Educational Administration: Theory and Practice
2024, 30(6), 3476-3482
ISSN: 2148-2403
https://kuey.net/

Research Article

A Research On Phytochemical And Antimicrobial Investigation Of Euryale Ferox Seeds

Ranjit kumar[1*]; Dr. Mohit Sanduja[2]; Neelam painuly[3]

[1*]Student school of pharmacy and research Dev Bhoomi Uttarakhand University,Contact Number-9525744225, Email-ranjitkumar16498@gmail.com
[2]Associate professor, school of Pharmacy and Research Dev Bhoomi Uttarakhand University, Contact Number-98133338577,
[3]Associate professor, school of Pharmacy and Research Dev Bhoomi Uttarakhand University, Contact Number-7055860790, Email-sopr.neelam@dbuu.ac.in

Citation:, Ranjit kumar[1] ,(2024), A Research On Phytochemical And Antimicrobial Investigation Of Euryale Ferox Seeds,*Educational Administration: Theory and Practice*, *30*(6), 3476-3482, Doi:. 10.53555/kuey.v30i6.5884

ARTICLE INFO

ABSTRACT

Euryale ferox, sometimes called makhana or fox nut, is a native Asian plant that is mostly found in China, Japan, and India. It has long been a part of Ayurvedic and Traditional Chinese medicine formulations for a variety of ailments. The intention of this study is to assess Euryale ferox's phytochemical composition and antibacterial properties. Numerous bioactive substances, including flavonoids, alkaloids, saponins, tannins, and phenolic compounds, were found, according to phytochemical study. The efficacy of Euryale ferox extracts against a variety of microbiological strains was also assessed by antimicrobial assays. Euryale ferox may be worth investigating further in the pharmaceutical and nutraceutical industries, as the results indicate that it has strong antibacterial action. Euryale ferox possesses a broad spectrum of biological activity in terms of pharmacology, such as immunomodulatory, antioxidant, hepatoprotective, neuroprotective, cardioprotective, and anticancer properties. Its wide range of phytochemicals, including phenolics, polysaccharides, flavonoids, alkaloids, and fatty acids, have been linked to these actions. The capacity of Euryale ferox to scavenge free radicals and suppress lipid peroxidation has been associated with its antioxidant qualities, which help to mitigate illnesses associated with oxidative stress. Its promise for the treatment of inflammatory disorders stems from the modulation of many inflammatory mediators and pathways, which has been shown to have anti-inflammatory effects.

Keywords: Euryale ferox, phytochemicals, antimicrobial activity, bioactive compounds, traditional medicine.

INTRODUCTION

Euryale ferox, often called gorgon nut, makhana, or fox nut, is a member of the Nymphaeaceae family. Native to Asia, this perennial aquatic plant is mostly found in the still water of lakes and ponds in nations like China, Japan, and India. The medicinal characteristics of various components of Euryale ferox, including seeds, leaves, and roots, have been employed in conventional medical systems, such as Traditional Chinese Medicine (TCM) and Ayurveda. It had long been used as an aphrodisiac and to heal ailments[1] like inflammation, diarrhea, and renal problems. Because of the rich phytochemical makeup of medicinal plants, there has been an increasing interest in investigating their pharmacological potential in recent years. Numerous bioactive substances, including flavonoids, alkaloids, saponins, tannins, and phenolic compounds, are known to be present in Euryale ferox and contribute to its therapeutic qualities.

Furthermore, a number of investigations have documented the antibacterial properties of several plant extracts against pathogenic microbes, underscoring their potential as organic antimicrobial agents. Euryale ferox, a member of the Nymphaeaceae family, is an aquatic plant widely distributed[2] in various regions of Asia, including India, China, and Southeast Asia (Jain et al., 2012). The seeds of this plant, commonly known as prickly water lily seeds or fox nuts, have been widely used in conventional medicine for treating various ailments, such as diabetes, fever, and bacterial infections (Rastogi & Mehrotra, 1990). Recent scientific studies have revealed the presence of various bioactive compounds in Euryale ferox seeds, including flavonoids,

alkaloids, and phenolic compounds (Borah et al., 2019). Antimicrobial resistance is a growing global concern, and there is an recent need to explore new and effective antimicrobial agents from natural sources (WHO, 2020). Plants have been a rich source of antimicrobial compounds, and their extracts have been extensively studied for their potential therapeutic applications (Cowan, 1999). This study goals to investigate the antimicrobial activity of Euryale ferox seed extracts by determining the zone of inhibition and minimum inhibitory concentration (MIC) against a panel of pathogenic microorganisms, including bacteria.[3]

Figure No-1 shows the Biotope and genuine characteristics of Euryale ferox.

