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Reproductive Health Benefits of *Euryale ferox* Salisb.: A Short Comprehensive Review

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Abstract

Medicinal plants are receiving increasing attention lately for their potential to cure many human illnesses. *Euryale ferox* Salisb., an aquatic cash crop that grows in ponds and is popularly known as makhana, foxnut, and gorgon nut, is one of these medicinal plants. It is also recognized as a delicious dry fruit. In view of their great nutritional and therapeutic values, *E. ferox* seeds are included in the Ayurvedic pharmacopeia. These benefits include curing chronic diarrhea, excessive leucorrhea, kidney failure, hepatic dysfunction, and other gastrointestinal issues. For many years, *E. ferox* has been used as a remedy for various illnesses affecting the digestive, respiratory, and reproductive systems. Antioxidant, anti-cancer, antibacterial, antihypertensive, immunomodulatory, anti-diabetic, anti-melanogenic, and anti-cytotoxic properties are only a few of the plant's many biological activities. Additionally, this article discusses, in brief, the anti-fatigue and antioxidant properties of the phenolics isolated from *E. ferox* seed coat. Besides these, the extracts show hepatoprotective and cardioprotective properties. It has been established that the bioactive components found in *E. ferox* are responsible for these benefits. This is a unique plant with great nutritional and therapeutic potential throughout, particularly in the seeds and plant shells. This article provides an overview of the recognized bioactive components discovered in the extracts with pharmacological characteristics present in different sections of the *E. ferox* plant. It also focusses on certain reproductive benefits in males as well.

Keywords: Bioactive Component, Foxnut, Nutritional Seed Coat, Supplement.

1. Introduction

One of the highly valuable aquatic cash crops, Foxnut (*Euryale ferox* Salisb., or in short, EFS), also known as Makhana, is now being grown in different parts of the world due to its high mineral content and significant therapeutic potential and has rapidly gained popularity over the years as a superfood on a global scale[1]. Moreover, Makhana is considered to have cultural, economic, nutritional, and medicinal benefits as well. than most othercrops (Figure 1). These all-around qualities have led to Makhana gaining nationaland global, unparalleled attention[2].

Rawmakhana seeds are referred to as "Black Diamond" seeds and are well-known for popping like a white ball. The flowering plant Makhana, botanically known as *E. ferox*, belongs to the family Euryalaceae (Nymphaeaceae). It goes by some other names too, such as Fox Nut, Gorgon Nut, and Euryale seeds as well (Figure 2). Makhana is also considered "God's Food" as it is offered to the God and because of its high protein and carbohydrate content, it is also used as a "Food for Fasting"[2]. Other names by which this superfood is known are "crop of ponds, fields, ditches, oxbow lakes, and land depressions," as they are generally grown in swamps and ponds[3]. Makhana is a high-value aquatic cash crop that is mainly farmed in eastern India[4,5]. While it is grown in a few other Indian states, including Bihar, West Bengal, Assam, Manipur, Tripura, and Madhya Pradesh, its commercial cultivation is primarily carried out in eight to ten districts in North Bihar, *viz*., Madhu

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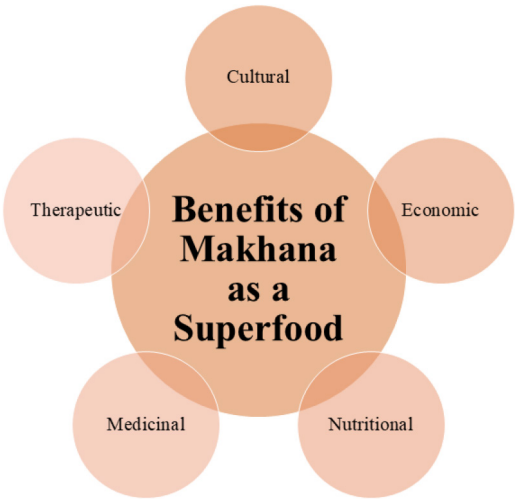


Figure 1: Different benefits of Makhana as a Superfood.

