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Research Article

UHPLC-HRMS-based mapping of metabolites from *Euryale ferox* Salisb. extract

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Background: *Euryale ferox* Salisb. (commonly known as makhana or foxnut) is an aquatic plant widely consumed for its nutritional and therapeutic value in traditional medicine. However, its bioactive components, responsible for the plethora of benefits it possesses for mankind, remain elusive.

Methods: Thus, in this study, we explored the bioactive components of foxnut extract by employing ultra-high-pressure liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS) to comprehensively profile the components.

Results: Our results showed that over 3000 compounds were initially detected and annotated, of which around 40 were selected based on spectral database comparisons. Out of all, fifteen bioactive metabolites were prioritized and classified into five therapeutic categories: vitamins, cardio-renal and anti-diabetic agents, anti-cancer, antioxidant, and anti-inflammatory compounds, neuroprotective agents, and reproductive health modulators. Notable compounds included thiamine, agmatine, betulin, docosahexaenoic acid (DHA), and testosterone undecanoate, all supported by literature evidence for their health-promoting roles.

Conclusion: These findings provide the detailed metabolite map of *Euryale ferox* and highlight its potential as a natural reservoir of pharmacologically relevant compounds for functional food and nutraceutical development.

Keywords: *Foxnut, bioactive, metabolites, functional food, plant-based therapeutics, mass spectrometry*

Introduction

Foxnut, scientifically known as Euryale ferox Salisb. (EFS), is an aquatic plant belonging to the family Nymphaeaceae, predominantly cultivated in the stagnant water bodies of South and Southeast Asia, with India and China being the primary producers (Kumar et al., 2025). In India, the state of Bihar accounts for over 85% of global production, where its seeds are harvested, dried, roasted, and popped to form what is commonly known as makhana (Kumari and Jha, 2018). This plant thrives in shallow ponds and wetlands, requiring low maintenance and minimal use of agrochemicals, thereby offering a sustainable source of nutrition. Traditionally, foxnut has been used in Ayurveda and Traditional

Chinese Medicine (TCM) for treating a variety of conditions, including renal dysfunction, chronic diarrhea, fatigue, and reproductive health issues (Jiang et al., 2023). Modern nutritional analyses show that foxnut is rich in carbohydrates, proteins, essential amino acids, dietary fiber, magnesium, potassium, and phosphorus but low in fat and calories, making it a popular health snack (Jha et al., 2018). Additionally, its phytochemical composition includes flavonoids, alkaloids, glycosides, sterols, saponins, and phenolic compounds, many of which exhibit antioxidant, anti-inflammatory, anti-hyperglycemic, anti-diabetic, neuroprotective, and hepatoprotective activities (Liu et al., 2021; Jana et al., 2024). Despite its rising popularity as a functional food, there is still a limited understanding of the specific bioactive compounds in foxnut and their molecular targets or mechanisms of action. While earlier studies have performed preliminary phytochemical screening or HPLC-based profiling, they often lacked comprehensive compound identification or quantification. Furthermore, the correlation between identified compounds and their known health-promoting effects remains underexplored. To address this, the present study employs ultra-high-pressure liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS) to conduct a detailed metabolomic profiling of Euryale ferox seeds. MS-based analysis is a powerful approach to identify both known and novel secondary metabolites, offering high sensitivity and specificity for compound detection even at trace levels. This rationale is supported by existing literature, which highlights the presence of key bioactives such as kaempferol, gallic acid, catechins, and chlorogenic acid in related plant species with similar uses (Joseph & Ramesh, 2023). A thorough mass spectrometric profile of foxnut could thus reveal phytochemicals with pharmacological relevance, especially those linked to antioxidant activity, metabolic regulation, and anti-inflammatory pathways. The results from this study may further pave the way for developing nutraceutical formulations or validating foxnut as a dietary intervention for managing lifestyle-associated diseases.

Materials and methods

Chemicals and reagents

All analytical-grade chemicals and reagents used in this study were sourced from Sigma-Aldrich (USA), unless specified otherwise. Additional laboratory consumables and instrumentation accessories were obtained from Thermo Fisher Scientific (USA). Broken foxnut (*Euryale ferox*) seeds were procured from a local supplier in Varanasi, India. Ultrapure water used throughout the experimental procedures was produced using a Milli-Q (MQ) water purification system (Millipore Corporation, Bedford, MA), ensuring both double distillation and deionization for high analytical purity.

