Phytochemical Screening, Total Phenolic contents, and Fatty Acid Composition of Litsea monopetala (ROXB.) Seeds

[1] Assistant Professor Dr. Shraddha Pandey, [2]Retd. Head of Department Dr. Kiran Vajpai, [3]Assistant Professor Dr. Nisha Tiwari

[1]Rishi Ms Institute Of Engineering & Technology For Women, Hyderabad
[2,3]Department of Chemistry Govt. Bilasa Girls P.G. College, Bilaspur

ABSTRACT: Studying the seeds for their chemical properties, especially in the forest regions of Chhattisgarh, is a valuable research endeavour. Such studies can contribute to our understanding of the chemical composition of the seeds and their potential applications, both in traditional medicine and various industrial sectors. The aim of this paper is to study the phytochemical properties along with Phenolic content & fatty acid profile of the seeds of Litsea monopetala (ROXB.) found in the forest region of Chhattisgarh. Popularly known Meda of Litsea monopetala (ROXB.) has been part of many traditional medicinal works found in literature in India. The oil obtained from the seeds of Litsea monopetala (ROXB.) is Redish Brown in color and was about 32.75 % of the weight. Phytochemical analysis of the seeds shows that refractive index of the seed is 1.44, Specific Gravity around 0.855, Saponification value of 28.05, Iodine value found was around 6.34 (gm/100gm), Acid Value of 13.07 (mg/g) & Free Fatty Acid content of around 6.53 %(mg/g). Ultimate analysis on the seed shows that the content percentage of Carbon is 48.44, Hydrogen is 6.66, Nitrogen is 6.42 and Sulphur is 0.3. Fatty acid analysis of the seeds shows that the content of Lauric acid (C12:0) & Capric acid (C10:0) was highest among the saturated fatty acid having values of 71.57 and 18.92 respectively. Lauric acid possesses antimicrobial property and can be affective against Acne (Nakatsuji, et al., 2009). In unsaturated fatty acids the content of Oleic acid was found to be the highest of around 4.05. Oleic acid a monounsaturated omega-9 fatty acid is considered heart-healthy and is associated with various health benefits. The seed were analysed for the presence of phenolic compounds like Flavonoids and Total phenol. The quantity of Flavonoids and Total Pheonl was found to be 322 mg of QE/100 gm and 139.13 mg of GAE/100 gm.
[Assistant Professor Dr. Shraddha Pandey, Retd. Head of Department Dr. Kiran Vajpai, Assistant Professor Dr. Nisha Tiwari. **Phytochemical Screening, Total Phenolic contents, and Fatty Acid Composition of Litsea monopetala (ROXB.) Seeds.** *Biomedicine and Nursing* 2026;12(2):1-5]. ISSN 2379-8211 (print); ISSN 2379-8203 (online). http://www.nbmedicine.org. 01. doi:10.7537/marsbnj120226.01

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These findings contribute valuable information about the chemical composition of Litsea monopetala (ROXB.), paving the way for potential applications in pharmaceuticals, traditional medicine, and other industrial sectors.

Introduction

The broad spectrum of phytochemicals present in plants has attracted considerable scientific attention owing to their potential health advantages and therapeutic uses (Imran, et al., 2024). Phytochemicals are bioactive compounds synthesized by plants to protect themselves from environmental stressors and pathogens. These compounds demonstrate a diverse array of biological functions, encompassing antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, rendering them indispensable for human health (Vishwakarma, Arora, & Dhobi, 2020). Understanding the phytochemical composition of plants is crucial for harnessing their therapeutic potential and exploring their role in disease prevention and treatment. In this paper, we delve into the multifaceted world of phytochemicals, examining their classification, biosynthesis, and biological activities. Through a comprehensive analysis of the current literature, we aim to shed light on the significance of phytochemicals in promoting health and well-being, emphasizing the importance of integrating plant-based remedies into modern medicine.

Native to South and East Asian countries like India, Myanmar, Thailand, and to China Litsea monopetala belongs to the family Lauraceae (Platform, 2024). This family is distributed across warm and tropical climates. The tree is evergreen and grows up to 18 m. The leaves alternate in pattern with a petiole of 1-3 cm, leaf blade can be ovate or obovate with 4-12 cm of length. Its bark surface is longitudinally fissured dark greyish, and its inner brown is mottled. Flowers in peduncled umbellules in short racemes, with (4-)6 tepals and 9- 12 stamens. Fruit oblong to ellipsoid, 0.7-1.2 cm long, seated on a small flat perianth cup. The fruit has a smooth & shiny seed coat and is inserted in a cup 5 mm in diameter (Orwa, Mutua, Kindt, Jamnadass, & Anthony, 2009).

The wood of the tree is used for furniture, tool handles, and planks. Muga silkworms' principal food can be the leaves of Litsea monopetala. Also, it is used as fodder in some places in Nepal. The seed contains oil of up to 30% and can be used for industrial oil. The leaves also have medicinal properties and can be used to cure arthritis and cosmetics. It also possesses some antimicrobial properties.

