Research Article

Preliminary Study of *Euryale ferox* Salisb. Seed Coat as a Potential Antioxidant and Antibacterial Source

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ABSTRACT

Agro-industrial waste generated each year causes detrimental effects on the environment and economy, but is a rich source of bioactive compounds. These compounds when recovered have various applications in food, pharmaceutical, cosmetic and packaging industry. In the present study, *Euryale ferox* Salisb. seed coat was used, a waste product of Makhana processing. Different solvents were used to extract the phytochemicals, the highest extraction value was found in ethanol:water. Phytochemical analysis, total phenols, flavonoids content, antioxidant and antibacterial activity was carried out of the seed coat extract. Phytochemical screening of extracts showed presence of phenols, tannins, alkaloids, glycosides, terpenoids, steroids and flavonoids. The total phenol content and flavonoid content was found to be 50.967±0.107 GAE mg/g and 16.274±0.73 QE mg/g respectively. The antioxidant activity of the ethanol: water extract was studied by using DPPH assay and IC_{50} value was found to be 1.62µg/mL. Antibacterial activity was carried out using agar cup method and was determined against *E. coli* and *S. aureus*. Ethyl acetate and ethanol extract showed higher zone of inhibition as compared to the other extracts. Development of HPTLC fingerprint profile for separation of active constituents was carried out. This study indicates that *Euryale ferox* seed coat might be a valuable bioactive source for antioxidant and antibacterial activity.

Key words: *Euryale ferox* Salisb., Seed coat, Antioxidant, Antibacterial, HPTLC fingerprinting.

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INTRODUCTION

Agro-industrial waste generated each year globally is posing a daunting and challenging task for various sectors such as agriculture, dairy processing, food and beverage industry, etc. Due to which community of researchers is striving to find cost-competitive solutions for recovery of biologically active compounds from these materials.[1] These bioactive compounds demonstrate various pharmacological activities such as antioxidant, antibacterial antifungal, anti-inflammatory, cardio-protective, hepato-protective, etc. Polyphenols, one of the largest class of phytochemicals having antioxidant properties can be extracted in different concentrations from agro-industrial waste and by-products. Recovered compounds can be re-utilized as food additives, functional foods, nutra-/pharmaceuticals, cosmeceuticals, beauty products and bio-packaging.[1]

Euryale ferox Salisbury (Nymphaeaceae), known as Makhana, is distributed in tropical and subtropical regions of south-east and east Asia.[2] It grows as an exclusive aquatic cash crop in shallow water bodies in north Bihar and lower Assam regions of India.[2] It has been widely used in traditional oriental medicine to cure a variety of diseases including kidney problems, chronic diarrhoea, excessive leucorrhoea and hypofunction of the spleen.[3] The seed coat, which accounts for about half of the fruit weight, was usually discarded in large

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quantities after the seeds had been harvested. It represents one of the major waste products from the *E. ferox* production that nowadays has a scarce use or value.[4]

As per the estimates of the National Research Center for Makhana, Darbhanga (ICAR), Bihar, total area under Makhana cultivation in India is estimated to be 15000 Ha (hectare). It yields 1,20,000 MT of Makhana seeds, which after processing yields 40,000 MT (metric tonne) of Makhana pop,[5] this data indicates the massive production of *E. ferox* (Makhana) and in turn the generation of large amount of waste in form of seed coat which is an under-utilized and underexploited part of the plant *E. ferox*.

Until now, as per our knowledge there are no published reports concerning the phytochemical analysis and biological activities of *E. ferox* seed coat in India yet. Therefore, in this report, we aimed to assess the phytochemical composition, antioxidant and antibacterial potential, also subsequently developing a HPTLC fingerprint profile of the *E. ferox* seed coat.

MATERIALS AND METHODS

Sampling of Plant material

Euryale ferox Salisb. also known as Gorgon nut, Fox nut is an aquatic crop belonging to the family of *Nympheaceae*. *Euryale ferox* seed with seed coat was procured from Darbhanga, Bihar. Seed coat was separated from seed and was grinded into fine powder. The fine powder was stored in clean and dry air-tight container and was used for further analysis.

