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Determination of *In-Vitro* Antioxidant and Radical Scavenging Activity of Root Extracts of *Barleria courtrallica* Nees (Acanthaceae)

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[1]A.Ponmathi Sujatha [2]A.Doss,
[3]S.Muthukumarasamy, *[2]V.R.Mohan

[1]Research Scholar (Reg. No: 12319), PG & Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamilnadu

[2]Ethnopharmacology Unit, PG & Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamilnadu

[3]Department of Botany, Sri K.G.S. Arts College, Srivaikundam, Tamilnadu

Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli, Tamilnadu, India

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ABSTRACT

The present study aimed at identifying the antioxidant capacity of the medicinal plant *Barleria courtrallica* Nees (Acanthaceae). Sequencing extracts of the plant were prepared in Petroleum ether, Hexane, Ethyl Acetate, Methanol and Ethanol respectively. These were then tested for their free radical scavenging activity (DPPH), hydroxyl radical scavenging activity, Superoxide radical scavenging activity, ABTS radical scavenging activity and reducing power assays. The crude extracts of *B. courtrallica* were found to have strong antioxidant properties. The results indicated that the methanol extract is the most potent antioxidant. This holds great promise for the use of *B. courtrallica* as a source of strong antioxidant compounds.

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INTRODUCTION

Oxidative damages caused by free radicals to living cells intervene the pathogenesis of huge number of chronic diseases, like atherosclerosis, Parkinson's disease, Alzheimer's disease, stroke, arthritis, chronic inflammatory diseases, cancers and other degenerative diseases. The free radicals generated in the body are neutralized by the body's natural antioxidant defenses, e.g. glutathione, glutathione peroxidase, catalase, and superoxide dismutase. Antioxidants are believed to play a very significant role in the body defense system against reactive oxygen species (ROS), which are dangerous by products generated during normal cell aerobic respiration. The importance of antioxidants in health and disease is now recognized by every branch of medicine and biological sciences. They can be either synthetic or naturally occurring. Synthetic antioxidants have been shown to possess carcinogenic activity, which leads to a need for the replacement of synthetic antioxidant with naturally occurring ones [1]. Natural antioxidants are shown to be safe and also possess anti-viral, anti-inflammatory, anti-cancer, antimutagenic, anti-tumour, and hepatoprotective properties. Natural oxidants namely phenols in medicinal and dietary plants can prevent the oxidative damage caused by free radicals. Plants produce a variety of antioxidants compounds to control the oxidative stress caused by sunbeam and oxygen, so they represent a source of compounds with antioxidant activity [2].

Genus *Barleria* belongs to the family Acanthaceae. The whole plant extract of Barleria contains a number of active compounds like alkaloids, terpenes, glycosides, flavonoids, lignins, phenolics *etc.* These compounds have exposed potent therapeutic activities against several diseases [3,4,5,6]. *Barleria* also shows various pharmacological effects such as antimicrobial, anthelminthic, antifertility, antioxidant, antidiabetic, antiarthritic, hepatoprotective, diuretic, cytoprotective, antidiarrhoeal, analgesic, antileukemic, anti-inflammatory and hypoglycemic properties without any toxic effects [7,8]. The current study, the crude extracts of *B. courtrallica* were investigated for their antioxidant properties of superoxide radical scavenging activity, ABTS radical scavenging activity, DPPH scavenging activity, Hydroxyl radical & reducing power assays.

MATERIALS AND METHODS

Collection of plants

The fresh plant parts of *Barleria courtrallica* (root) were collected from Agasthiar malai biosphere reserved, Western Ghats, Tirunelveli District, Tamil Nadu, India. The gathered samples were cut into small pieces and shade dried until the fracture is identical and even. The dried plant material was crushed or grinded by using a blender and separated to get uniform particles by using sieve No. 60. The final uniform powder was used for the extraction of active constituents of the plant material.