Bani, Darbhanga Samastipur, Saharsa, Supaul, Purnia, Kisanganj, Katihar, etc[4,6]. With an estimated 80% share of the world's makhana output, North Bihar is appropriately recognized as the global centre of commercial cultivation of makhana. China, Japan, South Korea, Bangladesh, Russia, and a few other nations have grown it outside of India[7] (Figure 3).

Up to 30 nations are recipients of India's makhana exports, with the United States and the United Arab Emirates being the top two importers[8]. Traditionally, the only areas where makhana was produced were ponds and other permanent bodies of water, and cultivated especially by fishing groups[4]. However, both productivity and the area under makhana cultivation need to be enhanced to fulfill the fast-expanding global demand for makhana farming and to maximize the potential income from it[9]. The introduction of makhana cultivation in field conditions, similar to rice, has recently been initiated to expand the area under makhana agriculture, as it is thought that the number of ponds and natural water bodies will steadily decrease.

The best way to raise Makhana is usually from seed each year. It possesses strong, fibrous roots that are divided into four to six clusters, each with roughly fifteen rootlets. It originates from a short, thick, erect rhizome and is a perennial plant whose leaves exhibit heterotrophic growth. There are roughly four basic varieties of leaves: sinuate, hastate, sagittate, and orbicular, which appear in chronological order. The final set of leaves is thorny, whereas the first three sets of leaves are not[10]. Their young seeds have a lumpy texture and vivid crimson, striped arils surrounding them. Fresh seeds lose their color and



Figure 2: The various names by which Makhana is known across India.

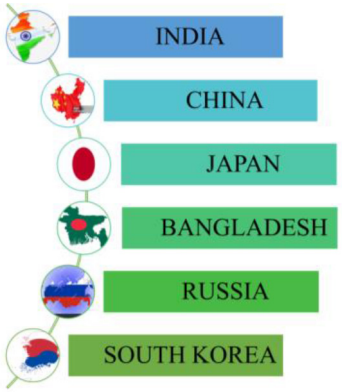


Figure 3: Global distribution of Makhana.

turn black after three to four days of decomposition. Seeds have a hard- outer shell and are sufficiently bold. The seed's diameter is in the range of 0.5 to 1.5 cm, and the perisperm, which is white and starchy, is the edible portion of the seed. The genus Euryale is monotypic. Therefore, this genus has no other species. Makhana has a chromosomal composition of 2n=2X=58 and is a diploid plant[1,10,11].

2. Use of Foxnut in Chinese Medicine

EFS or Makhana is referred to as Qian Shi in Chinese medicine. As stated in "Shen Nong's Classic of the Materia Medica", EFS was originally documented in China and

was described as having "sweet and astringent tastes and utilized for treating dampness and paralysis, pain in the knees and lumbar spine, tonifying and removing malignant diseases, benefiting the essence, strengthening the will, and making the ears and eyes wise". EFS is described as "sweet, astringent, and flat, attributed to the spleen and kidney meridians, benefiting the kidney and consolidating sperm, tonifying the spleen and inhibiting diarrhea, eliminating dampness and arresting leucorrhea" in the 2020 edition of the Current Chinese Pharmacopoeia[12,13]. It has been used to treat renal disorders, persistent diarrhea, excessive leucorrhea, and liver dysfunction in Ayurvedic and Chinese medicines sinceits bioactive substances have several properties such as immunomodulatory, anti-melanogenic, anti-ischemic, antioxidant, antimicrobial, and anticytotoxic (Figure 4)[13-17]. Interestingly, makhana is a nutraceutical supplement that patients with COVID-19 can take[18]. It is also thought to somewhat boost cell-mediated and humoral immunity when consumed regularly[18].