Extract preparation

Ten grams of dried foxnut seeds were accurately weighed and used for extract preparation. The seeds were dry-roasted using a microwave oven to simulate traditional processing, then manually ground into a fine powder using a sterile mortar and pestle. The resulting powder was suspended in 100 mL of MQ water, and the mixture was incubated in a shaker incubator at ambient temperature for a duration of 48-72 hours to facilitate maximum extraction of soluble phytoconstituents. Post-incubation, the aqueous extract was concentrated using a rotary evaporator (temperature maintained below 40°C) to prevent thermal degradation of heat-sensitive compounds. The concentrated extract was then transferred into a sterile container and subsequently lyophilized to obtain a stable, dry powdered form of the extract. For UHPLC-HRMS analysis, the lyophilized powder was reconstituted in MS-grade methanol (Merck, Darmstadt, Germany) to achieve a final concentration of 5 mg/mL. The solution was sonicated overnight at 4°C to ensure complete dissolution and homogenization of analytes before injection (Figure 1).

Metabolite profiling of the aqueous extract

To comprehensively characterize the metabolite composition of the EFS extract, the sample was analyzed using a high-resolution Orbitrap Eclipse™ Tribrid™ Mass Spectrometer (Thermo Fisher Scientific, USA) coupled to an ultra-high-pressure liquid chromatography (UHPLC) system (Dionex UltiMate™ 3000 RS UHPLC, Thermo Fisher Scientific). The instrument was equipped with an electrospray ionization (ESI) source operating in both positive and negative ionization modes. The entire analysis was carried out at the Central Discovery Centre (CDC), Banaras Hindu University, Varanasi, India. UHPLC separation was performed on a Thermo Fisher Hypersil GOLD™ C18 column (100 mm × 2.1 mm, 1.9 μm particle size), maintained at a flow rate of 0.3 mL/min. The chromatographic elution was carried out using a ternary solvent system (Solvent A: 100% water with 0.1% formic acid; Solvent B: 80% acetonitrile with 0.1% formic acid; Solvent C: 100% methanol with 0.1% formic acid). A total of 5 μL of the methanol-dissolved extract was injected per run. The mass spectrometric data were acquired using the default parameters of the Compound

Discoverer™ software (version 3.3.2.31), and metabolite annotation was performed using integrated online spectral databases, ensuring accurate identification of both known and potentially novel metabolites (Figure 1).

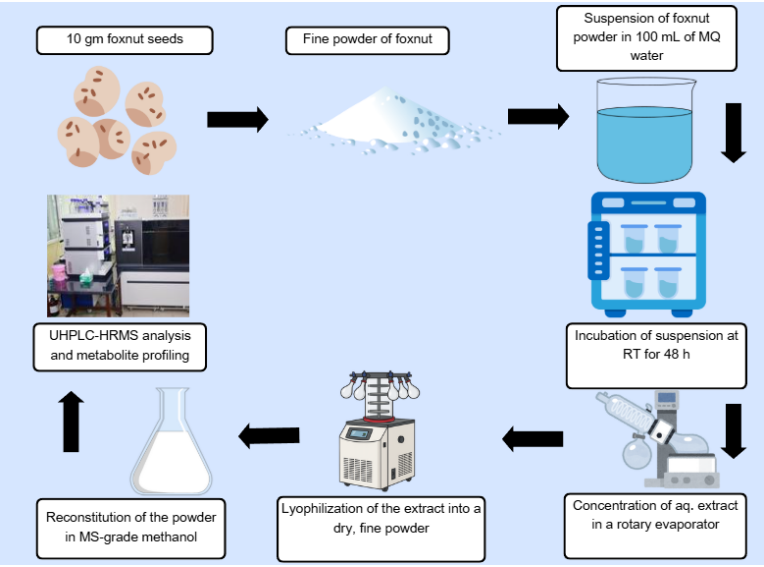

Figure 1. Schematic illustration of the workflow followed for the extraction of bioactive metabolites from foxnut seeds and their identification using UPLC-HRMS

Results

UHPLC-HRMS analysis of the aqueous extract of *Euryale ferox* yielded a comprehensive profile of over 3000 metabolites. Following spectral matching with online databases (mzCloud and ChemSpider) and manual cross-validation using literature sources, 41 compounds with known biological activity were shortlisted. Among these, 15 compounds were selected for detailed discussion based on their well-documented therapeutic potential and relevance to human health. The chemical structures of these 15 compounds are shown in Figure 2. From the full list of annotated features obtained via UHPLC-HRMS analysis, multiple compounds were shortlisted based on existing literature supporting their biological activity. Table 1 below and Table 1 of Supporting Information present the details of these selected metabolites, including their compound names, molecular formulas, molecular weights, retention times (RT), m/z values, functional classifications, described bioactivities, and corresponding literature references.

