

Identification of Nutraceutically Important Metabolites in Raw and Popped Makhana (*Euryale ferox* Salisb.) Seeds Using GC-MS Technique

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ABSTRACT

Euryale ferox, commonly known as fox nut or makhana, is an aquatic plant whose seeds are highly regarded for their nutritional and medicinal values. The plant has several health benefits, containing antioxidant, anti-cancer and anti-inflammatory qualities due to the presence of bioactive phytochemicals that include alkaloids, flavonoids, tannins, saponins and phenolics. Nutritionally, *E. ferox* seeds are high in proteins, essential amino acids, fiber, polyunsaturated fatty acids (especially omega-3), vitamins (B-complex), and minerals (Ca, Mg, P, etc.). Recent developments in analytical methods, particularly gas chromatography-mass spectrometry (GC-MS), can accurately identify and measure the complex phytochemical contents of *E. ferox*. GC-MS technology allows for a more detailed understanding of the volatile and non-volatile compounds present in the plant, providing insights into its antioxidant, antidiabetic, and anti-cancer activities. This communication highlights the potential of GC-MS in a comprehensive analysis of bioactive constituents in *E. ferox*. The metabolome obtained shows the presence of 36 metabolites. Major metabolites identified include glycerol, butanoic acid, decanoic acid, myristic acid, gallic acid, palmitic acid, myoinositol, linoleic acid, farnesol, tricosanoic acid, etc. The roles of these compounds on human metabolism have been deciphered.

This study takes into account results obtained that emphasise the need for further research using all possible methods to explore its medicinal and nutritional potentials. The application of GC-MS could significantly contribute to optimizing the use of *E. ferox* in both functional and therapeutic contexts.

Highlights

- GC-MS profiling identified bioactive secondary metabolites in raw and popped *Euryale ferox* seeds.
- Thermal processing led to notable changes in phytochemical composition.
- Thirty-six metabolites were detected, including fatty acids, esters, and phytosterols.
- Key compounds like palmitic acid, phytol, linoleic acid and stigmasterol showed health-promoting properties.
- Results support the nutraceutical value of popped makhana as a functional food.

Keywords: *Euryale ferox*, Fox nut Makhana, Metabolomics, Bioactive, Superfood, Nutraceutical.

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INTRODUCTION

Euryale ferox Salisb. belonging to family Euryalaceae (derived from the bigger family Nymphaeaceae) is now widely recognized as an aquatic crop that is extensively grown in the non-calcareous zone of Mithila area in north Bihar, of which about 10 districts are known to have a well-managed system of makhana cultivation, harvest and processing. These include -Madhubani, Darbhanga, Saharsa, Supaul, Madhepura, Purnea, Katihar, Araria, and Kishanganj. Districts like Khagaria and West Champaran have also been included to provide subsidies to growers by the Government of Bihar. Local fishermen have a traditional expertise in rearing fish in makhana ponds. Integrated aquaculture is helpful in meeting the requirement of dietary protein requirements of the associated human population. It is only the seeds of *E. ferox* lying at the pond bottom that are taken out of the system and all other parts are made to disintegrate and add to the organic matter forming the detritus. This provides an ideal system for the natural growth of air-breathing, detritivorous fishes under integrated makhana-based aquaculture (Jha and Kumar 2022, Jha and Sinha 2024, Sinha

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and Jha 2025b, etc.). Saxena *et al.* (2024) have emphasised the suitability of low-land agriculture for the cultivation of makhana to raise the income of farmers.

In 2025, India dominates worldwide makhana production, with Bihar accounting for a sizable chunk (approximately 90% of its total production). India accounts for 80% of the

world's demand for foxnuts. Bihar generates 90% makhana production in India. Bihar is India's major makhana producer, accounting for a sizable portion of the country's total. Bihar is a makhana production hub in India, accounting for a sizable share of the entire output. Makhana is becoming more and more pronounced as a "superfood" throughout the world due to its nutritional advantages. The formation of a "Makhana Board" in India has been announced in the Union Budget 2025–2026 with a view to improving its production, processing, and marketing. The global makhana market is expected to increase from \$43.56 million in 2023 to \$100 million by 2033. (<https://www.thehindu.com>, accessed on. Feb 26 2025)

After the COVID pandemic, makhana has fast emerged as a wonder food aimed at increasing the immune-boosting properties of the human body (Sinha and Jha 2025a). It is free of gluten and also possesses resistant starch. (Biswas *et al.*, 2020, Yang *et al.*, 2021). A food item being gluten-free helps in improved digestion (Reilly, 2016). It is also effective in the treatment of ailments like renal disease, diarrhea, severe leucorrhea and splenic hypofunction (Kumari *et al.*, 2019). Ayurveda, the Indian system of traditional medicine, speaks of the spermatogenic properties of makhana that have been experimentally substantiated in a recent study made by Shaw *et al.* (2025). There has been a concomitant increase in its cultivation and processing in recent years. The need to raise its productivity as well as bring more and more of the waterlogged areas under its cultivation is being increasingly felt. Darbhanga district alone has witnessed a considerable increase in its acreage. During the year 2023-24, 27,800-hectare land area was under its cultivation, which increased by about 1000 acres during 2025-26 in the area under makhana cultivation. (Source: Horticulture department, Darbhanga district).