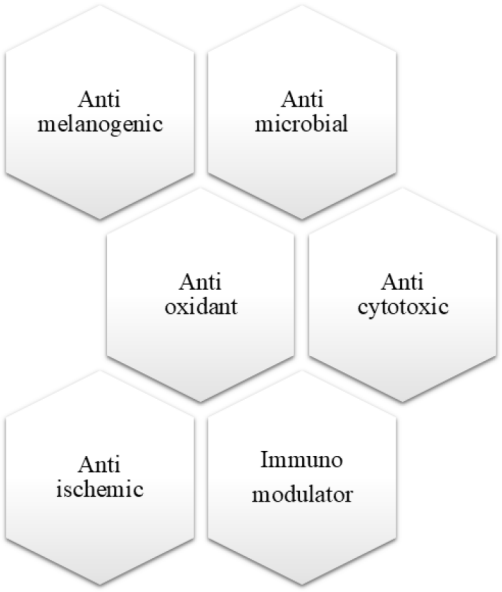


Figure 4: Properties of the active components of the Makhana, used in Ayurvedic and Chinese Medicines.

3. Nutritional Constituents of the Foxnut

The dietary advantages of foxnuts have led to a significant rise in their usage. Foxnuts have a highfat, little calorie, and high mineral composition. Micronutrient deficits can be less common if nutrient-dense foxnuts are regularly substituted in a diet[19]. Owing to its high nutrient content, foxnuts can be eaten in popped form or as a powder mixed with different food items. Popping foxnuts is said to improve their phytochemical profile and other bioactive components. A flavonoid called *kaempferol*, which is also found in almond skin, is present in foxnuts and adds to their phytochemical activity. Studies have indicated that its high amino acid index helps muscles recover after exercise, which is a crucial aspect of cell metabolism[19,20].

In Bihar, the nutritional value of popped fox nuts on the "Swarna Vaidehi" type is 358% calorific, 79.8% carbohydrate, 8.7% protein, 0.5% fat, and 0.2% crude fiber, and the estimated contents of iron and calcium are 1.4% and 18.5%, respectively[2,7,21]. In another study, raw *E. ferox* seeds from Bangladesh revealed protein concentration of 15.6%, 7.6% fiber, 61.2% carbohydrates, and 1.3% fat. Except for tryptophan and phenylalanine, foxnuts contain seven of the nine essential amino acids, giving them a high "amino acid index".

Research has shown that roasting popped foxnuts dramatically improves their nutrient bioavailability and acceptability[21,22]. According to a study, roasted foxnuts were demonstrated to possess a significantly higher mineral, protein, and fiber content than unroasted foxnuts[22]. When compared to the control group of unroasted nuts, the roasted nuts had a considerably reduced moisture content (4.17%), greater crude protein (14.57%), crude ash (0.66%), crude fat (0.68%), crude fiber (0.51%), and nitrogen-free extract (79.41%)[22]. The primary cause of this nutritional condensation is the high temperature-induced 65% moisture loss in roasted nuts as compared to the control. After roasting, pulses and nuts were shown to have a similar decrease in moisture content, falling to less than 3% [22]. When it comes to total phenolic content, foxnuts are greater than other nuts[22,23]. They range from 346.02 to 470.62 mg GAE/100 g. Macadamias, cashews, brazil nuts, and almonds are lower than fox nuts (46 mg GAE/100 g), while it is 137 mg GAE/100 g in cashews and 239 mg GAE/100 g in almonds, which is still lower. Pistachios (867 mg GAE/100 g) and walnuts (1625 mg GAE/100 g), on the other hand, show the opposite pattern, which means that these nuts rather exhibited a higher amount of phenolic contents when compared to roasted foxnut[22].