Preparation of extract

100 g of the coarse powder of *B. courtrallica* root was extracted successively with 250 ml of alcoholic and organic solvents (Petroleum ether, Benzene, Ethyl acetate, Methanol and Ethanol) in a Soxhlet apparatus for 24 hrs. All the extracts were filtered through Whatman No. 41 filter paper separately and all the extracts were concentrated in a rotary evaporator. All the concentrated extracts were subjected for *in vitro* antioxidant activity.

ANTIOXIDANT ACTIVITY

DPPH radical scavenging activity

The DPPH is a constant free radical and is extensively used to measure the radical scavenging activity of antioxidant component. This process is based on the reduction of DPPH in methanol solution in the company of a hydrogen donating antioxidant due to the arrangement of the non-radical form DPPH-H [9]. Using 1, 1- diphenyl-2-picryl-hydrazyl (DPPH) the free radical scavenging action of all the extracts was assessed as per the previously reported process [9]. 0.1 mM solution of DPPH in methanol was prepared. 1 ml of this solution was poured into 3 ml of the solution at different concentrations (50, 100, 200, 400 and 800 µg/ml). The mixtures were shaken dynamically and allowed to stand at room temperature for 30 minutes. After that, the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10s UV: Thermo electron corporation). Ascorbic acid was employed as the reference. The lesser absorbance values of reaction mixture specify higher free radical scavenging action. Using the following formula the ability to scavenge the DPPH radical was calculated.

DPPH scavenging effect (% inhibition) = (A0 - A1) / A0 X 100

Where, A0 is the absorbance of the control and A1 is the absorbance of the test samples and reference. All the tests were carried out in triplicates and the outcomes were averaged.

Hydroxyl radical scavenging activity

According to the modified method of Halliwell *et al.* [10], the scavenging ability for hydroxyl radical was projected. Stock answers of $FeCl_3$ (10 mM), Ascorbic Acid (1 mM), EDTA (1 mM), H2O2 (10 mM) and Deoxyribose (10 mM) were put in distilled deionized water. The assay was performed by adding 0.1 ml EDTA, 0.01 ml of FeCl3, 0.1 ml H_2O_2, 0.36 ml of deoxyribose, 1.0 ml of the extract of different concentration (50, 100, 200, 400 & 800 µg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.9), 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37°C for 1 hour. 1.0 ml of the incubated mixture was mixed with 1.0 ml of 10% TCA and 1.0 ml of 0.5% TBA (in 0.025 M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging action of the extract is reported as % inhibition of deoxyribose. The degradation is computed by using the subsequent equation

Hydroxyl radical scavenging activity = (A0 - A1) / A0 X 100

where, A0 is the absorbance of the control and A1 is the absorbance of the test samples and reference. All the tests were carried out in triplicates and the results were averaged.

Superoxide radical scavenging activity

The superoxide anion scavenging action was calculated as elucidated by Srinivasan *et al.* [11]. The superoxide anion radicals were made in 3.0 ml of Tris - HCl buffer (16 mM, pH 8.0) containing 0.5 ml of NBT (0.3 mM), 0.5 ml NADH (0.936 mM) solution, 1.0 ml extract of different concentrations (50, 100, 200, 400 & 800 µg/ml) and 0.5 ml Tris - HCl buffer (16 mM, pH 8.0). The reaction was started by adding 0.5 ml PMS solution (0.12 mM) to the mixture, incubated at 25^{o}C for 5 min and the absorbance was estimated at 560nm against a blank sample, ascorbic acid. The percentage inhibition was determined by using the following equation

Superoxide radical scavenging activity = (A0 - A1) / A0 X 100

where, A0 is the absorbance of the control and A1 is the absorbance of the test samples and reference. All the tests were performed in triplicates and the results were averaged.

Antioxidant Activity by Radical Cation (ABTS+)

ABTS assay was based on the slightly modified method of Huang *et al.* [12]. By reacting 7 mM ABTS solution with 2.45 mM potassium persulphate, ABTS radical cation (ABTS+) was prepared. This mixture is permitted to be in the dark at room temperature for 12-16 hrs previous to use. With ethanol to an absorbance of 0.70 + 0.02 at 734 nm, the ABTS+ solution was added. Following this trolox standard to 3.9 ml of diluted ABTS+ solution or addition of 100 µL of sample, absorbance was calculated at 734 nm by Genesys 10S UV-VIS (Thermo scientific) accurately after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC).

