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Phytochemical Screening and Evaluation of Antioxidant Potential in *Euryale ferox* Salisb. and *Eupatorium birmanicum* DC. of Manipur, India

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ABSTRACT

Introduction: *Euryale ferox* Salisb. popularly called "Foxnut" is a spiny aquatic plant locally named "Thangjing" in Manipur and is one of the most highly consumed aquatic crops while *Eupatorium birmanicum* DC. locally named "Langthrei" is an endemic ethnomedicinal plant found in Manipur, India. **Methods:** This study was carried out to examine the phytochemical content, evaluate the Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and the *in vitro* antioxidant potential (DPPH and ABTS assays) in the seeds, arils of *E. ferox* and leaves of *E. birmanicum*. **Results:** Phytochemicals such as phenols, flavonoids, saponins, tannins, alkaloids and steroids were detected from the plant samples studied. Among the samples, TPC was found in the range 21.95 ± 1.18 mgGAE/g ext to 119.80 ± 2.63 mgGAE/g ext while, TFC was recorded in the range 2.57 ± 0.07 mgQE/g ext to 7.27 ± 0.28 mgQE/g ext with the highest value of TPC and TFC in *E. ferox* seeds. In case of DPPH and ABTS assays, the recorded IC_{50} value were attained in the range 16.99 ± 0.49 µg/mL to 335.90 ± 2.19 µg/mL and 70.69 ± 1.83 µg/mL to 576.26 ± 4.41 µg/mL respectively with the lowest IC_{50} value recorded in *E. ferox* seeds. **Conclusions:** The results of our study revealed that seeds and arils of *E. ferox* as well as leaves of *E. birmanicum* contain a variety of phytochemicals and promising antioxidant activity which will contribute a scientific insight for exploration of their therapeutic potentials in the future.

Key words: Foxnut, Phytochemical, Phenol, Flavonoid, Antioxidant, Northeast India.

INTRODUCTION

Euryale ferox Salisb. commonly known as "prickly water lily" or "foxnut" is a spiny aquatic plant having circular floating leaves and it belongs to the family Nymphaeacea.[1] It is popularly known as "Makhana" in Hindi and locally named "Thangjing" in Manipur. It has been traditionally cultivated as a cash crop and is one of the most highly consumed aquatic crops found in Manipur[2] and is reported to be found abundantly in India, Japan, Korea, Southeast Asia, and China.[3,20] Seeds are widely used in Ayurvedic medicines and Chinese preparations for treating many health problems, such as chronic diarrhea, kidney failure, rheumatic disorders, and hepatic dysfunctions.[4,9] The fresh seeds along with the mucilaginous arils are eaten raw with Eromba (soup-based chutney) or Morok metpa (soupless chutney) and are considered as a delicacy in the traditional cuisine of Manipur.[8] On the other hand, *Eupatorium birmanicum* DC. is a wild shrubby plant that belongs to the family Asteraceae and locally named "Langthrei" in Manipur is among the endemic ethnomedicinal plants used by the various ethnic communities of the state for treating several ailments.[10] The fresh juice of the leaves is taken orally to treat gastroenteritis.[11,12] Consumption of leaf decoction in small amounts is believed to be useful in diabetes by lowering the blood sugar level.[13] Free radicals or Reactive oxygen species (ROS) such as superoxide anion radical, hydrogen peroxide and hydroxyl radical are produced as by-products of various physiological processes in our body.[6] An antioxidant is a substance which has the ability to inhibit or delay the oxidative damages to cells of the organisms by either scavenging or neutralizing harmful free radicals that can lead to several degenerative human diseases such as atherosclerosis, cardiac, kidney, liver diseases, and neurodegenerative disorders.[5] Manipur is a state in the northeast India and as a part of the Indo-Burma biodiversity hotspot, it has a very rich diversity of flora and fauna.[17] Different phytoconstituents vary depending on various elements including environmental factors and geographic factors.[18,19] The available literature review so far indicated very limited data on the phytochemical profile and antioxidant potential of *E. ferox* as well as *E. birmanicum* found in the northeastern states of India. Our study aimed to evaluate the phytochemical content and antioxidant potential of the hydroalcoholic extract of seeds, arils of *E. ferox* and leaves of *E. birmanicum*.

MATERIALS AND METHODS

Chemical Reagents

Aluminium Chloride, Sodium Acetate, Quercetin, Gallic Acid, Ascorbic Acid, Folin-Ciocalteu reagent, Methanol, Ethanol, Potassium persulfate, DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS ((2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid). Chemicals used in this research are all in analytical grade.

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Table 1: Details of the plant samples studied.

