

QUALITATIVE PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF *ELYTRARIA ACAULIS* LINDAU (ACANTHACEAE)

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ABSTRACT

Objectives: To analyze the phytochemicals quantitatively and screening the antioxidant efficacy of methanol extracts of *Elytraria acaulis* plant extract by *in vitro*.

Methods: The total phenols, flavonoids, and tannin contents analyzed by Folin-Ciocalteu method, aluminum chloride colorimetric and calculated as gallic acid equivalents/g (GAE/g) of dry weight. Free radical scavenging activity was screened using 1,1-diphenyl-2-picrylhydrazyl (DPPH), radical scavenging activities, and ferric reducing power assay was also analyzed.

Results: The total phenolic content of the aqueous leaf extract was 46.84 µg GAE/g of extract powder. Significantly similar levels of total flavonoid and tannin contents of the plant were 41.72 and 39.50 µg GAE/g, respectively. The plant extracts showed appreciable free radical scavenging activities at the highest concentration of 400 µg/mL superoxide anion radical with IC₅₀ values (107.7±1.081 µg/mL), and for DPPH and considerably high amount of radical scavenging activity was found with very low IC₅₀ values (4.3±0.88 µg/mL) compared with quercetin.

Conclusion: The phenolic and flavonoid compounds provide substantial antioxidant properties which could be effectively used for pharmaceutical, nutraceutical as well as anti-inflammatory applications.

Keywords: Phenols, Flavonoids, 1,1-diphenyl-2-picrylhydrazyl, Pharmaceuticals.

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INTRODUCTION

In search of novel sources of antioxidants from the last decades, medicinal plants have been extensively studied for their antioxidant activity. From ancient times, herbs have been used in many areas, including nutrition, medicine, flavoring, beverages, cosmetics, etc. The plants are a rich source of large amount of drugs comprising to different groups such as antispasmodics, emetics, anti-cancer, and antimicrobials. A large number of the plants are claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. It is now believed that nature has given the cure of every disease in one way or another. Plants have been known to relieve various diseases in Ayurveda. Therefore, the researchers today are emphasizing on evaluation and characterization of various plants and plant constituents against a number of diseases based on their traditional claims of the plants given in Ayurveda [1].

Extraction is an intimate process to isolate the components from plant cells, which may contain a complex mixture of many metabolites, such as alkaloids, glycosides, terpenoids, flavonoids, lignan, phenols, and saponins most of the researchers prefers hot continuous extraction (soxhlet extraction). The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled [2,3].

Oxidation is an essentially biological process for energy production in many living organisms. However, excessive reactive oxygen species, produced *in vivo* during some oxidative reactions, are not only strongly associated with lipid peroxidation but also involved in the development of some chronic diseases, such as cancer, cardiovascular disease, atherosclerosis, and diabetes [4]. The aim of the present study is to screen the antioxidant and phytochemical properties of *Elytraria acaulis* plant extracts.

METHODS

Collection of plant

The Indigenous plant variety *E. acaulis* from the Family of Acanthaceae was collected from the places in and around Bhuvanagiri, Cuddalore district and identified morphologically and taxonomically; the specimens were submitted to the Department of Botany, Annamalai University.

Preparation of plant extract

The collected plant material was washed cleanly in tap water and then air dried under shadow condition at room temperature (25°C) for 2-3 weeks until it becomes brittle. After complete drying, the plant material was grinded to a fine powder using an electrical blender. 50 g of dried powder was packed in the soxhlet apparatus with 300 mL of solvents (methanol, acetone, chloroform, and hexane) extracted until the extract was clear. The solvents from the extracts were evaporated using rotary vacuum evaporator, and the extract was stored in a refrigerator for further use [5].

Determination of total phenol

The total phenolic content of the methanol extract of *E. acaulis* plant was determined by using the Folin-Ciocalteu reagent according to the method of Singleton *et al.* [6]. About 1 mL of plant extract was mixed with 5 mL of Folin-Ciocalteu reagent at the ratio of 1:10, followed by the addition of 4 mL of Na₂CO₃ (0.7 M). Subsequently, the mixture was shaken for 2 hrs at room temperature and the absorbance was measured at 760 nm. All the tests were performed at triplicates. The concentration of total phenolic compounds was determined as µg gallic acid equivalents using the following equation obtained from a standard gallic acid graph:

$$\text{Absorbance} = 0.001 \times \text{pyrocatechol } (\mu\text{g}) + 0.0033.$$

Total flavonoid content

The total flavonoid content of the crude extract was determined by the aluminum chloride colorimetric method [7]. In brief, crude extract 1 mg/mL ethanol were made up to 1 mL with methanol, mixed with 4 mL of distilled water and then 0.3 mL of 5% NaNO₂ solution; 0.3 mL of 10% AlCl₃ solution was added after 5 minutes of incubation, and the mixture was allowed to stand for 6 minutes. Then, 2 mL of 1 mol/L NaOH solution were added, and the final volume of the mixture was brought to 10 mL with double-distilled water. The mixture was allowed to stand for 15 minutes, and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg rutin equivalent per g dry weight.

