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In Vitro antioxidant studies and quantitative phytochemical analysis of *Excoecaria Agallocha* L.

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Abstract
Excoecaria agallocha (Euphorbiaceae) a mangrove plant, latex is traditionally used for toothache, wood as anti-epileptic and root has anti-inflammatory activity. The study was conducted to investigate antioxidant activity of various extract. The Total Phenolic Content (TPC), total alkaloid content and Total Flavonoid Content (TFC) were quantitatively estimated. The method were employed for the evaluation of antioxidant activity are DPPH radical scavenging assay, Reducing power assay and Phospho molybdenum assay. Potent antioxidant activities were shown by the acetonitrile extract and water extract of leaf and stem in all the assays. Acetonitrile extract of leaf showing highest DPPH scavenging activity (IC_{50} =31.76). Acetonitrile extract of leaf showing good reducing power. The better reduction of molybdenum was shown by water extract of leaf (715.5±3.5). Highest TPC and TFC were obtained for the acetonitrile extract of leaf (240.30±0.62 and 363.49±3).

Keywords: *Excoecaria agallocha*, antioxidant activity, phenolics, flavonoids, alkaloids

1. Introduction
India has vast geographical area with rich culture of medicinal herbs, that is about more than 2000 thousand species and it is used in the traditional medicine system like Ayurveda, Siddha and Unani and millions of people in rural and remote places are still use herbal medicine to satisfy their healthcare needs [11]. Medicinal plants are very important in maintaining health care of people. Majority of the world population, medicinal plants are being used for their primary healthcare [13]. Traditional medicine based on plant origin depended by majority of population in the world. World's richest source of medicinal plants is in India because of its rich geographical diversity, varied climatic and ecological features. 80% of the people in the world is trusted on plant medicine and they are use traditionally for their primary healthcare needs [6].
Chemicals synthesize by different parts of the plants are called phytochemicals. Terpenoids, steroids, carotenoids, flavonoids, alkaloids and glycosides are the bioactive chemicals produced by them. These phytochemical have different bioactivities such as antimicrobial, antioxidant, antidiabetic and anti-inflammatory activities.
Molecular oxygen produces as a result of photosynthesis. Reactive oxygen species such as superoxide, hydroxyl and peroxyl radical produces as a consequence of this normal process. Bioactive compound that inhibit or reduce the oxidation of molecule can be defined as antioxidants. These may be natural or synthetic. Reducing of inflammation, delay of aging and prevention of certain cancers are the dynamic role of antioxidants such as beta carotene, phenolics and ascorbic acid [8].
Mangroves are seen in a stressful environment with the condition like high salinity, high solar radiation during low tide and low nutrition. By these condition, reactive oxygen species produces in mangrove plants and they are also rich in polyphenol compounds which act as antioxidants during the oxidative stress [2]. Mangroves protecting the adjacent land as a barrier against tidal waves and sea storm [10]. Study shows that root extract of mangrove have antioxidant activity and it is by the presence of flavonoid and polyphenol compounds [1]. Mangroves produces various types of secondary metabolites. 80% of the carbon is utilized for the production of naturally occurring phenolics and the rest is assumed for photosynthesis [18].
Climate, rainfall, altitude and other environmental factors may effect growth of plant and it will affect the bioactive compounds present in them [14]. Various types of chemical are synthesized by plants and it will estimated by the qualitative phytochemical screening.
Beneficial phytochemical present in the plant can be used for the needs of human body because it act as natural antioxidants [3]. Various types of bioactive compounds are produced by plants. Fruits and vegetables contains antioxidant compound including carotenoids, phenolics, toccopherol and anthocyanins [12]. Phenolic compounds present in plants such as tannins, flavonoids and lignin can act as antioxidants. Reactive oxygen species cause oxidative damage in foods and this is controlled or reduced by the antioxidants, so the quality and shelf life of the food is increased

2. Materials and Methods
2.1 Plant Collection
Excoecaria agallocha L. collected from Pazhayangadi, Kannur district, Kerala was taken for the study. The collected plants were washed thoroughly with tap water followed with distilled water for the removal of dust and soil particles. The plants cut into pieces and were shade dried at room temperature for 15 days then coarsely powdered and used for extraction.

