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Review Article

A Critical Review on Ethnobotanical and Pharmacological Aspects of *Euryale Ferox Salisb.*

Roopal Mittal[1,2,3,*] Shailesh Sharma[3], Amit Mittal[4]

ABSTRACT

Introduction: One of the relished dry fruits known by the names of Makhana, Phool Makhana, Gorgon Nut and Fox Nut in the Indian continent, *Euryale ferox Salisb.* (Nymphaeaceae) is the only plant that belongs to Euryale genus. It is found in abundance in Mithila, Darbhanga and Madhubani region of Bihar state. Since ages, in Ayurveda and Chinese practices, it has been used for the treatment of the renal disorder, chronic diarrhoea, excessive leucorrhea and hepatic dysfunctioning. Its bio-active compounds act as antioxidant, antimicrobial, anti-ischaemic, anti-diabetic, immunomodulatory, anti-melanogenic, anti-cytotoxic. **Methods**: The information has been collected from various scientific journals, reviews, books, reports and patent databases. **Results**: This review summarizes the isolated bioactive compounds in different extracts, patented compounds/formulations with pharmacological activities, present in different parts of *Euryale ferox Salisb.* Hence, it has been used as a remedy for numerous ailments since long and also proves itself as a panacea for humanity.

Key words: Anti-aging, Anti-cytotoxic, Anti-diabetic, Cerebrosides, *Euryale ferox*, Ethnobotanical.

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INTRODUCTION

India is a land of biodiversity where many plants are used as medicines in the form of herbs. Immunoboosters such as *Ocimum tenuiflorum* (Tulsi), *Mentha* (Pudhina), *Cinnamomum verum* (Dalchini), *Syzygium aromaticum* (Clove) and *Illicium verum* (Star anise) are vitals of our kitchen and means, as aromatic condiment and flavouring additives. Thereby the known medicinal effects of these herbs make them more dispensable in daily chores as well the researchers get interested in extracting active moiety to further explore their pharmacological actions. Further, these herbs have low-cost value with no side effects. Similarly, the *Euryale ferox* Salisb. (Nymphaeacae), is an aquatic rhizomatous flowering plant that belongs to Euryale genus. It is the only plant species that is native to Eastern and Southern Asia covering parts of India, Korea and Japan.[1] Foxnut, fox nut, gorgon nut, semen euryales, Qian shi and prickly water lily are the synonyms used in various parts of the world for the plant.[2,-19] In India it is known as makhana, and is relished as a dry fruit all over the country. Traditionally makhana is the delicacy of Mithila, Darbhanga and Madhubani regions of Bihar state.[4-5] *Euryale ferox* seeds have been reported earlier in their uses in Ayurveda and Chinese preparations for the treatment of the renal disorder, chronic diarrhoea, excessive leucorrhea and hepatic dysfunctioning.[6] In a daily diet, it was believed to increase humoral immunity and the cell-mediated immunity to some extent.[7] The ethnomedicinal use including halua (semisolid preparation), Laddus (Wheat flour balls) shared *Euryale ferox* (Makhana) one of the main ingredients in the post-pregnancy diet.[8] As per old saying-"The *Euryale ferox* seed is a grain dish, good for a baby and also for an old man- to prolong life," as it has the characteristics of strengthening the spleen, nourishing the stomach, supplementing the kidney, and controlling nocturnal enuresis. Thus, makhana is a neutraceutical supplement for a balanced diet.[9]

This review enlisted the details of the isolated bioactive compounds and different extracts of plant parts and various patented compounds/formulations along with their pharmacological activities which are present significantly in *Euryale ferox Salisb.* Thus, the *Euryale ferox Salisb.* is an important plant to be discussed in detail which can be used to protect human beings from serious ailments in their early stages and further help in alle*via*ting individual health. Therefore, there is a need to compile it in one place.

GEOGRAPHICAL INDICATION

Euryale ferox Salisb. is distributed all across north India encompassing the Kashmir valley from Northern Himalayan to Eastern Himalayan ranges, Manipur, Bihar, Assam, Orissa, West Bengal. Though now its cultivation is confined only to Bihar along with adjacent states i.e. Assam, Orissa and West Bengal.[5] Optimum atmospheric conditions to grow is tropical and sub-tropical climate, temperature ranging from 20°C-35°C, relative humidity between 50%-90% and rainfall between 100 cm-250 cm.[6] The *Euryale ferox* is a perineal plant, rooted in the soil with leaves that are broad, round and blade-shaped thus remaining afloat on the surface of pond water. The leaf lamina and vascular bundles have many sharp prickles whereas the rhizomes lie deep in the bottom of the pond with the help of dense fleshy roots. At a time, the plant bears 15-20 spongy fruits,