A large no. of farmers have taken up its cultivation after the announcements regarding its GI tagging, followed by the setting up of a "Makhana Board" in the union budget of 2025-26. Very recently, makhana has been awarded a separate HSN code as well, in order to have a clear picture regarding its export potential. A large no. of entrepreneurs have also joined the business of developing processed items. The Makhana system is being used for raising the integrated production of both captured and cultured fish. Introduced fishes help bring control of associated aquatic weeds (Sinha and Jha 2025b). *E. ferox* is held as a significant nutraceutical in view of its nutritional and medicinal properties. Its wide-ranging use in the primary health system of China speaks of the necessity to investigate its phytochemical constituents. *E. ferox* is also called as Gorgon nut. It is referred to as Quian Shi in Chinese medicine (Jha 1999). The plant holds evolutionary significance as it is classified as a eudicot and exhibits both monocot and dicot characteristics (Jha *et al.*, 1991). So far, research on *E. ferox* has focused on the total phenolic and flavonoid content, as well as antioxidant activity. *E. ferox* seeds contain flavonoids, while *E. ferox* arils include flavonoids, phenols and steroids. (Devi *et al.*, 2024).

Makhana pops are a commercial item. Perisperm in makhana is its edible part that is rich in starch and quality protein. It is almost fatless and, as such, is in high demand in affluent countries where a large section of the populace suffers from the problem of obesity (Jha *et al.*, 1991; Jana *et*

al., 2022). Makhana could be held as a significant nutraceutical on account of its nutritional and medicinal properties (Jha *et al.*, 1991a, b). A recent publication by Kumar *et al.* (2025) deals with bioactive compounds present in the edible parts of *E. ferox*. It is a good source of secondary metabolites that serve as bioactive compounds (Liu *et al.*, 2023; Kumar *et al.*, 2025, etc.). Leaves are reported to have flavonoids with widespread glycosylation. Makhana contains a high concentration of macro and micronutrients and minerals. Roasted fox nuts contain more polyphenols that boost antioxidant activity. In comparison to other nuts, makhana contains more phenolic content and as such, there is a possibility of its wider use in industrial medicine. The popped foxnut is notably high in carbohydrate, followed by proteins, ash and minerals like P, K, Fe, Ca, Mg, Na, Cu, Mn and Zn. Essential amino acids include glutamic acid, followed by arginine, leucine, valine, serine, glycine, histidine, threonine, alanine, proline, and aspartic acid. Makhana pops were in high demand during the COVID pandemic, mostly on account of the high presence of Zn in them. The limited supply of Zn tablets during this period triggered people to consume makhana on an enhanced scale. A high content of histidine as an essential amino acid in makhana promotes the development of the brain in infants (Jha *et al.*, 1987, 1991a, Bhatt *et al.*, 2017, Jana *et al.*, 2022).

Metabolomic study based on gas chromatography-mass spectrometry (GC-MS) is a powerful technique for accurately and thoroughly evaluating a variety of primary and secondary metabolites from extremely complex matrices, both qualitatively and quantitatively (Jorge *et al.*, 2016). With a view to studying genetic diversity and molecular markers, we were able to extract high-quality genomic DNA from its leaves (Kumar *et al.*, 2018). According to Yang *et al.* (2024), metabolomics, a thorough investigation of small molecule metabolites, sheds light on the wide variety of volatile and non-volatile metabolites that they found in *E. ferox*.

Nutraceutical and Medicinal Properties

The edible *E. ferox* seeds are traded and exported, both in raw and popped forms. According to nutritional research done to identify its components, carbohydrates are the most abundant of these components, which are found in varying levels. (Tehseen *et al.*, 2020; Saurav *et al.*, 2023). It is almost fatless and, as such, is a much sought-after food item in countries suffering from the problem of obesity.

Makhana is used for its nourishing properties. It contains a moderate proportion of proteins (10–12%), 78% starch-based carbohydrates, 0.5% minerals, and a high index of essential amino acids (EAAI), 89–93%. The distinctive quality of makhana is revealed by its high index of essential amino acids, which is greater than most staple foods. The percentage of phytochemical components of makhana ranges from Ca (0.02), P (0.9), and Fe (0.0014). Its seed has a moisture content of 12.8%. It has a good amount of sugar, phenol, and ascorbic acid. The plant has the capacity to absorb sufficient Na even from the low-sodium soil (Jha *et al.*, 2018; Desale, 2019).

Raw makhana seeds have 362 kcal/100g energy content, while the same in pops is 328 kcal/100g. The higher ratios of leucine to isoleucine in makhana seeds could be accounted for as a factor behind its low biological value of around 55.

