

**Original Research Article**

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STUDY OF ANTIOXIDANT ACTIVITY OF *BARLERIA COURTRALLICA*A. Ponmathi Sujatha¹, A. Doss², S. Muthukumarasamy³, V. R. Mohan^{2*}

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ABSTRACT: The present investigation is to evaluate the antioxidant activity of various extracts of leaf of *Barleria courtrallica* using different vitro methods. The antioxidant activity was studied by DPPH radical scavenging method, hydroxyl radical scavenging activity, superoxide radical scavenging activity, ABTS radical scavenging activity and reducing power methods. Ethanol extract of leaf showed very good antioxidant properties. The present study exposed that ethanol extract of *B.courtrallica* include effective potential source of natural antioxidant, which might be useful in preventing the progress of various oxidative stresses.

KEYWORDS: Traditional medicine, Free radicals, Antioxidant activity, DPPH, ABTS

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1.INTRODUCTION

Antioxidants are essential nutrients which safe haven the human body against free radical injure that cause several diseases such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degeneration, aging course and possibly dementias. Oxygen radicals are major inducer of oxidative stress that is believed to be a primary factor in various diseases as well as normal process of ageing. The medicinal plants and their derivatives have long been recognized as important sources of antioxidants in the prevention and treatment of various diseases. Several studies have shown the antioxidant properties of medicinal plants, foods and beverages which are

rich in phenolic compounds [1]. Number of herbs and spices has been reported to show antioxidant activity, which includes *Solanum trilobatum* [2], *Prosopis cineraria* [3], *Hygrophilia auriculata*, *Pergularia daemia* [4], *Alangium salvifolium* [5], *Daphniphyllum neilgherrense* [6], *Crateva magna* [7] and numerous Indian and Chinese plant. The significance of antioxidants in human health has become gradually clearer due to spectacular advances in accepting the mechanisms of their reaction oxidants [8]. Genus *Barleria* comes under 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 [9,10,11,12]. *Barleria* also shows various pharmacological effects such as antimicrobial, anthelmintic, antifertility, antioxidant, antidiabetic, antiarthritic, hepatoprotective, diuretic, cytoprotective, antidiarrhoeal, analgesic, antileukemic, anti-inflammatory and hypoglycemic properties without any toxic effects [13, 14]. In the current study, the crude extracts of *B. courtrallica* were investigated for their antioxidant properties of super oxide radical scavenging activity, ABTS radical scavenging activity, DPPH scavenging activity, Hydroxyl radical and reducing power assays.

2. MATERIALS AND METHODS

The fresh plant parts of *Barleria courtrallica* (leaf) 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* leaf 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 [15]. 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 [15]. DPPH of 0.1 mM solution 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 identify higher free radical scavenging action. Using the subsequent formula the ability to scavenge the DPPH radical was computed.

$$\text{DPPH scavenging effect (\% inhibition)} = (A_0 - A_1) / A_0 \times 100$$

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.* [16] the scavenging ability for hydroxyl radical was projected. Stock answers of FeCl₃ (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 executed by adding 0.1 ml EDTA, 0.1 ml H₂O₂, 0.01 ml of FeCl₃, 0.36 ml of deoxyribose, 1.0 ml of the extract of diverse 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 achievement of the extract is accounted as % inhibition of deoxyribose. The degradation is figured by using the succeeding equation

$$\text{Hydroxyl radical scavenging activity} = (A_0 - A_1) / A_0 \times 100$$

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.* [17]. 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°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.

$$\text{Superoxide radical scavenging activity} = (A_0 - A_1) / A_0 \times 100$$

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

Antioxidant Activity by Radical Cation (ABTS+)

ABTS assay was supported on the slightly modified technique of Huang *et al.* [18]. 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).

$$\text{ABTS radical cation activity} = (A_0 - A_1) / A_0 \times 100$$

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 [19]. 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° C for 20 minutes. Then 5ml of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5°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.

3. 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. The free radical scavenging action of plant extract was studied by its capacity to reduce the DPPH, a stable free radical and any molecule that can contribute an electron or hydrogen to DPPH. It can respond with it and thus bleach the DPPH absorption [20]. 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. When the plant extracts were tested for the DPPH free radical scavenging ability, the ethanol extract showed strong radical scavenging activity with percentage increase of 141.34%. The concentration of *B. courtrallica* leaf ethanol extract needed for 50 % inhibition (IC₅₀) was 42.31 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 were produced from the substrate

deoxyribose by the reaction of ferric EDTA in concert with H₂O₂ and ascorbic acid. It affects macromolecules like proteins, DNA, fatty acid in membranes and most biological molecules also [21] and is known to be capable of abstracting hydrogen atoms from membrane lipids [22] and brings about peroxidic reaction of lipids. When the leaf extract was incubated with the above reaction mixture, it can prevent the damage against sugar. 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* leaf ethanol extract needed for 50 % inhibition (IC₅₀) was found to be 32.84 mg/ml, whereas 28.23 mg/ml (Table 1) needed for ascorbic acid. The result obtained in the study indicates that the ethanol extract showed significant OH radical scavenging activity compared to standard Ascorbic acid (Figure 2).

