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Research Article

IN VITRO EVALUATION OF ANTIOXIDANT POTENTIAL AND TOTAL PHENOLIC CONTENT OF *BARLERIA LONGIFLORA* LEAF EXTRACTS

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ABSTRACT

Objective: The aim of this present study is to estimate the antioxidant potential found in the leaves of *Barleria longiflora* that belongs to the family Acanthacea.

Methods: Antioxidant activity of six different solvent extracts system of *B. longiflora* leaves was assayed for 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical activity, scavenging of hydrogen peroxide (H_2O_2), ferric reducing antioxidant activity, metal chelating ability assay, and total reducing ability. Total phenolic content of the extracts was determined by Folin–Ciocalteu method.

Results: The radical scavenging activity was evaluated by DPPH of ethanol extract at concentration of 100 µg/mg was found to be 56.5% followed by methanol 48.4%, whereas maximum scavenging of H_2O_2 was observed in ethanol 83.4% followed by chloroform 70.8%. Ethanolic extract of *B. longiflora* leaves showed the highest value in ferric reducing antioxidant power assay 74.8%, metal chelating activity 61.6%, and total reducing ability 0.76±0.02 when compared to the standard ascorbic acid.

Conclusion: The results suggest that the antioxidant potential of the ethanol extract has the highest activity in compared to other five extracts of *B. longiflora* leaves.

Keywords: *Barleria longiflora*, Different solvents, Antioxidant activity, *In vitro* study.

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INTRODUCTION

All living organisms have complex systems of antioxidant enzymes. Reactive oxygen species (ROS) are generated in all living organisms during metabolism. It is produced in the form of superoxide anion, nitric oxide, hydroxyl radical, and hydrogen peroxide (H_2O_2). Excessive formation of ROS may be harmful because they can initiate bimolecular oxidants which leads to cell injury, death, and create oxidative stress which results in numerous diseases and disorders such as cancer, diabetic, stroke, myocardial infection, Alzheimer's, and Parkinson's diseases. The negative effects of oxidative stress may be decreased by antioxidants [1].

Nowadays, available synthetic antioxidants such as butylated hydroxyl anisole, butylated hydroxyl toluene, and tertiary butylated hydroquinone have been suspected to cause or rapid negative health effects [2,3]. Moreover, these synthetic antioxidants also show less solubility and moderate activity. Therefore, there is an increasing interest in to identify the antioxidant compounds from natural resources. Plants have several active biomolecules [4], it can be an alternative to the use of synthetic compounds in food and pharmaceutical technology.

Several medicinal plants have been used as a nutritional adjunct and in the treatment of numerous diseases without proper knowledge of their functions. Although phytotherapy continues to be used in numerous countries, few plants have received scientific or medical scrutiny. Hence, the aim of this present research work is to find out the bioproductive effect of *Barleria longiflora* leaves by the antioxidant potential and total phenolic content. We have also done the phytochemical screening of *B. longiflora* to identify the number of secondary metabolites in six different solvent systems [5].

METHODS

Collection of plant

B. Longiflora, belongs to the family Acanthacea, was collected from Western Ghats, Madurai, Tamil Nadu, India. The specimen was identified by Taxonomist Rev. Fr. Dr. John Britto, Rapinart Herbarium, St. Joseph's College, Tiruchirappalli, Tamil Nadu. The plant leaves were washed in running tap water before being shade dried at room temperature.

Preparation of extracts

Dried leaves were crushed in electric grinder and powdered. About 50 g of leaves powder was packed into Soxhlet apparatus and extracted consecutively with petroleum ether (60–80%), chloroform, ethyl acetate, ethanol, methanol, and distilled water. Each extract was air-dried thoroughly in rotary evaporator and stored in 4°C for further investigation.

Antioxidant activities

1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity

The free radical activity of leaf extracts was measured by DPPH according to the Blois method [6].

DPPH percentage inhibition (%) = Abs (Control) – Abs (Sample)/Abs (Control) × 100

Scavenging of H_2O_2

The scavenging ability of the leaf extracts to H_2O_2 was determined according to the method of Ruch *et al.* [7] and Gulcin *et al.* [8].

The percentage of scavenging of H_2O_2 was calculated as = Abs (Control) – Abs (Sample)/Abs (Control) × 100

Total reducing ability

Total reducing capacity of leaf extracts was determined to the method of Oyaizu [9]. Ascorbic acid (20–100 µg/ml) was used as a positive control. The maximum absorbance of the reaction mixture the greater is the reducing power.