ABTS radical cation activity = (A0 - A1) / A0 X 100

where, A0 is the absorbance of the control and A1 is the absorbance of the test samples and reference. All the tests were repeated thrice and the end results were averaged.

Reducing Power

The reducing power of the extract was established by the method of Kumar and Hemalatha [13]. 1.0 ml of solution containing 50, 100, 200, 400 & 800 µg/ml of extract was mixed up with sodium phosphate buffer (5.0 ml, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 ml, 1.0%). The mixture was incubated at 50^{o} C for 20 minutes. Then 5ml of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5^{o}C) in a refrigerated centrifuge. The upper layer of the solution (5.0 ml) was diluted with 5.0 ml of distilled water and ferric chloride and absorbance read at 700 nm. The experiment was performed thrice and results were averaged.

RESULTS AND DISCUSSION

The antioxidant properties of *B. courtrallica* have been evaluated by measuring their DPPH, ABTS, Superoxide radical, Hydroxyl radical, reducing ability and ascorbic acid contents using crude extracts aerial parts of this plant. Antioxidant potential of the crude extracts was measured by DPPH radical scavenging activity. The results are expressed as % inhibition of DPPH and reported in Figure 1. Ethanol extract showed high antioxidant activity than that of

other extracts. The DPPH radical scavenging activity was found to be increasing as dose increases. The consequence of antioxidants on DPPH is considered to be due to their hydrogen donating activity [14]. As DPPH is considered as the lipophilic radical, it readily accept electron from the antioxidant compound and converts its colour from violet to yellow which is detected at 517nm. DPPH scavenging activity is influenced by the polarity of the reaction medium, chemical structure of the radical scavenger and the pH of the reaction mixture, sample concentration and reaction time. In this study, free radical scavenging activities of *B. courtrallica* roots ethanol extract and standard ascorbic acid were determined by using DPPH method. The concentration of *B. courtrallica* root ethanol extract needed for 50 % inhibition (IC_{50}) was 21.96 mg/ml, while ascorbic acid needed 21.59 mg/ml (Table 1). The result obtained in the study indicates that the extract exhibited good radical scavenging activity but was to a lesser extent compared to standard ascorbic acid.

Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous biological damage [15]. They were formed in this study by incubating ferric-EDTA with ascorbic acid and H_2O_2 at pH 7.4 and responded with 2-deoxy-2-ribose to generate a malondialdehyde (MDA)-like product. This compound forms a pink chromogen upon heating with TBA at low pH [10]. When *B. courtrallica* extract was added to the reaction mixture, it removed the hydroxyl radicals from the sugar and prevented the reaction (Figure 2). The concentration of *B. courtrallica* root methanol extract needed for 50 % inhibition (IC_{50}) was found to be 27.94 mg/ml, whereas 28.23 mg/ml (Table 1) needed for ascorbic acid. The result obtained in the study indicates that the methanol extract showed significant OH radical scavenging activity compared to standard Ascorbic acid (Figure 2).

Superoxide radical scavenging capacities of the crude extracts tested varied from 20.86 to 124.16 % which represents a variation of standard ascorbic acid. Methanol extract showed the highest antioxidant capacity (124.16%) followed by methanol (119.31%) and ethyl acetate (103%). In this assay, benzene (87.36 %) showed the lowest antioxidant potential (Figure 3). The IC_{50} value of methanol extract of *B. courtrallica* root on superoxide radical was found to be 38.13 mg/ml and 29.65 mg/ml for ascorbic acid, respectively (Table 1). Superoxide anion is one of the most representative free radicals. In cellular oxidation reactions, superoxide radicals have their initial effects magnified because they produce other kinds of cell-damaging free radicals and oxidizing agents, e.g., hydroxyl radicals. The

superoxide anion scavenging activities of the crude extracts were investigated and compared standard Ascorbic acid.