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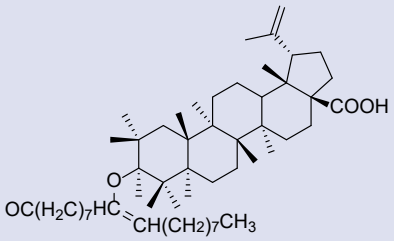
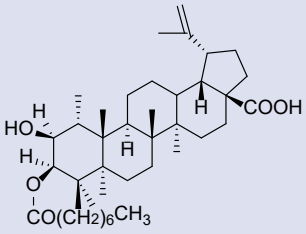
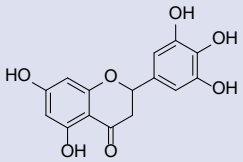
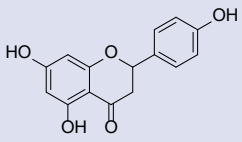
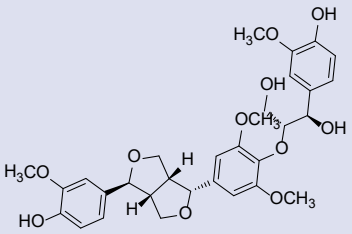
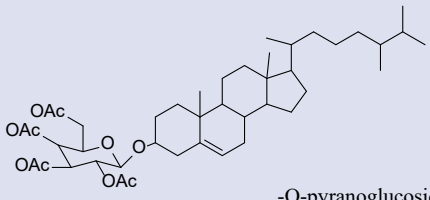
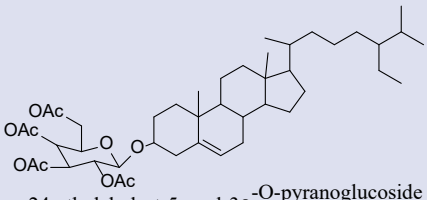
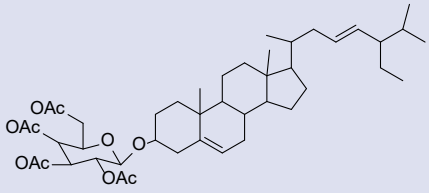
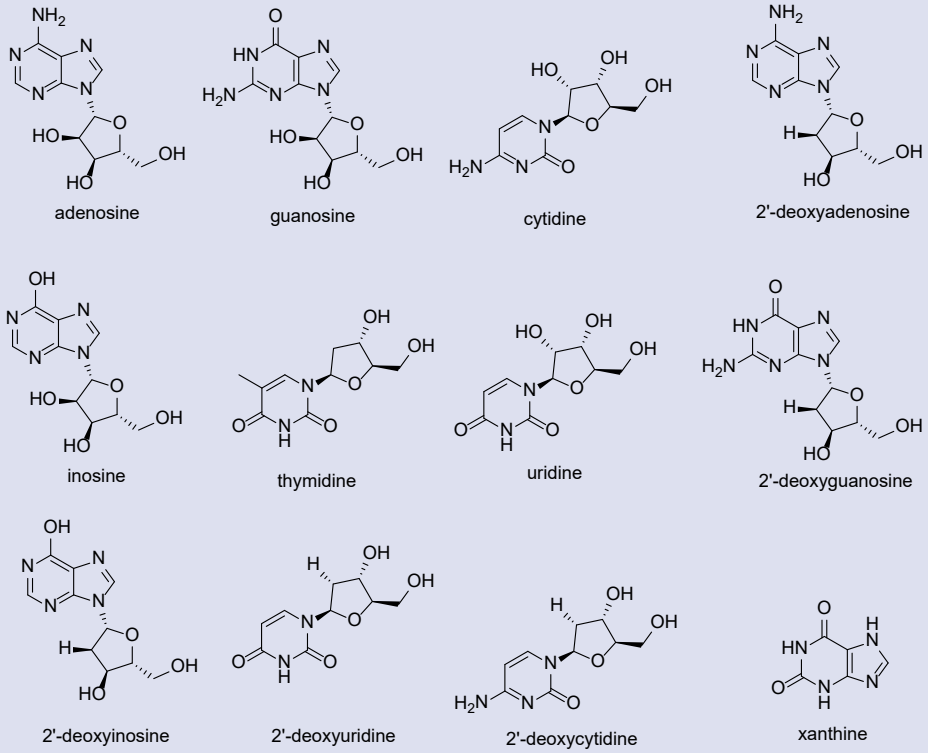
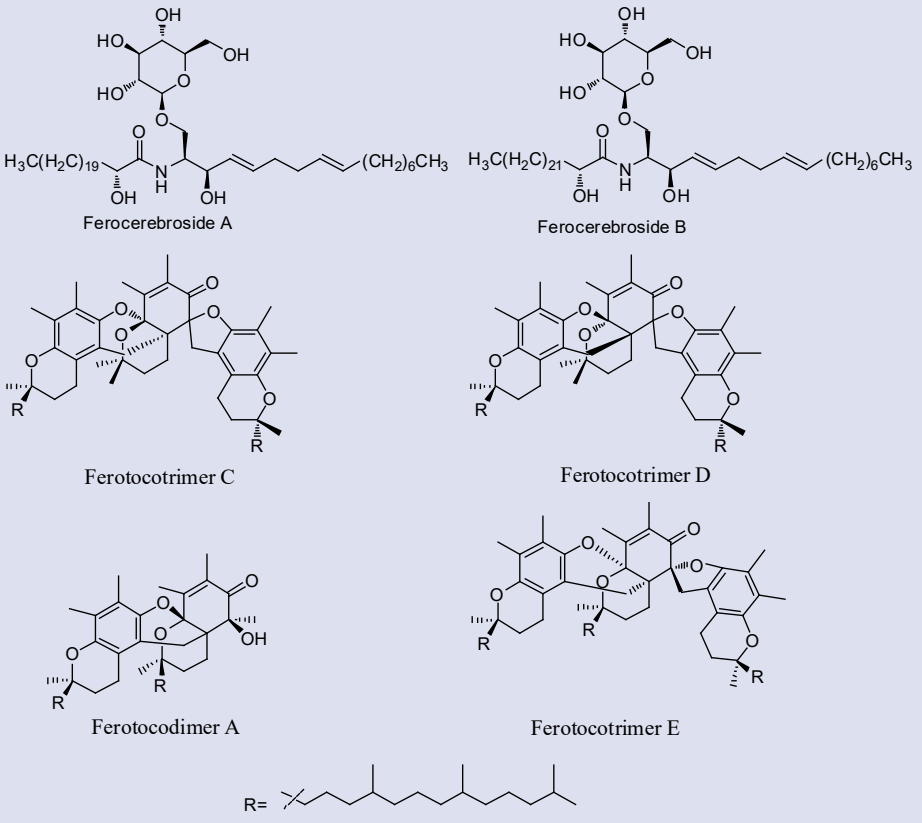
containing 30-40 nuts each. When the fruit ripens, it dehisces and releases the nuts, which encode within a mucilaginous coat.[5,10]

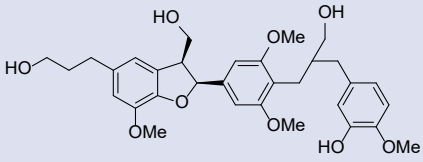
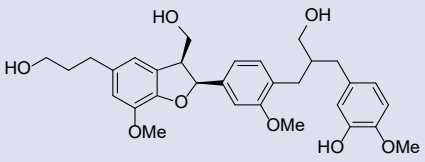
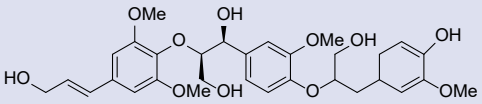
BIOACTIVE CONSTITUENTS

