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Research Journal of Agricultural Sciences
Volume 14; Issue 01; pp 167–170

Open Access

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Full Length Research Article

Evaluation of the Nutritional and Biological Activity of *Euryale ferox* Salisb Cultivated in Manipur

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Abstract

The present study dealt with the quantification of nutritional quality and deciphering the bioactivity of methanolic extract of three different parts of foxnut fruit viz; aril, seed coat, and seed kernel. The nutritional composition of aril, seed coat, and seed kernel aril, seed coat, and seed kernel was found to be different. The carbohydrate content was recorded in the seed coat as (74.6%), seed kernel (73.22%) and aril (46.68%). While highest protein content was recorded in aril (16.8%) followed by seed kernel (14%) and seed coat (6.82%). The ash content is highest in aril (19.4%) as compared to seed coat (7.6%) and seed kernel (1.8%). The methanolic extract of foxnut seed is rich in phenolic and flavonoid content. The extracts also exhibited antioxidant activity. The highest antioxidant activity was shown by seed coat as indicated by its lower EC_{50} value in the DPPH assay (EC_{50}=7.21±0.03 µg/mL) and ABTS assay (EC_{50}= 4.39 ± 0.25µg/mL). The extracts also showed α-amylase inhibitory activity, the EC_{50} value of the extracts was recorded as 149.55±0.91 µg/mL for seed coat, 577.65±4.1 µg/mL for seed kernel, and 895.61± 6.7 µg/mL for aril. The methanolic extract of seed coat exhibited antibacterial activity.

***Key words*:** *Euryale ferox* Salisb, Foxnut, Antioxidant, Antihyperglycemic, Antimicrobial

Euryale ferox Salisb. an aquatic herbaceous plant, is the only species in the genera Euryale of the family Nymphaeaceae. It is native to Southeast Asia, China, Japan, India and Korea. The plant is commonly known as foxnut in English, Qianshi in Chinese and Onibasu in Japanese [1]. The starchy seed kernel is the main edible part of *Euryale ferox* Salisb. Makhana is the process product of the dried seed and is available commercially worldwide. More than 80% of the World's makhana production is contributed by Bihar, a state of India [2-3]. The seed kernel can be used to make a variety of dishes, such as puddings, Kheer, snacks, and desserts. In Manipur foxnut has been traditionally cultivated as a cash crop from immemorable time in wetlands and private ponds. The young leaves and stems of plants, for example, can also be eaten as vegetables. The fruit is eaten either raw or cooked in the North-Eastern region of India, where it is more commonly regarded as a vegetable. In Manipur, people make chutney known as "eromba" by using tender leaves, seed arils, and fruit peel [4]. The foxnut seed is rich in nutrients are also known as a super dry fruit i.e. they are rich in carbohydrates, proteins, essential amino acids, fats and flavonoids [5]. Physicochemical properties and structure of starches of foxnut (*Euryale ferox* Salisb.) from India and its application. Moreover, the different plant parts have been used for the treatment of many diseases in traditional medicines from time immemorial. The foxnut seeds are used for the treatment of renal disorders, persistent diarrhoea, excessive leucorrhea, and hepatic dysfunction in Ayurvedic and Chinese treatments [2]. The petioles and pedicels of *E. ferox* have been used to cure polydipsia, mouth dryness, and dry throat, while the seed has been used to treat diarrhoea and spermatorrhea. Many researchers have reported that the plant exhibited antioxidant, antidepressant, immunostimulatory, hepatoprotective, antidiabetic and antimicrobial activity [6-7]. Foods having both nutritional superiority and pharmaceutical properties have gained the attention of functional foods/nutraceutical-producing companies. These foods occupy the major market share of the food industries. In this context, *Euryale ferox* Salisb seed is a potential candidate for the production of functional food. Even though it is a potential cash crop the cultivation of *Euryale ferox* Salisb is not well organized in Manipur and is still considered as an underutilized plant. Moreover, there is a very limited report on the nutritional and medicinal properties of *Euryale ferox* Salisb cultivated in Manipur. Hence the present study was taken to assess nutritional quality and biological activity such as antioxidant, antihyperglycemic and antimicrobial activity of methanolic extract of three different parts of foxnut fruit.

Received: 12 Nov 2022; Revised accepted: 08 Jan 2023; Published online: 20 Jan 2023

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Citation: Devi KR. 2023. Evaluation of the nutritional and biological activity of *Euryale ferox* Salisb cultivated in Manipur. *Res. Jr. Agril Sci*. 14(1): 167-170.