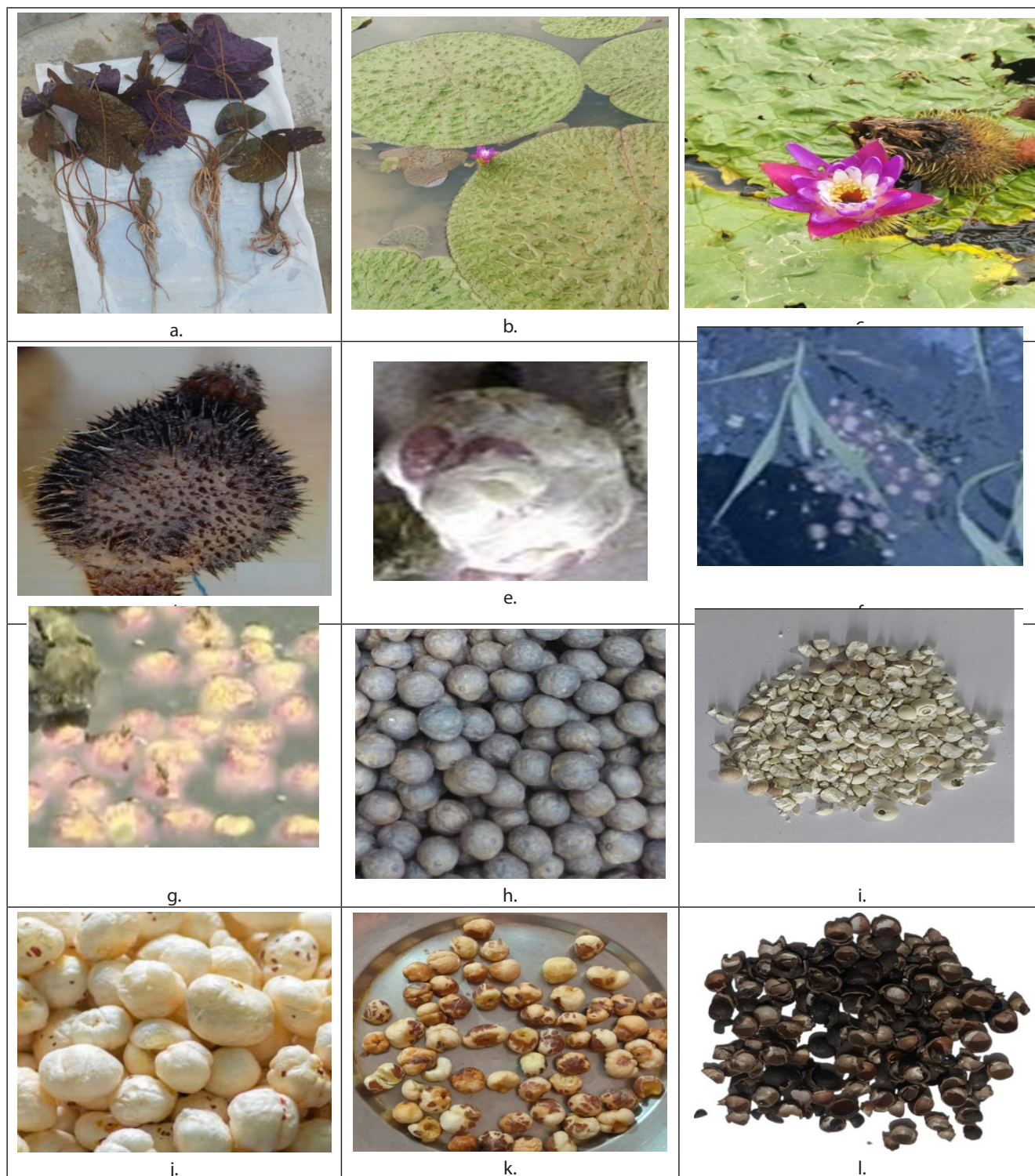


Fig. 1: Different stages of the Makhana crop and its usable product

Fig. (f) shows the oily layer spread on the water surface caused by the just released arillated seeds after natural dehiscence of the fruit. Fig.

(g) provides a close-up of slightly submerged seeds of *E. ferox* on the way to settling to the pond bottom on account of flattening of the seed aril. Fig. (h) shows raw makhana seeds, while Fig. (i) shows their raw kernels. Figs. (j) and (k) show grade 1 and low-grade pops (thurri), respectively. Fig.(l) shows the broken seed coats after removal of the kernel.

Secondary metabolites like alkaloids, glycosides, phenols, tannins, saponins, flavonoids, steroids, etc. have been obtained from the seed extract (Parrray *et al.*, 2011; Kumar *et al.*, 2016; Mummadi *et al.*, 2023; Kumar *et al.*, 2024). It includes kaempferol, which is recognized to have anti-aging, anti-inflammatory, and antioxidant activities. It enhances kidney function, strengthens the heart, and aids in the process of detoxification. In Ayurvedic and Unani medicine, it is frequently used to treat arthritis, sleeplessness and hypertension (Kaur *et al.*, 2014; Jana *et al.*, 2024).

Makhana is an excellent dry fruit with high nutritional value and is held as a component of “panchmeva” (i.e., the five auspicious dry fruits used during religious occasions. Starch is the main nutritional component (Nath and Chakraborty 1985a, 1985b, Jha *et al.*, 1991). Popped Makhana seeds have higher commercial value. It is a non-cereal food that is used extensively in Chinese and Ayurvedic medicines to treat a variety of ailments, including chronic diarrhea, kidney failure, splenic hypofunction, and in reduction of chronic fatigue and burning sensations. It also provides nourishment to the heart, uterus, testis, and ovary. The sperm count is increased when makhana is taken with milk. In both men and women, it boosts fertility and decreases debility (Sidh *et al.*, 2019). Makhana seeds and pops have aphrodisiac qualities. The seeds have emetic, expectorant, and heart-stimulating properties (Imanishi *et al.*, 2014). Makhana has anti-diabetes and anti-hyperglycemic properties. It has been utilized to treat human physiological problems like poor spermatogenesis leading to oligospermia (Ahmed *et al.*, 2018). A recent study conducted by Shaw *et al.* (2025) has experimentally substantiated the Ayurvedic reference on the presence of spermatogenic property in makhana. It also restores the respiratory system, aids digestion, and prevents excessive urination.