Common names: Meda, Kutmiro, Ghian, Singran, Muga,

Figure 1:Picture of seeds of Litsea monopetala (Roxb.) (Koley, 2015)

Materials and Methods

Seed samples were gathered from the woodland region of Chhattisgarh, then meticulously cleansed of dirt and extraneous matter, followed by manual removal of the husk shell. Subsequently, the seeds underwent grinding in a mixer grinder for analysis purposes.

Oil Extraction

Oil was extracted from Litsea monopetala (Roxb.) seeds using a Soxhlet extractor with petroleum ether as the solvent. A total of 239 grams of pulverized seeds were placed in the Soxhlet extractor with petroleum ether for approximately 10 hours, during which the boiling point ranged between 60-80°C. Excess solvent was subsequently removed through a rotary evaporation procedure. The oil yield was calculated, and the extracted oil was stored in a refrigerator for further analysis (Nibe, Sable, & Hinge, 2023)

Physicochemical Analysis

The extracted yellow oil yielded 32.75%. Subsequently, the oil underwent analysis for iodine value, saponification value, acid value, free fatty acid, and unsaponifiable matter using the standard method outlined in AOAC (A.O.A.C, 2005).

The refractive index of the oil, which increases with the rise in unsaturation and depends on the chain length of fatty acids, was determined slightly above room temperature using an Abbe refractometer (fssai, 2005)

To assess the density of the oil, the specific gravity was determined at a temperature slightly above room temperature using a specific gravity bottle (fssai, 2005).The results of the Physico-chemical analysis are captured in **Table *1***.

Assessing the physico-chemical parameters of seed oil is essential for determining its suitability for consumption, utilization, and application across different domains. These parameters also aid in determining whether the seed can be utilized for the production of high-grade oil.

Table 1:Physico-Chemical characteristics of oil

Properties	Value
Color	Reddish Brown
Oil Content (%)	32.75
Refractive Index	1.44
Specific Gravity	0.855
Saponification Value(mg KOH/gm)	28.05
Iodine Value (gm/100gm)	6.34
Acid Value(mg/g)	13.07
Unsaponifiable Matter	36.64
Free Fatty Acid (%)	6.53

Fatty Acid composition of seed oil

Following the trans-esterification process, fatty acids were analysed using a Gas Chromatograph (7890B by Agilent Technologies) equipped with a flame ionization detector and an Agilent – DBFFAP column (nitroterephthalic-acid-modified polyethylene glycol of high polarity for the analysis of volatile fatty acids) (Rosa, Maccioni, & Maxia, 2020), (Danish & Nizami, 2019). Calibrating the Gas Chromatograph for fatty acids required maintaining the column temperature at an initial temperature of 120°C for 5-6 minutes, then raising it to 240°C at a rate of 4°C/min. Nitrogen was used as the carrier gas at a column flow rate of 1.0 ml/min, and the detector temperature was maintained at 280°C. Standards used were 47885-U Supelco® 37 Component FAME Mix, 10 mg/mL in methylene chloride. Individual trans-fatty acid standards, such as Supelco trans-9-Eliadic methyl ester, 10 mg/ml in heptane, trans-9, 12-Octadecadienoic (linoleliadic) methyl ester, and trans-11-Vaccenic methyl ester, were employed. The sample's fatty acid composition was compared with the standard fatty acid composition, and percentages were calculated by normalizing peak areas. The results of the fatty acid analysis are shown in ***Table 2* &**

Table 3. Investigating the fatty acid composition of seed oil proves valuable across various domains, including nutritional assessment, health implications, quality evaluation, functional properties, and biotechnological applications.

Table 2:Saturated Fatty acid composition of oil

Saturated	Area (%)

Caprylic Acid (C8:0)	0.19
Capric acid (C10:0)	18.92
Lauric acid (C12:0)	71.57
Myristic Acid (C14:0)	1.27
Palmitic Acid (C16:0)	1.49
Stearic acid(C18:0)	0.18
Arachidic acid (C20:0)	0.17

Table 3:Unsaturated Fatty acid composition of oil

Unsaturated	Area (%)
Palmitoleic acid (C16:1)	0.37
Oleic acid (C18:1n9c)	4.05
Elaidic acid (C18:1n9t)	0.39
Linoleic acid (C18:2n6c)	0.96
y-Linolenic (C18:3n6)	0.13
Nervonic acid(C24:1n9)	0.19