MATERIALS AND METHODS

Extraction and sample preparation

Euryale ferox Salisb. seed parts viz; aril, seed coat, and endosperm are separated and dried in an oven at 45 °C. The dried samples were grounded and 10 gm of powdered sample was extracted in methanol for 48h at room temperature. The extract was then filtered through Whatman filter paper I. The filtrate was concentrated using a rotatory evaporator under reduced pressure and dried to remove the methanol completely. The samples were kept at 4ºC and for long-term storage, the samples were stored at -20°.

Proximate content

The proximate content (Moisture, total carbohydrate, protein, fat, and ash) was determined according to AOAC method [8].

Estimation of total phenolic content

Total phenolic content (TPC) was determined spectrophotometrically using the Folins Ciocaltaeu reagent [9]. 0.5 mL of the sample was taken in a test tube, and 1.5 mL of deionized water was added to it. Next, 0.25 mL of the Folins-Ciocaltaeu reagent was added, and the solution was kept for 3 minutes. 1 mL of 8% sodium carbonate was then added and incubated for 30 minutes at room temperature. Absorbance was read at 750 nm against a reagent blank. From a gallic acid standard curve, the amount of total phenolics was determined and expressed in milligrams of gallic acid equivalent (mg GAE/g extract).

Estimation of flavonoid content

Total flavonoid content (TPC) was determined spectrophotometrically with little modification from Barros *et al*. [10]. For this, 0.5 mL of the sample was taken in a test tube, and 0.5 mL of sodium nitrite (5%, w/v) was added. After four minutes, 0.5ml aluminum chloride (10%, w/v) was added and incubated for 5 minutes then 2ml 1M sodium hydroxide was added. The coloured complex was read at 510nm. Quercetin was used as standard and expressed in milligrams of quercetin equivalent per gram of extract (mg QE/g extract).

In-vitro antioxidant activity

2, 2-diphenyl 1- picrylhydrazyl (DPPH) assay

0.2 mL of each of the five different concentrations of the extracts dissolved in methanol were mixed with 3.8 mL DPPH reagent (A_{517}=1.1+0.01) prepared in methanol [11]. The mixture was incubated for 30 min in dark and then read at 517nm. Ascorbic acid was used as a positive control. The radical scavenging activity (RSA) of each extract was calculated as follows:

$$RSA\ (\%) = [(AbsControl - AbsSample) / AbsControl] \times 100$$

From the RSA%, 50% Effective concentration (EC_{50}) was calculated and expressed in µg/mL. EC_{50} is defined as the minimum amount of the extract required to scavenge half of the initial stable free radical in 30 min in 1 mL of reaction in dark.

2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay

The reaction mixture containing 0.2 mL of the extract dissolved in methanol was mixed with 3.8 mL ABTS radical (A_{734} =1.0 ± 0.01) then incubated for 30 min in dark and the absorbance was read at 734nm [11]. Ascorbic acid was used as a positive control. RSA and EC_{50} of the extract were calculated as described above.

Antihyperglycemic activity

α-amylase inhibitory assay

0.5 ml of different concentrations of the extract was mixed with 0.5 ml of porcine pancreatic α-amylase (0.5 mg/mL) was incubated at 25°C for 10 min. 0.5 ml of starch (1%) solution prepared in 0.02 M sodium phosphate buffer (pH- 6.9) was added to the previous mixture and was incubated for 10 min at 25 °C. Dinitrosalicyclic acid (1.0 mL) was added to the mixture to stop the reaction. The resultant solution was incubated for 5 min at 100 °C. The solution was allowed to cool and absorbance was measured at 540 nm [12]. The α-amylase activity was calculated and expressed as percentage inhibition using the following formula:

$$\%\ Inhibition = [(Abs_{Control} - Abs_{Sample}) / Abs_{Control}] \times 100$$

α-glucosidase inhibitory assay

100 µL of the enzyme solution (1.0 U/mL) was treated with 50 µL of the extract (100 µL) then incubated at 25 °C for 10 min. Then 50 µL of 4-nitrophenyl-α-D-glucopyranoside solution (5 mM) which was prepared in 0.02 M phosphate buffer (pH 6.9) was added. The reaction mixture was incubated at 37 °C for 10 min. The reaction was stopped by adding 4 ml of sodium carbonate and absorbance was measured at 405 nm [13]. The α-glucosidase inhibitory activity was expressed as percentage inhibition using the above formula.