Table 1. Physicochemical and biological information of the prioritized bioactive compounds identified in the aqueous extract using UHPLC-HRMS

Compound Name	Formula	Mol. wt.	m/z	RT (min)	Classification	Bioactive Functions	Reference
Agmatine	$C_5H_{14}N_4$	130.12247	131.1298	0.73	Primary amino compound, and is a natural metabolite of arginine	It reduces blood pressure, and heart rate, causes a little hypoglycemic state, lessens oxidative stress at cellular level, and augments glomerular filtration rate	(Rafi et al., 2024)
Thiamine	$C_{12}H_{16}N_4OS$	264.10558	265.1129	0.75	A primary alcohol and a vitamin B1	Acts as an important metabolite	(Lonsdale, 2006)
Pyridoxine	$C_8H_{11}NO_3$	169.0745	170.0819	0.81	Vitamin B6	Treat or prevent vitamin B6 deficiency anemia, seizure in babies	(Rabe, 1956)
Bethanidine	$C_{10}H_{15}N_3$	177.1273	178.1346	0.85	Guanidinium derivative (drug)	Anti-hypertensive agent	(Gifford, 1965b)
Nicotinamide	$C_6H_6N_2O$	122.0486	123.0559	1.00	Drug	Dietary supplement and medication for vitamin b3	(Biţă et al., 2023)
Docosahexaenoic acid	$C_{22}H_{32}O_2$	328.2418	329.2491	27.667	Omega-3 fatty acid	It plays a crucial role in the development process of eye and nerve tissues	(Salem et al., 2001)
Cannabichromevarin	$C_{19}H_{26}O_2$	286.1943	287.2016	27.659	1-benzopyran	Anti-cancerous and anti-inflammatory in nature	(Shoyama et al., 1975)
Testosterone decanoate	$C_{29}H_{46}O_3$	442.3465	443.3538	27.383	Androgen	Used in the treatment of male hypogonadism	(Brady et al., 2005)

Ergocalciferol	$C_{28}H_{44}O$	396.3411	429.3746	27.067	Vitamin D2	Vitamin D helps the body absorb calcium.	(Gravesen et al., 2013)
Betulin	$C_{30}H_{50}O_2$	442.3826	443.3899	26.176	Triterpenoid	Act as a metabolite, possess anti-viral, anti-neoplastic, and anti-inflammatory capabilities, and also serves as an analgesic	(Drąg-Zalesińska et al., 2017)
Cinnamaldehyde	C_9H_8O	132.0581	133.0653	23.267	Flavonoid	It has a role as a hypoglycemic agent	(Subash Babu et al., 2007b)
Xanthoxin; Methoxsalen	$C_{15}H_{22}O_3$	250.1578	251.165	22.344	Furanocoumarins	Employed for treating eczema, vitiligo, psoriasis, and few cutaneous lymphomas along with exposing the skin to ultraviolet light from sunlight or lamps	(Ivie, 1978)
Testosterone undecanoate	$C_{30}H_{48}O_3$	456.3622	457.3695	22.13	Prodrug/steroid ester	An androgen and anabolic steroid medication	(Swerdloff et al., 2020)
2,6-Di-tert-butylphenol	$C_{14}H_{22}O$	206.1678	207.1751	20.124	Phenols	It serves as an antioxidant	(Li et al., 2024)

Discussion

This study aimed to comprehensively map the bioactive metabolite profile of *Euryale ferox* (foxnut) aqueous extract using UHPLC-HRMS, with the goal of identifying compounds that may contribute to its traditional and emerging therapeutic uses. The obtained compounds were grouped into five major categories based on their physiological benefits: (i) Vitamins, (ii) Cardiovascular, renal, and anti-diabetic effects, (iii) Anti-cancer, anti-oxidant, and anti-inflammatory activity, (iv) Neuroprotective effects and (v) Reproductive health benefits.