Its main nourishing components are carbohydrates, proteins rich in essential amino acids, flavonoids, cyclic dipeptides, sterols, lipids and vitamin E.[1] The chemical composition of *Euryale ferox* is 75.4% carbohydrates, 11.16% protein, 0.028% Phosphorus, 0.042% Sulphur, 0.027% Calcium, 0.51% fat, 0.5% minerals, 11.82% moisture, 0.62% ash.[11-13] The raw seed and puffed seeds have 362 and 328 kcal/100gm of calorific value.[14] Table 1 shows the number of compounds identified and their pharmacological activity. Glycosylsterols (24-methylcholest-5-enyl-3β-O-pyranoglucoside, 24-ethylcholest-5-enyl-3β-O-pyranoglucoside, and 24-ethylcholesta-5,22E-dienyl-3β-O-pyranoglucoside) were identified in *Euryale ferox* by Nuclear Magnetic Resonance (NMR) and Mass Spectroscopic methods.[15] The cerebroside, an isomeric mixture of N-α-hydroxyl-cis-octadecaenoyl-l-O-β glucopyranosylsphingosine and its trans isomer were reported to be present in the rhizome extract of *Euryale ferox*.[16] Two cerebrosides, ferocerebroside A [(2*S*,3*R*,4*E*,8*E*,2'*R*)-1-*O*-(*β*-glucopyranosyl)-*N*-(2hydroxydocosanoyl)-4,8-sphingadienine] and ferocerebroside B [(2*S*,3*R*,4*E*,8*E*,2'*R*)-1-*O*-(*β*-glucopyranosyl)-*N*-(2'-hydroxytetracosanoyl)-4,8-sphingadienine], two tocopherol trimers, ferotocotrimers C and D, and two known tocopherol trimers had also been isolated in the seeds of *Euryale ferox*.[17,18] The three sesquineolignans, namely Euryalin A (7''-dehydroxyacernikol), Euryalin B (7''-dehydroxy-5'demethoxyacernikol) and Euryalin C (4'-O-(2-guaiacyl-2,3-propanediol)syringylglycerol-8-O-4-(sinapylalcohol) ether), and 16 known compounds, which were all first isolated from the plant.[19] The two tocopherol polymers, chroman-type dimer named ferotocodimer A and its spiro-type trimer named ferotocotrimer E were isolated from the seeds of *Euryale ferox*.[18] The three phenolic compounds (5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-chroman-4-one, 5,7,4-trihydroxyflavanone and buddlenol E) have been identified with the technique LC-ESI-MS/MS (Liquid Chromatography-Electro Spray Ionisation-Tandem Mass Spectrometry).[20] The crude polysaccharides were isolated from petioles and pedicels using ultrasound-assisted technique.[21] Recently the ribonucleosides and deoxyribonucleotides, a series of nitrogen-based molecules, which have been confirmed as indispensable nutritional ingredients and functional substances were also quantified in seed extract.[22] The 2β-hydroxybetulinic acid 3β-caprylate (HBAC)[23] and 2β-hydroxybetulinic acid 3β-oleiate (HBAO)[24] were isolated from the seeds of *Euryale ferox* ethyl acetate fraction. The phenyl ammonia-lyase (PAL) and cytochrome P450 encoded gene expression in phenylpropanoid biosynthesis) lead to the maturation of *Euryale*

Table 1: List of compounds reported in the various extract of *Euryale ferox* Salisb. (Nymphaeaceae).

Activity	Compound isolated	Structure
Anti-diabetic[23-24]	Pentacyclic triterpenes in metanolic extract from seeds	 2β-hydroxybetulinic acid 3β-oleiate  2β-hydroxybetulinic acid 3β-caprylate
Anti-fatigue activity[20]	Polyphenols in the ethanolic extract from seeds	 5,7-dihydroxy-2- (3,4,5 trihydroxyphenyl)chroman-4-one  5,7,4 trihydroxyflavanone  Buddlenol E
Ulceroprotective Activity[15]	Glycosylsterols reported in ethanolic extract	 24-methylcholest-5-enyl-3β-O-pyranoglucoside  24-ethylcholest-5-enyl-3β-O-pyranoglucoside  24-ethylcholesta-5 ,22 E-dienyl-3β-O-pyranoglucoside

Anti-cancer[22]	Nucleosides and nucleobases reported in the distilled water extract of seeds	
Anti-oxidant activity[17-18]	Cerebrosides and Tocopherol polymers reported in ethanolic extract from seeds	

Diabetic nephropathy[19]	Sesquineolignans reported in ethanolic extract	 Euryalin A  Euryalin B  Euryalin C

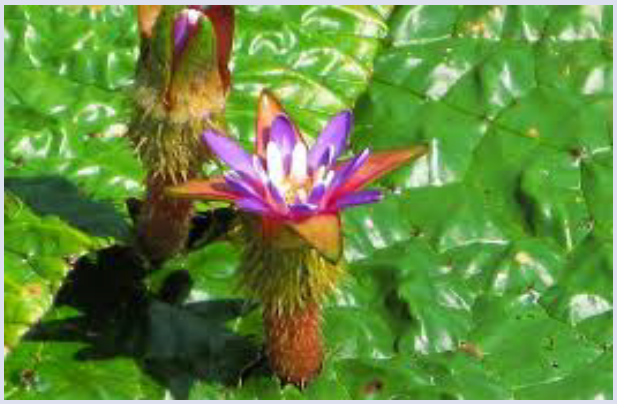


Figure 1: *Euryale ferox Salisb.*

Figure 3: Seeds of *Euryale Ferox.*

Figure 2: Rhizome containing seeds.

Figure 4: Makhana (Popped Form).

ferox seeds.[25] The reveratrol, alliin and gallic acid were detected in the ethanolic *Euryale ferox* extract by HPLC technique.[26]

PHARMACOLOGICAL ACTIVITIES OF *EURYALE FEROX* SEEDS

Activator and adsorbent activity of shell

Earlier *Euryale ferox* salisb., the shell was used as a fuel.[27] Although later a new application enlightened that the *Euyrale ferox* shell has been used to fabricate the activated charcoal with phosphoric acid, potassium hydroxide and zinc chloride as an activating agent *via* a chemical reaction.[28-30] The eco-friendly activated charcoal derived from *Euryale ferox* shell is used as a bio-adsorbent for chemical dyes. Hence it is a valuable source of remediating wetland water bodies polluted with colored water effluents such as basic fuschin, industrial waste.[31] The methanolic extract of *Euryale ferox* seeds and other plant parts showed significant antibacterial activity against nine clinically isolated bacterial strains (*Staphylococus aureus*, *Escherichia coli*, *Pseudomonas aureoginosa*, *Citrobacter freundi*, *Shigella flexneri*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella typhi and Salmonella typhimurium*), thereby providing a scientific basis for its use in urinary tract infections.[32]