Scientific Names	Local Names	Plant Parts	Therapeutic Uses
Euryale ferox Salisb.	Thangjing	Fruits, seeds, young petioles and pedicels.	Diabetes, heart diseases[4,13,21]
Eupatorium birmanicum DC.	Langthrei	Leaves	Diabetes, gastroenteritis[11,13]

Collection of Samples

Spiny fruits of *Euryale ferox* Salisb. were collected from its natural habitat at Mayang Imphal, Imphal West District, Manipur (GPS coordinate: 24°62′59″N & 93°89′19″E) from June to September 2023. Fresh plant leaves of *Eupatorium birmanicum* DC. were also collected from its natural habitats at Thoubal District, Manipur (GPS coordinates: 24°38'37"N & 93°59′27″E) during June to August 2023. The local names, parts of plant used and therapeutic uses of the two plants are given in Table 1. Seed kernels and mucilaginous aril part were removed from the spiny fruits of *E. ferox* and oven-dried at 40°C-45°C. After thorough washing, the leaves of *E. birmanicum* were also oven-dried at 35°C-40°C.

Preparation of Extracts

The dried plant materials were pulverized into coarse powder. The plant powder (100g) for each sample was extracted three times using 70% methanol (500mL) through the cold maceration method by keeping at room temperature for 72 h. Using Whatman filter paper, the extracts obtained were filtered, and then solvents were evaporated with the help of rotary evaporator at 40°C. The concentrated plant extracts were then stored at – 20°C for future use.

Qualitative Phytochemical Screening

The qualitative screening of phytochemicals present in the plant extracts was carried out using the standard methods. The presence of phytochemicals such as flavonoids, phenols, saponins, tannins, steroids, and alkaloids in the plant extracts was determined.[22, 23,24]

Total Phenolic Content and Total Flavonoid Content Estimation

Determination of Total Phenolic Content

Total phenol content (TPC) of the plant extracts was determined on a 96-well microplate using the Folin–Ciocalteu method described previously with slight modifications.[25] A calibration curve of the standard Gallic acid was constructed by preparing the dilutions of (0.01, 0.025, 0.05, 0.1, and 0.2) in methanol from the standard stock of 1mg/mL. 10 µL of each of the dilutions and plant extracts were placed in a 96-well plate containing distilled water (50 µL) and then added Folin-Ciocalteu reagent (10 µL) and kept for 6 minutes. Then, 7% Sodium carbonate (100 µL) and distilled water (50 µL) were added to the reaction mixture. The mixture was incubated for 90 minutes in the dark at room temperature and the absorbance was recorded at 760 nm and compared with the standard curve. The expression of TPC was given in milligrams of gallic acid equivalent (GAE) per gram of dry extract weight (mg GAE/g ext). All the samples were taken in three replicates and the results obtained are denoted as mean ± SD.

Determination of Total Flavonoid Content

Total flavonoid content (TFC) of the plant extracts was determined using a 96-well microplate by the Aluminium Chloride Colorimetric method with some minor modifications.[26] A standard calibration curve of Quercetin was constructed by preparing the dilutions of (0.005, 0.01, 0.025, 0.05, and 0.1) in ethanol from the standard stock 1mg/mL. 50 µL of each of the dilutions and each of the plant extracts were placed in a 96-well plate followed by adding 10 µL of 10 % $AlCl_3$. After that, 96 % ethanol (150 µL) and Sodium Acetate (10 µL) were added. Incubate the mixtures in the dark for 40 minutes at room temperature and the absorbance was recorded at 415 nm. The content of total flavonoids in the samples was measured by comparing to the standard curve and expressed as milligrams of quercetin equivalent (QE) per gram of dry extract weight (mg QE/g ext). Experiments were performed on three independent replicates.

Antioxidant Activity

DPPH (1, 1-diphenyl-2-picrylhydrazyl) Scavenging Assay

The potential of the plant extract to inhibit or neutralize the activity of DPPH free radicals was used to measure its antioxidant capacity following the previously described method with some modifications and ascorbic acid was used as the standard.[27,28] The assay is based on the reduction of alcoholic DPPH solutions caused by a hydrogen donating antioxidant. The reduction of DPPH is shown by drop in its absorbance. 100 µL of plant extract solution in methanol as well as the standard taken in different concentration ranges were mixed with 100 µL of freshly prepared methanolic solution of 0.1mM DPPH in a 96-well plate. The mixtures were then incubated for 30 min at room temperature in the dark. The absorbance was measured at 517 nm using a nano-spectrophotometer. The percentage of DPPH scavenging activity was calculated using the formula

% Scavenging of DPPH= {(Ac- As)/Ac} x 100

where, Ac is the absorbance of control (including all the reagents except the test sample) and As is the absorbance of the sample or the standard. The IC_{50} value of the antioxidant activity was calculated using the linear equation derived from the dose-inhibition curve of the extract concentration versus the corresponding percentage scavenging activity.