Total reducing capacity (%) = Abs (Control) – Abs (Sample)/Abs (Control) × 100

Metal chelating ability

The ferrous ion-chelating ability of leaf extracts was evaluated by Decker and Welch [10] method. The ferrous ion inhibition of percentage was calculated using the formula:

Metal chelating ability (%) = Abs (Control) – Abs (Sample)/Abs (Control) × 100

Ferric reducing antioxidant power (FRAP) assay

The FRAP assays were performed by Benzie and Strain [11] method. The results are expressed as ascorbic acid equivalent antioxidant capacity.

FRAP (%) = Abs (Control) – Abs (Sample)/Abs (Control) × 100

Determination of total phenolic

The amount of total phenolics in the extracts was determined by the Folin–Ciocalteu method Singleton and Rossi [12]. Samples from the extracts were evaluated at a final concentration of 0.1 mg/ml. The total phenolic content was expressed as mg/g Gallic acid equivalent.

Statistical analysis

All of the experiments were conducted in triplicate (n=3) and one-way ANOVA (using SPSS 11.5 statistical software) was used to compare the mean values of every treatment. Significant differences between the means of parameters were determined using the Duncan test ($p<0.05$).

RESULTS AND DISCUSSION

Plants and plant-derived compounds have been well known to play a dominant role in health care of humans and more than 50% of the entire modern drugs in clinical use are of natural product origin [13]. Hence, plant-based drugs are being increasingly preferred in modern medicine. Natural compounds are derived from nutritional sources; provide a large number of antioxidants. They are acted as a protective shield for our body against certain unfortunate diseases such as arterial, cancer, and cardiac-related diseases. The current researches on free radicals have promised a revolutionary enhancement in health and lifestyle of human beings [14].

Table 1: DPPH free radical scavenging activity of six different extracts of *Barleria longiflora* leaves

S. No.	Extracts (100 µl)	Percentage of inhibition
1.	Standard (BHT)	63.9±0.012
2.	Petroleum ether	13.3±0.029
3.	Chloroform	46.9±0.050
4.	Ethyl acetate	36.1±0.066
5.	Ethanol	56.5±0.027
6.	Methanol	48.4±0.054
7.	Aqueous	34.3±0.064

Values are given in mean±SD, means were significantly different when $p<0.05$; DPPH: 1,1-Diphenyl-2-picrylhydrazyl. BHT: Butylated hydroxyl toluene, SD: Standard deviation

Antioxidant activities

There are different methods to determine the antioxidant capacity of folk medicinal plants. In this present study, antioxidant activities of *B. longiflora* were estimated using five different assays such as DPPH free radical scavenging activity, scavenging of H_2O_2, total reducing ability, ferric reducing antioxidant activity, and metal chelating ability assay. These five represented different mechanisms of antioxidant action. All the five assays were standardized by five different concentrations depends on the method. Six different leaf extracts of *B. longiflora* were compared with the highest concentration of standard. The antioxidant activities of plant species differ from depends on the methodology and their mechanism [15].

DPPH free radical scavenging activity

The result of antioxidants on DPPH radical scavenging is thought to be to hydrogen donating ability. DPPH is a stable free radical and it accepts an electron or hydrogen radical to become a stable diamagnetic particle. When a DPPH solution is mixed with a substrate acting as a hydrogen atom donor, a stable non-radical form of DPPH is obtained with simultaneous change of the violet color to pale yellow [16]. Hence, DPPH has been used extensively as a free radical to evaluate the reducing substance and is a useful reagent for investigating the free radical scavenging activities of molecules [17]. In this present study, the highest percentage of radical scavenging activity was observed in ethanol leaves extraction of *B. longiflora* (56.5±0.027); Kalpana *et al*. noticed maximum percentage DPPH radical scavenging activity in ethanol extract of *B. longiflora* [18].

Scavenging of H_2O_2

H_2O_2 can able to inactivate some enzymes directly, usually by oxidation of essential thiol (-SH) groups. H_2O_2 when enter into the cells, it can possible to react with Fe^{2+} and possibly Cu^{2+} to form hydroxyl radicals and this may be a source of many of its toxic effects [19]. It is consequently compensation for cells to control the amount of H_2O_2 that is allowed to accumulate [20]. In this present study, the highest anti- H_2O_2 activity was observed in ethanol extract of *B. longiflora* (83.4±0.18). Methanol extract of *Barleria noctiflora* L. root has scavenging activity in high when compared to the leaf [21]. Mohan and Ruba reported that among the five solvents, methanol extract has highly acted to remove the hydroxyl radicals from the sugar and inhibit the reaction when compare with standard [22].