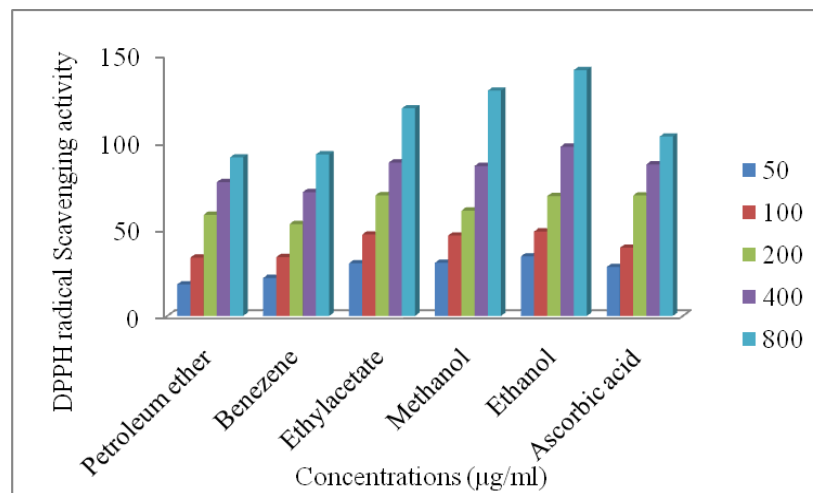


Figure 1: Effect of different solvent extract of *B. courtrallica* leaf on DPPH assay

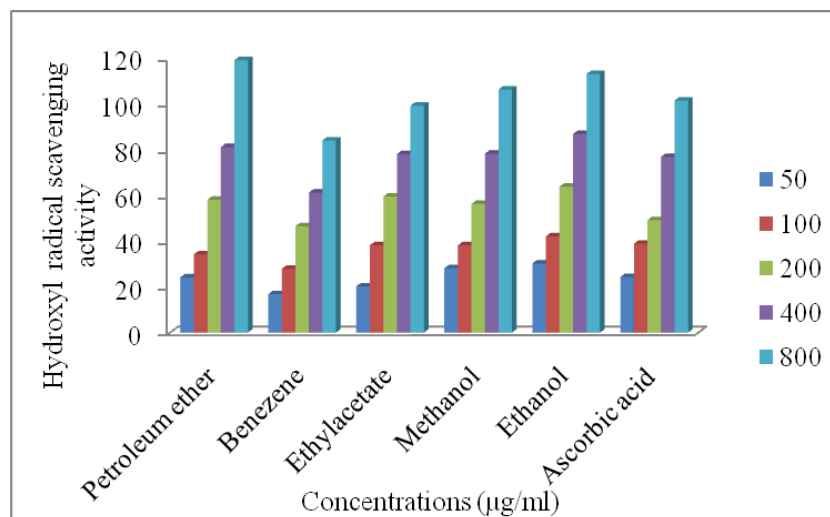


Figure 2: Effect of different solvent extract of *B. courtrallica* leaf on Hydroxyl assay

Superoxide radical scavenging capacities of the crude extracts tested varied from 19.54 to 136.92 % which represents a variation of standard ascorbic acid. Ethanol extract showed the highest antioxidant capacity (136.92%) followed by methanol (128.36%) and ethyl acetate (113.15%). In this assay, petroleum ether (91.36 %) showed the lowest antioxidant potential (Figure 3). The

IC₅₀ value of ethanol extract of *B. courtrallica* leaf on superoxide radical was found to be 34.56 mg/ml and 29.65 mg/ml for ascorbic acid, respectively (Table 1). By dissolving oxygen by phenazine methosulphate/NADH coupling effect diminishes nitro blue tetrazolium and superoxide anion is obtained. The decrease in the absorbance at 560nm with the fruit extract thus indicates the consumption of superoxide anion in the reaction mixture. The superoxide anion scavenging activities of the crude extracts were investigated and compared standard Ascorbic acid.

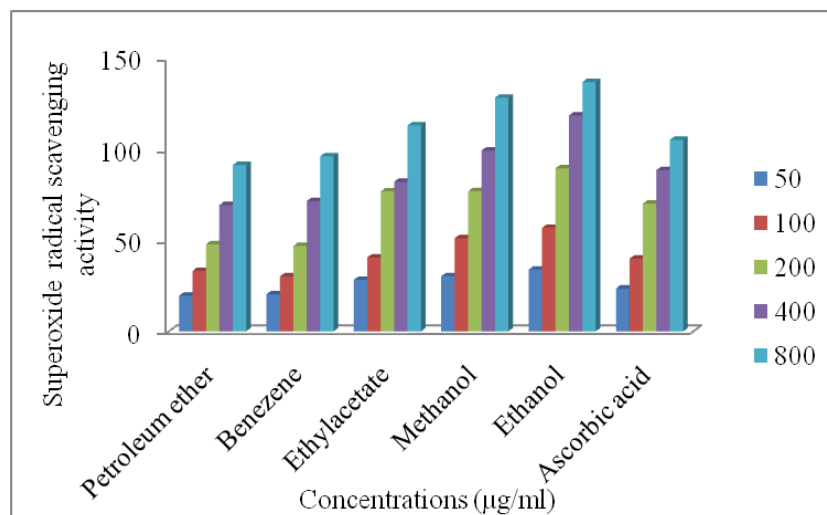


Figure 3: Effect of different solvent extract of *B. courtrallica* leaf on Superoxide anion assay