2.2 Preparation of Extract
The powder (50 gm) was extracted with petroleum ether, chloroform and acetonitrile in a soxhlet apparatus (3840; Borosil Glass works Ltd., Mumbai, India) in increasing order of their polarity. Finally the dried powder was macerated using water with constant stirring for 48 hours using the orbital shaker (Rivotek; Riviera Glass Pvt. Ltd.,

Mumbai, India) and the extract was filtered. The extracts were concentrated, dried and stored at -20 ºC in the deep freezer (RQV- 300; plus, REMI electro technik Ltd., Thane, Maharashtra, India) for further analysis.

2.3. Quantification Assays

2.3.1 Quantification of total phenolics

The total phenolics of the different plant extracts were determined according to the method described by Makkar [15]. In this method 50µL of different plant extracts were taken into a series of test tubes and made up to1mL with distilled water. A test tube with 1mL of distilled waterserved as the blank. Then, 500µL of Folin – Ciocalteau Phenol reagent (1N) was added to all the test tubes including the blank. After 5 minutes, 2.5mL of sodium carbonate solution (20%) was added to all the test tubes. The test tubes were vortexed well to mix the contents and incubated in dark for 40 minutes. The formation of blue colour in the incubated test tubes indicated the presence of phenolics. Soon after incubation the absorbance was read at 725nm against the reagent blank. Gallic acid standard was also prepared and the results were expressed as Gallic acid equivalents (GAE). The analyses were performed in triplicates.

2.3.2 Quantification of total flavonoids

The flavonoid contents of all the extracts were quantified according to the method described by Zhishen, *et al*. [25]. About 500µL of all the plant extracts were taken in different test tubes and 2mL of distilled water was added to each test tube. A test tube containing 2.5 mL of distilled water served as blank. Then, 150µL of 5% $NaNO_2$ was added to all the test tubes followed by incubation at room temperature for 6 minutes. After incubation, 150µL of 10% $AlCl_3$ was added to all the test tubes including the blank. All the test tubes were incubated for 6 minutes at room temperature. Then 2mL of 4% NaOH was added to all the test tubes which were then made up to 5mL using distilled water. The contents in all the test tubes were vortexed well and they were allowed to stand for 15 minutes at room temperature. The pink colour developed due to the presence of flavonoids was read spectrophotometrically at 510nm. Rutin was used as the standard for the quantification of flavonoids. All the experiments were done in triplicates and the results were expressed in Rutin equivalents (RE).

2.3.3 Quantification of total alkaloid

Alkaloid determination using Harborne [9] method. 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

2.4. In vitro Antioxidant Assays

2.4.1 DPPH˙ scavenging activity

The antioxidant activity of the extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH**,** according to the method of Braca *et al*. [4]. Sample extracts at various concentrations were taken and the volume was adjusted to 100 µL with methanol. About 3 mL of a 0.004% methanolic solution of DPPH was added to the aliquots of samples and standards (BHT and Rutin) and shaken vigorously. Negative control was prepared by adding 100 µL of methanol in 3 mL of methanolic DPPHsolution. The tubes were allowed to stand for 30 minutes at 27°C. The absorbance of the samples and control were measured at 517 nm against the methanol blank. Radical scavenging activity of the samples was expressed as IC_{50} which is the concentration of the sample required to inhibit 50% of DPPH˙ concentration.

2.4.2 Phosphomolybdenum assay

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method of Prieto *et al*., [21]. An aliquot of 100 µL of samples and standards (BHT and rutin) were taken in to a series of test tubes and were made up to 300 µL with methanol. About 300 µL methanol taken in a test tube was considered as the blank. All the test tubes were added with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and vortexed well to mix the contents. The mouth of the test tubes were covered with foil and incubated in a water bath at 95°C for 90 minutes. After the samples were cooled to room temperature, the absorbance of the mixture was measured at 695 nm against the reagent blank. Ascorbic acid was used as the reference standard and the results were expressed as milligrams of ascorbic acid equivalents per gram extract.