The different solvent extracts of *B. courtrallica* root were subjected to be ABTS radical cation scavenging activity and the results are shown in figure 4. The methanol extract of *B. courtrallica* root exhibited potent ABTS radical cation scavenging activity in concentration dependent manner. At 800µg/ml concentration, the methanol extract of *B. courtrallica* root possessed 129.16%**.** The quantity of *B. courtrallica* methanol extract required to produce 50% inhibition of ABTS radical 29.12 mg/ml whereas 23.29 mg/ml (Table 1) needed for trolox. ABTS radical cation scavenging activity respectively. ABTS radical cation scavenging assay engages a method that produces a blue/green ABTS chromophore via the reaction of ABTS and potassium persulfate. The ABTS radical cation is generated by the oxidation of ABTS with potassium persulfate. Its reduction in the company of hydrogen donating antioxidants is created spectrophotometrically at 745 nm.

The ferric reducing assay quantifies the ability of an antioxidant capacity to reduce a reactive oxygen species against that species oxidative power [16]. This is important to make the reactive oxygen species more stable and non-reactive. The results are showed in figure 5. As seen from the graph, the ethanol extract exhibited a reducing power almost equal to that of Ascorbic Acid. The ethanol extract showed a very good reducing power at lower concentrations. However, at higher concentrations, its reducing power was lesser than that of the ethanol extract.

CONCLUSION

Reactive oxygen species (ROS) generated by oxidative stress in large quantities is one of the most important reasons for cancer, ageing and for various disorders. To certain extent, to overcome the damage, every living being will have its own antioxidant protective mechanism. When the stress is more, it may require supplementing the system with antioxidants as a concentrate. Various forms of antioxidants are in use for therapy and still, there is a search to find few more sources for antioxidants. Based on the above experiments, we can conclude that methanol extracts of *B. courtrallica* are the most effective as antioxidants. These could find potential application in today's urban lifestyle which increases our exposure to various harmful oxidants. These findings indicate that in addition to its anti-diabetic effect, *B. courtrallica* has a potent antioxidant activity.

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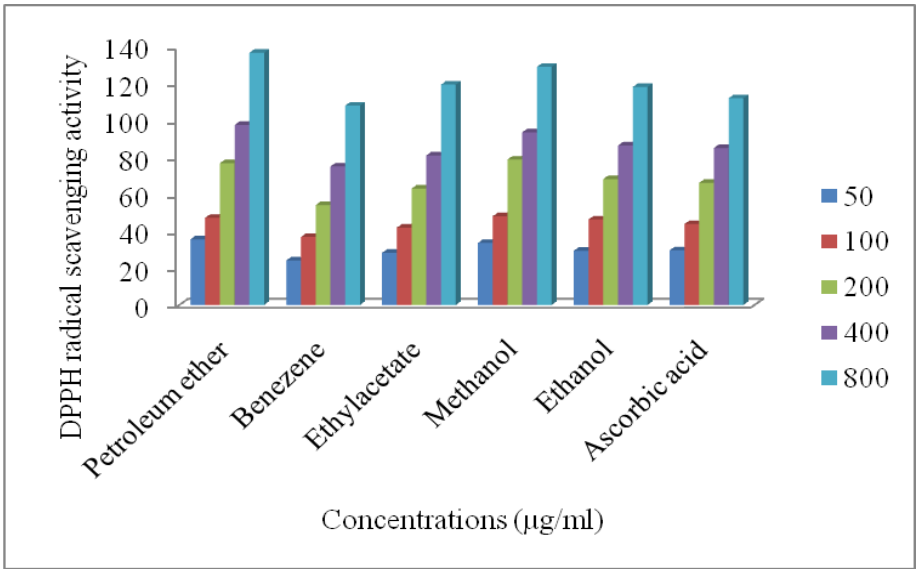