The novelty of this study to presenting a comprehensive GC-MS-based metabolomic profiling of *Euryale ferox* (makhana), offering new insights into its bioactive phytochemical composition in both raw and thermally processed forms. The study also emphasizes the impact of thermal processing on phytochemical composition, which has implications for nutraceutical formulation. By coupling advanced analytical techniques (GC-MS) with nutritional and pharmacological interpretation, the paper offers a novel, integrative approach to understanding and optimizing *E. ferox* as a functional food and Ayurvedic therapeutic agent.

Makhana is mistakenly held as a product of lotus (*Nelumbo nucifera*), of course, belonging to the same family, Nymphaeaceae. Rising awareness about the proper origin of this aquatic product as a superfood in recent years has helped the crop gain greater visibility towards its correct identity. Fig. (a) shows the young plantlets of *E. ferox* ready for transplantation. Fig. (b) shows its mature plants with large leaves. Fig. (c) shows a chasmogamous flower. Fig. (d) shows a mature fruit full of stout spines. Fig. (e) shows arillated seeds embedded in the placental mass and pieces of fruit sheath manually removed. Fig. f. shows the oily layer spread on water surface caused by the just released arillated seeds after natural dehiscence of fruit. Fig .g. provides a close up of slightly submerged seeds of *E. ferox* on way to settling to the pond bottom on account of flattening of the seed aril. Fig .h.

shows raw makhana seeds while Fig. i. shows its raw kernel. Figs. j and k. show grade 1 and low grade pops (thurri) respectively. Fig. l. shows the broken seed coats after removal of kernel.

MATERIALS AND METHODS

Makhana sample collection

The seeds of *E. ferox* of local variety “Swarn Vaidehi” and procured from the ICAR-RCER Research Centre for Makhana, Darbhanga, Bihar (26° 9'8 N, 85°53'50 E) and the pops were commercially obtained.

Sample preparation from makhana seeds and pops

One gram each of makhana seed and pop was crushed in liquid nitrogen and extracted with 5 mL of methanol at room temperature. The meat was homogenized for 2 minutes before shaking, which was performed overnight at 90 rpm in the dark. The extract was centrifuged at 14000 x g for 15 minutes. The supernatants were collected and analyzed for sugar, amino acid, organic acid, phenolics, fatty acids, and phytosterols using GC-MS.

METABOLOMIC STUDY OF MAKHANA SEEDS AND POPS USING GC-MS

Chemicals and reagents

Sigma-Aldrich Chemical Co. Ltd. (India) provided chemicals such as 4-phenylphenol (an internal standard) and N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA). Methanol (HPLC grade) was obtained from SRL chemicals (Mumbai, India).

Sample preparation for GC-MS analyses

A 1.5 mL plastic microcentrifuge tube was filled with 100 µL of methanolic extract that was subsequently dried using a vacuum concentrator (Eppendorf Concentrator Plus, Germany). The dried residue was derivatized for 1 hour at 37°C in a dry bath with 70 µL of N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA). After derivatization, the mixture was centrifuged at 12000 x g for 10 minutes. The supernatant was collected and stored in a GC glass vial for GC-MS analysis.

GC-MS run conditions

An Agilent 7890B gas chromatograph and Agilent 5975B mass detector (Agilent Technologies, CA, USA) were used to perform GC-MS analysis. An Agilent HP-5MS column with 5% phenyl methyl polysiloxane, measuring 30 m in length x 0.25 mm in diameter x 0.25 µm in thickness. Agilent Technologies, CA, USA, was employed to separate the chemicals using helium as a carrier gas at a rate of 1 milliliter per minute. Split-less mode was used and the injection volume was 1 µL. A temperature of 280°C was established for the injector. The oven temperature was set to 80°C for 2 minutes, then increased to 220°C at a rate of 10°C/min without holding, and finally to 310°C at a rate of 20°C/min and held for 10 minutes. The column flow rate was 1-mL/minute. The mass spectrometer's (MS) operating parameters were set as follows: The temperature of the ion source was 230°C, while the temperature of the MS quad was 150°C. Mass spectrum

data were collected using electron impact ionization (70 eV) in full scan mode (m/z 50-900) at a speed of 100 scans per second (Kumar *et al.*, 2021, 2022).

Identification of metabolites (GC-MS)

Metabolites were identified by comparing retention time, retention indices (RI), and fragmentation pattern with standard metabolites (when available), or by comparing calculated retention indices (RI) with literature and mass spectral data in a library search (Wiley; National Institute of Standards & Technology library - NIST17). Results have been presented in the form of 2 Tables, 2 graphs and one plate containing 12 photographs.