ABTS ((2, 2′ -azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) Assay

The capacity of the plant extract to stabilize free radicals was also measured with the help of ABTS assay following the already reported method with small changes and using ascorbic acid as the standard.[29,30] This assay measures the capacity of an antioxidant to neutralize the ABTS radical cation (ABTS •+) by the mechanism of electron transfer. ABTS radical cations (ABTS •+) which are green-blue chromophores are generated through a reaction between aqueous 7mM ABTS and 2.45 mM potassium persulfate ($K_2S_2O_8$) which was kept at room temperature in the dark for 12 to 16h before use. Adjustment of the ABTS solution absorbance was done by adding methanol till we got absorbance value (0.70 ± 0.02) at 734 nm. 30 µL of plant extract solution in methanol as well as the standard taken in different concentration ranges were mixed with 170 µL of ABTS solution in a 96-well plate. After 10 min of incubation in the dark, the absorbance was measured at 734 nm. The degree of discoloration is proportional to the antioxidant activity of plant extract or the ABTS cation inhibition. The percentage inhibition was evaluated using the formula given below

(ABTS •+) Inhibition (%) = {Ac-As)/Ac)} x 100

where, Ac is the absorbance of control (including all the reagents except the test sample) and As is the absorbance of the sample or the standard. The IC_{50} values of the antioxidant activity were determined in the same manner as described in the DPPH assay. Experiments were performed in triplicates and results obtained are denoted as Mean ± SD.

Statistical analysis

The statistical analysis and representation of data were done using the GraphPad Prism 8.4.3. All data are represented as mean ± SD from at least three independent replicas. Significant differences were determined by using One-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test at the 5% level ($p < 0.05$).

RESULTS

Phytochemical Screening

Qualitative phytochemical screening revealed the presence of flavonoids, alkaloids, phenols, saponins, and steroids from *E. ferox* seeds while, the presence of flavonoids, phenols, and steroids from *E. ferox* arils. The presence of flavonoids, phenols, saponins, steroids and alkaloids were detected from *E. birmanicum* leaves.

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Gallic acid and quercetin were used as the standards to obtain the calibration equations, y = 0.0046x + 0.215, R2 = 0.9916 and y = 0.0167x + 0.0036, R2= 0.9975 for the estimation of TPC and TFC of the plant extracts respectively. The values of TPC and TFC are denoted in terms of milligrams of gallic acid equivalent (GAE) per gram of dry extract weight (mgGAE/g ext) and milligrams of quercetin equivalent per gram of dry extract weight (QE mg/g ext) respectively. All the samples were taken in three replicates and the results obtained are given as mean ± SD. TPC was found to be highest in *E. ferox* seeds (119.80 ± 2.63 mgGAE /g ext) when compared with *E. ferox* arils (21.95 ± 1.18 mgGAE/g ext) and *E. birmanicum* (30.97 ± 1.19 mgGAE /g ext). *E. ferox* seeds also had the highest TFC value (7.27 ± 0.28 QE mg/g ext) when compared with *E. birmanicum* (4.7 ± 0.13 mg QE/g ext) and *E. ferox* arils (2.57 ± 0.07 mg QE/g ext).

Antioxidant Activity Analysis

Antioxidant potential of the plant samples was assessed using the DPPH and ABTS assays. The IC_{50} value of the DPPH antioxidant activity of the standard Ascorbic acid came out to be 5.38 ± 0.12 µg/mL. Among the three plant extracts, the highest DPPH radical scavenging activity or minimum IC_{50} value was found in *E. ferox* seeds (16.99 ± 0.49 µg/mL) followed by *E. birmanicum* (232.32 ± 2.99 µg/mL) and *E. ferox* arils (335.90 ± 2.19 µg/mL). The standard Ascorbic acid showed the IC_{50} value of 18.91 ± 0.81µg/mL. for the ABTS antioxidant activity. Similarly to that of DPPH, the highest ABTS radical scavenging activity or minimum IC_{50} was found in *E. ferox* seeds (70.69 ± 1.83µg/mL) followed by *E. birmanicum* (303.69 ± 3.77 µg/mL) and *E. ferox* arils (576.26 ± 4.41 µg/mL).

