

RESEARCH ARTICLE

IN VITRO ANTIOXIDANT ACTIVITY APPLYING AN ABTS^{•+} RADICAL SCAVENGING ABILITIES OF DIFFERENT EXTRACTS OF *EUPHORBIA ROUTHIANA* SPRENG. PLANT PARTS (EUPHORBIACEAE)

Anusuya Devi, R., B. Veena and K. Thenmozhi*

PG and Research Department of Botany, Kongunadu Arts and Science College,
Coimbatore - 641 029.**ABSTRACT**

The aim of this work was to determine antioxidant activity in various parts of different extracts of *Euphorbia routhiana* Spreng. In Indian system of medicine the plant is used as an antifungal. Antioxidant activities were determined by ABTS^{•+} assay in leaf, stem and root extract of *Euphorbia routhiana*. ABTS^{•+} method, all the sample extracts of were able to quench ABTS^{•+} radical more efficiently with their TEAC values ranging between 187.2 to 6089.2 μmol Trolox equivalent/g extract. In this context, ethanol of *E. routhiana* root were found to be fast and potent scavengers of ABTS^{•+} radicals, as they were able to quench ABTS^{•+} radicals more readily than the other solvent extracts. Therefore the present study reveals that the study species has a reliable source of bioactive compounds which were highly correlated to their therapeutic properties and thus confirm the traditional medicinal usage of the plant practiced by the traditional healers.

Keywords: *Euphorbia routhiana*, *in vitro* antioxidant, ABTS^{•+} radicals scavenging abilities.

1. INTRODUCTION

Natural antioxidants have great interest among scientist because of their anticarcinogenic and health promoting properties (1). Plants are good source of phytochemicals such as vitamin E, vitamin C, carotenoid, flavonoids, glutathione, ascorbic acid etc. which having antioxidant properties. Natural antioxidants have great interest among scientist because of their anticarcinogenic and health promoting properties (2). Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases (3, 4). ABTS is also frequently used by the food industry and agricultural researchers to measure the antioxidant capacities of foods (5).

Euphorbia routhiana (family Euphorbiaceae) is an annual erect, glabrous, profusely branched sub shrub of one-meter height and distributed in India (Maharashtra and Tamil Nadu). In Indian system of medicine the plant is used as an antifungal (6), hypotensive agent and anthelmintic. Acne vulgaris is a disease which affects more than 90% of young people, which leads to permanent marking on the skin, disfiguring of the face. In view of the above, we designed the study to evaluate the ABTS^{•+} antioxidant potential content in *Euphorbia routhiana* different solvent extracts of various parts.

2. MATERIALS AND METHODS**2.1. Description of the selected plant**

Euphorbia laeta Heyne ex Roth. syn. *Euphorbia routhiana* Spreng. Belonging to the family

Euphorbiaceae, comprises of 300 genera with more than 6500 species. It is an annual or perennial erect herb with copiously branched stem, Leaves alternate, linear-lanceolate or oblanceolate. In India they are wide spread in an open sunny grasslands and evergreen forests at an attitude of 1000-2500m. They are commonly distributed in the regions like Madhya Pradesh, Gujarat, Maharashtra, Karnataka, Kerala and Tamil Nadu.

2.2. Collection of Plant material

Fresh leaves, stem and root parts of *Euphorbia routhiana* were collected from Dhottabeta hills, Niligri District. The authenticity of the selected plant material was confirmed by comparing with the reference specimen preserved at Botanical Survey of India, Southern Circle, Coimbatore. The leaf, stem and root parts were dried in shade at room temperature, chopped and ground to a fine powder in a mechanical blender. For extraction (50 g) of coarsely powdered plant samples were subjected to successive solvent extraction with petroleum ether, chloroform, ethyl acetate and ethanol using Soxhlet apparatus. The extracts were concentrated to dryness under reduced pressure using rotary vacuum evaporator, lyophilized and stored at -20°C for further phytochemical and *in vitro* antioxidant studies.

2.3. Preparation of crude plant extracts

50 g of coarsely powdered plant samples were subjected to successive solvent extraction with petroleum ether, chloroform, ethyl acetate and ethanol. The extracts were concentrated to dryness

*Correspondence: Dr. K. Thenmozhi, Assistant Professor, Department of Botany, Kongunadu Arts and Science College, Coimbatore- 641029, Tamil Nadu, India. E-mail ID: thenmozhi_05@yahoo.co.in

under reduced pressure using rotary vacuum evaporator (Supervac R-185, India), lyophilized to remove traces of water molecules and the lyophilized powders were stored at -20°C for further studies.