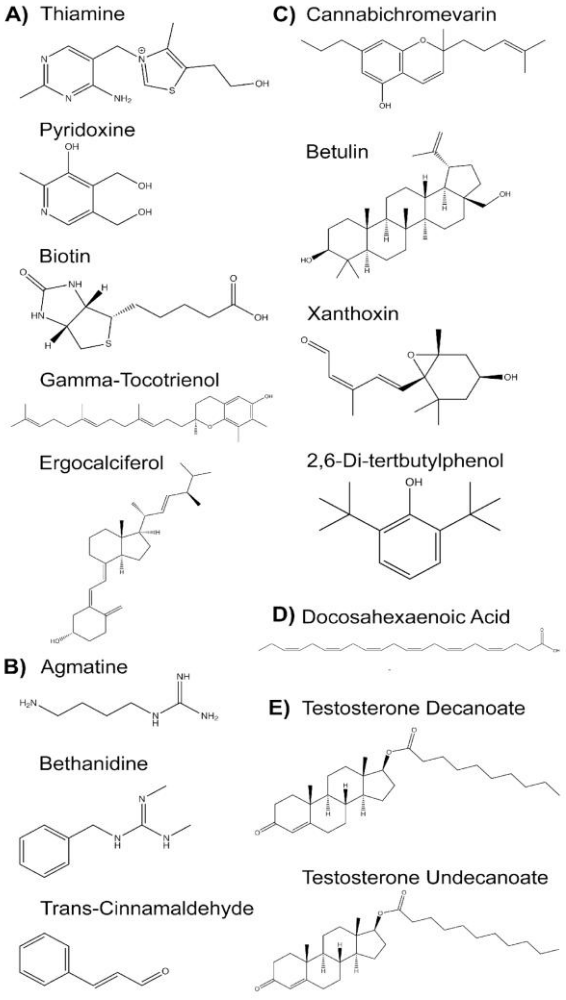

Figure 2. Chemical structures of 15 bioactive compounds identified from using UHPLC-HRMS, grouped according to their reported biological activities.
A) Vitamins: Thiamine, Pyridoxine, Biotin, γ-Tocotrienol, and Ergocalciferol, known for their roles in metabolic regulation, antioxidant defense, and skeletal health. B) Cardio-Renal and Anti-Diabetic Agents: Agmatine, Bethanidine, and Cinnamaldehyde, associated with vasodilation, blood pressure regulation, and glycemic control. C) Anti-Cancerous, Antioxidant and Anti-Inflammatory Compounds: Cannabichromevarin, Betulin, Xanthoxin, and 2,6-Di-tert-butylphenol, reported to modulate oxidative stress, suppress inflammatory signaling, and induce cancer cell apoptosis. D) Neuroprotective Agent: Docosahexaenoic acid (DHA), an omega-3 fatty acid essential for neuronal function, synaptic plasticity, and protection against neuroinflammation. E) Reproductive Health Enhancers: Testosterone decanoate and testosterone undecanoate, which are androgenic steroids involved in sexual development, reproductive function, and endocrine regulation.

