making any modification in the hardware of the engine.

The effect of injection opening pressure (IOP) for 20% blend (B20) of Mahua oil methyl ester (MOME) and 22.5 litres per minute (lpm) of hydrogen dual fuel mode was investigated by Syed *et al.* (2017). A single cylinder, four stroke, direct injection (DI) diesel engine (3.3 Kw, 1500 rpm) was studied for its efficiency, combustion, and emission characteristics. The maximum brake thermal efficiency, minimum brake specific fuel combustion, and lowest HC, CO, and smoke emissions with increased NO<sub>x</sub> concentrations were obtained at IOP of 250 bar for B20-hydrogen dual fuel mode (Syed *et al.*, 2017).

Pradhan *et al.* (2017) investigated the processing, characterization, and potential applications of Mahua oilseed bio-oil. The process of pyrolysis was carried out between 450°C and 600°C in a semi-batch style reactor for the development of Mahua pyrolysis oil (MPO). At an optimum temperature of 525 °C, the MPO yield was found to be about 50%. The MPO was also characterized for its suitability as an alternative fuel for internal combustion engines.

The efficiency and emission characteristics of a direct injection (DI) diesel engine with cerium oxide nanoparticles additives in diesel and biodiesel blends were studied by Seela *et al.* (2019). Transesterification was used to make Mahua methyl ester, which was then mixed with diesel. According to the findings, the thermal efficiency of B20 + 100 ppm cerium oxide brakes increased by 1.8, with a 1% reduction in real fuel consumption. Hydrogen and carbon monoxide emissions were lower than with diesel fuel.

Vijay Kumar *et al.* (2019) investigated two methods of transesterification to produce Mahua methyl ester (MME) from raw Mahua seed oil. The physical properties of the samples were compared to the ASTM D-6751 requirements. In an unmodified diesel engine, the obtained MME and its blends of B20, B40, B60, and B80 were investigated. It was observed that brake-specific fuel consumption and thermal efficiency are slightly improved (B20 and B40) at part-load conditions and approach diesel at full-load conditions based on the results of the performance. From combustion analysis, it was found a shorter ignition delay for biodiesel and its blends compared with the diesel fuel. Both of the fuel blends reduced carbon dioxide, hydrocarbons, and smoke opacity emissions, but at high temperatures, they undergo an endothermic reaction that produces various nitrogen oxides.

Bai *et al.* (2021) investigated the production of methyl ester from Mahua oil through esterification and transesterification. The methyl ester of Mahua oil was

prepared under a variety of conditions (methanol to oil molar ratio, concentration of catalyst, effect of temperature, and reaction time). The results shown that 6:1 liquor to oil molar proportion with 1% KOH impetus, 60 °C response temperature and time 120 min gives 92% ideal yield are the optimum conditions for esterification. The ASTM standards were used to compare the different fuel properties of Mahua oil methyl ester. To recognise and confirm the existence of fatty acids methyl esters, GCMS and FTIR analysis were used. The Mahua oil was found to be a possible raw material for the methyl ester. It is affordable and could become a viable renewable fuel in the near future.

The reduction of thermal efficiency by Mahua mixed biodiesel has major challenge (Ansari *et al.*, 2022). They reported the performance and emission characteristic of Mahua as blended biodiesel. The natural diesel blended with the proportion of starting increasing from (5 to 20 %) of Mahua seeds oil and starting reduction from (95 to 80 %) of natural diesel oil. The diesel engine was operated on the basis of different Mahua seeds oil quantity with natural diesel, and it was observed the highest thermal efficiency of blended biodiesel were at (20 %:80 %) of Mahua seeds oil and natural diesel oil respectively.

#### 4. Process Development of value - added food products

A good source of value-added products can be made from the Mahua flowers, fruits and seed oil (Dwivedi *et al.*, 2022).