4. Phytochemical Constituents in Foxnut and Their Activities

Alkaloids, terpenoids, tannins, and glycosides were among the phytochemicals found in a study, along with phenols and flavonoids in the foxnut's seed coat[24] (Figure 5). Caffeine and atropine are two of the many alkaloids that are commonly investigated in plant roots and seeds[25]. According to reports, they are potent central nervous system stimulants, anesthetics, analgesics, hypoglycemic agents, and antioxidant agents. To fight infections, they are also endowed with antibacterial, anti-helminthic, antiseptic, analgesic, antiplatelet, and anticoagulant qualities in addition to muscle relaxant qualities[25-29]. One of the biggest classes of phytochemicals, terpenoids, has also demonstrated anti-inflammatory, anti-carcinogenic, anti-oxidant, and anti-malarial properties as a defense against various viral and bacterial diseases[30]. Water-soluble polyphenols called tannins have anti-oxidant, anti-carcinogenic, and anti-mutagenic qualities[31]. Glycosides can potentially prevent cancer and neurological and cardiovascular disorders by increasing immunity and reducing inflammation[25].

5. Health Benefits of Foxnut

5.1 Foxnut as an Antioxidant

Foxnut is a notable source of natural antioxidant. The primary benefit of makhana is its antioxidant activity, which is linked to conditions like diabetic nephropathy and proteinuria inhibition. When 2,2-diphenyl-1-picrylhydrazyl (DPPH), Catalase (CAT), and Superoxide Dismutase (SOD) activity are examined, foxnut extracts show elevated rates of radical scavenging behavior and are proven to be an effective antioxidant because they prevent lipid peroxidation as well[25,27,29,32,33]. According to laboratory investigations, foxnut extracts exhibit strong anti-oxidant properties based on the IC_{50} values found after performing different experiments[25]. Additionally, it boosts the activity of specific antioxidant enzymes, protects against H_2O_2-driven apoptosis, and enhances cell viability[27,32].

The total protein and fat contents of fox nuts are greatly impacted by roasting, which in turn changes the antioxidant activity that the nuts display after roasting. When roasted nuts (470.62 mg GAE/100 g) were compared to the control (346.02 mg GAE/100 g), the total protein content was 36% higher. When roasted nuts were compared to the control (4.15 mgCE/g, a similar rise in total fat content (7%) was also seen. Regarding antioxidant activity, roasted fox nuts are more effective in scavenging DPPH free radicals (79.13%) than roasted cashews (59.99%), pistachios (74.20%), and other nuts[22]. In conclusion, different nuts have varying amounts of total phenols and flavonoids; however, roasted foxnuts have more antioxidant capacity than most commonly ingested nuts (i.e., cashews and pistachios).

5.2 Antibacterial and Cytotoxic Potential of Foxnut

In a recent study of bio-fabrication of silver nanoparticles from foxnut leaves and evaluating their antibacterial, antioxidant[38], and cytotoxic potential[37], a rapid and reliable method for synthesizing AgNPs from a hot hydrothermal leaf extract of foxnut (ELAgNPs) was established. The cytotoxic, antioxidant, and antibacterial properties of these nanoparticles have also been assessed. The pathogenic bacteria *B. subtilis* and *E. coli* were employed for determining their antibacterial activity using the agar well diffusion method[36,37]. Using a sterile cotton swab, fresh overnight cultures with an OD of 0.5 McFarland (about 1-2 × 10^8 CFU/mL) were utilized as inoculums and seeded on agar plates. Using sterile tips, 6 mm wells were drilled into the agar medium plate's surface. Various concentrations (1, 10, 50, 100, 250, and 500 ppm) of ELAgNPs with four replicates were employed in a volume of 100 uL. After being incubated for 12 and 24 hours, the Zone Of Inhibition (ZOI) was measured. ELAgNPs demonstrated cytotoxicity in a dose-dependent manner and performed better in their anti-bacterial activity than

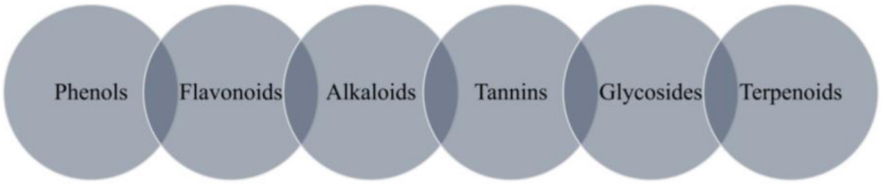


Figure 5. The various types of phytochemicals found in foxnuts.

the controls[36,37]. Thus, the foxnut leaves were confirmed to possess excellent anti-bacterial and cytotoxic activity.