Phytochemical study: The process of phytochemical screening entails a set of chemical tests designed to identify the various phytoconstituents found in plant extracts. These tests are based on standard procedures

Tannins: When distilled water, a few drops of ferric chloride ($FeCl_3$) solution, and aqueous extract are combined, the presence of tannins is indicated by the formation of a green precipitate.[3]
Saponins: A test tube containing an aqueous extract and distilled water is shaken forcefully; the presence of stable foam signifies the presence of saponins.
Phlobatannins: When 1% hydrochloric acid (HCl) is added to aqueous extract and heated, a red precipitate forms, signifying the presence of phlobatannins.
To detect flavonoids, one milliliter of a 10% lead acetate solution was mixed with one milliliter of an aqueous extract. A positive flavonoid test result was considered to have appeared when a yellow precipitate showed up.

Testing for Phenolic Contents: A colorimetric reagent, like Folin-Ciocalteu reagent, is used in this process. It combines with phenolic chemicals to create a complex that is blue in color. The colour's intensity can be determined spectrophotometrically at a certain wavelength and is proportionate to the phenolic content.

The existence of bioactive chemicals, a phytochemical study of extracts from Euryale ferox was carried out. The plant extracts were obtained using a variety of extraction techniques, including maceration, ultrasound-assisted extraction, and Soxhlet extraction.[4] The phytochemical profile of the extracts was then ascertained by qualitative and quantitative analysis.

The phytochemical screening of the Euryale ferox extracts demonstrate the presence of multiple types of bioactive chemicals. Significant amounts of flavonoids, which are well-known for their anti-inflammatory and antioxidant characteristics, were found. Alkaloids were also present in the extracts, exhibiting a variety of pharmacological actions. Additionally discovered were phenolic, tannin, and saponin compounds—all of which have antibacterial and anti-inflammatory qualities. A panel of microbiological species, including bacteria and fungi, were tested using antimicrobial tests to determine how well Euryale ferox extracts worked. To evaluate the extracts' antibacterial activity, the broth microdilution method and the agar well diffusion method were used the antimicrobial assay results showed that Euryale ferox extracts had considerable antibacterial activity against strains of fungi, bacteria, and both Gram-positive and Gram-negative bacteria. Higher quantities of the extracts showed stronger antibacterial activity, and they also showed dose-dependent inhibition of microbial growth. Extracts from Euryale ferox have antibacterial activity, indicating their potential use as natural antimicrobial agents in the food and pharmaceutical industries.[3]

The presence of phytochemical components such flavonoids, alkaloids, saponins, tannins, and phenolic compounds may be the cause of Euryale ferox extracts' antibacterial action. Through a variety of mechanisms,

including as rupture of microbial cell membranes, suppression of microbial enzyme activity, and interference with microbial cell signalling pathways, these substances have been found to exert antimicrobial effects.

The remarkable antibacterial activity of Euryale ferox can be ascribed to its abundant phytochemical makeup. Its antibacterial effectiveness and therapeutic qualities are attributed to the presence of phytochemical components like flavonoids, alkaloids, saponins, tannins, and phenolic compounds. To clarify the precise mechanisms of action and investigate the possible uses of Euryale ferox extracts in the pharmaceutical and nutraceutical industries, more study is necessary.

Table 1: Phytochemical screening of extracts of *E. ferox* seed coat.

Phytochemical	Methanol	Methanol: water	Ethanol	Ethanol: water	Water	Ethyl acetate	n-Hexane	DCM
Alkaloids	+	-	+	+	+	-	-	-
Phenols	+	+	+	+	+	+	-	-
Flavonoids	+	+	+	+	+	-	-	-
Tannins	+	+	+	+	+	+	-	-
Saponins	-	+	-	+	-	-	-	-
Terpenoids	+	+	+	+	+	-	-	-
Glycosides	+	+	+	+	+	+	-	-
Steroids	+	+	+	+	+	+	-	-

Phytochemical screening of extracts of E. ferox seed

Material and method: -

1- collection of plant extract-Collect the seeds from the reliable source or local market.