RESULT AND DISCUSSION

The present investigations were done to analyze the metabolites in raw and popped makhana. Several secondary metabolites were found to be present in both stages. New compounds also obtained from popped makhana may be formed due to the application of heat during the process to form popped makhana. Out of 36 metabolites, 24 are obtained from raw seeds while 14 are from popped makhana. Table 1 provides the details of metabolites in raw seeds and popped makhana (*E. ferox*) of local variety "Swarn Vaidehi" by using the GC-MS technique. Two metabolites d-allofuranose and palmitic acid are common as they remain same in both forms. It is generally the pops of *E. ferox* that are consumed as a supplementary food item. However, *E. ferox* is reported to have a significant presence in the primary health care system of China where the plant is available only in wild form. A northeastern state like Manipur has an old practice of utilization of raw vegetative parts of *E. ferox* (locally called as thanging) in its traditional cuisines. It is under this pretext that there is a need to take up detailed investigation on the secondary metabolites found in the entire parts of *E. ferox* plant and promote a wider utilization of its non-pop vegetative parts, on the pattern described above.

Variation in the metabolite profile of *E. ferox* from raw seed to processed makhana pops

This present investigation used metabolomic research-based GC-MS to identify changes in the chemical profile of popped makhana and *E. ferox* seeds. Table 2 provides information on the principal metabolites discovered in raw and popped makhana, including their chemical formula, molecular weight, retention time (RT), mass spectrum coverage and functions. According to their abundance in the acquired metabolome, these bioactive compounds are presented in Table 2. Metabolomics has been widely used in many thorough studies to examine the metabolites found in both forms. Of the main 36 metabolites that were identified in makhana seeds and makhana pops, 14 were fatty acids/fatty acids-like molecules, seven were lipids, five were sugar alcohols and derivatives, six were sugars and some other metabolites, including sugar acids, phenolic acids, gallic acids, triterpenoids, sesquiterpenoids, alkaloids, flavonoids and derivatives. Several metabolites, including those identified during the present study, have been shown to have a variety of physiological effects on the human body, including anti-

Table 1. Metabolites in Raw seeds and Popped Makhana (*Euryale ferox* Salisb.) of local variety "Swarn Vaidehi" by using GC-MS technique

Metabolites	Present (+)/Absent (-)	
	<i>Makhana Seed</i>	<i>Makhana Pops</i>
Phosphoric acid	+	-
Glycerol	-	+
Glyceric acid	-	+
Butanoic acid	-	+
Decanoic acid	+	-
Dodecanoic acid	+	-
Methyl tetradecanoate	+	-
Xylitol	-	+
D-Allofuranose	+	+
Myristic acid	+	-
Methyl L-sorboside	-	+
Hexadecanoic acid	+	-
Methyl galactoside	-	+
D-Sorbitol	-	+
Gallic acid	+	-
D-Glucose	-	+
Heptadecanoic acid	+	-
Palmitic Acid	+	+
Methyl stearate	+	-
Myo-Inositol	-	+
Linoleic acid/9,12-Octadecanoate	-	+
Stearic acid	+	-
Farnesol	+	-
1-Monomyristin	+	-
Pentadecanoic acid	+	-
Docosanoic acid	+	-
2-Palmitoylglycerol	+	-
1-Monopalmitin	+	-
13-Docosanoic acid	+	-
Tricosanoic acid	+	-
Tetracosanoic acid	+	-
Lactulose	-	+
Lactose	-	+
2-Monostearin	+	-
Glycerol monostearate	+	-
Squalene	+	-

Table 2 Major metabolites from the metabolomics of raw makhana seeds and popped makhana.