Anti-oxidant activity and antifatigue activity

The first methanolic extract of *Euryale ferox* seeds was prepared and then this methanolic extract was further fractioned into n-hexane, dichloromethane, ethyl acetate and n-butanol in a series. The DPPH free radical scavenging assay showed IC_{50} value 5.6, 1.5 and 2.2 µg/ml of crude extract, ethyl acetate and n-butanol fractions. The crude, ethyl acetate and n-butanol extracts showed a high level of inhibition with IC_{50} value of 20.5, 5.1 and 6.3µg/ml in lipid peroxidation assay. The crude, n-butanol and ethyl acetate fractions at 100 µg/ml were found to have been increase by 24%, 26% and 34% respectively in SOD inhibitory activity, 23%, 32% and 26% in CAT activity and 50%, 65% and 58% in GPX activity in a dose-dependent manner. Thus, in all enzyme inhibitory activity, the ethyl acetate showed highest endogenous enzyme inhibition thereby helps in removing oxidative stress.[33]

The antioxidant activity of *Euryale ferox* ethanolic extract was observed by further fractioning it into petroleum ether, ethyl acetate and n-butanol successively. These extracts were subjected to DPPH free radical scavenging activity using gallic acid and ascorbic acid as positive controls. The SC_{50} (Scavenging) values of *Euryale ferox* ethanolic , petroleum ether, ethyl acetate, n-butanol extract, gallic and ascorbic acid were found to be 68.7, 29, 20.4, 78.8, 10.7 and 20.8 µM respectively. The ethyl acetate fraction was found to be the most potent antioxidant activity (SC_{50}=20.4 µM). Among isolated compounds from ethyl acetate fraction, the Euryalin A, Euryalin B and Euryalin C have SC_{50} value of 237.2, 6.8 and 162.3µM. The authors described that Euryalin B possesses significant activity due to lack of methoxy group as compared to Euryalin A. The antioxidant activity was further evaluated in mesangial cells which were already treated with Euryalins and then exposed to oxidative stress *via* glucose stimulation. The Euryalin B, rel-(2α,3β)-7-O-methylcedrusin, (+)-syringaresinol and buddlenol E were found to have significant antioxidant action.[20] The three compounds (5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-chroman-4-one, 5,7,4-trihydroxyflavanone and buddlenol E) were identified from the seed coat and found responsible for the antioxidant activity using DPPH free radical scavenging assay.[20] The polysaccharide EPJ was isolated from semen Euryales by water extraction and found to have strong antioxidant properties (superoxide ion, reducing power, DPPH free radical scavenging activity). In DPPH activity, the polysaccharide EPJ posses IC_{50} value of 0.026mg/ml when compared with vitamin C and tertiarybutyl hydroquinone (TBHQ) having IC_{50} value 0.001 mg/ml. In superoxide ion scavenging activity, the polysaccharide EPJ, vitamin C and TBHQ exhibit equal inhibiting activity with IC_{50} value 0.783, 0.129 and 0.217 mg/ml respectively. Similarly, in reducing power, at 2mg/ml, the polysaccharide EPJ was equipotent to Vitamin C and TBHQ. *In-vivo* D-Galactose model was used where Vitamin E acted as a positive control. The SOD, CAT and GSH were observed to a significant decrease ($P<0.05$ vs control group) while Malondialdehyde (MDA) had a marked increase ($P<0.01$). Thus the polysaccharide derived from seed shell might help in increasing endogenous enzymes and decreasing lipid peroxidation.[2]

Ho *et al.*, 2012 carried out the estimation of antioxidant properties[34] and total phenolic content of wetland medicinal plants in Taiwan and reported the following values of *Euryale ferox Salisb.* (Nymphaeaceae) as mentioned in Table 2.

Antidiabetic activity

The *Euryale ferox* seed shell ethanolic extract was evaluated for antidiabetic activity, by measuring fasting blood glucose, fasting serum insulin and insulin sensitivity index. The Streptozotocin injection led to the development of hyperglycaemia and then the groups were administered the dosage of ethanolic extract at various concentrations for 4 weeks. A significant reduction was observed in a high dose (600 mg/kg) group by 41% which was compared to standard glimiperide (51% reduction). The fasting serum insulin was found to be 7.31, 6.11 and 5.90 mIU/L in the middle, high dose and glimiperide group. The insulin sensitivity index was significant ($P<0.01$) in the both high dose feeding group and glimiperide group. The total cholesterol was observed at low levels in a high dose below the normal levels. The improvement in HDL was not significant in both middle and high dose groups.[35] The ethanolic extract of *Euryale ferox* seed was also evaluated by glycaemic parameters. The blood glucose level, oral glucose tolerance test value and plasma insulin level for the group (400 mg/kg) was 91.8 mg/dl, 88.45 mg/dl, 11.66 µM as compared to control group (81.8mg/dl, 138.15 mg/dl, 12.39 µM).[36] Similarly, the 2β-hydroxybetulinic acid 3β-caprylate (HBAC) and 2β-hydroxybetulinic acid 3β-oleiate (HBAO) were isolated from *Euryale ferox* seeds extract and further evaluated at 20, 40, 60 mg/kg dosage for blood glucose, plasma insulin and glycosylated haemoglobin (A1c) level. The blood plasma glucose level of HBAC and HBAO were evaluated as 100.3 and 114.7 mg/dl in 60 mg/kg, p.o. as compared to control (94.8 and 97.8 mg/dl). Successively the plasma insulin level of HBAC and HBAO were found to be 18.2 and 13.6 µM as compared to control (19.9 and 16.1 µM). This was further confirmed by the glycosylated haemoglobin (A1c) level of HBAC and HBAO found

Table 2: Activities and constituents reported in Aqueous and Methanolic Extract of plant *Euryale ferox Salisb.* (Nymphaeaceae).