Preparation of plant extracts

Kinetic Maceration method[6] was adopted with some modifications to obtain different extracts. 1g of fine powder was added to 10ml of 8 different solvents ranging from polar, mid-polar to non-polar. Solvents used were Ethanol, Methanol, Ethanol:Water(1:1), Methanol:Water(1:1), Water, Ethyl acetate, n-hexane and Dichloromethane. The mixture was left on rotary shaker for 24 hr at 30 rpm. The resulting mixture was filtered through Whatman Filter paper no. 1 and the extracts were preserved for further use.

Soxhlet method was adopted to obtain Ethanol: Water(1:1) extract.[7] The powdered material of the seed coat was kept in thimble and extraction was carried out using Soxhlet apparatus. The collected residue was kept on water bath for solvent evaporation. The dried extract was stored at 4°C for further determination of extractive value, phytochemical analysis, antioxidant and antibacterial activities.

Determination of Extractive values

The filterate obtained from Kinetic Maceration and Soxhlet exraction were used for determination of extractive values. The estimation was performed in two trials and the results were expressed in mean ± SD. The extractive value in percentage was calculated by using following formula and recorded.[8]

$$\text{Extractive value (\%)} = \left[\frac{\text{Weight of dried extract}}{\text{Weight of plant material (sample)}}\right] \times 100$$

Phytochemical Analysis

A. Qualitative Phytochemical Screening

All 8 extracts were subjected to preliminary phytochemical screening for their detection. The presence or absence of different phytochemicals such as alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, glycosides and steroids were detected by standard protocols with some modifications.[9-11]

B. Quantitative Phytochemical test

Total Phenol Content (TPC): Total Phenol Content was estimated using Folin-Ciocalteau assay[12] with some modifications. Dried extract of Ethanol:Water was reconstituted in distilled water (0.1mg/mL). 0.5ml of test extract and diluent was added to make the total volume upto 1 ml, followed by 1 ml diluent and 0.5ml of Folin-Ciocalteau reagent. The tubes were incubated at room temperature for 3 min and 2 ml of 20% sodium bicarbonate was added and kept on boiling water bath for 1 min. The intensity of blue colour developed was measured at 650nm using UV-visible spectrophotometer. The standard calibration curve (0.01-0.05mg/mL) was plotted using Gallic acid as standard. TPC results were expressed in terms of milligram Gallic acid equivalents per gram of dried extract (mg/g of dried extract). The estimation was performed in triplicates and the results were expressed in mean ± SD.

Total Flavonoid Content (TFC): Aluminium chloride assay[9] was used to estimate flavonoid content with some modifications. Dried extract of Ethanol:Water was reconstituted in methanol (0.2mg/mL). 0.5mL of test extract and diluent was added to make the total volume upto 1mL, followed by addition of 0.1ml of 1M potassium acetate and incubation at room temperature for 6 min. 0.1ml of 10% aluminium chloride and 2.8mL of distilled water was added to the reaction mixture and was incubated for 30 min at room temperature. The absorbance was measured at 400nm using colorimeter. The standard calibration curve (0.02-0.1mg/mL) was plotted using quercetin as standard. TFC results were expressed in terms of milligram quercetin equivalents

per gram of dried extract (mg/g of dried extract). The estimation was performed in triplicates and the results were expressed in mean ± SD.

Evaluation of Antioxidant activity by DPPH assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of the Ethanol:Water extract was determined using DPPH assay.[6,13] Sample concentration ranging from 1,1.5, 2, 2.5, 3µg/mL was mixed with diluent methanol to make total volume up to 3ml. 1ml of 0.1mM methanolic DPPH reagent was added and shaken vigorously, followed by 30 min incubation in dark. Absorbance was then measured at 517nm against DPPH alone in methanol as blank using UV-Vis spectrophotometer. Ascorbic acid was used as standard at concentration ranging from 1-5µg/mL. The percent of DPPH discoloration of the samples was calculated and results were expressed as percentage inhibition using the formula shown below:

$$\% \text{ Inhibition} = [A_{control} - A_{sample} / A_{control}] \times 100$$

Where $A_{control}$ and A_{sample} are the absorbance values of the control and test sample respectively.

The inhibitory concentration of sample required to scavenge DPPH radical by 50% (IC_{50}) was obtained by linear regression analysis by plotting graph between % inhibition and concentration.