2.4.3 Reducing power assay

The reducing power of sample extracts was determined according to the method of Oyaizu [20]. Different aliquots of extracts (50–250 mg) and standards (BHT and rutin) were taken into a series of test tubes and were made up to 1ml with methanol. A test tube with 1 mL of methanol served as the blank. Then each test tubes including the blank were added with phosphate buffer (2.5 ml, 0.2 M, pH-6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 650 x g for 10 minutes at room temperature. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

2.5 Evaluation of Antidiabetic activity by using *in vitro* assay

2.5.1 Alpha -amylase inhibitory assay

The Alpha-amylase inhibitory assay for different solvent extracts *Excoecaria agallocha* were evaluated according to a previously described method by Malik and Singh *et al*., [16] with slight modification. In brief, 0.5 ml of extract was mixed with 0.5 ml of α-amylase solution (0.5 mg/ml) with 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl).The mixture was incubated at room temperature for 10 min and 0.5 ml of starch solution (1%) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added. The resulting mixture was incubated at room temperature for 10 min, and the reaction was terminated using 1mL of

dinitrosalicylic acid color reagent. At this time, the test tubes were placed in a water bath (100 ^{0}C and 5 min) and cooled until room temperature was reached. The mixture was then diluted with 10 ml of deionized water, and absorbance was determined at 540 nm. The absorbance of blank (buffer instead of extract and amylase solution) and control (buffer instead of extract) samples were also determined. Acarbose was used as standard drug. The inhibition of α-amylase was calculated using the following equation.

% inhibition of α-Amylase = (Abs $_{control}$ – Abs $_{sample}$) / (Abs $_{control}$) × 100

Where Abs $_{control}$ corresponds to the absorbance of the solution without extract (buffer instead of extract) and with α -amylase solution and Abs $_{sample}$ corresponds to the solution with extract and α -amylase solution.

2.6 Statistical Analysis

Measurements were recorded in triplicates for all the analysis. Results were calculated as the mean (n=3) ± SD (standard deviation) for each sample. One-way ANOVA followed by student's t- test was performed using GraphPad Prism version 7.02 for Windows (GraphPad Software, La Jolla California, USA). $p < 0.05$ were considered significant. IC_{50} values were determined using nonlinear regression method.

3. Result

3.1 Quantification Assays

3.1.1 Quantification of total phenolics

Total phenolic contents of the various extract was determined using Folin-Ciocalteu's method and represented in terms of gallic acid equivalent. The total phenolics of different extract of leaf and stem of *E. agallocha* were analysed and are tabulated (Table 1). In *E. agallocha*, phenol content in the extracts ranged from 3.98 to 240.30 mg GAE/g. Acetonitrile extract of leaf of shows highest amount of phenolics (240.30 µg of GAE/g) among other extracts.

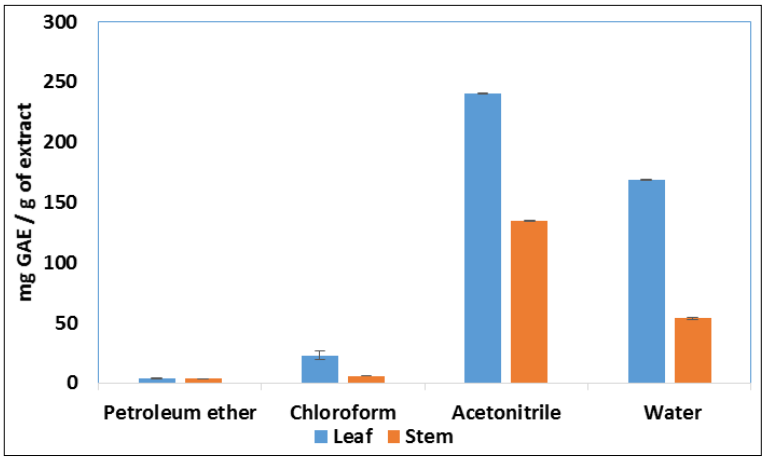
3.1.2 Quantification of total flavonoids

The flavonoid content of different extract in the selected plants were analysed. The present investigation revealed the presence of broad range of flavonoid content with high significance in leaf extracts. Total flavonoid content was determined from standard (+)- rutin regression curve and expressed as (+)- rutin equivalents per gram of extracts. Total flavonoid content of leaf and bark of *E. agallocha* are shown in Table 2. Flavonoid content of leaf extracts *E. agallocha* varied from 54.86 to 363.49 mg RE/g of extracts. Flavonoid content of stem extracts varied from 57.10 mg RE/g to 312.15 mg RE/g. In leaf and stem the acetonitrile extract shows good result. Water and chloroform extracts have adequate amount of flavonoid. Petroleum ether extract showed poor flavonoid content.