DISCUSSION

Our study has shown that the seeds and arils of *E. ferox* as well as leaves of *E. birmanicum* are rich in a variety of phytochemicals and serve as good sources of natural antioxidants. *In E. ferox*, the presence of flavonoids, alkaloids, phenols, saponins, and steroids was detected in seeds while, the presence of flavonoids, phenols, and steroids was detected in arils (Table 2). Analysis of phytochemical content on the extract of *E. birmanicum* leaves revealed the presence of flavonoids, phenols, saponins, steroids and alkaloids (Table 2). Phytochemicals are chemical compounds naturally found in plants that determine their medicinal or therapeutic potential.[14] Some of the most important phytochemicals naturally present in plants are alkaloids, phenolics, flavonoids, tannins, saponins, and steroids which are distributed in different parts of the plants.[15] The most generally found phytoconstituents in medicinal plants and vegetables which have very high antioxidant activities are phenols and flavonoids. Flavonoids and phenols are known to promote good health due to their high antioxidant potentials caused by their anion radicals[16] which supports the use of our studied plants in the traditional treatment of various metabolic disorders and will induce health benefits to the consumers. The presence of saponin in the plants indicated their potential inhibitory effect on inflammation[35] and this supports the use of *E. ferox* seeds as anti-inflammatory agent in Ayurvedic and Chinese medicines. Plant sterols such as sitosterol, stigmasterol and campesterol have the capacity for lowering cholesterol in our body which will help in reducing various complications of cardiovascular diseases [36] and the used of *E. ferox* plant in treating heart diseases is justified because of the presence of the plant sterols mentioned *per se* in our plant samples. Alkaloids are also important phytochemicals known for various medicinal properties such as cytotoxicity and anti-microbial[23] and this supports the use of our studied plants in the preparation of various traditional medicines. The reported phytoconstituents of the genus *Eupatorium* include monoterpene derivatives, sesquiterpenes, diterpenes, triterpenes, flavonoids, alkaloids, essential oil, and some others which exhibit various biological activities.[31] From the *E. ferox* also, many studies have isolated and determined various phytochemicals which can be mainly classified into polysaccharides, polyphenols, flavonoids, cyclic dipeptides, phytosterol, and triterpenoids.[4] The values of TPC, TFC, and antioxidant obtained from our study were found to be highest in *E. ferox* seeds followed by *E. birmanicum leaves* and *E. ferox arils*. The value of TPC (119.80 ± 2.63 GAE mg/g ext) and TFC (7.27 ± 0.28 QE mg/g ext) in *E. ferox seeds* (Table 3) were found in concordant with previous reported study.[32] There is no reported data of TPC and TFC for the *E. birmanicum* leaves as well as *E. ferox* arils. Free radicals when

Table 2: Result of Phytochemical Screening of the plant extracts.

Phytochemicals	Test Performed	*E. ferox* seed	*E. ferox* aril	*E. birmanicum* leaves
Flavonoids	Alkaline Reagent test	Positive	Positive	Positive
	Ferric Chloride test	Positive	Negative	Negative
Phenols	Folin-Ciocalteu Reagent test	Positive	Positive	Positive
	Ferric Chloride Test	Positive	Negative	Positive
Saponins	Foam test	Positive	Negative	Positive
Tannins	Ferric Chloride test	Negative	Negative	Negative
Steroids	Salkowski test	Positive	Positive	Positive
Alkaloids	Mayer's Reagent test	Positive	Negative	Positive

Table 3: Values of Total Phenolic Content (TPC), Total Flavonoid Content (TFC) of the plant extracts.

Sample	TPC (mg GAE/g dry extract wt)	TFC (mg QE/g dry extract wt)
E. ferox seeds (EFS)	119.80 ± 2.63	7.27 ± 0.28
E. ferox arils (EFA)	21.95 ± 1.18	2.57 ± 0.07
E. birmanicum leaves (EBL)	30.97 ± 1.19	4.7 ± 0.13

Results are expressed as mean ± SD (n=3). GAE= Gallic acid equivalent; QE= Quercetin equivalent

Table 4: Antioxidant activity (DPPH and ABTS) of the plant extracts.

Sample	DPPH Assay IC_{50} (µg/mL)	ABTS Assay (µg/mL)	IC_{50}
Ascorbic Acid (AA)	5.38 ± 0.12	18.91 ± 0.81	
E. ferox seeds (EFS)	16.99 ± 0.49	70.69 ± 1.83	
E. ferox arils (EFA)	335.90 ± 2.19	576.26 ± 4.41	
E. birmanicum leaves (EBL)	232.32 ± 2.99	303.69 ± 3.77	

Results are expressed as mean ± SD (n=3). DPPH= 1, 1-diphenyl-2-picrylhydrazyl; ABTS = ((2, 2′ -azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