Figure 1: Effect of different solvent extract of *B. courtrallica* root on DPPH assay

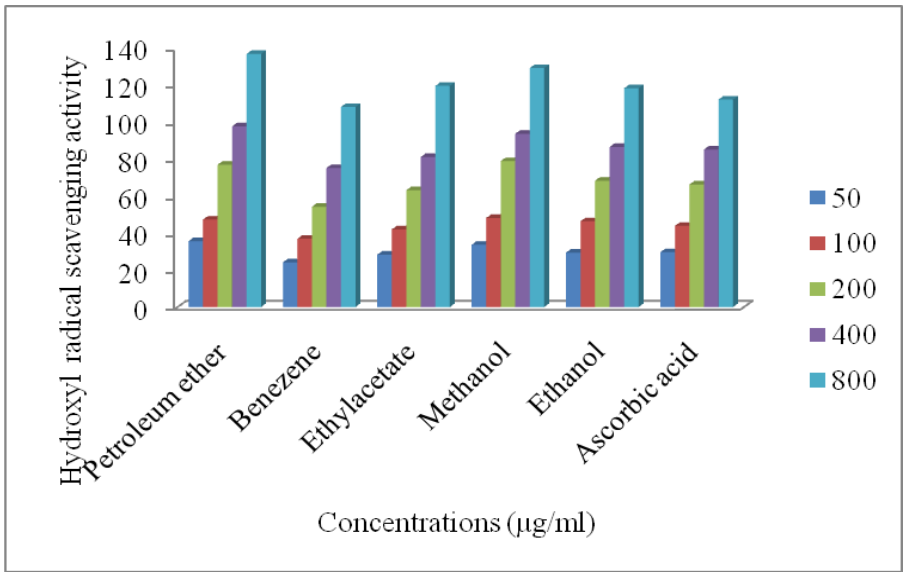

Figure 2: Effect of different solvent extract of *B. courtrallica* root on Hydroxyl assay

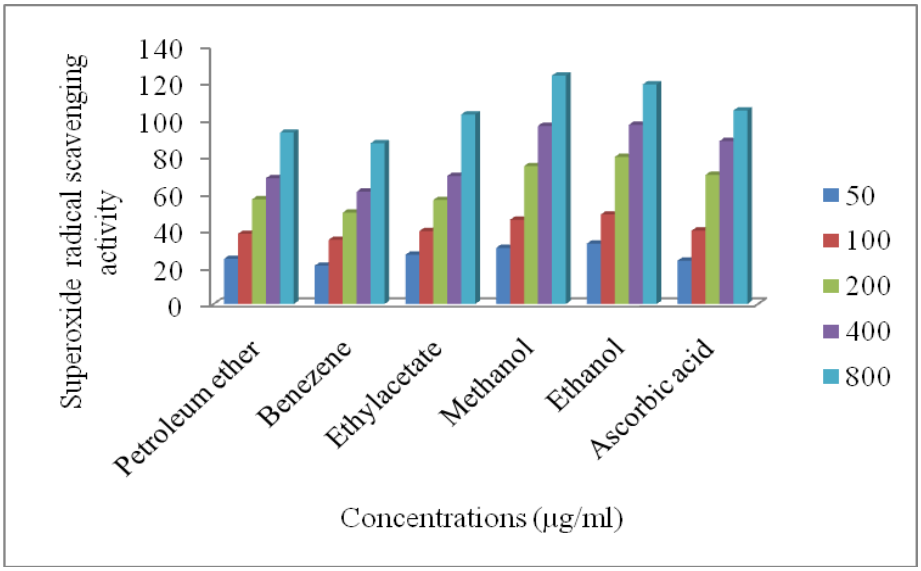

Figure 3: Effect of different solvent extract of *B. courtrallica* root on Superoxide anion assay