Besides this, anti-apoptotic chemicals were also found to be present in the ethanolic extract of foxnut, indicating its anticarcinogenic effect, and the concentrations of several compounds, such as gallic acid, resveratrol, and allicin, were measured employing HPLC. Additionally, tumor size decreased in the A549 Human Caucasian Lung Carcinoma Cancer cell line-induced tumor in male immunodeficient mice model[26]. Thus, this superfood is also endowed with cytotoxic potential as well.

5.3 Anti-diabetic Properties of Foxnut

Foxnut is a nutrient-dense food with therapeutic value in treating diabetes, too. According to Ayurvedic science, foxnut satisfies the Pitta and Vata doshas, strengthening the body, easing chronic weariness, and nourishing the ovaries, heart, testicles, and uterus. Its Pitta-reducing qualities help to lessen the burning sensation that arises with any illness. Ultrastructural and histopathological studies confirm that the *E. ferox* seed extract increases antioxidant enzyme levels and lowers hyperglycemia, which may be caused by the pancreatic release of insulin from remnant and restored β-cells in STZ-induced diabetic rats, protecting β-cells against ROS-mediated death[21-24]. The seeds of *E. ferox* are a good source of naturally occurring antioxidants and can help avoid diabetic nephropathy. It has been postulated that 2β-hydroxybetulinic acid 3β-caprylate (HBAC), an anti-diabetic active principle found in fox nuts, might be responsible for the probable release of insulin from the pancreatic β-cells, enhancing glycaemic control in diabetes[21-24]. A plethora of secondary complications occur in terms of reproductive health as well due to diabetes; thus, foxnut has indirect reproductive benefits as well.

5.4 Anti-ageing Activity

The body's metabolism is reflected in the skin, which is known to be sensitive to changes. An internal repair mechanism for coarse pores, allergies, and skin infections is provided by the right balance of amino acids. Numerous studies have confirmed the value of amino acids in preserving healthy, firm skin and hair[39-41]. Amino acids nourish the skin, hair, and nails inside the body and give the body's cells an antioxidant[38]. The most potent antioxidants are amino acids, which can also operate as a mediator, remove intermediates of free radicals, and break chain reactions. Leucine, methionine, isoleucine, cysteine, and arginine are among the other amino acids found in this superfood[25]. The amino acids that are creatine precursors and necessary for skin, nails, and hair sustenance are methionine and arginine. Healthy skin is largely provided by the creatine that is derived from arginine and glutamine. Proline and isoleucine are two more amino acids that support the body's growth and development. Raw foxnut contains the highest concentration of glutamic acid[25]. Higher amounts of anti-aging amino acids such as methionine, glutamine, and cysteine are found in this food item. Significant extra amino acid levels are present in raw and popped seeds for optimum bodily function. Because *E. ferox* contains a wealth of antioxidants, the skin appears younger and more beautiful for a longer period, functioning as an age-lock mechanism. This *E. ferox* has proven to be more helpful for someone with early aging symptoms, such as wrinkles, prematurely white hair, and other indicators of aging[29]. Foxnut has also been demonstrated to have anti-aging properties by helping arteries and veins regain flexibility and supporting the upkeep of healthy tissues[29,39,40]. Its usage has been shown in conventional medical treatment as well to stop the accumulation of toxins and make it easier for the body to eliminate dangerous chemicals[29].