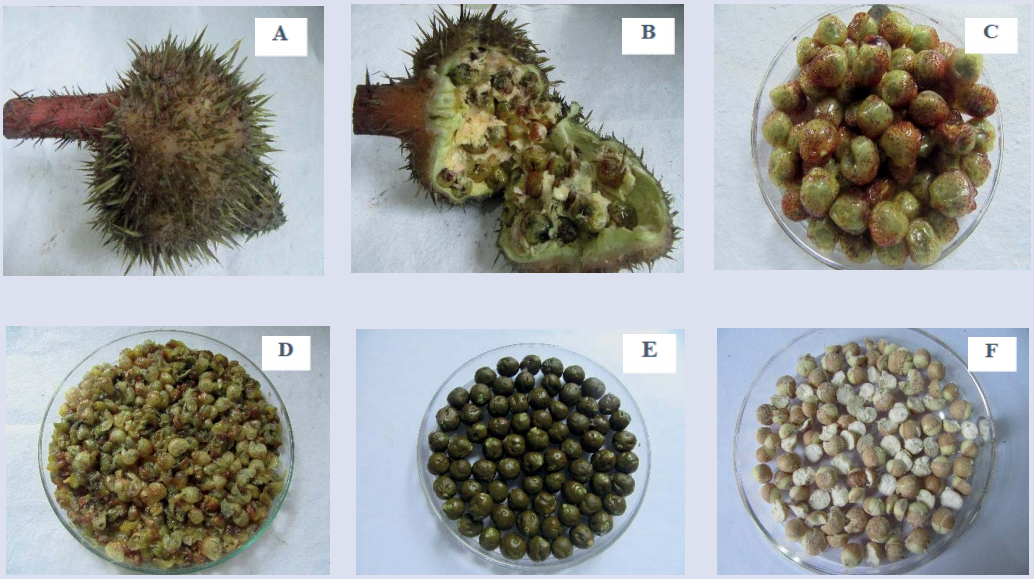

Figure 1: A) *E. ferox* (fruit) B) *E. ferox* (opened fruit) C) *E. ferox* (seeds covered with arils) D) *E. ferox* (arils) E) *E. ferox* (seeds with hard shells) F) *E. ferox* (seed kernels).

Figure 2: A) *E. birmanicum* (young plant) B) *E. birmanicum* (matured plant) C) *E. birmanicum* (flowering plant).

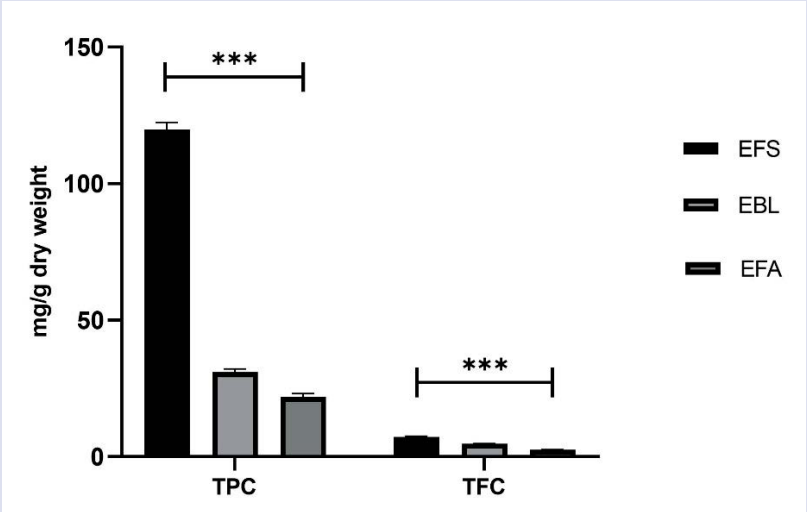

Figure 3: TPC and TFC values of the plant extracts. Values are denoted as mean ± SD of independent triplicates of experiments. Statistical significance between EFS and EFA was measured by using One-way ANOVA set at $p < 0.05$ (***$p < 0.001$). TPC is expressed as mg GAE/g dry extract wt. and TFC is expressed as mg QE/g dry extract wt. EFS = *E. ferox* seeds EBL= *E. birmanicum* leaves EFA = *E. ferox* arils.