Activities and constituents	Aqueous Extract	Methanolic Extract
TEAC (Trolox Equivalent Antioxidant Capacity) Assay antioxidant activity estimation	14.58 ± 1.11	350.73 ± 4.13
IC_{50} value DPPH radical scavenging activity (µg/ml)	>2000	307.35 ± 1.61
Reducing power	0.07 ± 0.01	0.76 ± 0.12
Polyphenol (µg CE[a]/mg)	28.16 ± 0.49	213.58 ± 7.29
Flavanoid (µg RE[a]/mg)	3.28 ± 0.21	16.70 ± 2.31
Flavanol (µg CE[a]/mg)	1.93 ± 0.02	3.43 ± 0.01

[a]Where CE is Catechin equivalent and RE is Rutin equivalent

to be 8.95% and 8.42% in 60 mg/kg, p.o. treated rats as compared to controls (8.5% and 7.09%). The A1c level remained constant in normal rats which showed that HBAC and HBAO did not cause hypoglycaemia in normal rat groups. The activity of SOD, CAT, reduced glutathione and glutathione peroxidise increased significantly when compared with control. The histopathological examination of pancreas, liver and kidney also proved that damage caused by diabetes was also reversed by *Euryale ferox* extract.[23- 24] The *Euryale ferox* seed's ethyl acetate extract had phenolic substances such as gallic acid, digallic acid, hexaglycone, epicatechin gallate, procyanidine, catechin, gallic acid derivatives. The Euryale extract was evaluated as a tablet, capsule and steamed bun which were taken 30 min before the meal, resulted in a decrease of glucose level by 26.5%, 28.7% and10.3% respectively. *In vitro* alpha amylase assay was also carried where IC_{50} value of Euryale extract was 1.36 mg/dl as compared to standard acarbose (1.08 mg/dl). The extract exhibited good alpha amylase inhibition thus resulting in postprandial glucose decrease.[3] The deproteinized polysaccharides extracted from *Euryale ferox* seeds were evaluated for an insulin resistance model. The Hep G2 and 3T3-L1 adipocytes were used to check the rate of glucose consumption. Crude polysaccharides as a test, Dexamethasone (1μM/l) as standard and a control group were used in the experiment. The glucose consumption rate of crude polysaccharide, standard and control were 100.4%, 100.4% and 102.4% respectively. Therefore researchers concluded that polysaccharides not only improve uptake of glucose by insulin resistant cells but also induce insulin to reduce blood glucose levels.[37- 38] The crude polysaccharide tested above was found to be Glucan composed of (1→4)-α-D-Glcp with branches substituted at O-6 position and terminated with T-α-D-Glcp. The Glucan decreased blood glucose level *via* upregulation of GLUT-4 which might follow PI3/Akt pathway in insulin resistance cells.[39] The popped makhana was treated with amylopullulanase (Lactobacillus amylophilus GV6) to obtain a floor that could be used as a pre/diabetic diet or as a therapeutic ingredient in preparations. The ash, moisture, amylase, starch content and water holding capacity, oil absorption capacity, swelling power, *in-vitro* digestibility test were carried out between enzyme resistant floor and simple makhana flour. The total dietary fibre content was found to increase significantly by 0.005 p-value. Thus it can be used in the food processing industry.[40]

Anti-hyperlipidemic activity

The total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride levels were found to be 151.4, 58.3, 32.03 and 74.77 mg/dl as compared to control (137.7, 63.19, 23.54 and 67.57 mg/dl) in rats after administration of ethanolic seed extract at 400 mg/kg.[36] Further HBAO and HBAC isolated from ethyl acetate fraction from the same plant were subjected to lipid estimation. The total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides values of HBAO were found as 121.4, 49.14, 37.10 and 84.17 mg/dl as compared to control (118.9, 55.7, 24.4 and 71.9 mg/dl) and HBAC were found as 133.6, 46.3, 34.05 and 81.9 as compared to control (126.2, 54.6, 25.7 and 74.8 mg/dl).[23-24]

Hepatoprotective activity

The ethanolic extract of *Euryale ferox* seed coat was evaluated for non-alcoholic fatty liver disease (NAFLD) characterised by hepatic steatosis, which was inducted in Wistar rats *via* high-fat diet for 4 weeks. The body weight, adipose tissue and ratio of liver to body weight increased in NFALD rats as compared to control. The total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride values of ethanolic extract at 30 mg/kg dose were 6.4, 1.3, 0.2 and 4.8 mmol/l as compared to control (4.2, 0.6, 0.15 and 3.2 mmol/l). The SOD and MDA value at 30 mg/kg were found to be 0.23 U/ml and 23.2 mmol/l as compared to control (0.21 U/ml and 10.4 mmol/l). Further liver activity parameters alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were found to be 40.4 and 42.6 IU/ml as compared to control (21.3 and 19.4 IU/ml). The expression of IRS-1 (insulin resistance substrate-1) and CYP2E1 were analysed *via* western blotting. There was an increase in the expression of CYP2E1 as compared to control. Thus the author concluded that overexpression of CYP2E1 leads to hepatoprotective activity.[42] The ethanolic extract of seed was evaluated for hepatic enzymes as a consequence of diabetes impact on the liver. The hepatic hexokinase, glucose-6-phosphatase, fructose-1-6-biphosphatase and glucose-6-phosphate dehydrogenase levels were found as 0.202, 0.040, 0.037 and 0.134 units/min/mg of protein as compared to control (0.236, 0.035, 0.032 and 0.162 units/min/mg of protein).[36] Similarly, after diabetes these enzymatic parameters were studied in HBAC and HBAO compounds. In HBAC, the hepatic hexokinase, glucose-6-phosphatase, fructose-1-6-biphosphatase and glucose-6-phosphate dehydrogenase levels were estimated at 0.196, 0.197, 0.030 and 0.105 units/min/mg of protein as compared to control (0.232, 0.174, 0.028 and 0.125 units/min/mg of protein).[23] In HBAO, the hepatic hexokinase, glucose-6-phosphatase, fructose-1-6-biphosphatase and glucose-6-phosphate dehydrogenase levels were 0.20, 0.04, 0.02 and 0.09 units/min/mg of protein as compared to control (0.24, 0.03, 0.01 and 0.15 units/min/mg of protein).[24] The 2β-hydroxybetulinic acid 3β-caprylate (HBAC) moiety exhibits an excellent hepatoprotective activity. They concluded that the HBAC and HBAO at 60 mg/kg administration could significantly increase the activity of glucose-6-phosphate dehydrogenase which was considered to be an essential enzyme for Hexose Mono Phosphate (HMP) shunt.[24]