#### 4.1. Nutritional Value

Sugar profiling of Mahua flower revealed that it is a rich source of both reducing sugar (48–75%) and non-reducing sugars (3–18%) such as inositol, sorbitol, dextrose, fructose, sucrose, raffinose, and maltose (Singh *et al.*, 2020; Dwivedi *et al.*, 2022). The nitrogen content of the flower varies from 0.65-1.1% being apparently higher in the younger than in the flowers varies from 4.4-7%. Similarly, the fat content of dry flowers varies from 0.09-1.3%. Calcium, phosphorus, iron, potassium, sodium, and magnesium are among the several minerals found in the flowers (Pinakin *et al.*, 2020). Adequate quantity of other vitamins, including as thiamine, riboflavin, niacin, folic acid, and ascorbic acid, are also found in the flowers. Enzymes like amylase, maltase, invertase, catalase and oxide was detected due to the high rate of degradation of the vitamin c content in the storage experiments (Bakhara *et al.*, 2016; Singh *et al.*, 2020; Dwivedi *et al.*, 2022).

#### 4.2. Food Value

The juice extracted from the Mahua flower can be used as a natural sweetener in a variety of foods, including biscuits, cookies, cakes, jam, jelly, juice, and squash (Figure-1). Furthermore, Mahua flowers are commonly used by locals to make alcohol and are processed by many locals for use as a food source in the winter (Singh *et al.*, 2020; Dwivedi *et al.*, 2022). There exists a tremendous scope for development and value addition of Mahua flower for economic development of the tribal community (Dwivedi *et al.*, 2022).



Figure 1. Value added food products from Mahua flowers

## 5. Other developed Products

In addition, to value added food products, the other marketable value products like Mahua oil cake has been prepared from Mahua flower which has been well appreciated by the consumers. Mahua deoiled cake (MDC), a by-product obtained during extraction of oil from Mahua seed is a plentiful resource of protein, nitrogen, lipids and carbohydrate etc. (Gupta *et al.*, 2012). MDC is widely used in several industrial applications such as biogas production, biotechnological application for mushroom cultivation, lipase production or other biochemical production (Ramachandran *et al.*, 2007; Biswal *et al.*, 2019). MDC is also used as substitute for protein hydrolysates during the treatment of protein malnutrition for animals (fish, cattle etc.) (Biswal *et al.*, 2019).

### 5.1. Mahua Oil Cake in Aquaculture

Feed is the most important factor that significantly affects aquaculture production and profitability. Aquaculture's net revenue would grow if feed costs is reduced and techniques that promote nutrient efficiency has implemented (Rath *et al.*, 2017). Mahua oil cake (MOC) is one of the non-conventional ingredients which are generally used as manure for horticultural crops. MOC is rich in protein (24%) and energy (19.0 KJ/g) with high levels of fatty acids comprising saturates (45%), monoenes (42%) and polyunsaturated fatty acids (PUFA, n-6) (7%) (Rath *et al.*, 2017). They reported that MOC added to the diet of rohu (*Labeorohita*) fingerlings at a rate of 300 g/Kg and caused without having any negative effects on growth, survival, or nutrient uptake. However, Mahua oil cake can also be used as cheap source of nutrient for fish replacing conventional plant ingredient by solid state fermentation (SsF) (Gupta *et al.*, 2018; Das *et al.*, 2022). Gupta *et al.* (2018) reported the de-oiled Mahua seed cakes, via solid state fermentation (SsF), producing proteases and cellulases as value added products. The SsF provided a suitable method to degrade their anti-nutritional factors, along with producing enzymes as value added products. The detoxified cakes can be further employed as quality manure and/or animal feed with improved digestibility.

The SsF of Mahua oil cake was mixed strain of microbes (*Sachharomyces cerevisiae* and *Bacillus subtilis*) which improved the quality of Mahua oil cake was reported by Das *et al.* (2022). The increase in crude protein, decrease in crude fibre and decrease in anti-nutritional factors like total tannin and saponin was claimed in SsF mixed Mahua oil cake, without interfering growth, digestibility and immunological parameters in rohu (*L. rohita*) fingerlings (Das *et al.*, 2022). Mahua oil cake act as a major piscicide

and the extract possess saponins, which are poisonous and are used for fish killing (Rajput & Gaur, 2015). More recently, Saha and Saha (2022) reported the use of Mahua oil cake (MOC), as a piscicide in pre-stocking pond preparation for composite fish culture.