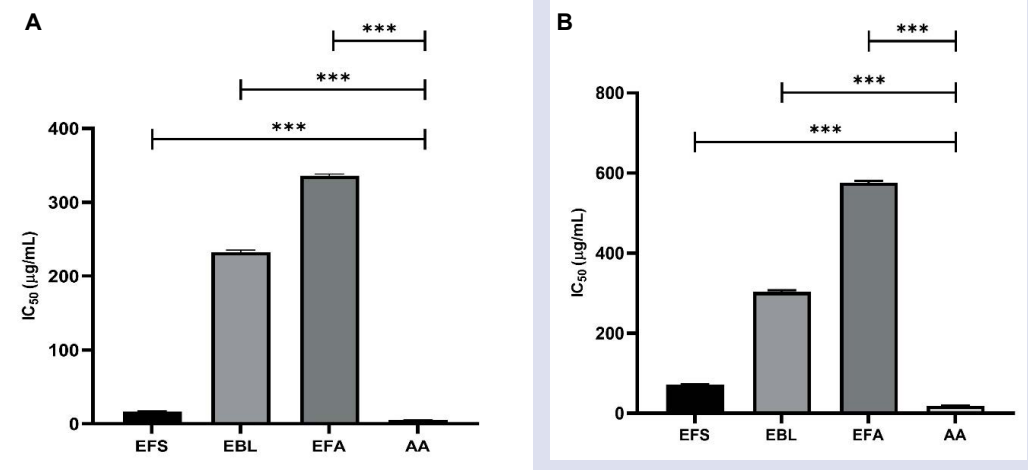

Figure 4: Antioxidant activity of the plant extracts. (A) DPPH assay (B) ABTS assay. Values are expressed as mean ± SD (n=3). Statistical comparison of the samples with the standard AA was carried out by using One-way ANOVA set at $p < 0.05$ (***$p < 0.001$). EFS = *E. ferox* seeds EBL= *E. birmanicum* leaves EFA = *E. ferox* arils AA= Ascorbic acid.

produced in excess amount can lead to several degenerative human diseases such as atherosclerosis, cardiac, kidney, liver diseases, and neurodegenerative disorders.[5] IC_{50} is the most accepted and frequently used parameter to determine the antioxidant potential of a substance and it is defined as the concentration of plant extracts that can neutralize or inhibit the free radical formation by 50%. So, higher the value of IC_{50}, the lower is the antioxidant activity of the plant samples and vice versa.[7] The IC_{50} value of *E. ferox* seed kernels for DPPH (16.99 ± 0.49 µg/mL) obtained from our study as shown in Table 4 was found to be less as compared to the value (22.95 ± 0.25 µg/mL) of the previous study conducted in Korea[33] and higher than that of the value of another study (5.6 µg/mL) conducted in China.[34] There is no reported data on the antioxidant potential for *E. birmanicum* leaves and *E. ferox* arils. The results of DPPH and ABTS assays from our study indicates that the two plants have promising antioxidant potential and their antioxidant potential may be due to the presence of certain bioactive compounds as recorded in the present study that can donate hydrogen to neutralize the harmful free radicals. Our study thus implies that these two plants may be used as natural agents of antioxidant and can be further studied for identifying biologically active compounds for future investigations in their therapeutic potentials.

CONCLUSION

Medicinal plants form a very important part of traditional medicine, and also a thrust area of research to be investigated for the discovery of many important phytochemicals for therapeutic purposes. There is an increased demand of local plants that can be used as herbal medicines as they are easily available and mostly free from side-effects as compared to the modern synthetic drugs. The hydroalcoholic extracts of the seeds and arils of *Euryale ferox* Salisb. as well as the leaves of *E. birmanicum* showed an appreciable amount of TPC, TFC, and promising antioxidant activity. So, they have the potential to be used as natural agents of antioxidants in the field of health products. To our knowledge till date, no comprehensive studies have been carried out on the phytochemical profile and antioxidant potential of *Euryale ferox* Salisb. widely consumed by the natives of Manipur. Also, there has been very limited data reported on *Eupatorium birmanicum* DC. which is an ethnomedicinal plant of Manipur. Thus, the findings of our study will contribute a scientific insight for identifying biologically active compounds and antioxidant potential in such traditional food as well as medicinal plant used by the natives of North East India for exploration of their therapeutic potentials in the future. Our study will also help to make people aware of the importance of these plants and help to take up initiatives for their better production which ultimately will help in their conservation which is needed most at present.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests.

ABBREVIATIONS

TPC: Total Phenolic Content**; TFC:** Total Flavonoid Content; **DPPH:** 1, 1-diphenyl-2-picrylhydrazyl**; ABTS:** 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); **IC_{50}:** Half maximal inhibitory concentration; **EFS:** *E. ferox* seeds; **EFA:** *E. ferox* arils, **EBL:** *E. birmanicum* leaves; **AA:** Ascorbic Acid; **GAE:** Gallic acid equivalent; **QE:** Quercetin equivalent.