Anti-melanogenic activity

The *Euryale ferox* seed's ethyl acetate extract was evaluated for anti-melanogenic activity. In-vitro antioxidant activity was determined by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt (ABTS) radical scavenging assays which exhibited results in a dose-dependent manner. The ferric reducing antioxidant power (FRAP) and cupric ion reducing capacity (CUPRAC) assay revealed 820 fold and 669 fold increase at 30 μg/ml as compared to control (20 fold increase). The oxygen radical absorbance capacity (ORAC) assay, the trolox acted as a positive control. The area under the curve for ethyl acetate extract (6.25 μg/ml) and trolox (10 μg/ml) were found to be 86.1 and 6.3 respectively. Further, the mushroom tyrosinase inhibition was determined where arbutin acted as standard. The ethyl acetate extract (300 μg/ml) inhibited activity by 87.4±5.4% as compared to arbutin (57.0 ± 3.9%) and copper chelating effect of ethyl acetate extract (500 μg/ml) was found to be 49.6 ±0.7% as compared to PTU (n- phenylthiourea) (27.2 ± 4.0%). Thus indicated that ethyl acetate extract inhibit tyrosinase enzyme by copper chelation. The cell *via*bility and melanin level analysed for ethyl acetate extract (30 μg/ml) and Arbutin (2mM) were found to be 44.1± 2.5% decrease and 33.4± 2.3% decrease as compared to control. The ethyl acetate extract effect on tyrosinase was further confirmed by L-DOPA zymography. In western blotting technique, the tyrosinase, TYRP-1(Tyrosinase Related Protein-1) and TYRP-2 (Tyrosinase Related Protein-2) proteins reduced by 100%, 41% and 91% respectively as compared to control. The lysosomal degradation was analysed by using hexoaminidase as substrate through western blotting. The ethyl acetate (30μg/ml), arbutin (2mM) and PTU (n-phenylthiourea) did not show any lysosomal degradation whereas the chloroquine decreased drastically which suggested that ethyl acetate extract showed proteosomal degradation. Thus tyrosinase inhibition and copper chelating activity collaborated in the anti-melanogenic property, thereby suggested that the ethyl acetate extract act as a biomedical whitening agent.[42,43]

Anti-cytotoxicity

The *Euryale ferox* seeds possess an anti-cancer activity property as these were believed to induce apoptosis with the internal regulation of mitochondrial membrane's reactive oxygen species, caspase proteins

and p-53 factors. The reveratrol, alliin and gallic acid were detected in the *Euryale ferox* ethanolic extract by HPLC technique. Firstly, anti-cancer activity was carried in all types of cells with ethanolic extract (150 µg/kg) but promising results were shown by A549 Human Caucasian Lung Carcinoma Cancer Cells. The cytotoxic effects were observed at different concentrations (50-150µg/kg dissolved in 1% DMSO). A549 human Caucasian lung cancer cells and MRC-5 human lung fibroblast cells were chosen for cell proliferation assay (Lactate dehydrogenase (LDH) cytotoxicity assay and MTT calorimetric assay) and Pifithrin (p-53 inhibitor) as standard. The ethanolic extract (150µg/ml) showed significant activity through 60% of control cell *via*bility in A549 Human Caucasian Lung Carcinoma Cancer Cells whereas 90% of control cell *via*bility was observed in MRC-5 human lung fibroblast cells. Similarly, the LDH released in A549 Human Caucasian Lung Carcinoma Cancer Cells is 60% of control and 20% of control in MRC-5 human lung fibroblast cells. Thereby the ethanolic extract (150µg/ml) had cytotoxic effect in A549 Human Caucasian Lung Carcinoma Cancer Cells. The Annexin V staining and Hoechst 33342 staining were performed to check the pathway followed by destructive cancer cells. The ethanolic extract showed increased apoptotic bodies of A549 Human Caucasian Lung Carcinoma Cancer Cells rather than necrosis in the Annexin V staining technique. Further, the Hoechst staining involved in staining of nucleus led to the determination of DNA fragmentation. Both techniques revealed that the cells followed the apoptotic pathway and DNA fragmentation in a dose-dependent manner. The cell cycle arrest assay was performed through a flow cytometer. The DNA content index revealed that the percentage of ethanolic extract treated cells in the G1 phase increased as compared to control whereas there was a decline in percentage of ethanolic extract treated cells in the S phase of the cell cycle. In mitochondrial membrane potential assay and Caspase 3/7 activity revealed that the ethanolic extract promoted apoptosis in a dose-dependent manner. The molecular changes were determined by western blotting through the expression of various proteins (COX-2, Bcl-2, Bak, Bax, PARP, MDM2, Akt and P-53), thus confirmed the suppression of Akt signaling and p-53 induced apoptosis in ethanolic extract treated cells in a dose-dependent manner. *In-vivo* experiment was performed by transplanting A549 Human Caucasian Lung Carcinoma Cancer Cells in male Balb/c nu/nu mice and constructed a Xenograft model. The tumour size was found reduced in ethanolic extract (100mg/kg) as well as the H&E staining and tunnel assay showed high levels of p53 and degraded DNA by 50% of control. Altogether, results expressed the anti-cancer effects in a p53-dependent manner.[26,44]

Cardioprotective activity

The cardioprotective activity of *Euryale ferox* seeds aqueous and alcoholic extracts were estimated. *In-vitro* ROS scavenging activity, at 0.005% and 0.01% were used for SOD analysis and it was observed that the extracts scavenged 39% and 52% of O_2^- radicals as compared to 100% SOD and at 0.01% extracts scavenged 93% and 100% of O_2^- radicals. The male Sprague Dawley rat heart was isolated for the myocardial reperfusion model and kerbs-Henseleit bicarbonate buffer as a physiological salt solution in the Lagendroff assembly. The hearts were perfused with makhana ethanolic extract (25, 125 and 250 µg/ml). Another feeding model was also set up in which rats were fed with 250 and 500 mg/kg for 21 days and the hearts of rats were isolated, subjected to 30 minutes ischaemia and 2-hour reperfusion. The coronary flow and aortic flow were determined. Firstly no difference was observed in control and makhana treated rats, later on, 60 and 120 min of reperfusion significant difference was noticed. Myocardial Infarct size was reduced in both the acute and feeding models. Later antibody array technique was used to determine the functional proteins responsible for an action. The thioredoxin-1 (Trx-1) and thioredoxin related protein-32 (TRP32) were found in makhana treated hearts. Taken together, potent free radical scavenging activity and increased expression of Thioredoxin-1 and Thioredoxin Related Protein-32 (TRP-32) were responsible for the cardioprotective action.[45-46]

Anti-depressant activity

The petroleum ether fraction of *Euryale ferox Salisb.* was administered in chronically unpredictable stressed female Balb/c mice and subjected to several depressant experiments (sucrose preference test, open field test, tail suspension test, and forced swim test). The behavioural performance was not found different but the sucrose performance test showed significant improvement in pleasure at 100 and 150mg/kg of petroleum ether extract. The phosphorylated AMPK(serine/threonine protein kinase adenosine monophosphate-activated protein kinases)-Thr 172 and ULK1(mammalian autophagy-initiating kinase)-Ser 137 were found to be upregulated in the petroleum ether fraction treated mice and mTOR downregulation, lead to autophagy, ultimately resulted in maintenance of high quality proteins, organelles and sufficient energy. The presence of vitamin E by 69.62% (GCMS analysis), the most abundant compound in the extract was found responsible for the neuroprotective behaviour in *Euryale ferox* petroleum ether extract treated mice.[47]