Table 1: Total phenolic content of *E. agallocha* (mg GAE/g extract)

Solvents	Leaf	Stem
Petroleum ether	3.98±0.35	3.57±0.25
Chloroform	23.02±3.90	5.73±0.09
Acetonitrile	240.30±0.62	134.64±0.06
Water	168.54±0.65	53.81±0.82

Values are mean of triplicate determination (n=3) ± standard deviation
GAE-Gallic Acid Equivalents *statistically significant at P<0.05



Values are mean of triplicate determination (n=3) ± standard deviation
GAE-Gallic Acid Equivalents, * statistically significant at P<0.05

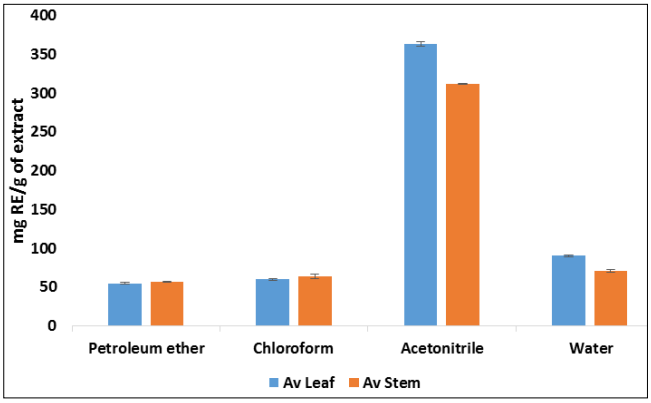
Fig 1: Total phenolic content *Excoecaria agallocha*

Table 2: Total flavonoid content of *E. agallocha* (mg RE/g extract)

Solvents	Leaf	Stem
Petroleum ether	54.86±0.96	57.10±0.87
Chloroform	59.83±1	63.84±2.77
Acetonitrile	363.49±3	312.15±0.73
Water	90.47±0.96	71.22±1.66

Values are mean of triplicate determination (n=3) ± standard deviation

RE-Rutin Equivalents *statistically significant at P<0.05



Values are mean of triplicate determination (n=3) ± standard deviation

RE-Rutin Equivalents *statistically significant at P<0.05

Fig 2: Total flavonoid content of *Excoecaria agallocha*

3.1.3 Quantification of total alkaloid

The total alkaloid content of leaf and stem of *E. agallocha* has been estimated and results are given in Table 3. The alkaloid content was 0.04 mg / 1g powder in both leaf and stem.

Table 3: Total alkaloid content (mg / g powder)

Excoecaria agallocha	Leaf	0.043±2.76
	Stem	0.048±1.62

3.2 In vitro antioxidant assay

3.2.1 Phosphomolybdenum assay

The phosphomolybdenum assay is a reduction based analysis in which the reduction of Mo (VI) to Mo (V) occurs. The reduction of molybdenum occurs in the presence of antioxidant compound. The green phosphomolybdenum complex is formed at high temperature and in acidic pH. The phosphomolybdenum antioxidant capacity of different solvent extracts of leaf and stem were analysed and are shown in Table 4.

In *E. agallocha* the better reduction was by the water extract of leaf followed by acetonitrile extract of stem. The phosphomolybdenum method detects antioxidants like ascorbic acid, some phenolics, tocopherols and carotenoids. The total antioxidant activity of the extract were compared with AAE, a standard antioxidant and the results are given below.

Table 4: Phosphomolybdenum assay of *E. agallocha* (mg AAE/g extract)

Solvents	Leaf	Stem
Chloroform	285±4.5	175.66±3.51
Acetonitrile	412.66±1.15	609.33±2.30
Water	715.5±3.5	156.66±1.15

Values are mean of triplicate determination (n=3) ± standard deviation

AAE-Ascorbic Acid Equivalents *statistically significant at P<0.05

3.2.2 Reducing power assay

Antioxidant potential was estimated according to their reducing capabilities and results are represented in figures. Reducing power of all the extract were evaluated. The reducing power of petroleum ether, chloroform, acetonitrile and water extract of leaf and bark shown in Figure 3 and Figure 4 respectively. Ascorbic acid is used as standard to evaluate the reducing activity. *E. agallocha* leaf shows better reducing power activity when compared to leaf. Ascorbic acid is used as standard to evaluate the reducing activity.