REFERENCES

1. Bana M, Gupta RK. Formulation, nutritional and phytochemical analysis of ready to mix infant Food using Gorgon Nut, Samak Rice and Banana powder. Journal of Pharmacognosy and Phytochemistry. 2015;4(4):76-81.
2. Jain A, Singh HB, Kanjilal PB. Economics of Foxnut (Euryale ferox Salisb.) cultivation: a case study from Manipur in North eastern India. Indian Journal of Natural Products and Resources. 2010;1(1): 63-67.
3. Zhang L, Chen Y, Zeng J, Zang J, Liang Q, Tang D, *et al.* Digestive and physicochemical properties of small granular starch from Euryale ferox seeds growing in Yugan of China. Food Biophysics. 2022; 17: 126-135. doi:10.1007/s11483-021-09706-7. DOI:10.1007/s11483-021-09706-7

4. Jiang J, Ou H, Chen R, Lu H, Zhou L, Yang Z. The Ethnopharmacological, Phytochemical, and Pharmacological Review of Euryale ferox Salisb.: A Chinese Medicine Food Homology. Molecules. 2023;28(11):4399. **https://doi.org/10.3390/molecules28114399**
5. Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, *et al.* Oxidative stress, prooxidants, and antioxidants: The Interplay. BioMed Research International. 2014; 2014: 1-19. doi: 10.1155/2014/761264
6. Bardaweel SK, Gul M, Alzweiri M, Ishaqat A, ALSalamat HA, Bashatwah RM. Reactive oxygen species: The dual role in physiological and pathological conditions of the human body. The Eurasian journal of medicine. 2018;50(3):193. doi: 10.5152/eurasianjmed.2018.17397
7. Rivero-Cruz JF, Granados-Pineda J, Pedraza-Chaverri J, Pérez-Rojas JM, Kumar-Passari A, Diaz-Ruiz G, *et al.* Phytochemical constituents, antioxidant, cytotoxic, and antimicrobial activities of the ethanolic extract of Mexican brown propolis. Antioxidants. 2020;9(1):70. doi: 10.3390/antiox9010070
8. Singh AV, Singh PK. Nutritional, ethnotherapeutics and socioeconomic relevance of Euryale ferox Salisb. Manipur, India. Life Sciences Leaflets. 2011; 22:1104-15.
9. Kumar N, Shikha D, Kumari S, Choudhary BK, Kumar L, Singh IS. SSR-based DNA fingerprinting and diversity assessment among Indian Germplasm of Euryale ferox: an aquatic underutilized and neglected food crop. Applied biochemistry and biotechnology. 2018; 185:34-41. doi: 10.1007/s12010-017-2643-9
10. Singh LW. Traditional medicinal plants of Manipur as anti-diabetics. J Med Plant Res. 2011;5(5):677-87.
11. Chongtham R, Rani M. Eupatorium birmanicum: A Medicinal Plant of Ritualistic Importance in the North-East Indian State of Manipur. In: Singh A, Singh P, Bithel N, Editors. Advanced Pharmacological Uses of Medicinal Plants and Natural Products. India: IGI Global, p. 286-301; 2020. DOI:10.4018/978-1-7998-2094-9.ch015
12. Ningombam DS, Devi SP, Singh PK, Pinokiyo A, Thongam B. Documentation and assessment on knowledge of ethno-medicinal practitioners: a case study on local Meetei healers of Manipur. Int J Pharma Biosci. 2014; 9:53-70. DOI:10.9790/3008-09115370
13. Devi AR, Devi YS, Sharma HM. Plants used in the treatment of diabetes by the Meitei community in Imphal east district of Manipur (India). Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences. 2019;5(1):913-31. DOI:10.26479/2019.0501.77
14. Ezeonu CS, Ejikeme CM. Qualitative and quantitative determination of phytochemical contents of indigenous Nigerian softwoods. New Journal of Science. 2016; 2016:1-9. **https://doi.org/10.1155/2016/5601327**
15. Sheel R, Nisha K, Kumar J. Preliminary phytochemical screening of methanolic extract of Clerodendron infortunatum. IOSR Journal of Applied Chemistry. 2014;7(1):10-3. DOI:10.9790/5736-07121013
16. Scalbert A, Johnson IT, Saltmarsh M. Polyphenols: antioxidants and beyond. The American journal of clinical nutrition. 2005;81(1):215S-7S. DOI: 10.1093/ajcn/81.1.215S
17. Ningthoujam SS, Talukdar AD, Potsangbam KS, Choudhury MD. Traditional uses of herbal vapour therapy in Manipur, North East India: an ethnobotanical survey. Journal of ethnopharmacology. 2013;147(1):136-47. DOI: 10.1016/j.jep.2012.12.056
18. Mukherjee PK, Bahadur S, Chaudhary SK, Kar A, Mukherjee K. Quality related safety issue-evidence-based validation of herbal medicine farm to pharma. In: Mukherjee PK, Editor. Evidence-based validation of herbal medicine. India: Elsevier, p. 1-28; 2015. DOI:10.1016/B978-0-12-800874-4.00001-5