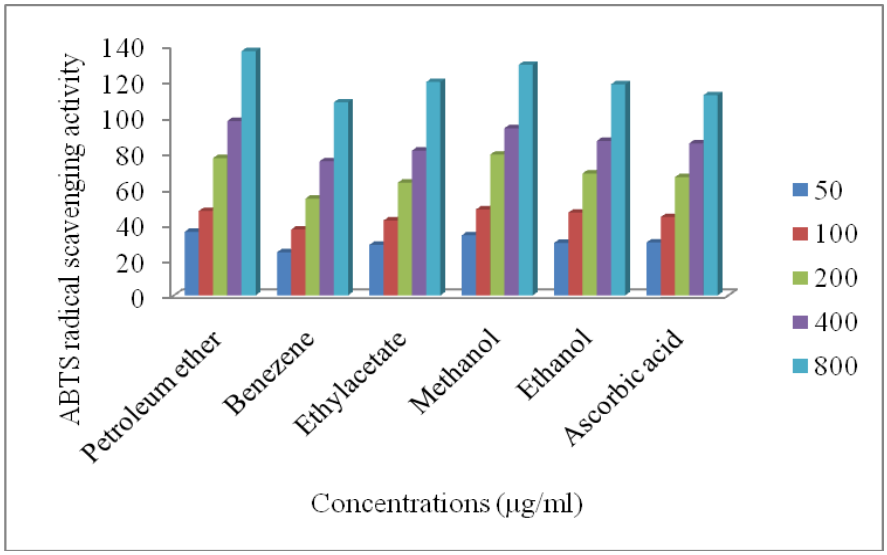

Figure 4: Effect of different solvent extract of *B. courtrallica* root on ABTS assay

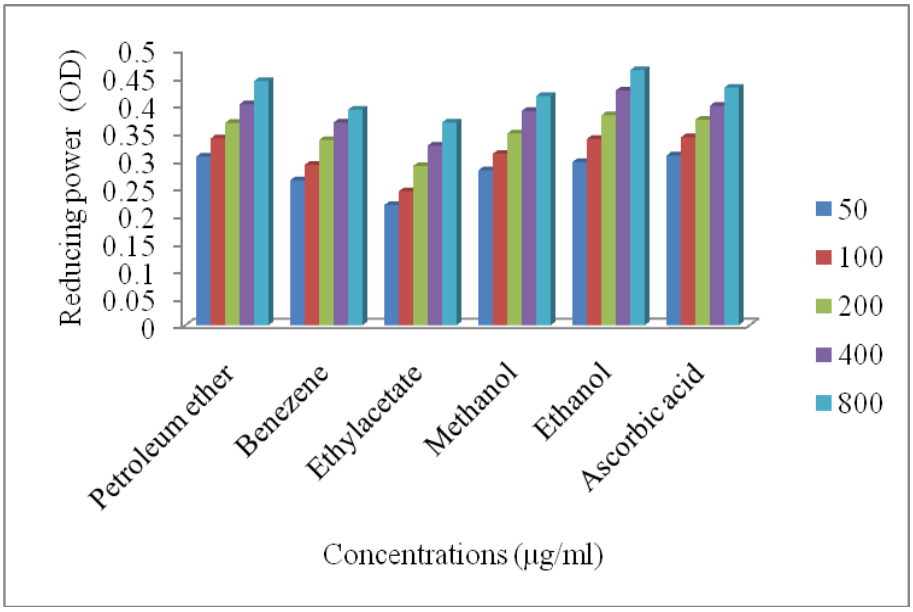

Figure 5: Effect of different solvent extract of *B. courtrallica* root on reducing power assay

Table 1: IC_{50} values of different solvent extracts of the root extracts of *B. courtrallica*

Solvents	IC_{50} (µg/ml)			
	DPPH	Hydroxyl	ABTS	Superoxide anion
P. ether	20.88	29.31	32.86	25.23
Benzene	21.98	28.11	22.18	23.65
Ethyl acetate	20.04	26.78	28.64	28.23
Methanol	22.47	27.84	29.12	38.13
Ethanol	21.96	27.47	26.88	34.86
Ascorbic acid	21.59	28.23	-	29.65
Trolox	-	-	23.29	-