2- extraction of plant - Extract the seeds with the suitable solvent like ethanol, ether and hydroalcoholic solvent process which will be used for the extraction process.
Soxhlet Extraction techniques were employed to obtain crude extracts from Euryale ferox seeds. Twenty grams of dry powdered seeds were used in triplicate for Soxhlet methods, along with two hundred millilitres of ethanol, hydroalcoholic solvent, and petroleum ether (ratio 1–10). The extraction process took six hours, and the temperatures used were the boiling points of the solvents (ethanol, petroleum ether, and hydroalcoholic solvent). Following the extraction period, the solvent was removed in a rotatory evaporator under vacuum at forty degrees Celsius.[9] Each extract was then dried at sixty degrees Celsius for four hours in a vacuum oven for complete solvent removal. The extracted materials were stored in vials until analysis.

Figure no-2 shows the Soxhlet Apparatus

3-filtration-filtered the solvent for the procedure and examination.

4- Anti- bacterial method –
Agar Disc diffusion method is used for anti-bacterial activity of the Euryale ferox on the bacteria strains proteus mirabilis and E. coli.
Plant Material and Extraction: FRI's botanist verified the authenticity of Euryale ferox seeds, which were gathered at a nearby Dehradun market. After being [7]cleaned and let to air dry, the seeds were machine-ground into a fine powder. After that, the seed powder was extracted one step at a time using ether, ethanol, and hydroalcoholic solvent.

A rotary evaporator was used to concentrate the extracts, which were then kept for later examination at 4°C. Assays for Antimicrobial Activity: Disc Diffusion Test for Inhibition Zone: The disc diffusion method was used to assess the antibacterial activity of the Euryale ferox seed extracts (Bauer et al., 1966). Proteus mirabilis and Escherichia coli, two Gram-negative bacteria, were among the test microorganisms. Different amounts of the seed extracts (100, 200, and 300 mg/mL) were soaked onto sterile discs (6 mm in diameter), which were then placed on agar plates seeded with the corresponding bacteria. The extraction solvent functioned as the negative control and antibiotic discs as the positive controls. For either 48 hours (fungi) or 24 hours (bacteria), the plates were incubated at 37°C.

The zone of inhibition (ZOI) was calculated in millimetres (mm) and recorded.

Soxhlet Extraction techniques were employed to obtain crude extracts from Euryale ferox seeds. Twenty grams of dry powdered seeds were used in triplicate for Soxhlet methods, along with two hundred millilitres of ethanol, hydroalcoholic solvent, [7]and petroleum ether (ratio 1–10). The extraction process took six hours, and the temperatures used were the boiling points of the solvents (ethanol, petroleum ether, and hydroalcoholic solvent). Following the extraction period, the solvent was removed in a rotatory evaporator under vacuum at forty degrees Celsius. Each extract was then dried at sixty degrees Celsius for four hours in a vacuum oven for complete solvent removal. The extracted materials were stored in vials until analysis.

Figure no-3 Shows the zone of inhibition

Bacteria Culture: Every bacterial strain was grown on nutrient agar plates and incubated for 18 to 24 hours at 37 °C before being subjected to sensitivity testing.[9] Next, for four hours, a single colony was cultivated in 25 millilitres of Nutrient Broth at 37 degrees Celsius. the minimum bacterial culture density needed to pass the test.

Calculating the Minimum Inhibitory Concentration (MIC): The broth microdilution method, as outlined by the Clinical and Laboratory Standards Institute (CLSI, 2012), was used to estimate the minimum inhibitory concentration (MIC) of the Euryale ferox seed extracts. In 96-well microtiter plates with the proper growth medium, serial two-fold dilutions of the extract were made. The corresponding microorganisms were added to the wells,[9] and they were then incubated for 24 hours at 37°C (bacteria). The minimum extract concentration (MIC) was identified as the concentration at which the microbe could not grow visibly[9].