### 5.2. Essential oil from Mahua flowers

The corolla of flower is fleshy, and cream colored having characteristics odor and source of essential oil (Suryawanshi & Mokhat, 2019). Suryawanshi & Mokhat (2019) isolated the essential oil Mahua flowers and identified its chemical compositions. The chemical composition of isolated essential oil was investigated by using gas chromatography coupled with mass spectrometry (GC/MS). The isolated essential oil was found to be abundant in sesquiterpenes and could be a good source/precursors of Farnesol and Farnesene. Further studies are needed to investigate the biological activities of this essential oil.

### 5.3. Mahua flowers as Carbon source

#### 5.3.1. Bacterial polyhydroxyalkanoate (PHA)

Mahua flowers are an organic substrate that are about 60% sugar and also include organic acids that are necessary for the synthesis of copolymers (Anil Kumar *et al.*, 2007). Anil Kumar *et al.* (2007) reported that the utilization of Mahua flowers, as a carbon source for bacterial (*Bacillus* sp-256) fermentation intended to produce polyhydroxyalkanoate (PHA). In PHA production medium, nearly 50% of the cost is due to carbon sources such as sugar and organic acids. This can be economized by using industrial by products or natural substrates. Mahua flower can be viewed as a less expensive source of carbon for the production of PHA copolymers, second only to molasses.

#### 5.3.2. Synthesis of Fumaric acid

6. The use of commercial carbon sources, which are expensive, is the main cost factor affecting the manufacture of fumaric acid. By exploiting naturally occurring, less expensive substrates rich in carbon, production costs can be decreased (Singh *et al.*, 2021). Singh *et al.* (2021) reported the utilization of Mahua flowers as a cheaper carbon source and low cost production medium for cost-effective fumaric acid production using *Rhizopus oryzae*. The fumaric acid production using Mahua (24.1 g/L) was comparable to the fumaric acid obtained from glucose. The result claimed that the feasibility of low-cost natural substrate for cost-effective fumaric acid production using *R. oryzae*.

## 7. Scope for Economic Development

Mahua-based products have given numerous opportunities rural economies and are regarded as an

important source of income for tribal people, particularly in India. Mahua-based products are a major source of basic necessities for a sizable portion of India's rural population. A large number of rural populations are dependent on the Mahua based value added food products for fulfilment of their basic needs (Nayak & Sahoo, 2020). From an economic stand point, the oil yield is always the main consideration when determining if a plant is suitable for industrial use. The seeds of Mahua are among those that are underutilised for oil production, as is typical with many other seeds from tropical fruits. This can be because there is not enough technical knowledge about its characteristics and possible applications (Ramadan *et al.*, 2016).

## 8. Conclusion and Future Prospective

The Mahua plant plays a crucial role in the tribes' way of life and serves as one of their most important and main sources of food. The Mahua products, however, are not widely marketed or used. It was observed that tribal dwellers are selling the Mahua flowers and seeds to local markets or intermediaries due to lack of post-harvest management and not having the potential for restoration and lack of access to market. The price of Mahua flowers and their products is higher in metropolitan and global markets than in the local markets. However, the most advantages from Mahua based products for local communities can be achieved by using participatory community-based development initiatives. Taking proper care of the flowers in the post-harvest phase to maintain the quality and preparing different value added food products from it in small scale could contribute for improving the economy of tribal people.

Expanding the market for Mahua flowers, diversifying the uses of Mahua flowers and seeds, building vital infrastructures for Mahua flower processing, developing the skills of Mahua flower and seed collectors, and planting high-yielding Mahua trees are some suggested actions that could be taken to improve the rural economy, particularly for those who live in the forest.

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