Estimation of total tannins

Tannins content of *E. acaulis* was estimated following the method of Siddhuraj and Manian [8]. 500 µL of the extracts were taken in test tubes and treated with 100 mg of polyvinylpyrrolidone and 500 µL of distilled water. This solution was incubated at 4°C for 4 hrs. Then the sample was centrifuged at 5000 rpm for 5 minutes, and 20 µL of the supernatant was taken. This supernatant has only simple phenolics free of tannins - the tannins would have been precipitated along with the polyvinylpyrrolidone. The phenolics content of the supernatant was measured at 725 nm and expressed as the content of free phenolics on a dry matter basis. From the above results, the tannins content of the extract was calculated as follows:

$$\text{Tannin (mg/g extract)} = \text{Total phenolics (mg/g extract)} - \text{free phenolics (mg/g extract)}$$

-free phenolics (mg GAE / g extract)

1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity

The free radical scavenging activity of extracts was determined using the DPPH method [9]. Different concentrations of extracts and positive control in methanol solution (100 µL) were added to 4 mL of DPPH methanol solution (0.1 mM). An equal amount of methanol was used as a blank. After incubation at room temperature for 30 minutes in the dark, the absorbance was measured at 517 nm using a UV spectrophotometer. The activity of scavenging (%) was calculated using the following formula:

$$\text{DPPH radical scavenging (\%)} = \frac{[(\text{OD control} - \text{OD sample}) / \text{OD control}] \times 100\%}{}$$

Superoxide scavenging activity

The superoxide anion radical scavenging activity of methanol extracts was performed according to NTB reduction method [10]. The reaction was initiated by adding 100 µL of phenazine methosulfate solution (60 µM, PMS) in phosphate buffer (pH 7.4) to the reaction mixture followed by incubation at 25°C for 5 minutes and the absorbance at 560nm was measured against blank.

Ascorbic acid was used as the standard.

$$\text{Superoxide scavenging activity (\%)} = \frac{\text{Abs(control)} - \text{Abs(sample)}}{\text{Abs(control)}} \times 100$$

Where, Abs (control): Absorbance of the control and Abs (test): Absorbance of the extracts/standard.

Determination of ferric reducing power assay (FRAP)

The reducing power was performed according to the method of Oyaizu [11], ferric-tripyridyl-triazine (TPTZ-Fe³⁺) complex is reduced to TPTZ-Fe²⁺ in the presence of antioxidants. Various concentrations of samples (25-200 µg/mL) in 200 µL of methanol were mixed with 0.5 mL phosphate buffer (0.2 M, pH 6.6) and 0.5 mL potassium ferricyanide (1% w/v). The mixture was incubated at 50°C for 20 minutes. After terminating the reaction by adding trichloroacetic acid (10% w/v), the mixture was centrifuged at 1000 ×g for 10 minutes. The supernatant

of solution 0.5 mL was mixed with 0.5 mL distilled water and 0.1 mL FeCl₃ (0.1% w/v). After 5 minutes, the absorbance was measured at 700 nm in a microplate reader. Ascorbic acid is used as a positive control. FRAP value is expressed as the number of equivalence of gallic acid.

RESULTS

Quantitative analysis of phytochemical compounds

The phytochemical analysis of the plant extract showed the presence of various biologically active chemical compounds such as alkaloids, amino acids, catachols, flavonoids, phenols, saponins, steroids, and tannins and the results were presented in Table 1. The amount of phytochemical compounds present in the *E. acaulis* extract was quantitatively determined by standard procedures. All the extracts of *E. acaulis* showed different amount of phytochemicals. The total phenolic content was high in the methanol extract of *E. acaulis*.

DPPH scavenging activity

The DPPH radical scavenging activity of methanol extracts of EAM at five different concentrations (3.125, 6.25, 12.5, 25, and 50 µg/mL) was tested, and the results were shown in Fig. 1. All the extracts exhibited a dose-dependent increase in activity. The highest DPPH scavenging activity was shown *E. acaulis* Lind extracts (93.06% and 92.47% for precipitate and liquid fractions respectively) at 50 µg/mL concentration. The IC₅₀ value of the *E. acaulis* extract (4.3 µg/mL) was comparable to that of ascorbic acid standard (3.2 µg/mL).

Superoxide scavenging activity

Methanol extract of *E. acaulis* was found to scavenge the superoxide radicals generated by riboflavin photoreduction method. The highest superoxide anion scavenging potential as indicated by the lowest IC₅₀ concentration was IC₅₀ - 107.7 µg/mL. This sample compares well with the ascorbic acid standard of IC₅₀ 91.2 µg/mL.