Identified bioactive compounds and their functional categorization

Group A: Vitamins

Among the diverse array of bioactive metabolites detected in the aqueous extract of *Euryale ferox*, five notable compounds were identified that serve as essential vitamins, reinforcing the nutritional and therapeutic potential of this plant. Thiamine (Vitamin B1), a primary alcohol and vital coenzyme in carbohydrate metabolism, was found to be present in our extract. Thiamine plays a critical role in energy production through its involvement in the decarboxylation of α-keto acids and the pentose phosphate pathway, which is particularly important in maintaining neural and cardiac function. Its presence in makhana extract corroborates previous nutritional studies indicating thiamine's role in supporting metabolic activity and preventing neurological deficits such as Wernicke's encephalopathy and beriberi (Chandrakumar et al., 2018). Another essential vitamin identified was pyridoxine (Vitamin B6), known for its coenzyme activity in amino acid metabolism and neurotransmitter synthesis. Pyridoxine is extensively used to treat or prevent vitamin B6 deficiency, which can manifest as microcytic anemia, peripheral neuropathy, and seizures, especially in newborns with genetic metabolic disorders (Parra et al., 2018). The presence of pyridoxine in *Euryale ferox* supports the traditional use of makhana in diets aimed at improving cognitive and hematological health. Biotin (Vitamin B7), another compound found in the extract, is crucial for gluconeogenesis, branched-chain amino acid catabolism, and fatty acid synthesis. It acts as a cofactor for several carboxylases and is especially important for maintaining healthy skin, hair, and nails, as well as embryonic development (Taniguchi & Watanabe, 2007). The inclusion of biotin in makhana further substantiates its value as a dietary supplement, especially in populations at risk of deficiency due to malnutrition or long-term anticonvulsant therapy. A particularly interesting compound identified in our profiling was gamma-tocotrienol, one of the isoforms of the Vitamin E family. Unlike tocopherols, tocotrienols such as gamma-tocotrienol exhibit stronger antioxidant activity and possess additional health benefits. These include anti-cancer properties through the modulation of apoptosis, radiation-protective effects, and hepatoprotective potential (Al-Baiaty et al., 2021). Its detection in *Euryale ferox* is significant, as it links makhana not only to antioxidant defence but also to possible adjunctive roles in cancer prevention and liver protection. Lastly, ergocalciferol (Vitamin D2), a secosteroid and plant-derived form of Vitamin D, was also found in the extract. Ergocalciferol is known to support calcium and phosphorus homeostasis by enhancing their intestinal absorption, making it essential for bone mineralization and the prevention of rickets and osteoporosis. The presence of ergocalciferol in makhana extract aligns with its potential as a dietary supplement for maintaining musculoskeletal health, especially in populations with limited sun exposure or dietary sources of Vitamin D. Together, the identification of these vitamins through UHPLC-HRMS not only confirms the nutritional richness of *Euryale ferox* but also supports its therapeutic relevance across multiple physiological systems, including neurological, metabolic, skeletal, and dermatological health.

Group B: Cardiovascular, renal, and anti-diabetic effects

Several compounds with documented cardiovascular, renal, and anti-diabetic properties also surfaced. Notably, agmatine, a primary amino compound derived from the decarboxylation of arginine, was detected. Agmatine is known to exert beneficial effects on cardiovascular and renal physiology by promoting vasodilation, leading to modest reductions in heart rate and blood pressure, alongside enhancing glomerular filtration rate (GFR) (Klahr & Morrissey, 2004). Additionally, it has been associated with improved insulin sensitivity and secretion, contributing to mild hypoglycemic effects, while also mitigating oxidative stress in tissues (Kamel et al., 2024). Its presence in makhana extract suggests potential roles in blood pressure regulation, glycemic control, and renal protection. Further, Bethanidine, a guanidinium derivative historically used as an anti-hypertensive agent, was also identified. This small-molecule sympatholytic drug acts by inhibiting adrenergic (sympathetic) nerve transmission, leading to a reduction in vascular tone and lowering of systemic blood pressure. Although less commonly used today, bethanidine's detection in makhana introduces an intriguing natural source of a compound with potential anti-hypertensive properties, meriting further investigation into its pharmacokinetic behaviour when derived from plant extracts. Lastly, cinnamaldehyde, an aromatic aldehyde and the principal component of cinnamon essential oil, was also observed in the extract. Well-studied for its hypoglycemic activity, cinnamaldehyde has demonstrated improvements in glycemic and lipid profiles in diabetic animal models by upregulating GLUT4 expression, activating insulin signaling pathways, and inhibiting advanced glycation end-products (AGEs) formation. Thus, the detection of cinnamaldehyde in *Euryale ferox* extract underscores makhana's potential as a natural source for managing blood glucose and protecting against metabolic disorders. These findings highlight makhana's potential as a dietary source of functional metabolites that support cardiovascular health, renal function, and metabolic balance.