The assay was performed in triplicates and the results were expressed in mean ± SD.

Antibacterial Activity

The four extracts of seed coat viz. Ethanol, Water, Ethyl acetate and Ethanol:Water were screened for antibacterial activity against one Gram positive bacteria *Staphylococcus aureus* and one Gram negative bacteria *Escherichia coli*. Both the strains were collected from Microbiology Department of Guru Nanak Khalsa College of Arts, Science and Commerce (Autonomous), Matunga. Antibacterial activity was done using slight modification of standard method of agar cup diffusion assay.[14]

Agar Cup Diffusion Assay

The solvents after Kinetic Maceration was evaporated on boiling water bath and four dried extracts were dissolved in 10% Dimethyl Sulfoxide (DMSO)[15] to make a stock concentration of 5% w/v[6,15] and was stored at 4°C. The optical densities of the actively growing bacterial cultures were then adjusted to 0.1OD at 620nm using colorimeter which were similar to 0.5 McFarland standard according to CLSI guidelines (Ninth Edition).[16] 1 ml of inoculum were added in 20 ml of sterile molten Mueller and Hinton Agar butts which was poured into sterile petri plate after thorough mixing. After solidification, wells (7mm) were made using sterile well borer and 50µl of test extracts were added to them. Plates were incubated at 37±2°C for 18-24 hr. Ciprofloxacin (0.2mg/mL) served as positive control while 10% DMSO served as negative control. The experiment was performed in triplicates and the results (diameter of zone of inhibition) were expressed in mean ± SD.

HPTLC Fingerprinting

100mg/mL of ethanol and ethyl acetate extracts were prepared and then filtered through Whatman filter paper No.1 and these sample extracts were used further for analysis.10 µL of both the sample extracts i.e. ethanol extract and ethyl acetate extract were applied on the 4×10 cm TLC silica gel 60 F_{254} plate using Linomat 5 applicator with the help of Hamilton syringe (10-100 µL) in the form of two bands. A number of solvent systems were tried for each extract, for better resolution and maximum number of spots, but satisfactory resolution was obtained in the solvent Ethyl acetate: Glacial acetic acid: Formic acid: Water- 10:1:1:1. The plate was then developed in the twin trough chamber where the solvent front ran up to 80mm distance from the bottom edge of the plate. The plates were air dried and later scanned using CAMAG TLC Scanner 3 at 254 nm using winCATS software.

RESULTS

Extractive value

Percent Extractive yields using Kinetic maceration method was calculated and the results are displayed in Figure 1, the highest extractive yield was found in Ethanol:water extract (8.48±0.013%) followed by Methanol extract (7.127±0.013%). Ethanol: water extract subjected to Soxhlet Extraction method rendered 14.32% extraction yield.

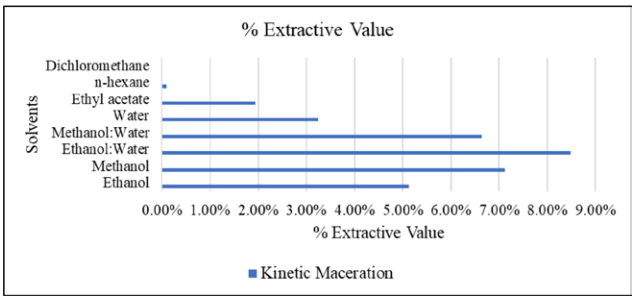


Figure 1: Percent Extraction yields of *E. ferox* seed coat by Kinetic Maceration method.

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	+	+	+	+	+	+	-	-

(+)-present; (-)-absent, DCM-Dichloromethane.

Qualitative phytochemical analysis

The qualitative phytochemical screening of *E. ferox* seed coat extracts is presented in Table 1. The results indicated the presence of phenols, tannins, glycosides and steroids in almost all the extracts except hexane and dichloromethane extract. Alkaloids, flavonoids, terpenoids and saponins were also present in some of the extracts.