Reducing power is associated with antioxidant activity by the compounds present in the sample. The compound with electron donating capacity have reducing power ability and they can reduce the oxidised intermediate and also act as good antioxidant. Reduction of ferric to ferrous is by the activity of the reducers present in the plant extracts and colour change occurs from green to blue. Iron reduction is the important mechanism in the phenolic antioxidant activity (Manach *et al*., 2004).

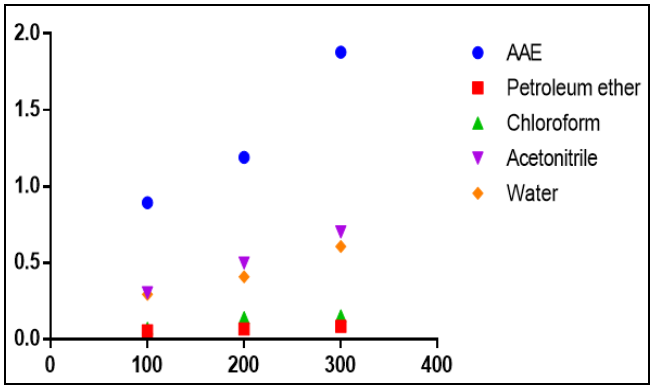


Fig 3: Reducing power of *E. agallocha* leaf

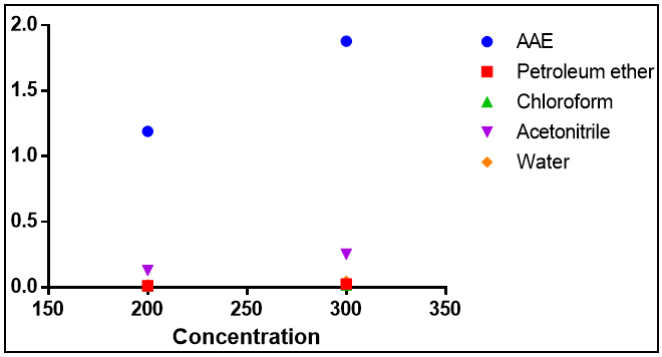


Fig 4: Reducing power of *E. agallocha* stem

3.2.3 DPPH• scavenging activity

The radical scavenging activity of the stable 1, 1,-diphenyl-2-picryl- hydrazyl (DPPH) is the basis for the evaluation of antioxidant activity of plant extract as well as the standard (Braca *et al*., 1958). The lower value of IC_{50} indicates higher antioxidant activity of extracts. Both leaf and stem of *E. agallocha* shows good inhibition activity. Acetonitrile and water extract of leaf have low IC_{50} value 31.76 and 44.05 respectively and it is shown in Table 5. Stem extract shows good radical scavenging activity, among them acetonitrile extract have low IC_{50} value i.e., 41.02. In *E. agallocha* the leaf extract showed good inhibition compared to the stem extract shown in table 5 and 6 respectively.

Table 5: DPPH• scavenging activity of *E. agallocha*

Plant part	Solvent	Concentration	Inhibition (%)	IC $_{50}$
Leaf	Acetonitrile	50	76.26±0.51	31.76
		100	93.60±1.01	
		150	95.06±0.19	
		200	95.36±0.25	
		250	95.88±0.78	
	Water	50	61.46±0.96	44.05
		100	88.06±1.50	
		150	89.67±0.11	
		200	90.38±0.64	
		250	91.09±0.74	

Table 6: DPPH• scavenging activity of *E. agallocha*

Plant part	Solvent	Concentration	Inhibition (%)	IC $_{50}$
Stem	Acetonitrile	30	35.02±0.65	41.02
		50	60.72±0.55	
		80	78.01±0.60	
		100	88.52±0.18	
		150	93.22±0.36	
	Water	30	9.03±0.76	119.67
		50	12.17±0.65	
		80	18.62±0.69	
		100	34.27±0.69	
		150	56.04±0.96	