Antimicrobial activity

Antibacterial activity of the methanolic extract of the seed coat was performed by Kirby-Bauer disk diffusion [14] with some modification. Four test organisms *Salmonella typhimurium (*ATTC13311), *Escherichia coli* (MTCC 739), *Bacillus subtilis* (MTTCC 121*)* and *Staphylococcus aureus* (ATCC 1026) were grown in Mueller-Hinton broth (MHB) at 37 °C and 160 rpm and brought to its exponential phase. The extracts were dissolved in 50% dimethyl sulfoxide to attain the concentration of 10 mg/mL and filtered through a (0.22 µm) syringe filter. The bacterial broth was brought to 0.5 McFarland turbidity and 100µL of each bacterial broth was spread over sterile Mueller-Hinton agar (MHA) plate using a sterile spreader. Sterile discs (6 mm diameter) were placed on the freshly spread MHA plates and impregnated with 20µL sample (0.2 mg). The plates were incubated for 24 h at 37 °C. Chloramphenicol 0.025mg was used as a positive control. The zone of inhibition was recorded.

Statistical analysis

All the experiments are performed in triplicate and data are expressed as mean ± standard. Analysis of variance (ANOVA) was done considering significance at $P < 0.05$ followed by Tukey's test. Statistical analysis was done with help of SPPS 16 software.

RESULTS AND DISCUSSION

The proximate analysis of the different parts of *Euryale ferox* Salisb seeds revealed that the nutritional composition of aril, seed coat, and seed kernel aril, seed coat, and seed kernel was found to be different (Table 1). The total carbohydrate content was recorded highest in the seed coat (74.6±3.6%) and kernel (73.22±1.5%) followed by aril (46.68±2.1%). On the other hand, the highest protein content was recorded in aril followed by seed kernel and seed coat. Fat content was found to be highest in aril. The ash content was found to be highest in aril (19.4%) as compared to seed coat (7.6%) and seed kernel (1.8%). The methanolic extract of foxnut seed was rich in phenolic and flavonoid content. The seed coat showed the

highest phenolic (148.6±24 mg GAE/g) and flavonoid content (86.5±6 QE/g). Similarly, Wu *et al*. [15] reported that the acetone extract of foxnut seed coat contained 113.30 mg GAE of polyphenols per gram dry weight of the extract. While Kadu *et al*. [16] observed that the ethanol: water extract of foxnut seed coat contained a polyphenol content of 50.967±0.107 GAE mg/g and flavonoid content of 16.274±0.73 QE mg/g. The polyphenol and flavonoid content of *Eurayle ferox* Salisb seed in the present study is higher than those reported in the literature. The variances may be due to the difference in the variety of the foxnut or the difference in the solvent used for the extraction.

Table 1 Nutritional composition

Parameters	Seed coat	Kernel	Aril
Moisture (%)	10.3±1.4 [a]	10.42±0.83[a]	13.7±2.1[b]
Carbohydrate (%)	74.6±3.6[a]	73.22±1.5[a]	46.68±2.1[b]
Protein (%)	6.82 ±0.62[a]	14.2 ±2.1[b]	16.8±2.6[b]
Fat (%)	0.55±0.02[a]	0.54±0.24[a]	3.12±0.84[b]
Ash (%)	7.6±1.6[a]	1.8±0.6[b]	19.4±2.03[c]
Phenolics (mg GE/g sample)	148.6±24 [a]	33.0±4.6 [b]	109.4±12.02 [c]
Flavonoids (mg QE/g sample)	86.5±6[a]	20.8±3.2[b]	80.6±10.05[a]

Values with the same alphabet within the same raw is not statistically significant at p<0.05

Table 2 In-vitro antioxidant activity

Samples	ABTS (EC_{50}) (µg/mL)	DPPH(EC_{50}) (µg/mL)
Seed coat	4.39±0.0.25[a]	7.21±0.03[a]
Kernel	157.9±1.82[b]	423.5±1.05[b]
Aril	84.95±1.7[c]	51.2±1.5[c]
Ascorbic acid	4.4±0.25[a]	7.4±0.06[a]

Values with the same alphabet within the same raw is not statistically significant at p<0.05

Table 3 In-vitro α- amylase and α-glucosidase activity

Parameters	Seed coat (EC_{50}) (µg/mL)	Kernel ((µg/mL) EC_{50})	Aril ((µg/mL) EC_{50})	Acarbose (E(µg/mL) C_{50}
α-amylase	149.5±10.9[a]	577.65±4.1[b]	895.61± 6.7[c]	14.06±1.62[d]
β-glucosidase	74.6±2.08[a]	73.22±0.98[a]	46.68±1.26[b]	122.8±4.82[c]

Values with the same alphabet within the same raw is not statistically significant at p<0.05