Total Phenol Content and Total Flavonoid Content

The total phenolic content (TPC) of *Euryale ferox* seed coat ethanol: water extract was estimated spectrophotometerically using Folin-Ciocalteau method and was found to be 50.967±0.107 mg GAE/g. The result was derived from the calibration curve (y = 0.0186x, R^2 = 0.9859) of gallic acid (0-50µg/ml) and expressed in gallic acid equivalents (GAE) per gram dry extract weight.

The total flavonoid content of ethanol:water extract was estimated colorimetrically using aluminium chloride method and was found to be 16.274±0.73 mg QE/g. The result was derived from the calibration curve (y = 0.0034x, R^2 = 0.8848) of quercetin (0-100µg/ml) and expressed in quercetin equivalents (QE) per gram dry extract weight. The results of both total phenolic content (TPC) and total flavonoid content (TFC) is displayed in Table 2.

Antioxidant activity

Figure 2 illustrates the combined graph of concentration vs % DPPH inhibition of the ethanol: water extract and ascorbic acid standard. The antioxidant activity was expressed in terms of the IC_{50} value which is defined as a concentration (µg/mL) of a sample that inhibits the DPPH radical by 50%. IC_{50} value was calculated from concentration vs% DPPH inhibition graph using linear regression analysis. The IC_{50} value of ethanol:water extract was found to be 1.620µg/ml and that of ascorbic acid was 2.288µg/ml.

Table 2: Total Phenol Content and Total Flavonoid Content of ethanol: water extract of *E. ferox* seed coat.

Extract Used	Total Phenol Content	Total Flavonoid Content
Ethanol: Water	50.967±0.107 mg GAE/g	16.274±0.73 mg QE/g

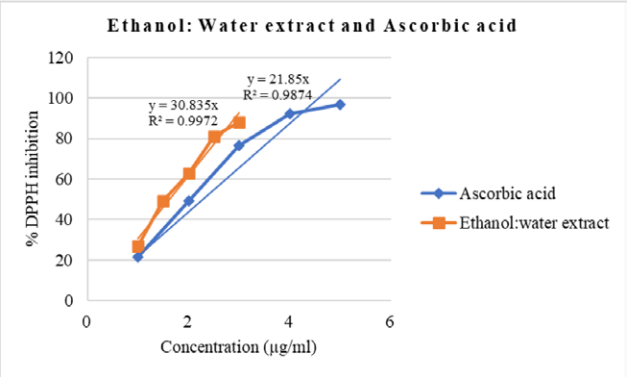


Figure 2: Combined graph of concentration vs % DPPH inhibition of ethanol:water of *E.ferox* seed coat and ascorbic standard.

Antibacterial activity

The results for agar well diffusion assay is expressed as mean diameter of zone of inhibition (mm)±standard deviation for all the *E. ferox* seed coat extracts in Table 3. All the four seed coat extracts exhibited broad spectrum antibacterial activity i.e. against both the Gram positive as well as Gram negative organism. Ethanol:Water and Ethyl acetate extracts of *E. ferox* seed coat showed larger zone of inhibition when compared to other extracts against Gram negative organism *Escherichia coli*; whereas Ethanol and Ethyl acetate extracts of *E. ferox* seed coat showed larger zone of inhibition when compared to other extracts against Gram positive organism *Staphylococcus aureus*.

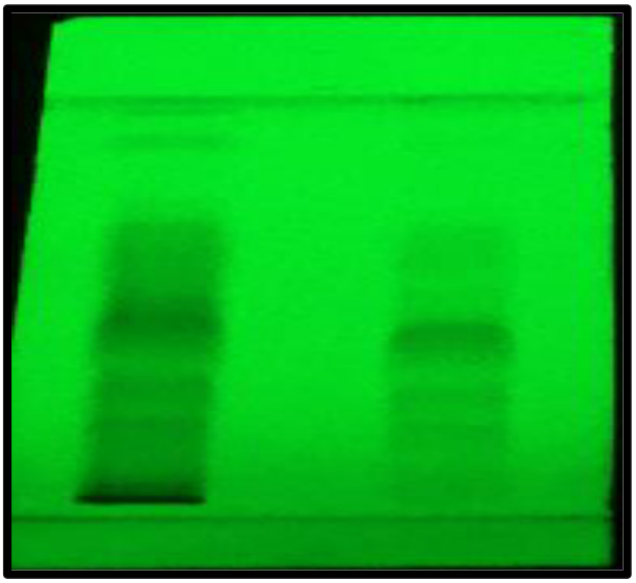
Table 3: Zone of inhibition obtained for extracts of *E. ferox* seed coat against *Escherichia coli* and *Staphylococcus aureus*.