5.5 Foxnuts as a Part of the Daily Dietary Regime

Foxnut seeds can prolong the life of an infant or an elderly person. It has characteristics that nourish the stomach and support the kidneys and the spleen. Therefore, for people impacted by COVID-19, these seeds can be used as a supplement, primarily as a nutraceutical. It is also believed to strengthen humoral and cell-mediated immunity when taken regularly[18]. The agent responsible for the COVID-19 pandemic, SARS-CoV-2, possesses an enzyme called ACE2, a functional receptor that physically blocks the renin-angiotensin-aldosterone system (RAAS) activation. Via docking-based evaluation, it has been seen that different compounds like ferotocodimer A, Buddlenol E, [(2S,3R,4E,8E,2'R)]-1-O-(β- glucopyranosyl)-N-(2-hydroxy-docosanoyl)-4,8- sphingadienine] found in foxnut can be potent ACE2 inhibitors that may be useful in balancing the RAAS network and possibly minimizing organ damage[44]. It is well-known to be used as a daily diet to prevent complications from SARS-COVID-19 or to increase strength while recovering. ICU patients on a

ventilator can receive foxnut seeds through a nasogastric tube as part of their nutritional routine[18]. Therefore, foxnut may be a great addition for COVID-19 patients. Additionally, it lessens weariness and palpitations while increasing strength and endurance. A 1:3 ratio of sticky rice to foxnut seeds in China is used to make rice dumplings[42]. When used in diet therapy, these function as extremely natural and superfoods.

5.6 Spermatogenetic Activities of Foxnut

Foxnut is demonstrated to be used in certain preparations to enhance the sperm count in the body and thus might play a crucial role in improving male reproductive health. Moreover, a traditional Chinese preparation in powder form consists of makhana to treat spermatorrhoea[23]. Fox nuts aid in maintaining the water balance and hydration of our body. This keeps the secretions of the body moist. Thus, foxnut helps the male reproductive system by making secretions stickier and raising bodily moisture levels. It boosts the quantity and quality of sperm; therefore,the reproductive system is also thought to benefit from makhana[29]. The powdered seed is recommended to be taken twice a day in Ayurvedic medicine at a quantity of 5 to 10 grams. For adults, the dosage can be increased to 30 grams per day for enhancing the reproductive system[29]. It has a low glycemic value but a high amino acid index. Male infertility has traditionally been treated with makhana due to its spermatogenic and aphrodisiac properties, which has resulted in making it a culturally important crop. Our recent study investigated the role of *Euryale ferox* Salisb., against cadmium-induced testicular pathophysiology, oxidative stress, hormone levels, and other parameters of testis damage. Results indicated that its extract ameliorates cadmium-induced testicular impairments, modulating testicular histoarchitecture, steroid hormone, and oxidative stress[44].

6. Conclusion

This review provides an overview of the plethora of virtues foxnuts possess in treating a wide range of diseases. It also deals with the recent advancements made in the field of employing foxnut extracts for various purposes. This plant cultivation has reached several countries, such as China and Japan, to name a few, and the north-eastern part of India. This article provides a critical analysis of various extracts and chemical components that are present in *E. ferox* and their respective biological activities. Because the shell is an adsorbent, its effects outweigh the entire plant's. Additionally, it offers a variety of bioactivities, such as anti-oxidant, anti-diabetic, anti-cancer, anti-bacterial, anti-depressant, anti-aging, anti-inflammatory, and anti-melanogenic properties. Some mitigating implications provided by *E. ferox* include neuroprotective, hepatoprotective, and cardioprotective actions. Makhana has several health benefits, including enhancement of weight loss, overcoming infertility, keeping one's heart healthy, reducing the aging process, maintaining the health of the digestive system, improving kidney health, aiding people suffering from arthritis, etc. If further research is done on these plant components, amazing therapies for many ailments might be found, and they might also be able to alleviate human suffering in other ways. Conventional studies and methodologies should be used to examine further the new pharmacological qualities and biological entities of the *E ferox* plant. Last but not least, foxnut also possesses reproductive benefits as well. Lastly, employing the several activities of this superfood in the various biotechnological and medical applications should be the prime focus for future work in clinical studies since several bioactive ingredients found in foxnut are patented to play key roles in different bodily systems[23]. Additionally, the reproductive benefits of the foxnut should be explored further clinically.

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