Anti-aging activity

A unique assemblage of amino acids in *Euryale ferox* was reported to have a strong antioxidant property which could remove intermediate free radicals and chain reactions. The leucine to isoleucine ratio, arginine and lysine to proline ratio of raw *Euryale ferox* were 1.7 and 9.6% respectively. The essential amino acid index was observed to be greater than any other cereal (wheat, rice, soya, amaranth and Bengal gram). The amino acids particularly arginine and methionine are considered to be the precursor of creatine protein which is an essential element for beautiful skin, nails and hair. The glutamine for firm skin, carnitine for fat burning, arginine for vasodilation *via* NO release, taurine released from cysteine effective in diabetic cells were found to be in abundance in *Euryale ferox* plant. The higher protein and amino acid value created a window for further development of research in considering its neutraceutical value and improvement in horticultural management practices.[13]

Gastro-retentive non-effervescent tablets

The non-effervescent floating matrix tablets of ciprofloxacin were developed by using *Euryale ferox* seeds powder (150 mg), HPMC K4M (180 mg), 0.5% magnesium stearate and polyvinyl pyrolidine (PVP) (29 mg) and ciprofloxacin (291 mg) offered a hardness (5.1kg/cm^2), thickness (0.59 cm), apparent density (0.974 g/cm^3), buoyancy lag time (0 min) and floating duration (12 hrs) following zero-order kinetics. The buoyancy showed by tablet was due to the natural porous structure of *Euryale ferox* seed powder at a particle size of 302 µm thus proved to be gastro-retentive prolonged delivery tablets. The advantage of this effervescent tablet was to replace 50% of HPMC K4M with *Euryale ferox* seeds powder which is natural in origin and increased the floating time by 4 hrs as compared to standard.[48]

PATENTS

The *Euryale ferox* seeds have high medicinal value that was used in the number of preparations to lower the postprandial blood glucose level, as a component of whitening agent, and to cure diabetic nephropathy. The *Euryale ferox* green stems were used to prepare healthcare vinegar which helps in improving stomach flatulence, clear heat of the body. Similarly, a composite powder of walnut and *Euryale ferox* seeds were used to prepare a soup that prevents constipation. The *Euryale ferox* seeds were also reported to have been processed with glutinous rice to have rice dumplings. The *Euryale ferox* seeds were found to have been used in preparation that tonify the kidney, revitalize spleen,

cure nocturnal enuresis, improve sperm count and treat insomnia. The *Euryale ferox* shell was used as a natural cotton dying agent and functional fabric finishing agent as well. Some of the patents showing the importance of wetland medicinal plant *Euryale ferox* are listed in Table 3.

DISCUSSION

Euryale ferox seeds, Makhana (Popped form) is most commonly used as dry fruit, apart from being used as salty roasted makhana or instant makhana porridge. In a daily diet, it was believed to increase humoral immunity and the cell-mediated immunity to some extent. In *Euryale ferox*, several bioactive compounds comprising euryalins, glycocylsterols, buddlenol E, cerebrosides and tocopherol polymers in ethanolic extract; 2β-hydroxybetulinic acid 3β-caprylate, 2β-hydroxybetulinic acid 3β-oleiate, and phenolic compound in ethyl acetate fraction; nucleosides and nucleobases, polysaccharides in aqueous extract have been reported. The seeds having fractions of ethylacetate, depicted the antioxidant results, which make it an ethno-medicinal valued plant. The 2β-hydroxybetulinic acid 3β-caprylate and 2β-hydroxybetulinic acid 3β-oleiate were found potential anti-diabetic compound that could be used as adjuvant therapy in diabetes. The essential amino acids index (EAAI) of *Euryale ferox* raw was highest at 93.63% as compared to other essential amino acids rich feeds (Soybean 85.59%; Rice 82.88%; Wheat 65.18%). The seeds alongwith medicinal values, also have industrial applications as they are used as adsorbent, activator and fabric finishing agents. The gastro-retentive ciprox floating tablet had also been manufactured by replacing 50% of HPMC with *Euryale ferox* seeds powder (302 μm). The *Euryale ferox* plant has been reported for various pharmacological activities comprising antimelanogenic, antihyperlipidaemic, hepatoprotective, immunomodulatory action and anticytotoxicity. Many patents have been registered for the traditional use of *Euryale ferox* seeds in treating diabetic nephropathy, enuresis, chronic diarrhoea and liver dysfunctioning.

CONCLUSION

This review projects detailed account of different extract and some of the chemical compounds in *Euryale ferox Salisb.* that could be identified, i.e. Glycosylsterols, ferocerebroside A, ferocerebroside B, ferotocotrimers C and D, sesquineolignans, Euryalin A, Euryalin B and Euryalin C, 5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-chroman-4-one, 5,7,4-trihydroxyflavanone and buddlenol E, 2β-hydroxybetulinic acid 3β-caprylate (HBAC) and 2β-hydroxybetulinic acid 3β-oleiate (HBAO), reveratrol, alliin and gallic acid. Further, there were several biological activities afforded by *Euryale ferox Salisb.,* i.e. activator and adsorbent activity, antioxidant, antihyperlipidemic, hepatoprotective, antidiabetic, antimelanogenic, anticytotoxic, cardioprotective and antiaging activity. In spite of having neutraceutical values, the cultivation of this plant has been limited to North-Eastern part of Himalayan range in India, China and Taiwan. If more and more research is done on this plant parts than amazing solutions for different diseases can be found and it can provide relief to humanity in multiple ways. There is a further need to explore the *Euryale ferox* plant for its new biological entities and their pharmacological activities with the aid of standard experiments.

Table 3: List of patents by different inventors detailing the medicinal uses of *Euryale ferox Salisb.* (Nymphaeaceae).