FRAP

The reducing power of different methanol extracts of *E. acaulis* which may serve as a significant reflection of the antioxidant activity was evaluated using potassium ferricyanide reduction method, and the results were noted in Fig. 2. All the studied extracts exhibited considerable reducing ability when compared with the standard BHT and the results were documented in the Fig. 2.

The absorbance value and concentration of extracts showed linear relationship. Therefore, increasing OD values indicate increasing trend in reducing power.

Table 1: Qualitative phytochemical screening of *E. acaulis*

S. No.	Phytochemical compounds in methanol extract	Quantitative analysis µg/mg
1	Total flavonoids	41.72
2	Total phenolic	46.84
3	Tannins	39.50

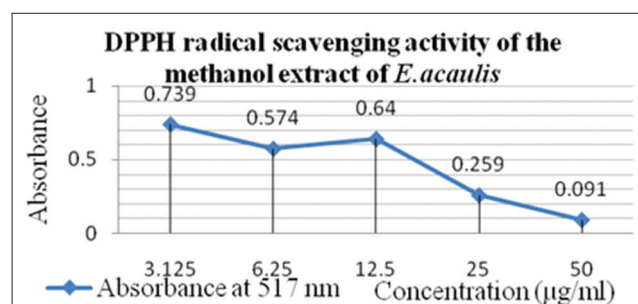


Fig. 1: 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity of methanol extract

Table 2: Superoxide anion scavenging activity

Sample	Concentration ($\mu\text{g/mL}$)	Absorbance at 560 nm	% inhibition	IC ₅₀ ($\mu\text{g/mL}$)
Control		1.125		
<i>E. acaulis</i> extract	25	0.629 \pm 0.001	39.35 \pm 0.137	107.7 \pm 1.081
	50	0.567 \pm 0.014	52.62 \pm 2.760	
	100	0.457 \pm 0.030	59.57 \pm 1.159	
	200	0.326 \pm 0.005	71.02 \pm 0.051	
	400	0.217 \pm 0.008	80.70 \pm 0.009	

Values shown are mean \pm SEM for four tests. $p < 0.001$, as compared to control. SEM: Standard error of mean

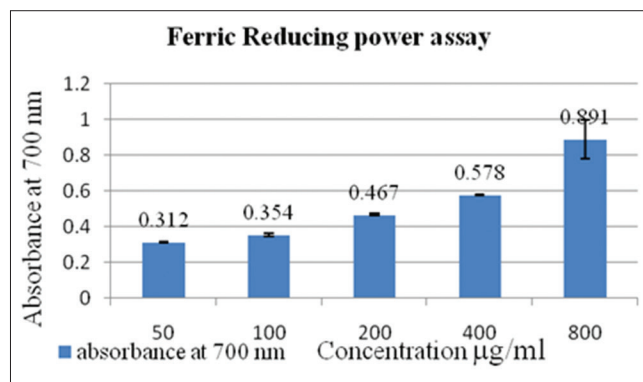


Fig. 2: Ferric reducing power assay

DISCUSSION

The antiradical activity of the methanol extract was measured by the ability to scavenge DPPH free radicals with higher scavenging activity at various concentrations ranging from 3.125 $\mu\text{g/mL}$ to 50 $\mu\text{g/mL}$. This result proved that the extracts are capable of donating an electron or hydrogen which could react with DPPH radical and the IC₅₀ value of *E. acaulis* extract (4.3 $\mu\text{g/mL}$) was comparable to that of ascorbic acid standard (3.2 $\mu\text{g/mL}$). FRAP absorbance value and concentration of extracts showed linear relationship. Therefore, increasing OD values indicate increasing trend in reducing power.

The availability of phenolic compounds such as total phenols, tannins, and flavonoids in high range consists of medicinal values including anticancer [12,13], antioxidant [14,15], antimicrobial [15,16], wound-healing [17], and anti-inflammatory [18], which in turn helps for improvement of novel drugs from our present investigation.

Phenolic compounds consist of redox properties which induce them to act as antioxidants; their hydroxyl groups facilitate their free radical scavenging ability, and the total phenolic concentration could be used as base for rapid screening of antioxidant activity [19].

Superoxide is a reactive oxygen species, damage cells, and DNA, leading to various diseases in human [20]. Superoxide scavenging activity, determined by the nitroblue tetrazolium assay, was 62.16% for 500 $\mu\text{g/mL}$ of the root extract and 89.36% for ascorbic acid. In methanolic root extract of *E. acaulis* a high free radical scavenging activity of 79.38%, 82.92%, and 83.5% in relation to concentrations of 200, 300, and 400 $\mu\text{g/mL}$, respectively [21].