The different solvent extracts of *B. courtrallica* leaf were subjected to be ABTS radical cation scavenging activity and the results are shown in figure 4. The methanol extract of *B. courtrallica* leaf exhibited potent ABTS radical cation scavenging activity in concentration dependent manner. At 800µg/ml concentration, the methanol extract of *B. courtrallica* leaf possessed 129.16%. The quantity of *B. courtrallica* methanol extract required to produce 50% inhibition of ABTS radical 29.78 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. ABTS assay is an exceptional tool for forming the antioxidant activity of hydrogen-donating antioxidants and of chain-breaking antioxidants [23]. For the measurement of the reducing power of the plant extracts, we investigated the transformation of Fe³⁺ to Fe²⁺ ion in the presence of the plant extracts, which served as an indicator of their antioxidant activity [24, 25]. 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.

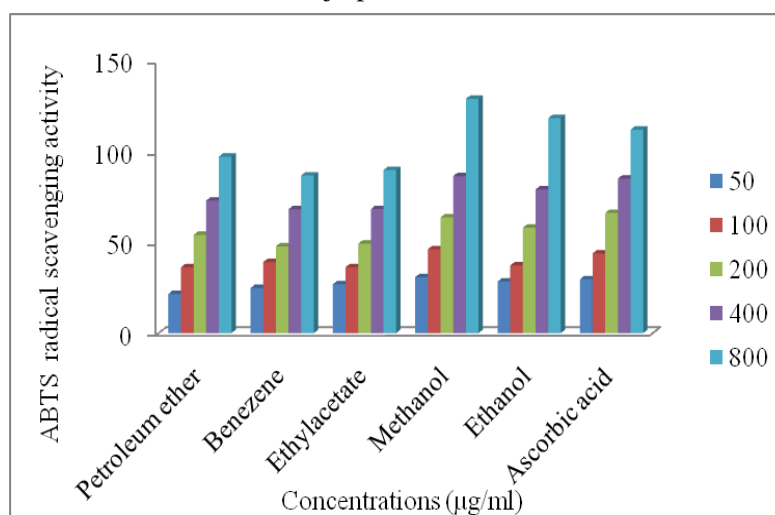


Figure 4: Effect of different solvent extract of *B. courtrallica* leaf on ABTS assay

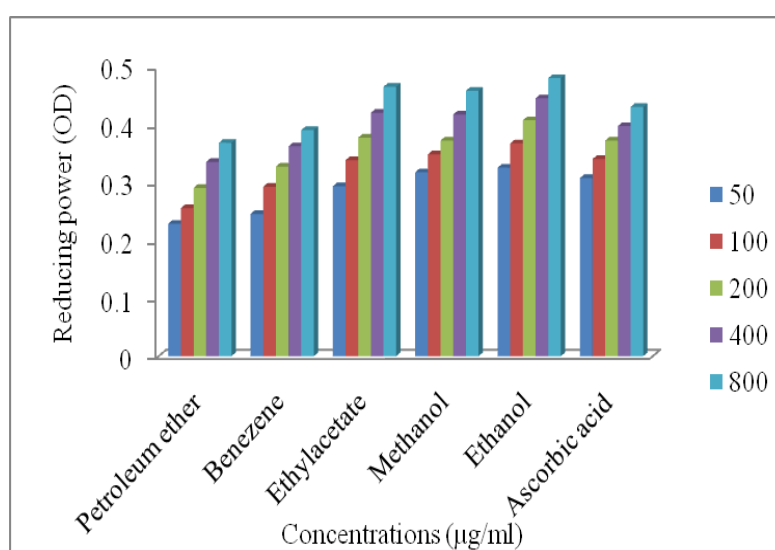


Figure 5: Effect of different solvent extract of *B. courtrallica* leaf on reducing power assay

Table 1: IC₅₀ values of different solvent extracts of the leaf extracts of *B. courtrallica*

Solvents	IC ₅₀ (µg/ml)			
	DPPH	Hydroxyl	ABTS	Superoxide anion
P.ether	20.16	33.81	20.23	25.88
Benzene	20.89	26.16	18.16	27.46
Ethyl acetate	29.13	27.84	19.47	34.63
Methanol	39.16	31.13	29.78	32.92
Ethanol	42.31	32.84	24.89	34.56
Ascorbic acid	21.59	28.23	-	29.65
Trolox	-	-	23.29	-

4. CONCLUSION

From the above results it can be inferred that *B. courtrallica* ethanol extract has got better free radical scavenging potential in comparison to other crude extracts. The present study supports the in vitro antioxidant potential of *B. courtrallica* but further studies of their activity in biological systems is necessary to confirm their usefulness as natural source of antioxidants.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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