Test organism	Set No.	Zone of inhibition (mm)						
		Extracts of *E. ferox* seed coat				Negative control	Positive control	
		Water	Ethanol	Ethanol:water	Ethyl acetate	10% DMSO	Ciprofloxacin (0.2mg/mL)	Sterile d/w
Escherichia coli	Set 1	10	12	13	12	-	24	-
	Set 2	10	13	13	13	-	23	-
	Set 3	10	12	13	13	-	23	-
	Mean ±S.D.	10±0	12.33±0.47	13±0	12.66±0.47	-	23.33±0.47	-
Staphylococcus aureus	Set 1	15	17	16	17	-	21	-
	Set 2	15	16	17	17	-	20	-
	Set 3	15	17	16	17	-	20	-
	Mean ± S.D.	15±0	16.66±0.47	16.33± 0.47	17±0	-	20.33±0.47	-

(-): No zone of inhibition.

HPTLC Fingerprinting profile

The results from HPTLC fingerprint profile of both the extracts showed presence of 7 polyvalent phytoconstituents under 254nm (Figure 3-5). The R_f values for the ethanol extract ranged from 0.18 to 0.97, components with R_f values 0.55 and 0.74 covered predominant percentage area of 52.02% and 19.35% respectively corresponding to their concentration. The R_f values for the ethyl acetate extract ranged from 0.13 to 0.92, components with R_f values 0.52 and 0.19 covered predominant percentage area of 51.06% and 13.36% respectively corresponding to their concentration.



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Track 1- Ethanol extract, Track 2- Ethyl acetate extract

Figure 3: HPTLC fingerprint profile of seed coat extracts of *E. ferox* under 256nm.

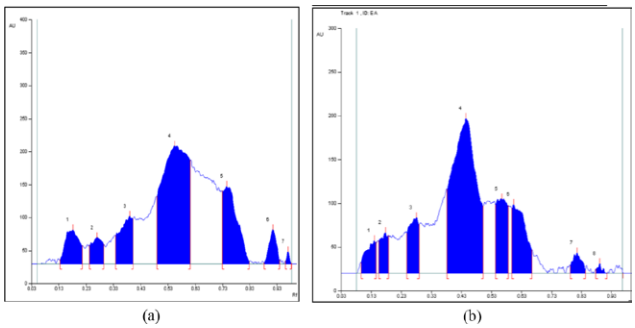

Figure 4: Chromatogram of ethanol extract(a) and ethyl extract (b) of *E. ferox* seed coat at 254nm.

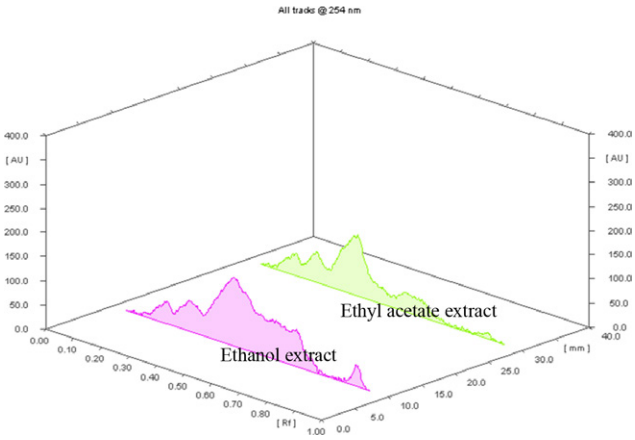

Figure 5: Three-dimensional representation of HPTLC chromatogram of *E. ferox* ethanol and ethyl acetate extract measured at 254 nm.

DISCUSSION

The present study is a step towards the exploration of bioactives, natural antioxidants, antimicrobial activity and HPTLC fingerprinting of seed coat extracts of *Euryale ferox* Salisb.

R_f values of ethanol (a) and ethyl extract (b) of *E. ferox* seed coat.