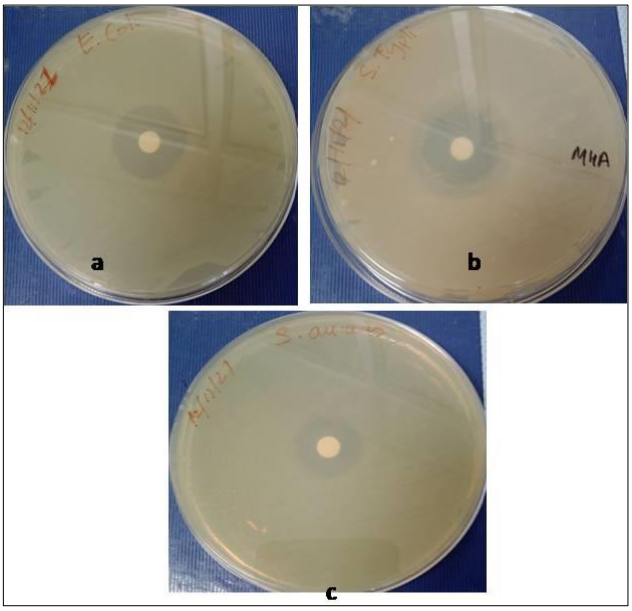

Fig 1 Antibacterial activity of methanolic extract of *Euryale ferox* Salisb seed coat (a) *Escherichia coli* (b) *Salmonella typhimurium* (c) *Staphylococcus aureus*

The antioxidant activity of the extracts was evaluated by two different in-vitro antioxidant assay methods viz; DPPH assay and ABTS assay. The highest antioxidant activity was shown by the seed coat as indicated by its lower EC_{50} value in the DPPH assay and ABTS assay (Table 2). Aril also showed good antioxidant activity. The least antioxidant activity was shown by the seed kernel. This may be correlated with the phenolic and flavonoid contents. Phenols and flavones are known to be good antioxidant agents. The seed coat contained the highest phenolic and flavone content and thus showed the highest antioxidant activity. The antioxidant activity of the seed coat is comparable with the standard ascorbic acid. A similar observation was also reported by Kadu *et al*. [16] that the IC_{50} value of foxnut seed coat in DPPH assay was found to be 1.620µg/ml and that of ascorbic acid were 2.288µg/ml. In addition, Lee *et al*. [17] reported that the crude methanolic extract of *Eurayle ferox* Salisb showed relatively high-level radical scavenging activity toward DPPH assay as indicated by its lower IC_{50} value 5.6 µg/ml. Further, they observed that the extract also showed stimulating activity on antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase in V79-4 cell lines in a dose-dependent manner. All the *Euryale ferox* Salisb seeds extracts (seed coat, kernel, and aril) also exhibited antihyperglycemic activity as evidenced by its α-amylase and α-glucosidase inhibitory activity (Table 3). The EC_{50} value of the extracts revealed that the extracts are more effective in α-glucosidase inhibition as indicated by their lower EC_{50}. The EC_{50} of all the extracts was lower than the acarbose in α-glucosidase inhibitory activity. The antihyperglycemic activity of *Euryale ferox* Salisb has been reported by other researchers [18-19]. The methanolic extract of the *Euryale ferox* Salisb seed coat showed potential antibacterial activity against gram +ve and gram –ve pathogens (Fig 1, Table 4). While the seed kernel and aril did not show any antibacterial activity against the test pathogens. The highest zone of inhibition was recorded in *Salmonella typhimurium* (24.0±2.0 mm), *Staphylococcus aureus* (22.1±2.0 mm), *followed by Escherichia coli* (18.0±1.0 mm). The antibacterial activity of *Euryale ferox* Salisb against various pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella flexneri* had been reported in the literature [20-21].

Table 4 Antibacterial activity of *Euryale ferox* Salisb seed

Extracts	Zone of inhibition (mm)		
	Escherichia coli	*Salmonella typhimurium*	*Staphylococcus aureus*
Seed coat	18 ±1[a]	24±2[b]	22±2[b]
Kernel	-	-	-
Aril	-	-	-
Chloramphenicol (Positive control)	26±2	28±3	24±2

Values with the same alphabet within the same raw is not statistically significant at p<0.05

CONCLUSION

These days food is not only considered a source of nutrition but also considered a medicine. And this warrants the increased demand for healthy foods such as nutraceutical and functional foods in the global market. Hence the search for new foods having both superior nutritional quality and health-beneficial activity is the need of the hour. The present study revealed that the *Euryale ferox* Salisb seed is not only rich in nutrients but also showed antioxidant and antihyperglycemic activity. Therefore, it will be a potential candidate for the formulation and marketing of nutraceutical/functional foods.

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