Plants rich in flavonoids and phenolics are good source of natural antioxidant and antibacterial activities. Qualitative and quantitative analysis of major individual phenolics in these leaves explains the relationships between total antioxidant capacity and total phenolic contents in these plants [22]. The presence of phenolic content in plants strongly reveals the antioxidant activity [23].

CONCLUSION

The antioxidant properties of phenols, flavonoids, and tannin content of *E. acaulis* plant extracts showed maximum free radical scavenging

activities. This result is, in turn, posing new ways of natural sources of antioxidant and phytochemical compounds and explains the relationships between the total antioxidant and total phenolics of the plants.

REFERENCES

- Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and Extraction: A Review. *Int Pharm Sci* 2011;1(1):98-106.
- Handa SS, Khanuja SP, Longo G, Rakesh DD. Extraction technologies for medicinal and aromatic plants. *Int Cent Sci High Tech Trieste* 2008;2:21-5.
- Nikhil SB, Dambe PA, Ghongade DB, Goupale DC. Hydroalcoholic extraction of *Mangifera indica* (leaves) by soxhletion. *Int J Pharm Sci* 2010;2(1):30-2.
- Moskovitz J, Yim MB, Chock PB. Free radicals and disease. *Arch Biochem Biophys* 2002;397(2):354-9.
- Chessbrough M. *Medical Laboratory Manual for Tropical Countries*. Linacre House, Oxford: Jordan Hill; 2000. p. 260.
- Singleton V, Orthofer R, Lamuela-Raventos R. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In: Packer L, editor. *Oxidants and Antioxidants, Part A. Methods in Enzymology*. Vol. 299. San Diego: Academic Press; 1999. p. 152-78.
- Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal* 2002;10(3):178-82.
- Siddhuraju P, Manian S. The antioxidant activity and free radical scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) Seeds. *Food Chem* 2007;105(3):950-8.
- Saha K, Lajis NH, Israf DA, Hamzah AS, Khozirah S, Khamis S, et al. Evaluation of antioxidant and nitric oxide inhibitory activities of selected Malaysian medicinal plants. *J Ethnopharmacol* 2004;92(2-3):263-7.
- Nishikimi M, Appaji N, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem Biophys Res Commun* 1972;46(2):849-54.
- Oyaizu M. Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn J Nutr* 1986;44(6):307-15.
- Lin Y, Shi R, Wang X, Shen HM. Luteolin, a flavonoid with potential for cancer prevention and therapy. *Curr Cancer Drug Targets* 2008;8(7):634-46.
- Khacha-ananda S, Tragoolpua K, Chantawannakul P, Tragoolpua Y. Antioxidant and anti-cancer cell proliferation activity of propolis extracts from two extraction methods. *Asian Pac J Cancer Prev* 2013;14(11):6991-5.
- Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr J Biotechnol* 2006;5(11):1142-5.
- Sengul M, Yildiz H, Gungor N, Cetin B, Eser Z, Ercisli S. Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. *Pak J Pharm Sci* 2009;22(1):102-6.
- Hendra R, Ahmad S, Sukari A, Shukor MY, Oskoueian E. Flavonoid analyses and antimicrobial activity of various parts of *Phaleria macrocarpa* (Scheff.) Boerl fruit. *Int J Mol Sci* 2011;12(6):3422-31.
- Nayak BS, Pinto Pereira LM. *Catharanthus roseus* flower extract has wound-healing activity in Sprague Dawley rats. *BMC Complement Altern Med* 2006;6:41.
- Rathee P, Chaudhary H, Rathee S, Rathee D, Kumar V, Kohli K. Mechanism of action of flavonoids as anti-inflammatory agents: A review. *Inflamm Allergy Drug Targets* 2009;8(3):229-35.
- Soobrattee MA, Neerghen VS, Luximon-Ramma A, Aruoma OI,

- Bahorun OT. Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. *Mutat Res Fundam Mol* 2005;579(1-2):200-13.
20. Shukla S, Mehta A, Bajpai VK, Shukla S. *In vitro* antioxidant activity and total phenolic content of ethanolic leaf extract of *Stevia rebaudiana* Bert. *Food Chem Toxicol* 2009;47(9):2338-43.
 21. Jagadeesan K, George T, Kamalanathan MN, Ilayaraja T. Studies on the anthelmintic and antioxidant activity of root extracts of *E. acaulis*. *Adv Anim Vet Sci* 2014;2(8):457-63.
 22. Saiah H, Allem R, El Kebir FZ. Antioxidant and antibacterial activities of six Algerian medicinal plants. *Int J Pharm Pharm Sci* 2015;8(1):367-74.
 23. Chandrika M, Chellaram C. Efficacy of antioxidation and anti-inflammation of the leaf extracts of *Borreria hispida*. *Int J Pharm Pharm Sci* 2016;8(8):369-72.