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.13 Rf	6.9 AU	0.18 Rf	52.0 AU	9.78 %	0.21 Rf	27.3 AU	2166.4 AU	8.09 %
2	0.24 Rf	27.5 AU	0.27 Rf	40.1 AU	7.53 %	0.29 Rf	29.4 AU	1370.3 AU	5.12 %
3	0.33 Rf	42.7 AU	0.39 Rf	72.3 AU	13.58 %	0.40 Rf	38.2 AU	2818.5 AU	10.52 %
4	0.49 Rf	102.3 AU	0.55 Rf	178.9 AU	33.62 %	0.61 Rf	56.7 AU	13932.5 AU	52.02 %
5	0.73 Rf	110.6 AU	0.74 Rf	117.5 AU	22.08 %	0.82 Rf	3.4 AU	5183.0 AU	19.35 %
6	0.88 Rf	1.3 AU	0.91 Rf	52.4 AU	9.85 %	0.94 Rf	5.8 AU	1145.9 AU	4.28 %
7	0.96 Rf	4.1 AU	0.97 Rf	19.0 AU	3.58 %	0.98 Rf	0.8 AU	168.3 AU	0.63 %

(a)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.09 Rf	5.1 AU	0.13 Rf	20.4 AU	3.81 %	0.13 Rf	19.6 AU	492.4 AU	1.86 %
2	0.15 Rf	26.8 AU	0.19 Rf	85.4 AU	15.94 %	0.23 Rf	50.8 AU	3530.3 AU	13.36 %
3	0.25 Rf	44.4 AU	0.31 Rf	65.1 AU	12.16 %	0.32 Rf	31.4 AU	2433.5 AU	9.21 %
4	0.43 Rf	82.2 AU	0.52 Rf	174.8 AU	32.64 %	0.58 Rf	73.8 AU	13540.5 AU	51.26 %
5	0.63 Rf	67.6 AU	0.68 Rf	82.6 AU	15.42 %	0.68 Rf	76.0 AU	2779.1 AU	10.52 %
6	0.70 Rf	78.5 AU	0.71 Rf	85.2 AU	15.91 %	0.77 Rf	29.9 AU	3077.8 AU	11.65 %
7	0.88 Rf	1.0 AU	0.92 Rf	22.0 AU	4.11 %	0.94 Rf	7.1 AU	562.4 AU	2.13 %

(b)

Biologically active compounds usually occur in low concentration in plants. Extraction is an important step in the itinerary of phytochemical processing for the discovery of bioactive constituents from plant materials.[12] Polar solvents produced higher yields, the highest being Ethanol:Water, implying that polar compounds are getting extracted. Soxhlet extraction results showed that yield of the Ethanol:Water extract was better when extraction was done under reflux. This indicates that Soxhlet Extraction is more efficient than Kinetic Maceration, thus offering higher extract yields which is in agreement with the previous findings.[17]

Phytochemical screening test is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance, to make the best and judicious use of available natural wealth.[18] The study reveals the presence of important phytochemicals such as alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, glycosides and steroids to be present in *E. ferox* seed coat under study. Polar extracts showed more phytochemicals than that of mid-polar. The different test extracts of *Euryale ferox* seed coat demonstrated presence of an array of phytoconstituents in qualitative analysis.

Phenolics compounds are the most abundant secondary metabolites in plants, playing a key role in pigmentation, growth and reproduction of the plant, together with resistance to pathogens and predators. They have been shown to provide anti-allergic, anti-inflammatory, antioxidant,[19] hepatoprotective, antiviral and anti-carcinogenic activities[20] and antimicrobial activity.[19] Flavonoids can prevent injury caused by free radicals in various ways and one way is the direct scavenging of free radicals. Flavonoids stabilize the reactive oxygen species by reacting with the reactive compound of the radical.[21] Several high-quality investigations have examined the relationship between flavonoid structure and antibacterial activity and these are in close agreement.[22] The quantification of both phenol and flavonoid content revealed that they may be one of the main constituents in the seed coat. Hence, taking this into consideration, the seed coat shows the presence of both phenols and flavonoids which are powerful antioxidants and antimicrobial agents as it has been established through numerous research findings.