S. no	Patent number	Description
1)	CN 110090243	The Euryale seed extract can be used for lowering postprandial blood glucose level.[3]
2)	CN108424469	The polysaccharide isolated from Euryale seed extract consist a molar ratio of glucouronic acid: mannose: glucose: galactose: arabinose in 0.55: 0.6: 52.7: 1: 4.3 and had a molecular weight of 8.7*103 Da. The polysaccharide could promote insulin resistance and glucose uptake form cells, which was natural and non-toxic.[37]
3)	CN109645177	*Euryale ferox* leaves are used in preparing tea in which golden flower generation concept was preferred so that referred tea would be rich in Eurotium cristatum which regulate immunity, clear heat and invigorate stomach.[49]
4)	CN106511479	The Euryale seed shell extract was used to regularise lipid profile as well to normalise the body weight. Thus proves to be an anti-hyperlipidemic and anti-obesity.[50]
5)	WO/2015/152564	The mixture oil of *Euryale ferox Salisb*., *Euphorbia lathyris l*., and *Rosa multiflora thumb*. possess a stimulating property of Peroxisome Proliferator Activated Receptors (PPAR) -α nuclear receptors that helps in alleviating skin sensitivity.[43]
6)	KR100899847*	A skin whitening composition containing Poria cocos, Dioscorea opposite, Nelumbo nucifera and Euryale ferox is prepared and its main action is inhibition of tyrosinase enzyme and melanin production.[51]
7)	CN103638137	A traditional Chinese powder preparation involving *Euryale ferox* as one was capable of treating spermatorrhea, improve sleep quality.[52]
8)	746/DEL/2008	The ready to mix Makhana's kheer instant anywhere in any conditions, either in hot or cold water.[53]
9)	CN105671935	The leaves and stem extract of Euryale ferox is used as a functional finishing agent in fabric development.[54]
10)	CN106261716	The Euryale ferox seeds and glutinous rice are used in 1:3 ratio to prepare rice dumplings. These act as super natural food in diet therapy.[55]
11)	CN106047630	Gordon Euryale leaves extract have been used in preparation of vinegar that helps in clearing heat, diuretic and blood purifier.[56]
12)	CN107760056	The *Euryale ferox* seed shell is used in preparation of natural dye for colouring the cotton fabric.[57]
13)	CN109527352	A nerve soothing and sleep assisting powder have been prepared with the components of black seasame, oats, brown rice, lotus seeds, lily, Euryale ferox and walnut kernels.[58]
14)	JP2003113100	The polar extract of *Euryale ferox, Nelumbo nucifera* and *Mactonia amazonica* can inhibit excessive fat accumulation by promoting the expression of heat producing protein (32 Da molecular weight) in brown adipose cell.[59]
15)	CN101057671	The Chinese dietry composition containing Euryale ferox, red dates and walnut are used to make soup, has evidence of curing nocturnal enuresis.[60]
16)	CN102716458	The medicinal liquor containing 20-40% Chinese medicine liquor, 25-38% Euryale ferox seed powder, 20-35% coix seed powder and 5-13% of yeast is reported to possess anti-rheumatoid property.[61]
17)	IN201621011361	The neutraceutical composition is prepared by using *Foeniculum vulgare, Plantago ovata, Terminilia chebula, Terminilia belerica, Euryale ferox, Glycine max and Saccharum officinarum* is reported to cure constipation.[62]
18)	CN102379978A	The Euryale ferox plants have been used in treatment of diabetic nephropathy.[63]

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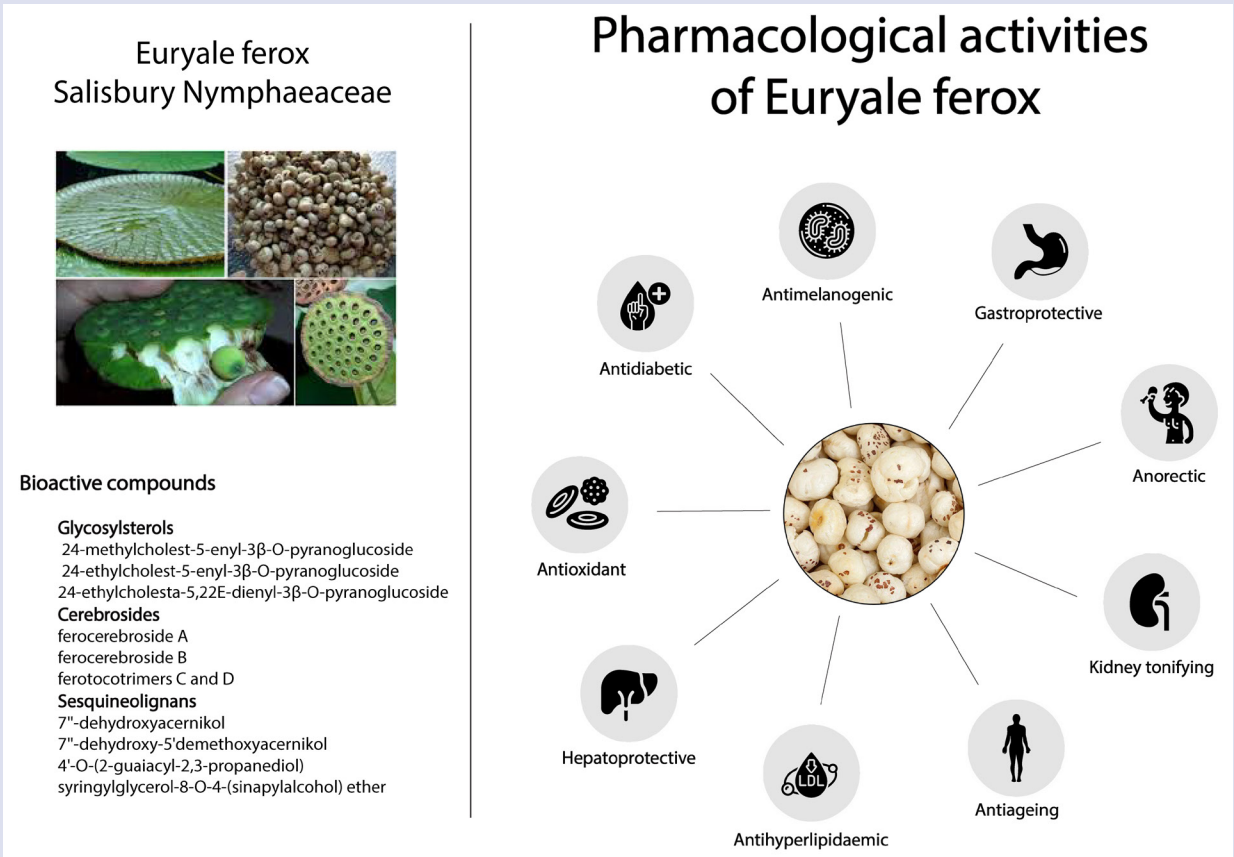
GRAPHICAL ABSTRACT



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