S. No.	Metabolite name	GROUP	MW	RT (min)	Formula	Bioactive function
1.	Phosphoric acid	Oxo acid	98	6.48	H ₃ PO ₄	Storage and consumption of energy and help kidney function
2.	Glycerol	Sugar alcohol	92.09	7.539	C ₃ H ₈ O ₃	Skin hydration, wound healing, and treating high intracranial pressure.
3.	Glyceric acid	Sugar Acid	106.08	8.196	C ₃ H ₆ O ₄	Role in metabolic activity, Energy production
4.	Butanoic acid	Fatty acid	88.11	9.207	C ₄ H ₈ O ₂	Helps in digestion, anti-cancerous, anti-inflammation
5.	Decanoic acid	Fatty Acid	172.26	9.612	C ₁₂ H ₂₄ O ₂	Anti-bacterial, AIF, Manufacture of ester, fruit flavor, Volatile component
6.	Dodecanoic acid	Fatty acid	200.31	11.918	C ₁₂ H ₂₄ O ₂	Anti-microbial property
7.	Methyl tetradecanoate	Fatty acid	242.40	12.654	C ₁₅ H ₃₀ O ₂	Transport anti-cancer drug, flavoring agent and fragrance
8.	Xylitol	Sugar alcohol	152.15	12.973	C ₅ H ₁₂ O ₅	Reduce the amount of bacteria in tooth decay, reduce ear infection, help in digestion to produce Saliva
9.	Myristic acid	Fatty acid	228.38	14.035	C ₁₄ H ₂₈ O ₂	Post-translation protein changes, control metabolic process
10.	Methyl L-sorbose	Sugar	212.20	14.063	C ₇ H ₁₄ O ₆	Production of Vit. C, Sweeteners
11.	Hexadecanoic acid	Fatty acid	256.42	14.834	C ₁₆ H ₃₂ O ₂	Antioxidants, hypocholesterolemic
12.	Methyl galactoside	Sugar	194.18	14.834	C ₇ H ₁₄ O ₆	Hydrolyze the Enzyme, Regulate metabolic process
13.	D-Sorbitol	Sugar alcohol	182.17	15.204	C ₆ H ₁₄ O ₆	Laxative, foo, humectants used as indomethacin, relieve constipation
14.	Gallic acid	Phenolic acid	170.12	15.322	C ₇ H ₆ O ₅	Anti-inflammatory, antioxidants, anti-tumor, anti-bacterial, anti-obesity
15.	D-Glucose	Sugar	180.15	15.617	C ₆ H ₁₂ O ₆	The primary source of energy,
16.	Heptadecanoic acid	Fatty acid	270.45	15.719	C ₁₇ H ₃₄ O ₂	Promoting mammalian metabolite
17.	Palmitic Acid	Fatty acid	256.43	15.904	C ₁₆ H ₃₂ O ₂	Cell function and structural integrity.
18.	D-allofuranose	Sugar	180.16	13.811	C ₆ H ₁₂ O ₆	Energy provider, and help in biological process
19.	Methyl stearate	Lipids	298.50	16.498	C ₁₉ H ₃₈ O ₂	Help in neuroprotection, CVD, and mitochondrial function.
20.	Myo-Inositol	Sugar	180.16	16.502	C ₆ H ₁₂ O ₆	Co-factor of enzyme, as a messenger molecule in signal transduction
21.	Linoleic acid/ 9,12-Octadecanoate	Polysaturated omega-6 fatty acid, Lipids	280.44	17.108	C ₁₈ H ₃₀ O ₂	Prevention of nerve blood flow, decrease the risk of CVD, prevention of arrhythmias, decrease thrombosis
22.	Stearic acid	Fatty acid	284.48	17.254	C ₁₈ H ₃₆ O ₂	Food additive, negatively regulates type-2 diabetes
23.	Farnesol	Sesquiterpenoid alcohol	222.37	17.36	C ₁₅ H ₂₆ O ₂	Anti-cancer, anti-inflammatory
24.	1-Monomyristin	Lipid	302.45	18.139	C ₁₇ H ₃₄ O ₄	Anti-bacterial activity
25.	Pentadecanoic acid	Fatty acid	242.39	18.596	C ₁₅ H ₃₀ O ₂	Protecting cardiometabolic immune and liver health

26.	Docosanoic acid	Fatty acid	340.58	18.721	C ₂₂ H ₄₄ O	Cause heart lipidosis, neurotransmitter
27.	2-Palmitoylglycerol	Fatty acid ester	330.51	18.891	C ₁₉ H ₃₈ O ₄	Modulate pain sensitivity
28.	1-Monopalmitin	Lipids	330.50	19.044	C ₁₉ H ₃₈ O ₄	Drug delivery, lipid-based product inhibits cell proliferation synthesis
29.	13-Docosenoic acid	Fatty acid	338.56	19.162	C ₂₂ H ₄₂ O ₂	Impair heart function
30.	Tricosanoic acid	Fatty acid	354.61	19.198	C ₂₃ H ₄₆ O ₂	Human metabolism, help in hair growth and cardiovascular health
31.	Tetracosanoic acid	Fatty acid	368.63	19.575	C ₂₄ H ₄₈ O ₂	The human metabolite, reduce the risk of neurocarcinogenesis
32.	Lactulose	Sugar	342.29	19.552	C ₁₂ H ₂₂ O ₁₁	Treatment of constipation and hepatic encephalopathy
33.	Lactose	Sugar	342.3	19.603	C ₁₂ H ₂₂ O ₁₁	Energy source, inhibit pathogenic bacteria
34.	2-Monostearin	Lipid	358.56	19.682	C ₂₇ H ₄₂ O ₄	Drug delivery vehicle
35.	Glycerol monostearate	Lipids	358.57	19.843	C ₂₁ H ₄₂ O	Emulsify the foods, hydration powder in exercise formulas.
36.	Squalene	Triterpenoid	410.73	26.107	C ₃₀ H ₅₀	Skin lubrication and protection, drug delivery application

Source: - Ahmed et al., (2015, 2018), Biswas et al., (2016,2020), Choo et al., (2009), Devi et al., (2020), He et al., (2019), Huang et al., (2018), Kadu et al., (2020), Liaquat et al., (2022), Lotti and Aversa (1971), Row et al., (2007), Song et al., (2011), Yuan et al., (2014), Zhao et al., (1989) etc. MW: Calculated molecular weight, RT: Retention time, CVD Cardiovascular disease, AIF: Anti-inflammatory.