Group C: Anti-cancer, anti-oxidant, and anti-inflammatory activity

Our UHPLC-HRMS analysis of makhana extract revealed several bioactive metabolites with notable anticancer, antioxidant, and anti-inflammatory properties. One such compound is Cannabichromevarin, a rare Phyto cannabinoid structurally similar to cannabichromene, which has recently been shown to possess anti-inflammatory, anti-convulsant, and anti-cancer activity without psychoactive effects (Hong et al., 2023; Sepulveda et al., 2024; Iqbal & Matsabisa, 2024). Its detection in our extract of makhana is particularly intriguing, suggesting potential modulatory effects on the endocannabinoid system and promising anti-inflammatory actions. We also identified betulin, a pentacyclic triterpene commonly found as a metabolite in birch bark and other plants. Betulin demonstrates a diverse spectrum of biological and functional activities, such as anti-cancer, antiviral, analgesic, and anti-inflammatory properties. Betulinic acid, derived from betulin, has been known to induce mitochondrial apoptosis in diverse cancer cell lines, including colon cancer, via caspase-3 and -9 activation and cytochrome c release. Moreover, ester derivatives of betulin demonstrated potent cytotoxic effects against melanoma cells in vitro, inhibiting proliferation at micromolar concentrations (Drąg-Zalesińska et al., 2017). Hence, our detection of betulin underscores makhana's potential as a natural source of triterpenoid compounds with promising oncological and anti-inflammatory properties. Among the bioactive metabolites identified in the *Euryale ferox* extract, Xanthoxin, which is a known precursor of abscisic acid (ABA), was of particular interest due to its emerging therapeutic relevance. ABA has been shown to ameliorate symptoms of type II diabetes by targeting peroxisome proliferator-activated receptor gamma (PPARγ), in a manner akin to thiazolidinedione-class antidiabetic drugs, supporting its potential role in metabolic regulation. Moreover, recent pharmacological studies have revealed that Xanthoxin functions as a T2R4 (bitter taste receptor 4) agonist, while ABA serves as its antagonist, suggesting that this precursor itself may have unique signaling properties. The presence of Xanthoxin in makhana thus opens new avenues for investigating its role in glucose homeostasis and chemosensory-mediated signaling pathways. Lastly, we observed 2,6-di-tert-butylphenol, a phenolic compound widely recognized for its strong antioxidant capacity (Li et al., 2024). This compound acts as a phenolic free radical scavenger, inhibits lipoxygenase, and contributes to anti-inflammatory and anticancer effects, including use as a vitamin E analogue in medicinal applications (Milaeva et al., 2020). Its detection highlights the extract's potential for fending against oxidative damage and chronic inflammation.

Group D: Neuroprotective effects

Our results showed the presence of docosahexaenoic acid (DHA), a long-chain omega-3 fatty acid widely studied for its neuroprotective properties. DHA is a structural component of neuronal membranes, particularly enriching synaptic and photoreceptor phospholipids, which support neurotransmission and sustain membrane integrity. Experimental models of traumatic brain injury (TBI) in rodents have shown that post-injury administration of DHA significantly restores neurobehavioral function and reduces neuronal apoptosis; this is achieved through activation of the Nrf2-ARE antioxidant pathway, reduction of oxidative stress markers (such as malondialdehyde and caspase-3 activation), and enhanced expression of cytoprotective proteins like Bcl-2, NQO-1, and HO-1. In focal cerebral ischemia models, DHA both prevents blood–brain-barrier disruption and limits infarct volume, while enhancing synthesis of neuroprotectin D1. Additional studies in multiple sclerosis models demonstrate that DHA supplementation improves clinical scores by reducing oxidative stress and modulating neuroinflammatory responses through Nrf2 activation and suppression of reactive oxygen species (ROS) (Muñoz-Jurado et al., 2024). Mechanistically, DHA is incorporated into neuronal membranes, enriching phosphatidylserine-rich domains that facilitate pro-survival signaling (Akt, PKC, Raf-1), enhance synaptogenesis, neurogenesis, and promote antioxidant and anti-inflammatory docosanoid metabolites like resolvins and neuroprotectin D1 (Kim et al., 2022). Its detection via HRMS in EFS extract suggests that makhana may harbour neuroprotective omega-3 compounds capable of contributing to brain health, warranting future exploration of its potential as a plant-based neurotherapeutic.

Group E: Reproductive health benefits

Our UHPLC-HRMS profiling revealed the presence of several androstenedione esters and derivatives, namely testosterone decanoate and testosterone undecanoate compounds with established roles in male reproductive physiology. Testosterone decanoate, identified via its characteristic ester-specific ions, shares structural similarity with undecanoate (Turza et al., 2023). Structural analysis confirmed that decanoate esters form stable crystalline arrangements conducive to sustained-release profiles. Its detection in makhana suggests the plant may supply ester derivatives that mimic long-acting androgenic effects, potentially enhancing endogenous testosterone availability. A pivotal multicenter Phase IIb study combining etonogestrel implants with intramuscular testosterone decanoate (400–600 mg every 4–6 weeks) demonstrated profound suppression of spermatogenesis in over 80% of participants, confirming the compound's potent androgenic activity and influence on the reproductive axis.