19. Singh AK, Rai SN, Maurya A, Mishra G, Awasthi R, Shakya A, *et al.* Therapeutic potential of phytoconstituents in management of Alzheimer's disease. Evidence-based complementary and alternative medicine. 2021; 2021:1-9. DOI: 10.1155/2021/5578574
20. Ahmed D, Kumar V, Verma A, Shukla GS, Sharma M. Antidiabetic, antioxidant, antihyperlipidemic effect of extract of Euryale ferox salisb. with enhanced histopathology of pancreas, liver and kidney in streptozotocin induced diabetic rats. Springerplus. 2015 ;4(1):1-7. DOI: 10.1186/s40064-015-1059-7
21. Tehseen S, Sarfraz F, Muntaham S, Ateeq N, Ashfaq F, Yasmin I, *et al.* Foxnut (Euryale ferox Salisb.): A health promising fruit. Acta Scientific Agriculture. 2020;4(12):68-72.
22. Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies. 2020; 8(2):603-8. DOI:10.22271/chemi.2020.v8.i2i.8834
23. Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of Helichrysum longifolium DC. BMC complementary and alternative medicine. 2010;10(1):1-8. DOI: 10.1186/1472-6882-10-21
24. Yadav M, Chatterji S, Gupta SK, Watal G. Preliminary phytochemical screening of six medicinal plants used in traditional medicine. Int J Pharm Pharm Sci. 2014;6(5):539-42.
25. Shirazi OU, Khattak MM, Shukri NA. Determination of total phenolic, flavonoid content and free radical scavenging activities of common herbs and spices. Journal of pharmacognosy and phytochemistry. 2014;3(3):104-8.
26. Nurcholis W, Putri DN, Husnawati H, Aisyah SI, Priosoeryanto BP. Total flavonoid content and antioxidant activity of ethanol and ethyl acetate extracts from accessions of Amomum compactum fruits. Annals of Agricultural Sciences. 2021; 66(1):58-62. DOI:10.1016/j.aoas.2021.04.001
27. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants. 2019 ;8(4):96. DOI: 10.3390/plants8040096
28. Vidya B, Palaniswamy M, Sowmya S, Priyanga S, Perumal PC, Devaki K, Gopalakrishnan VK. Evaluate the antioxidant activity and FT-IR spectroscopic analysis of purified β-galactosidase from Aspergillus terreus. Indo-American Journal of Pharmaceutical Research. 2016; 6:6383-92.
29. Kokina M, Salević A, Kaluševiċ A, Lević S, Pantić M, Pljevljakušič D, *et al.* Characterization, antioxidant and antibacterial activity of essential oils and their encapsulation into biodegradable material followed by freeze drying. Food technology and biotechnology. 2019; 57(2):282. doi: 10.17113/ftb.57.02.19.5957
30. Benkhaira N, Koraichi SI, Fikri-Benbrahim K. In vitro methods to study antioxidant and some biological activities of essential oils: a review. Biointerface Res. Appl. Chem. 2022;12(3):3332. DOI:10.33263/BRIAC123.33323347
31. Zhang ML, Wu M, Zhang JJ, Irwin D, Gu YC, Shi QW. Chemical constituents of plants from the genus Eupatorium. Chemistry & biodiversity. 2008 ;5(1):40-55. DOI: 10.1002/cbdv.200890014
32. Nehal N, Mann S, Gupta RK. Two promising under-utilized grains: A review. Indian Journal of Traditional Knowlegde. 2015; 14(3):416-422.
33. Jin KS, Oh YN, Lee JY, Son BY, Choi W, Lee EW, *et al.* Anti-oxidative and anti-inflammatory activities of seven medicinal herbs including Tetrapanax papyriferus and Piper longum Linne. Microbiology and Biotechnology Letters. 2013;41(2):253-62. DOI:10.4014/kjmb.1207.07022
34. Lee SE, Ju EM, Kim JH. Antioxidant activity of extracts from Euryale ferox seed. Experimental & molecular medicine. 2002 ;34(2):100-6. DOI: 10.1038/emm.2002.15

35. Liu J, Henkel T. Traditional Chinese medicine (TCM): are polyphenols and saponins the key ingredients triggering biological activities? Current Medicinal Chemistry. 2002;9(15):1483-5. DOI: 10.2174/0929867023369709

36. Piironen V, Lindsay DG, Miettinen TA, Toivo J, Lampi AM. Plant sterols: biosynthesis, biological function and their importance to human nutrition. Journal of the Science of Food and Agriculture. 2000;80(7):939-66. DOI:10.1002/(SICI)1097-0010(20000515)80:7<939::AID-JSFA644>3.0.CO;2-C

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