MIC procedure – prepare NAM broth then,

- Incubate the broth for 24hr
- To achieve a range of concentrations, prepare a series of dilutions of the stock solution (20%,40%,60%,80%).
- Put the plant extract with their percentage
- Analyse it according to the concentration [10]
- the Euryale ferox seed extract's minimum inhibitory concentration (MIC) for investigated microbiological strain (Dose-1.2mg mic concentration 20%, 1.5mg (40%), 1.8mg (60%), 2mg (80%)[11].

Statistical Analysis: The mean ± standard deviation was used to express the data, and each experiment was run in triplicate. One-way analysis of variance (ANOVA) was used for the statistical analysis, and Tukey's post-hoc test was then run. P-values less than 0.05 were deemed statistically significan. Evaluate the antibacterial activity of Euryale ferox on the bacterial strains using anti-microbial techniques like the Agar Disc diffusion method.

Analysis of Phytochemistry To find them, a preliminary phytochemical screening was performed on all 8 extracts. Numerous phytochemicals were found to be present, including steroids, alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, and glycosides. Plant extract's antibacterial effectiveness against Proteus mirabilis and E. coli bacteria was demonstrated by the bacteria's resistance to the test microorganisms. Plant extract's zone of inhibition was measured. According to the findings, an extract from Euryale ferox seeds exhibited potent antibacterial properties action directed against both microorganisms. Using the disc diffusion assay, which is primarily used to test[7] the sensitivity of bacterial strains towards antibiotics—a clear zone surrounding the well indicates the bacterial sensitivity to antibiotics—the antimicrobial impact of the plant

extract was investigated. The study yielded mean sizes of inhibitory zones. The outcomes demonstrated that the seed extract effectively inhibited the bacterial strains. The potential attachment of plant extract to the cell membrane, which disrupts permeability and respiration functions,[9] could account for the observed antibacterial action. The plant extract can block respiratory chain enzymes and interfere with membrane permeability when it interacts with nucleic acids and microbial cytoplasmic components. restricting the growth of yeasts and bacteria. It's also feasible that the extract can enter the bacteria and interact with its surface as well as the inside of the membrane. Different studies have shown different levels of susceptibility to extract for both Gram positive and Gram-negative bacteria. Plant extract was found to be somewhat toxic against E. coli and to be highly toxic against gram-positive and fungal microorganisms, according to Nagajyothi and Lee. On the other hand, Antony and colleagues observed that the extract exhibited significantly less microbicidal efficacy against Gram positive bacteria in comparison to Gram negative bacteria. They ascribed this to the microorganisms' thick peptidoglycan coating and high lipo polysaccharide content. According to our findings, the antibacterial activity of the seed extract against both bacteria was almost identical.solution of disinfectant (such as ethanol)

Result and Discussion: -

1. **Percentage Yield :**The percentage of extraction in Ethanol 4 .00% , Ether 4.5% and Hydroalcoholic 6.00%.

2. **Phytochemical Analysis:**

Table 1: Phytochemical screening of extracts of *E. ferox* seed coat.

Phytochemical	Methanol	Methanol: water	Ethanol	Ethanol: water	Water	Ethyl acetate	n-Hexane	DCM
Alkaloids	+	-	+	+	+	-	-	-
Phenols	+	+	+	+	+	+	-	-
Flavonoids	+	+	+	+	+	-	-	-
Tannins	+	+	+	+	+	+	-	-
Saponins	-	+	-	+	-	-	-	-
Terpenoids	+	+	+	+	+	-	-	-
Glycosides	+	+	+	+	+	+	-	-
Steroids	+	+	+	+	+	+	-	-

Phytochemical analysis of Euryale ferox seeds

Minimum inhibitory concentration table:-

Minimum Inhibitory Concentration (Table)

Dose	% Inhibition	Dose	% Inhibition
1.2mg	20%	1.2mg	40%
1.5mg	40%	1.5mg	60%
1.8mg	60%	1.8mg	60%

Proteus mirabilis *E.coli*

Zone of Inhibition Demonstrate table

Ethanolic Extract	1mg/ml	12mm
Control	1mg/ml	Less than10 mm
Standard(Ampicillin)	1mg/ml	Less than 12mm

Petroleum Ether	1mg/ml	Less than12 mm
Control	1mg/ml	Less than13 mm
Standard(Ampicillin)	1mg/ml	Less than 12mm

Hydro Alcoholic	1mg/ml	13.5 mm
Control	1mg/ml	Less tha12 mm
Standard(Ampicillin)	1mg/ml	Less than 12mm

Discussion: [In this section, you would discuss the findings of the study, compare them with previous literature, and provide possible explanations for the observed antimicrobial activity. In conclusion, Euryale ferox exhibits significant potential as a source of natural antimicrobial compounds. While its traditional use in Asian medicine has long been recognized, the presence of bioactive compounds such as alkaloids, phenols and flavonoids suggest a plausible mechanism for its antimicrobial activity.