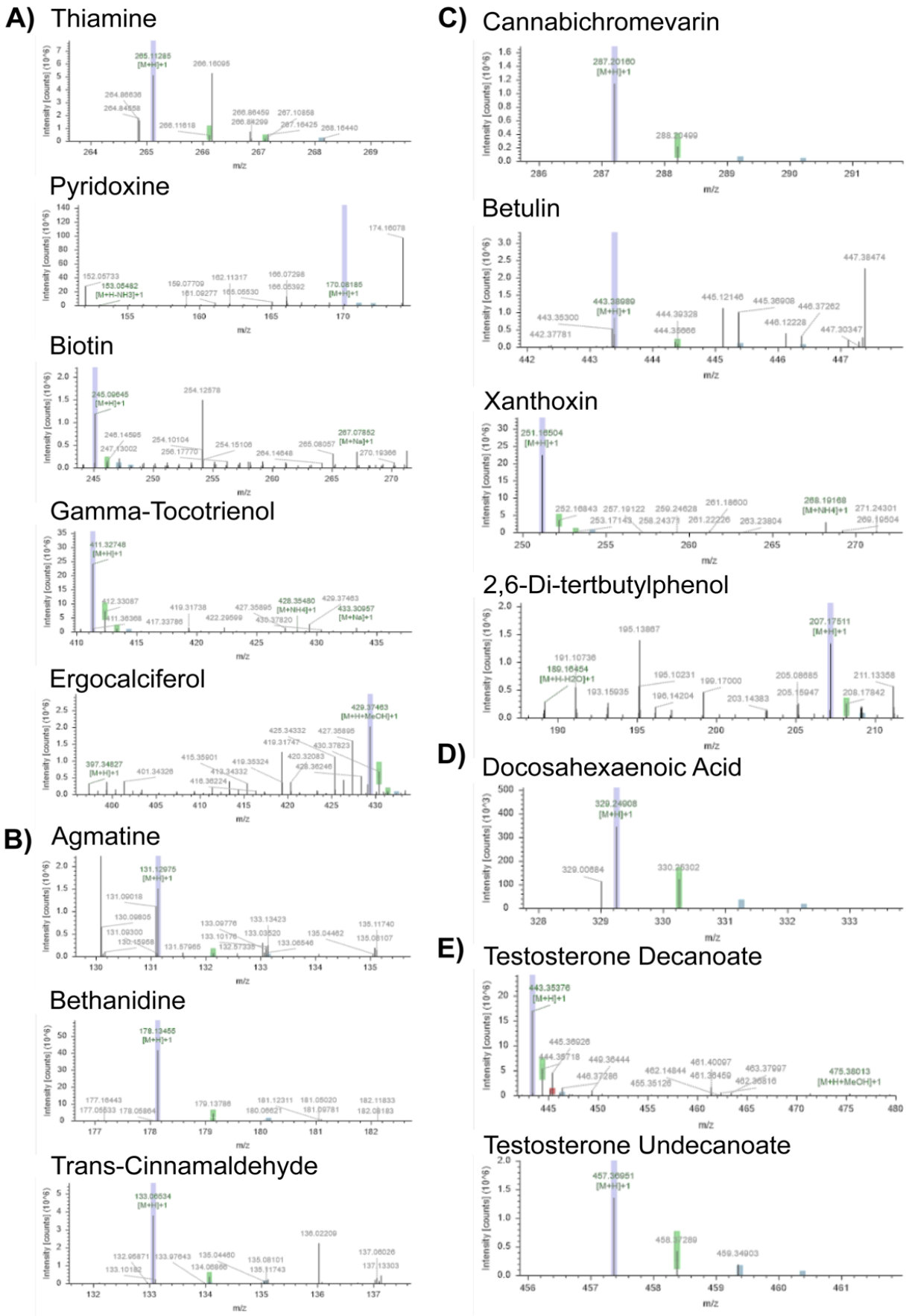

Figure 3. Representative UHPLC-HRMS spectra of 15 key bioactive compounds identified from the aqueous extract. All spectra represent positive ion mode detection, with molecular ion peaks annotated using accurate mass-to-charge (m/z) ratios and corresponding intensities.