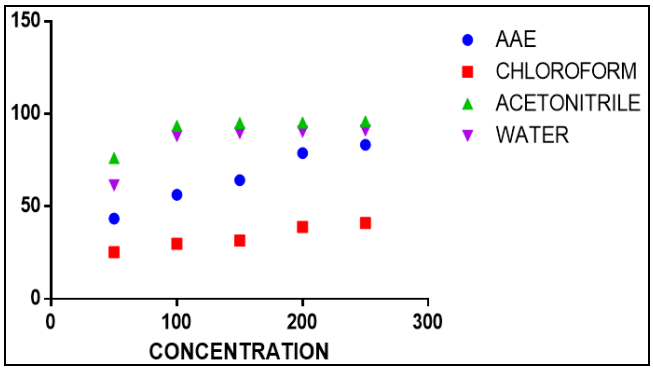


Fig 5: DPPH scavenging activity of *E. agallocha* leaf

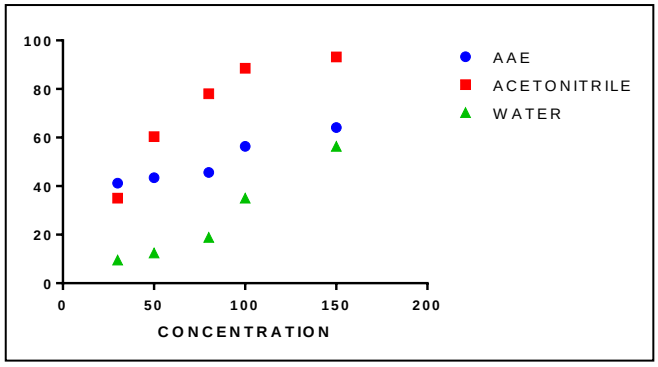


Fig 6: DPPH scavenging activity of *E. agallocha* stem.

3.3 In vitro α-amylase inhibition

In the present study chloroform, acetonitrile and water extract of leaf and stem were evaluated for antidiabetic activity by using standard *in vitro* technique, Alpha-amylase inhibition assay. Alpha-amylase is the enzyme involved in the metabolism of carbohydrates i.e. hydrolysis of starch and disaccharides to glucose.

Table 7: *In vitro* α-amylase inhibition activity of *E. agallocha* leaf

Samples	Concentration (µg/ml)	Inhibition %
Chloroform	500	11.49±0.39
Acetonitrile	500	47.75±0.56
Water	500	31.97±0.79
Acarbose	500	70.87±0.65

Table 8: *In vitro* α-amylase inhibition activity of *E. agallocha* stem

Samples	Concentration (µg/ml)	Inhibition %
Chloroform	500	7.20±0.39
Acetonitrile	500	33.69±1.05
Water	500	37.25±0.53
Acarbose	500	70.87±0.65

4. Discussion

Vegetables and food are rich in antioxidants minimize the risk of numerous diseases such as cardiovascular and cancer [24]. In plants, phenols is the main constituent. They have antioxidant activity by their redox property so they can adsorb and scavenge oxidants [19]. In total phenolics about 60% is account for flavonoid. They are the largest group of naturally occurring phenolics. Flavonoids have free radical scavenging activity and this is the basis for their biological activity such as anticancer antioxidant and anti-inflammatory [23]. Functional quality of plant can be evaluated by the antioxidant assay and it can be characterize the plant species [22]. Antioxidant compound is complex in nature so all the antioxidant capacity may not be determined by single assay and for the better evaluation multiple assay should be done [5].

Antioxidants repress lipid oxidation through free radical scavenging. DPPH, a stable free radical is used to evaluate the scavenging ability of plant which are rich in antioxidant [7]. The property of reducing power is generally related with the occurrence of reductones. The reductones present in the plant extract is responsible for antioxidant activity.

Reducing power is associated with antioxidant activity by the compounds present in the sample. The compound with electron donating capacity have reducing power ability and they can reduce the oxidised intermediate and also act as good antioxidant. Reduction of ferric to ferrous is by the activity of the reducers present in the plant extracts and colour change occurs from green to blue. Iron reduction is the important mechanism in the phenolic antioxidant activity [17].

5. Conclusion

The findings of the study gives that *E. agallocha* is a good source of natural antioxidants with acetonitrile. Antioxidant activity may be ascribed to phenolics and flavonoids. This plant could be useful in treating the disease associated with oxidative stress, so they are the good source of antioxidant molecule. So it can be used for the production of drugs for the treatment of various diseases.