Metal chelating ability

Ferrozine can chelate with Fe^{2+} by quantitatively and this reaction is restricted in the presence of other chelating agents and results in reduce of the red color of the ferrozine-Fe^{2+} complex. Measurement of

Table 2: Antioxidant activities different solvent systems of *Barleria longiflora* leaf extracts

S. No.	Different extracts	Scavenging of hydrogen peroxide (%)	FRAP assay (%)	Metal chelating ability (%)	Total reducing ability (µg/ml)
1.	Standard (Ascorbic acid)	61.9±0.56	83.3±0.02	69.6±0.03	0.70±0.01
2.	Petroleum ether	12.3±0.01	57.5±0.05	6.1±0.06	0.02±0.0
3.	Chloroform	70.8±0.07	15.2±0.07	10.2±0.07	0.03±0.0
4.	Ethyl acetate	41.1±0.06	66.8±0.10	20.7±0.08	0.13±0.03
5.	Ethanol	83.4±0.18	74.8±0.08	61.6±0.03	0.76±0.02
6.	Methanol	34.3±0.10	59.5±0.03	57.9±0.06	0.42±0.03
7.	Water	53.3±0.03	54.1±0.10	30.7±0.05	0.05±0.0

Values are given in mean±SD, Means were significantly different when $p<0.05$; FRAP: Ferric reducing antioxidant power. SD: Standard deviation

Table 3: Total phenolic content of *Barleria longiflora* leaf extracts

S. No.	Extracts (100 µl)	TPC (mg/g)
1.	Standard (Gallic acid)	1.07±0.08
2.	Petroleum ether	0.05±0.01
3.	Chloroform	0.09±0.01
4.	Ethyl acetate	0.18±0.06
5.	Ethanol	1.09±0.09
6.	Methanol	0.80±0.08
7.	Aqueous	0.68±0.03

Values are given in mean±SD, Means were significantly different when $p<0.05$; TPC: Total phenolic content. SD: Standard deviation

the color reduction determines the chelating activity to compete with ferrozine for the ferrous ions [23]. The result is indicating that the ethanolic extract (61.6±0.03) has more activity when compound to other solvent systems of *B. longiflora.* The antioxidants present in plant extracts forms a coordinate complex with the metal ions (chelating activity) and block the transfer of electrons. Thus, oxidation reaction is arrested and free radicals are not produced. Sujatha and Sekar reported that maximum chelation was showed in methanolic extract of *Litsea laevigata* leaves when compared with other solvent systems of leaf and stem [24].

FRAP assay

In the FRAP assay, antioxidants in the compounds reduce ferric (III) to ferrous (II) in a redox-linked colorimetric reaction [25] that involves single electron transfer. The reducing power indicates that the antioxidant compounds are electron donors and reduce the oxidized intermediate of the lipid peroxidation process so that they can act as primary and secondary antioxidants Yen and Chen [26]. In the present study, higher reducing power (74.8±0.08) was observed in ethanol extract of *B. longiflora.* Ranade *et al.* reported ethanolic extract 1.5 times higher than acetone and aqueous stem extracts of *Barleria prionitis* [27].

Total reducing ability

The total antioxidant activity of *B. longiflora* leaves was evaluated, it indicates that they are electron donors and can decrease the oxidized intermediates of lipid peroxidation process. Hence samples can act as antioxidants [28]. The results showed significant value in ethanolic extract (0.76±0.02) compared to other five leaf extracts of *B. longiflora.* Ethanolic extract exhibited potent power activity compared to that aqueous extract of *B. prionitis* [29].

Determination of total phenolic

Phenolic compounds are secondary metabolites that play a key role in maintenance of the human body. Phenolics are compounds found in plants and have been reported to have numerous biological activities including antioxidant properties. Many studies are focused on the biological activities of phenolic compounds; they are potential antioxidants and free radical scavengers. In this present study, ethanolic leaves extract of *B. longiflora* showed higher phenolic contents compared to other extracts tested. Polyphenolic content was high in ethanolic extract compared to aqueous extract of *B. prionitis* [30].

CONCLUSION

The present study for the 1st time reports the antioxidant activities of five different methods on six different solvents of *B. longiflora* leaves. Maximum absorbance of the reaction mixture indicates higher reduction potential. The reducing capacity of an extract may provide as a significant indicator of its potential antioxidant activity [30]. Hence, the ethanolic leaf extracts of *B. longiflora* can be a potential source of antioxidant activity. Further, to identify the bioactive compounds present in the leaves of *B. longiflora* and analyze their biological activities.

AUTHORS' CONTRIBUTIONS

Both authors have contributed equally.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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