This confirms that makhana's metabolite profile includes bioactive androgen precursors capable of modulating gonadal function, with potential implications for male reproductive health. Additionally, testosterone undecanoate was identified in the extract. This long-chain esterified form of testosterone is approved for treating hypogonadism and is notable for its favorable pharmacokinetic profile.

In a phase 3 clinical study, a new oral testosterone undecanoate (TU) formulation successfully raised hypogonadal individuals' T levels to mid-eugonadal levels (Swerdloff et al., 2020). Notably, in female reproductive health, oral testosterone undecanoate supplementation during estrogen therapy improved sexual function in postmenopausal women. The identification of this compound in *Euryale ferox* highlights the plant's capacity to serve as a natural source of bioavailable androgenic steroids, with potential roles in hypogonadal therapy or sexual health enhancement. The detection of this androgenic metabolite in makhana further supports its androgenic potential and suggests that *Euryale ferox* may influence androgen-dependent physiological processes, such as fertility and sexual development. The presence of androgenic metabolites in makhana suggests that *Euryale ferox* is a natural source of compounds capable of impacting hormone-driven reproductive processes. Notably, a recent in vivo study has shown that *Euryale ferox* Salisb. significantly ameliorates cadmium-induced testicular and hepatic impairments in male mice, reinforcing its potential role in supporting male reproductive and hepatic health at the organismal level (Shaw et al., 2025; Shaw et al., 2024). The representative high-resolution mass spectra (HRMS) of these compounds are shown in Figure 3, depicting the m/z values and ion intensities that confirmed their identity and structural match with database references.

Conclusion

In this study, we employed UHPLC-HRMS to comprehensively profile the aqueous extract of *Euryale ferox* Salisb., a traditionally valued aquatic plant widely consumed across South Asia. The metabolomic screening revealed the presence of over 3000 chemical features, all of which were annotated based on accurate mass and database comparisons; from these, 41 compounds were selectively shortlisted based on literature evidence for their known health benefits. From this dataset, 15 key bioactive compounds were selected and discussed in detail, based on literature-supported therapeutic relevance and grouped into five major functional categories: vitamins, cardio-renal and anti-diabetic agents, anti-cancer/anti-oxidant/anti-inflammatory compounds, neuroprotective agents, and reproductive health-related metabolites. Among these, we identified essential vitamins such as thiamine, pyridoxine, and biotin, along with compounds like agmatine and cinnamaldehyde known to modulate blood pressure and glycemic balance. Anti-cancer and antioxidant molecules such as betulin and 2,6-di-tert-butylphenol were also present, highlighting the plant's potential role in oxidative stress reduction and tumour suppression. The detection of DHA underscores its potential for promoting neural development and counteracting neurodegeneration. Furthermore, the presence of androgenic metabolites, including testosterone decanoate and testosterone undecanoate, links *Euryale ferox* to reproductive endocrinology and hormonal regulation. Collectively, this study provides the first comprehensive HRMS-based chemical mapping of *Euryale ferox*, validating its traditional usage and positioning it as a potential source of pharmacologically relevant biomolecules. The findings also pave the way for future investigations into bioactivity-guided fractionation, dose standardization, and preclinical validation. By decoding its complex phytochemical matrix, this work supports the development of *Euryale ferox* as a functional food or nutraceutical with multi-system therapeutic benefits.

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Author contributions

Ranjit Shaw: Conceptualization, Investigation, Methodology, Data curation, Formal Analysis, Validation, Writing – original draft, Writing - review & editing, Figure and structure making. **Anmol S Kamath**: Data curation, Formal Analysis, Validation, Writing - original draft, Writing - review & editing. **Reeta Ragini**: Conceptualization, Investigation, Methodology, Data curation, Formal Analysis, Validation, Writing - review & editing, Figure and structure making. **Sachin Shaw**: Methodology, Figure and structure making, Writing - review & editing. **Shikhar Deep**: Methodology, Writing - review & editing. **Radha Chaube**: Conceptualization, Writing - review & editing, Formal analysis, Project administration, Resources, Supervision, Funding acquisition.

Conflict of interests

The authors declare no conflict of interest.

Ethics approval

Not applicable.

AI tool usage declaration

No AI tools have been used in manuscript preparation.

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