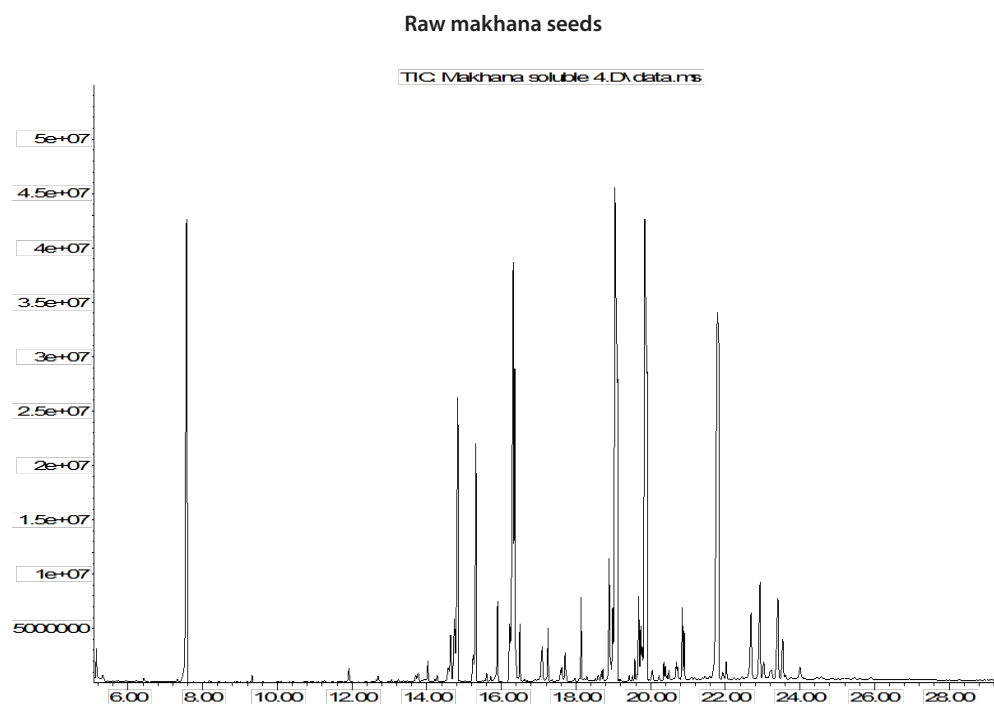


Figure: 2(a)- Typical TIC (total ion chromatogram) from GC-MS analysis of metabolites (after derivatization) from makhana seed.

depressive, antidiabetic, antimicrobial, antioxidative, anti-biotic, anti-pyretic anti-septic anti-angiogenic, anti-carcinogenic, anti-inflammatory, neuroprotective and immunomodulatory properties (Joseph *et al.*, 2023). Figs. 2(a) and (b) identify 36 highly expressed molecules among the total number of annotated peaks in the collected mass spectrum, representing

distinct substances from primary and secondary categories of metabolites. This study was used to examine changes in the metabolite composition of raw *E. ferox* seeds and pops using GC-MS-based metabolite profiling. Due to its high sensitivity, reproducibility and ability to separate and quantify a wide range of distinct metabolite classes (primary and secondary

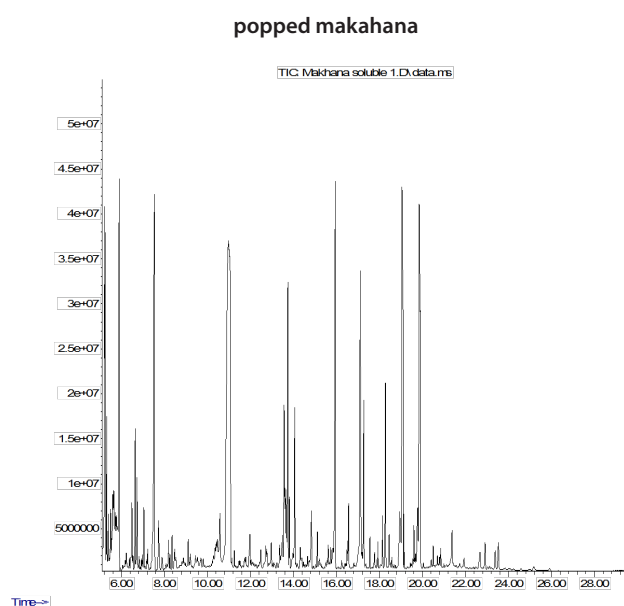


Figure 2: (b) Typical TIC (total ion chromatogram) from GC-MS analysis of metabolites after derivatization) from makhana pop

metabolites) in a single extraction step followed by suitable derivatization. GC-MS is a better technique for metabolite profiling from plant tissues (Hanifah *et al.*, 2018).

Present investigations are confined to the raw seeds and pops of *E. ferox*. Other parts of the plant have been investigated elsewhere for the contents of metabolites. Fatty acids and their derivatives, glycerophospholipids, carbohydrates and their conjugates, flavonoids and their derivatives, fatty amines, lignans, terpenoids and their derivatives, glycerolipids, cinnamic acids and their derivatives, sphingolipids, and other compounds were found in the seeds of *E. ferox*, according to research conducted by Xie *et al.*, (2025) using LC-HRMS. Other techniques previously used for identifying compounds in *E. ferox* include liquid chromatography with ultraviolet detection (LC-UV), nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy (FTIR), and liquid chromatography high-resolution mass spectrometry (LC-HRMS) (Song *et al.*, 2011, Wang *et al.*, 2014, 2016, Liu *et al.*, 2023). The main metabolites found in *E. ferox* seeds and popped makhana were flavonoids, phenolics, fatty acids, organic acids, and amino acids. Peak areas from the GC-MS total ion current (TIC) data were used to derive the components.