Total Phenols and Flavonoids

Folin-Ciocalteu assay (Singleton & Rossi, 1965)(Singleton & Rossi, 1965), (Martins, Monteiro, do Amaral, & da Silva, 2021) procedure was utilized to determine the total content of phenolic compounds. However, it is important to note that this reagent is not specifically designed to detect all phenolic groups in the seed extracts. The assessment of total phenolic compounds was conducted calorimetrically. In this process, 0.2 ml of seed extracts were transferred into a test tube, and subsequently, the extract was treated with 0.4 ml of 10% diluted Folin-Ciocalteu reagent. After three to four minutes, 0.8 ml of a 10% solution of sodium carbonate was added as a buffer, and the solution was thoroughly mixed. The tubes were allowed to stand for one hour at room temperature. Absorbance measurements were taken at 725 nm using a spectrophotometer (CE CELL, model 1020). A blank solution of reagents without the sample or seed extracts was employed. The phenolic compound contents were expressed as mg GAE/100g, i.e., gallic acid equivalents on a dry weight basis. The calibration curve was prepared using gallic acid. For the standard curve, 200 mg of gallic acid was mixed with 20 ml of distilled water, and solutions with varying concentrations of 0, 10, 25, 50, 100, 200, and 400 ppm were prepared. The same procedure as mentioned above was repeated using all reagents, and the absorbance was read at 725 nm. The result of total phenol and flavonoids of the seeds of Litsea monopetala (Roxb.) is shown in **Table *4***

Table 4:Total Phenolic Content & Flavonoid contents of seeds

Total Phenols (mg of GAE/100 gm)	Flavonoids (mg of QE/100 gm)
195.59	2390

Results & Discussions

Oil Content shows analysis is useful to determine if the seed can be used to check the economic value, oil yield and processing efficiency. The oil content in the seeds of Litsea monopetala (Roxb.) shows that the oil yield is good. **Iodine value** was checked to find the degree of unsaturation for the selected seeds varied was found to be 6.34 in the seed of Litsea monopetala (ROXB.). This value indicates that the oil falls in the category of non-drying oil. Oil from the seed of Litsea monopetala (ROXB.) seems to be highly saturated (Amin, et al., 2019) and is non-drying and can be used for making soap and are edible. Oils exhibiting higher iodine values tend to possess reduced stability and are more susceptible to oxidation. **Specific Gravity** is an important element in determining the purity of the oil. The result shows that specific gravity of seed oil is around 0.855 which indicates that the oil is less dense as compared to water suggesting its suitability for cream production. This characteristic facilitates smooth flow and even spreading of the oils on the skin (Aremu, Ibrahim, & Bamidele, 2015). The **acid value** & the **free fatty acid** is an important factor to determine the quality of the oil. Higher value is an indication of lower oil quality (Metrohm India, 2020). It also can indicate any pre-treatment required for the seed oil. Acid value of 13.07 and free fatty acid value of 6.53 makes the seed oil comparable to that of Palm oil (Metrohm India, 2020). These values also indicate that the oil might not be used as vegetable oil (Muangpratoom P, 2023) and might need additional treatment.

The **fatty acid analysis** conducted on the seed oil of Litsea monopetala (Roxb.) indicates a predominance of saturated fatty acids, constituting approximately 93% of the total content. ***Figure 2*** shows the comparison of the saturated and unsaturated fatty acid contents seed oil. This analysis shows the close resemblance of coconut oil which has almost 80-90% of saturated fatty acid (Nisitthichai, 2024)

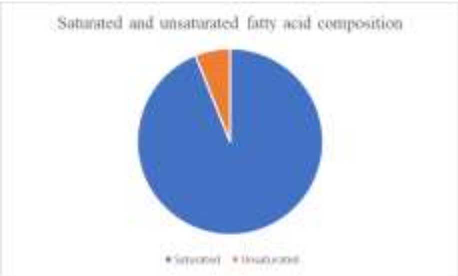

Figure 2: Saturated and Unsaturated fatty acid comparison of seed oil.

As compared to coconut oil the content of Lauric acid is higher in seeds of Litsea monopetala (Roxb.) (The Nutrition Source Coconut oil, 2024). Lauric Acid, classified as a medium-chain fatty acid, is recognized for its numerous health-promoting properties (Rumy kotoky, 2007). The result is also in accordance to other seeds from Litsea family (Rumy kotoky, 2007).

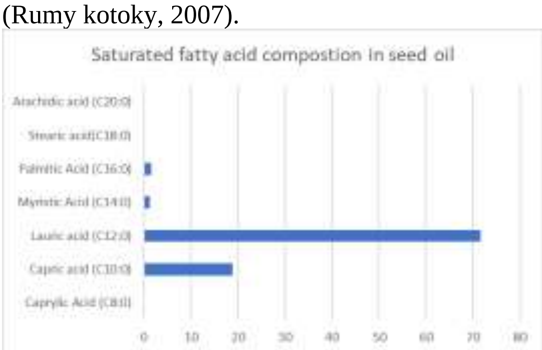

Figure 3: Saturated fatty acid composition in seed oil.

The seeds were also analysed for their contents of total Phenols and Flavonoids. The results shows the content as 195.59 (mg of GAE/100 gm) of total phenols and 2390 (mg of QE/100 gm) for the flavonoids.

The abundance of Lauric acid in the seed oils indicates their suitability for use in soap, detergent, and cosmetic industries, as well as to produce Lauric-based products. As per the results, seeds of Litsea monopetala (ROXB.) is seems to be an excellent source of flavonoids and can be used for its antioxidant properties. This seed and seed oil should be studied further for other health benefits (Phuyal, Jha, Raturi, & Rajbhandary, 2020).

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