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7. Conflict of Interest

The authors declare no known conflict of interest

8. References

1. Asha KK, Mathew S, Lakshmanan PT. Flavonoids and phenolic compounds in two mangrove species and their antioxidant property. Indian Journal of Geo-Marine Science, 2012; 41:259-264.
2. Banerjee D, Chakrabarti S, Hazra AK, Banerjee S, Ray J, Mukherjee B, *et al*. Antioxidant activity and total phenolics of some mangroves in Sundarbans. African Journal of Biotechnology. 2008; 7(6):805-810
3. Boots AW, Haenen GR, Bast A. Health effects of quercetin from antioxidant to nutraceutical. European journal of pharmacology. 2008; 585(2-3):325-337.
4. Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I, *et al*. Antioxidant principles from *Bauhinia tetrapotensis*. Journal of Natural Products, 2001; 64: 892-895.
5. Chu YH, Chang CL, Hsu HF. Flavonoid content of several vegetables and their antioxidant activity. Journal of the Science of Food and Agriculture. 2000; 80:561-566.
6. Dubey NK. Flora of BHU campus. Banaras Hindu University, BHU press, Varanasi India, 2004.
7. Dubey RK, Singh V, Upadhyay G, Pandey AK, Prakash D. Assessment of phytochemical composition and antioxidant potential in some indigenous chilli genotypes from North East India. Food Chemistry. 2015; 188:119-125.
8. Duthie SJ, Ma AG, Ross MA, Collins AR. Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. Cancer research. 1996; 56:1291-1295.
9. Harborne JB. Phytochemical methods. Chapman and Hill, Ltd London, 1973, 49-188.
10. Hogarth PJ. The Biology of Mangroves and Sea grasses. Oxford University Press, New York, 2007, 273.
11. Jain SK. Ethno botany, its scope and study. Indian Museum Bull, 1967; 2:39-43.
12. Jakubowski W, Bartosz G. Estimation of oxidative stress in *saccharomyces cerevisae* with fluorescent probes. International Journal of Biochemistry and Cell Boilogy. 1997; 29:1297-1301.
13. Kamboj VP. Herbal Medicine. Current Science, 2000; 78:35-9.
14. Kokate CK, Purohit AP, Gokhale, SB. Practical pharmacognosy. 2nd edition, Vallabh Prakashan, New Delhi, 2004, 466-470.
15. Makkar HPS. Quantification of tannin in tree and shrub foliage. A laboratory manual. Dondrecht, The Netherlands, Kluwer academic publishers, 2003.
16. Malik CP, Sing MB. Plant Enzymology and Histo enzymology. Kalyani Publishers, New Delhi, 1980, 278.
17. Manach C, Scalbert A, Morand C, Rémés C, Jimenez L. Polyphenols: Food sources and bioavailability. American Journal of Clinical Nutrition. 2004; 79:727-747.
18. Metcalf RL. Plant volatiles as insect attractants. Critical Reviews in Plant Science. 1987; 5:251-301.
19. Ohikhena F, Wintola O, Afolayan AJ. Quantitative phytochemical constituents and antioxidant activities of the mistletoe, *Phragmanthera capitata* (sprengel) balle extracted with different solvents. Pharmacognosy Research. 2018; 10(1):16-23.
20. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Japanese Journal of Nutrition. 1986; 44:307-315.
21. Prieto P, Pineda M, Anguilar M. Spectrophotometric quantification of antioxidant capacity through the formation of a phosphomolybdenum complex; specific application to the determination of Vitamin E. Annals of Biochemistry. 1999; 269:337-341.
22. Sharma K, Assefa AD, Kim S Ko, EY, Lee ET, Park SW, *et al*. Evaluation of total phenolics, flavonoids and antioxidant activity of 18 Korean onion cultivars: A comparative study. Journal of the Science of Food and Agriculture. 2014; 94(8):1521-1529.
23. Singh R. Chemotaxonomy: a tool for plant classification. Journal of Medicinal Plants Studies. 2016; 4(2):90-93.
24. Tsao R, Khanizadeh S, Dale A. Designer fruits and vegetables with enriched phytochemicals for human health. Canadian Journal of Plant Science. 2006; 86(3):773-786.
25. Zhishen J, Mengecheng T, Jianming W. The determination of flavonoid content on mulberry and their scavenging effects on superoxide radical. Food chemistry. 1999; 64:555-559.