The saturated fatty acid tetracosanoic acid, also called lignoceric acid, provides several health advantages, including lowering the risk of heart disease, supporting brain function, shielding the skin from harm and preserving hormone balance. It promotes liver function and reduces inflammation associated with arthritis. In contrast to glucose polymers, which have a higher GI (110), lactose has a low GI (46). The non-insulinogenic reaction to galactose is the main source of the low GI of lactose. Healthy gut flora is facilitated by lactose. Lactulose has a significant effect in the treatment of a person suffering from constipation. People with epilepsy get benefit from the anticonvulsant qualities of decanoic acid, also known as caprylic acid, a derivative of fatty acids. One derivative metabolite that

is utilized to treat colon cancer is butanoic acid, which has also food additive qualities. One polyunsaturated omega-6 fatty acid with numerous health advantages is linoleic acid. It may lessen the chance of developing diabetes and heart problems. Myo-inositol is an extra metabolite that reduces the risk of gestational diabetes, metabolic syndrome, and the symptoms of polycystic ovarian syndrome (PCOS). The obtained results indicate the presence of bioactive compounds that lead to the spermatogenesis capacity in human beings after consumption. Intake of makhana promotes healthy functioning of male sexual performance. This is because it improves both the quality and quantity of semen. There is a centuries-old custom in the Mithila region of consuming makhana on an extensive scale. Ayurveda, the ancient system of Indian medicine, speaks of the spermatogenic potentials of makhana that has found corroboration in the presence of a number of precursor metabolites (Shaw *et al.*, 2025). An ancient Indian system of Ayurvedic medicine speaks of makhana being helpful in raising human fertility (Sharma, 1969). While its impact on spermatogenic property has been experimentally substantiated, the same should also be verified in the case of female fertility.

Present findings on seed include the compounds present in the seed coat. These could be enlisted as phenolics, including gallic acid, ellagic acid, etc. (Wu *et al.*, 2013, 2021, 2023; Zhang *et al.*, 2023). Similarly, leaves of the plant have also been found to contain nine compounds in purified anthocyanin extracts (Wu *et al.*, 2020). These findings are more pertinent in view of the fact that non-seed vegetative parts are widely used as supplementary food items by the indigenous population in the northeastern state of Manipur (Singh *et al.*, 2003). There is a need to popularise such a type of usage of *E. ferox* in the area of the present investigation as well. During popping, the non-edible seed coat is separated. This is otherwise a waste item that is used as fuel during popping. Of late, the seed coat of *E. ferox* is being used by the fishing community as fish fodder, by mixing its powder with oil cake. This provides a better fish yield. However, a no. of studies indicate its phytotherapeutic potential and as such there is a need to devise wider beneficial uses of the same (Kadu *et al.*, 2020; Jiang *et al.*, 2023; Zhang *et al.*, 2023; Xie *et al.*, 2025).

After all, we found that the lipid content changed significantly. There are a number of reasons for this: First of all, frying at high temperatures can break down plant cell walls, allowing lipids to escape the cells more easily (Yang *et al.*, 2024). Second, some lipids in raw medicinal materials undergo enzymatic catalysis to form compounds with proteins and polysaccharides. The stability of these complexes decreases when high temperatures degrade enzyme function, causing bound lipids to become free lipids and a significant rise in the observed values (Tang *et al.*, 2022). Third, Enzymes found in traditional Chinese remedies, such as lipases and esterases, may aid in the breakdown and metabolism of fats. These enzyme proteins are denatured and rendered inactive by high-temperature frying which restricts the breakdown of lipids (Huang *et al.*, 2025).

CONCLUSION

The study reports a comparative analysis of metabolites present in *E. ferox* seeds and popped makhana. It describes the no. of

metabolites present in raw and popped makhana. Out of 36 metabolites, 24 are obtained from raw seeds, while 14 are from popped makhana. Two of the metabolites, d-allofuranose and palmitic acid, are common as they remain the same in both forms. Essential fatty acids are important for preventing cancer, reducing cardiovascular morbidity and mortality, promoting healthy brain and vision development, preventing diabetes mellitus, reducing hypertension, reducing arthritis, and preventing neurological and neuropsychiatric diseases.

Present findings are indicative of the fact that the essential oils of *E. ferox* seeds are an important component because of their physiological and therapeutic advantages. To validate the oil's pharmacological effect in further detail, more research is required. *E. ferox* is processed in a variety of ways and as such several phytochemical constituents get transformed during the popping process. There is a need to take up further investigations on the role of compounds identified in this study on human metabolism.

A total of 36 significant secondary metabolites have been identified (Table 2). Of these, there are 14 fatty acids and their derivatives, 7 lipids, 6 sugars, 5 sugar alcohols, 1 sugar acid, 1 oxoacid, 1 phenolic acid and 1 triterpenoid. It is important to have a treatment for numerous human ailments, including culinary, digestive, renal, and reproductive issues. The entire plant is used medicinally to treat problems related to spermatogenesis, rheumatism, polyurea, parturition, and biliary dysfunction. It lowers debility, boosts fertility in both men and women and raises sperm count. The pops are easily digestible and beneficial to human health due to their low-fat content.

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