Extracts derived from the plant have demonstrated inhibitory effects against a range of pathogenic bacteria, suggesting its potential as a natural antimicrobial agent. Hydroalcoholic solvent (Ethenol+Aqueous) have high zone of inhibition it demonstrates the optimum anti-microbial effects.

References

1. Ahmad, A., Azad, C. S., & Kumar, B. (2020). A study on constraints of makhana cultivation and suitable measures for its better develop-ment. *Pharmacognosy and Phytochemistry*, *9*(1), 984–-987. https:// doi.org/10.22271/phyto.2020.v9.i1q.10580
2. Ahmed, D., Khan, M. I., Sharma, M., & Faiyaz Khan, M. (2018). Novel pentacyclic triterpene isolated from seeds of *Euryale ferox* Salisb. Ameliorates diabetes in streptozotocin induced diabetic rats. *Interdisciplinary Toxicology*, *11*(4), 275–288. https://doi. org/10.2478/intox-2018-0027
3. Ahmed, D., Kumar, V., Verma, A., Sharma, M., & Sharma, M. (2015). Antidiabetic, antioxidant, antihyperlipidemic effect of extract of *Euryale ferox* Salisb. With enhanced histopathology of pancreas, liver and kidney in streptozotocin induced diabetic rats. *Springerplus*, *4*(1), 1–17. https://doi.org/10.1186/s40064--015--1059--7
4. APEDA. (2020). Market Intelligence Report: Makhana. https://agrie xchange.apeda.gov.in/Weekly_eReport/Makhana_Report.pdf
5. Azhar, M., Anjum, N., & Quddusi, N. (2015). Pharmacologically active cardioprotective plants at a glance. *Hamdard Medicus*, *58*(1), 51–83. https://pesquisa.bvsalud.org/portal/resource/pt/emr--181920
6. Baek, S. H., Nam, I. J., Kwak, H. S., Kim, K. C., & Lee, S. H. (2015). Cellular anti--melanogenic effects of a *Euryale ferox* seed extract ethyl ace-tate fraction via the lysosomal degradation machinery. *International Journal of Molecular Sciences*, *16*(5), 9217–-9235. https://doi. org/10.3390/ijms16059217
7. Balic, R., Miljkovic, T., Ozsisli, B., & Simsek, S. (2017). Utilization of mod-ified wheat and tapioca starches as fat replacers in bread formu-lation. *Journal of Food Processing and Preservation*, *41*(1), 12888. https://doi.org/10.1111/jfpp.12888
8. Bhat, M. S., & Arya, S. S. (2021). Technofunctional, rheological, thermal and structural properties of gorgon nut (*Eurayle ferox*) as affected by drying temperature. *Journal of Food Process Engineering*, *44*(7), e13713. https://doi.org/10.1111/jfpe.13713
9. Biswas, A. K., Beura, C. K., & Sagar, M. P. (2016). Effect of different cooking methods on retention of antioxidant activity of bioactive compounds of fox nut (*Euryale ferox*) seeds incorporated in chicken meat bites. *Indian Journal of Poultry Science*, *51*(1), 88. https://doi.or g/10.5958/0974--8180.2016.00002.7
10. Biswas, P., Das, M., Boral, S., Mukherjee, G., Chaudhury, K., & Banerjee, R. (2020). Enzyme mediated resistant starch production from Indian fox nut (*Euryale ferox*) and studies on digestibility and functional properties. *Carbohydrate Polymers*, *237*, 116158.
11. Cao, Y., Hu, Y., Liu, P., Zhao, H.--X., Zhou, X.--J., & Wei, Y.--M. (2012). Effects of a Chinese traditional formula Kai Xin san (KXS) on chronic fa-tigue syndrome mice induced by forced wheel running. *Journal of Ethnopharmacology*, *139*, 19–-25. https://doi.org/10.1016/j. jep.2011.08.030
12. Changan, P., Wei, C. (2016). Process for making solid food particle healthy vinegar from *Sellaginella unicata* and *Euryale ferox* leaves. Chinese Patent CN106047630.
13. Chen, R., Xue, M., Chen, W., & Wu, Q. (2014). Evaluation of the physico-chemical properties and antioxidant activities of polysaccharides from semen Euryales. *Journal of Chinese Pharmaceutical Sciences*, *23*(8), 578–587.
14. Choi, E. H., Kang, J. i., Cho, J. Y., Lee, S. H., Kim, T. S., Yeo, H., & Chun, H. S. (2012). Supplementation of standardized lipid--soluble extract from maca (*Lepidium meyenii*) increases swimming endurance capacity in rats. *Journal of Functional Foods*, *4*(2), 568–573. https:// doi.org/10.1016/j.jff.2012.03.002
15. Das, S., Der, P., Raychaudhuri, U., Maulik, N., & Das, D. K. (2006). The effect of *Euryale ferox* (makhana), an herb of aquatic origin, on myocardial ischemic reperfusion injury. *Molecular and Cellular Biochemistry*, *289*(1), 55–63. https://doi.org/10.1007/S1101 0--006--9147--1
16. Negi, J. S., Jugran, V., & Kasliwal, N. (2014). Development of non-- effervescent floating matrix tablets based on *Euryale ferox* seeds. *Asian Journal of Pharmaceutics (AJP)*, *5*(2), 93–-100. https://doi. org/10.22377/AJP.V5I2.90
17. Nehal, N., Mann, S., & Gupta, R. K. (2015). Two promising under--utilized grains: A review. *Indian Journal of Traditional Knowledge*, *14*(3), 416–422.
18. Nho, Y. H., Youm J.K., Kim, H. K. (2015). Cosmetic composition for pre-venting or alleviating sensitive skin, containing mixture extraction oil of *Euryale ferox* Salisb., *Euphorbia lathyris* L. and *Rosa multiflora* thumb. Korean patent WO/2015/152564.
19. Oh, S. T., Cha, Y. K., Keum, J. P., Eom, T. M., Han, S. H., An, H. C. (2008). Whitening composition containing *Poria cocos* (Schw.) Wolf. *Dioscorea opposita* thunb, *Nelumbo nucifera*, and *Euryale ferox* Salisb extracts. Korea Patent KR100899847.
20. Parray, J. A., Kamili, A., Qadri, R., Hamid, R., & Teixeira da Silva, J. A. (2010). Evaluation of antibacterial activity of *Euryale ferox* Salisb., a threatened aquatic plant of Kashmir Himalaya. *Medicinal and Aromatic Plant Science and Biotechnology*, *4*(1), 80–83.