Previous study suggested that lower IC_{50} value indicates high antioxidant activity.[23] In this study the IC_{50} value of Ethanol: Water extract of *E. ferox* seed coat was 1.621μg/mL, indicating the extract as a potential effective antioxidant, which corroborates a previous study[4] presenting significant DPPH radical scavenging activity of the phenolic extract of *E. ferox* seed coat.

Antibacterial assay results observed that all four extracts were more potent against *Staphylococcus aureus* (Gram positive bacteria) than *Escherichia coli* (Gram negative bacteria). According to the antibacterial assay done for screening purpose all extracts in general are more effective against Gram positive bacteria than against Gram negative bacteria.[15] The results agree with observations of previous researchers and could be explained by the different cell wall structures of these bacteria.[15] The outer membrane of Gram-negative membrane comprising of phospholipid bilayers and lipopolysaccharide acts as a protective barrier against hostile environment and threats like antibiotics.[15,24,25]

The plants with high antioxidant properties have high antimicrobial activity. The antibacterial activity of flavonoids and polyphenols has been attributed to inhibition of synthesis of RNA and DNA[26] and metabolic processes.[22] Based on this, high antioxidant activity, total phenol and total flavonoid content of Ethanol:Water extract reported correlates with the high antibacterial activity of Ethanol:Water extract against *Escherichia coli* and *Staphylococcus aureus*.

To the best of our knowledge, HPTLC profile of the *E. ferox* seed coat was generated for the first time in order to establish the presence of phytoconstituents in the extract. The fingerprint profile revealed presence

of several polyvalent phytoconstituents in ethanol and ethyl acetate extract, this study can help in further characterization of bioactive compounds responsible for the antioxidant and antibacterial action. Furthermore, other mobile phases and extracts can also be used for better resolution and separation of bands.

CONCLUSION

The present findings showed that *Euryale ferox* seed coat has a potential to be explored further to identify the bioactive compounds responsible for its antioxidant and antibacterial action. The results provide a solid background for the identification, isolation, purification and characterisation of the bioactive components present in the seed coat responsible for its biological activities, which can be undertaken using bioassays and hyphenated techniques. The *Euryale ferox* seed coat which is an agro-industrial waste can be utilized as a potential source of bioactive components leading to their bioprospection into commercially viable products.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

ICAR: Indian Council of Agricultural Research; **HPTLC:** High-Performance Thin-Layer Chromatography; **TLC:** Thin-Layer Chromatography; **SD:** Standard Deviation; **OD:** Optical Density; **CLSI:** Clinical and Laboratory Standards Institute; **IC_{50}:** Half maximal Inhibitory Concentration; **R_f:** Retention factor; **RNA:** Ribonucleic acid; **DNA:** Deoxyribonucleic acid.

SUMMARY

The study revealed the presence of phytochemicals such as alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, glycosides and steroids in the seed coat. The Ethanol: Water extract of the *E. ferox* seed coat exhibited strong free radical scavenging activity and considerable antibacterial activity against both gram-positive and gram-negative organism, these activities could be due to the presence of phytoconstituents such as phenols, flavonoids in the seed coat which are bioactive in nature. Also, HPTLC fingerprinting profile was successfully developed which can serve as a tool in bioassay guided approach in further screening of the bioactive components. The study promisingly denotes *E. ferox* seed coat as a potential source of antioxidant and antibacterial compounds.