2.4. Determination of *in vitro* antioxidant activity

2.4.1. ABTS^{•+} radical scavenging activity

Antioxidant activity was performed using an improved ABTS^{•+} method proposed by Siddhuraju and Manian (7). The ABTS radical cation (ABTS^{•+}) was generated by a reaction of 7 mmol/ L ABTS and 2.45 mmol/ L potassium persulfate after incubation for 16 h at laboratory temperature in dark. Blue – green ABTS^{•+} was formed at the end of this period. Prior to assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30°C to obtain an absorbance of 0.700 ± 0.02 at 734 nm, the wavelength of maximum absorbance in the visible region. The stock solution of the sample extracts in ethanol were diluted such that, after introduction of 10 µL aliquot of each dilution into the assay, they produced between 20- 80% inhibition of the blank absorbance. After the addition of 1.0 mL of diluted ABTS^{•+} solution to 10 µL of sample extracts or Trolox standards (final concentration 0-15 µM) in ethanol, absorbance was recorded at 30°C, exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicate determinations were made at each dilution of the standard, and the percentage inhibition of the blank absorbance at 734 nm was plotted as a function of Trolox concentration. The unit of total antioxidant activity (TAA) was defined as the concentration of Trolox having the equivalent antioxidant activity expressed as µmol/ g sample extracts on dry weight basis.

2.5. Statistical analysis

For *in vitro* antioxidant activity of the extracts, the result were recorded as mean ± standard deviation (SD) (n=3) and were subjected to one-way analysis of variance (ANOVA) followed by post-hoc Duncan's multiple range test using SPSS (Version 9, SPSS Inc Chicago, USA). P<0.05 was chosen as the criterion for statistical significance.

3. RESULTS

3.1. ABTS^{•+} radical scavenging activity

Trolox equivalent antioxidant capacity (TEAC) was measured using the improved ABTS^{•+} radical decolorization assay. The decolorization of ABTS^{•+} cation radical is an unambiguous way to measure the antioxidant capacity of test drugs or plant samples. Since, TEAC is a measurement of the effective antioxidant activity of the extract; a higher

TEAC value would imply greater antioxidant activity of the sample. This assay was calibrated with the water-soluble α-tocopherol analogue, Trolox. In the evaluation of total antioxidant capacity by ABTS^{•+} method, all the sample extracts of both the species were able to quench ABTS^{•+} radical more efficiently with their TEAC values ranging between 187.2 to 6089.2 µmol Trolox equivalent/ g extract. In this context, ethanol of *E. rothiana* root (Table 1) were found to be fast and potent scavengers of ABTS^{•+} radicals, as they were able to quench ABTS^{•+} radicals more readily than the other solvent extracts (Table 1).

Table.1. ABTS^{•+} radical scavenging activities of *E. rothiana* plant in different part extracts*

Parts	Extracts	ABTS ^{•+} scavenging activity
Leaf	Petroleum ether	1387.1±30.9 ⁱ
	Chloroform	4572.5±91.9 ^f
	Ethyl acetate	5088.4±56.4 ^d
	Ethanol	6082.5±46.4 ^b
Stem	Petroleum ether	187.2±61.9 ⁱ
	Chloroform	1443.7±65.9 ⁱ
	Ethyl acetate	3084.3±30.4 ^h
	Ethanol	5586.8±74.6 ^c
Root	Petroleum ether	288.4±76.1 ^k
	Chloroform	3262.6±77.3 ^g
	Ethyl acetate	4675.0±86.1 ^e
	Ethanol	6089.2±71.8 ^a

*Values are mean ± standard deviation (SD) of three independent experiments.

Values not sharing a common letter in a column are significantly (P<0.05).

Values are expressed as TEAC (Trolox equivalent antioxidant capacity/in µmol/g extract).

4. DISCUSSION

ABTS radical scavenging activity is relatively a recent one, which involves a more drastic radical chemically, produced and is often used for screening complex antioxidant mixture such as plant extract, beverage and biological fluids (8). In the presented investigation, the ethanolic extract of *E. rothiana* root (6089 µmol Trolox equivalent/gm extract) present appreciable antioxidant activity (Table 1). Therefore, the high radical scavenge activities of the extract reported in the study might be due to its ability to scavenge free radical, thereby preventing lipid oxidation via a chain breaking reaction (9).

5. CONCLUSION

Plants with medicinal properties have been known for thousands of years and have been used as traditional medicine for the people to treat diseases. The antioxidant potential of the extracts were assessed by employing similarly in ABTS^{•+}, radical scavenging assay, the ethanol extract of stem were

found to be fast and potent scavenger of ABTS^{•+}. In reducing power assay, through all the extract exhibited significant activity, ethyl acetate of *Erothiana* stem exerted stronger reductive abilities. Their therapeutic properties and thus confirm the traditional medicinal usage of the plant practiced by the traditional healers of southern district of Tamil Nadu, India.

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