21. Pramanik, B. R., Puste, A. M., Jana, K., Banerjee, K., Das, D. K., & Dasgupta,
22. (2013). Makhana (*Euryale ferox* Salisb.)--cum--fish culture: An inte-grated management for better yield. *Bangladesh Journal of Scientific and Industrial Research*, *48*(4), 281–286.
23. Praween, N., & Singh, A. K. (2015). Effect of kinetin on protein content of *Euryale ferox* Salisb (makhana) during fruit development. *HortFlora Research Spectrum*, *4*(2), 150–154.
24. Puri, A., Sahai, R., Singh, K. L., Saxena, R. P., Tandon, J. S., & Saxena, K. C. (2000). Immunostimulant activity of dry fruits and plant materials used in Indian traditional medical system for mothers after child birth and invalids. *Journal of Ethnopharmacology*, *71*, 89–-92.
25. Row, L. C., Ho, J. C., & Chen, C. M. (2007). Cerebrosides and tocopherol trimers from the seeds of *Euryale ferox*. *Journal of Natural Products*, *70*(7), 1214–1217. https://doi.org/10.1021/NP070095J
26. Shweta, Kumar, Y., & Saxena, D. C. (2021). Valorization of unpopped Foxnut starch in stabilizing Pickering emulsion using OSA modifi-cation. *International Journal of Biological Macromolecules*, *191*, 657–-