REFERENCES

1. Fierascu RC, Fierascu I, Avramescu SM, Sieniawska E. Recovery of natural antioxidants from agro-industrial side streams through advanced extraction techniques. Molecules. 2019;24(23):1-29.
2. Verma AK, Banerji BK, Chakrabarty D, Datta SK. Studies on Makhana (*Euryale ferox* Salisbury). Curr Sci. 2010;99(6):795-800.
3. Das S, Der P, Raychaudhuri U, Maulik N, Das DK. The effect of *Euryale ferox* (Makhana), an herb of aquatic origin, on myocardial ischemic reperfusion injury. Mol Cell Biochem. 2006;289(1-2):55-63. doi:10.1007/s11010-006-9147-1
4. Wu C, Chen R, Wang XS, Shen B, Yue W, Wu Q. Antioxidant and Anti-Fatigue Activities of Phenolic Extract from the Seed Coat of *Euryale ferox* Salisb. and Identification of Three Phenolic Compounds by LC-ESI-MS/MS. Molecules. 2013;18(9):11003-21.
5. Makhana F. Market Intelligence Report: Makhana. Retrieved from https://agriexchange.apeda.gov.in/Weekly_eReport/Makhana_Report.pdf
6. Barbade KD, Datar AG. Innovare Academic Sciences Extraction, Bioactivities, Phytochemical Investigation and *in-vivo* Toxicity Studies of *Mesua ferrea* L. Stamens. 2015;7(4):93-7.
7. Nielsen SS, Carpenter C. Fat Content Determination. In: Food Analysis Laboratory Manual. Cham: Springer International Publishing; 2017;121-9. [cited 2020 Jul 5] Food Science Text Series). Available from: http://link.springer.com/10.1007/978-3-319-44127-6_12
8. Pawar SS, Jadhav MG. Determination of Extractive value and Phytochemical analysis of *Bacopa monnieri* (L). Recent Adv life scinces. 2015;(4):9. Available from: https://www.researchgate.net/publication/296701173_Determination_of_Extractive_value_and_Phytochemical_analysis_of_Bacopa_monnieri_L--
9. Yadav R, Agarwala M. Phytochemical analysis of some medicinal plants. Journal of Phytology. 2011;5.
10. Pandey A, Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and Phytochemistry. 2014;2(5):115-9.
11. Silva GD, Abeysundara AT. Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. American Journal of Essential Oils and Natural Products. 2017;5(2):29-32.
12. Dhanani T, Shah S, Gajbhiye NA, Kumar S. Effect of extraction methods on yield , phytochemical constituents and antioxidant activity of *Withania somnifera*. Arab J Chem 2017;10:S1193-9. Available from: http://dx.doi.org/10.1016/j.arabjc.2013.02.015
13. Barbosa GB, Peteros NP. DPPH Radical Scavenging Potential of Ginger Leaves and Rhizomes. AJBLS. 2018;7(3):87-92.
14. Ghatage SL, Navale SS, Mujawar NK, Patil S, Patil V. Antimicrobial screening. IJOD. 2014;2(3):84-88.
15. Dahiya P, Purkayastha S. Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical isolates. Indian J Pharm Sci. 2012;74(5):443-50.
16. Wikler MA. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard. Ninth Edition. 2012;32.
17. Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules. 2009;14(6):2167-80.
18. Obouayeba AP, Diarrassouba M, Soumahin EF, Kouakou H. Phytochemical Analysis, Purification and Identification of *Hibiscus* Anthocyanins. J Pharm Chem Biol Sci. 2015;3(8):156-68. Available from: http://www.jpcbs.info

19. Proestos C, Boziaris IS, Nychas GJE, Komaitis M. Analysis of flavonoids and phenolic acids in Greek aromatic plants: Investigation of their antioxidant capacity and antimicrobial activity. Food Chem. 2006;95(4):664-71.
20. Noreen H, Semmar N, Farman M, McCullagh JSO. Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant *Coronopus didymus*. Asian Pac J Trop Med. 2017;10(8):792-801.
21. Panche AN, Diwan AD, Chandra SR. Flavonoids: An overview. J Nutr Sci. 2016;5.
22. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005;26(5):343-56.
23. Molyneux P. The Use of the Stable Free Radical Diphenylpicryl-hydrazyl (DPPH) for Estimating Antioxidant Activity: Songklanakarin J Sci Technol. 2004;26(12):211-9.
24. Hancock REW, Bell A. Antibiotic uptake into gram-negative bacteria. Curr Top Infect Dis Clin Microbiol. 1989;2(6):21-8.
25. Silhavy TJ, Kahne D, Walker S. The Bacterial Cell Envelope1. Cold Spring Harb Perspect Biol. 2010;2:1-16. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2857177/pdf/cshperspect-PRK-a000414.pdf
26. Sardarodiyan M, Arianfar A, Sani AM, Naji-Tabasi S. Antioxidant and antimicrobial activities of water-soluble polysaccharide isolated from Balangu seed (*Lallemantia royleana*) gum. J Anal Sci Technol. 2019;10(1):17.

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