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## Antioxidant activity of different solvent extracts of *Barleria longiflora* Linn

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### Abstract

This present study is designed to assess the different solvent extracts of *Barleria longiflora* (leaves) for their antioxidant activities with reference to 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), FRAP and ABTS<sup>+</sup> assay by using UV-double beam spectrometer. Among the different extracts tested, the ethanol extract showed significant antioxidant activity. Thus, the plant revealed promising antioxidant activity, and require further studies to throw light on their chemical composition and other pharmacological properties.

**Keywords:** *Clausena excavate*, DPPH, FRAP, NO<sub>2</sub>, UV absorption, ROS.

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### 1. Introduction

Now days, there is an increased occurrence of various diseases like cardiovascular disease, neurological disorders, cancer, diabetes and autoimmune disease due to the presence of free radicals. (Bandyopadhyay *et al.*, 1999). Antioxidant is defined as the agent that neutralizes the effect produced by free radical. (Fang *et al.*, 2002). The antioxidant can be classified into two categories, namely enzymatic and non-enzymatic. The enzymatic antioxidants are produced itself in our body, whereas most of non-enzymatic antioxidants are obtained from either natural plants or synthetics which are used for the treatment for various diseases (Lee J *et al.*, 2004 and Cuzzocrea S *et al.*, 2001). A number of side effects like liver damage and mutagenesis are associated with the use of antioxidants obtained from synthetic sources (Grice HC *et al.*, 1986). Thus, researchers are exploring natural resources to find out newer and safer natural antioxidants. The potentially reactive derivatives of oxygen, attributed as reactive species (ROS), are continuously generated inside the

human body. The generated ROS are detoxified by the antioxidants present in the body. However, overproduction of ROS and/or inadequate antioxidant defense can easily affect and persuade oxidative damage to various bio molecules, including proteins, lipids, Natural antioxidants lipoproteins and DNA. The WHO estimates that up to 80% of people still rely mainly on traditional remedies such as herbs for their medicine (Tripathi and Tripathi, 2003). India has been identified as a major resourceful area in the traditional and alternative medicines globally. Multi-factorial health beneficial activity of these plant extracts has been attributed to multi-potent anti-oxidant, anti-microbial, anti-cancer, anti-ulcerative and anti-diabetic properties. Several medicinal plants (Rasayana) have also been extensively used in the Indian traditional (Ayurveda) system of medicine for the treatment of number of diseases<sup>18</sup>. Some of these plants have shown potent antioxidant activity. (Kaur *et al.*, 2002 and Aqil F *et al.*, 2006). The WHO estimates that

up to 80% of people still rely mainly on traditional remedies such as herbs for their medicine (Tripathi and Tripathi, 2003).

Currently, the search for plant sources of antioxidants is gaining momentum with *B. longiflora*, its common name is long flowered *barleria* and kattu mullai or semmulli in Tamil. It is a small shrub that grows up to 1-2 m. Stems are covered with glandular hairs and oppositely arranged ovate long pointed leaves with densely hairy on both sides. This is a medicinal plant widely distributed in Tamil Nadu and the plant species distributed throughout the southern part of India are used in folklore medicine for the treatment of several illnesses such as malaria (Chennaiyan *et al.*, 2016), headache, abdominal pain, dysentery, pulmonary tuberculosis, diarrhea, cold, wound, snakebite, and poisoning. Recent studies showed that the plant also possessed immune-modulatory (Manosroi *et al.*, 2004), analgesic (Rahman *et al.*, 2002), anti-inflammatory, antiviral, anticancer (Sharif *et al.*, 2011), antioxidant (Guntupalli *et al.*, 2012), antimycobacterial (Sunthitikawinsakul *et al.*, 2003), and antifungal (Kumar *et al.*, 2012) activities.

## 2. Materials and Methods

### 2.1 Antioxidant activity - DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay

The Radical Scavenging Activity (RSA) of different extracts was determined by using DPPH assay following the method of Chang *et al.* (2008) with small modification. The decrease in the absorption of the DPPH solution at 517nm after the addition of antioxidant was measured in a cuvette containing 2.960µl of 0.1mm ethanolic DPPH solutions; 40µl of 20 to 200µg/mL of plant extracts after vortexing the mixture thoroughly. The setup was left at dark in room temperature and the absorption was monitored after 20 minutes by using double beam UV spectrophotometer. Ascorbic acid and Butylated hydroxytoluene (BHT) were used as references. The ability of the plant extract to scavenge DPPH radical was calculated by the following equation

$$\%RSA = \frac{A_c - A_s}{A_c} \times 100$$

Where

A<sub>c</sub> = Absorbance of control;

A<sub>s</sub> = Absorbance of sample

Abs. control = Absorbance of DPPH radical + ethanol;

Abs. sample = absorbance of DPPH radical + plant extract. Measurements were performed in triplicates.

Absorbance values were corrected for radical decay using blank solutions.

### 2.2 Ferric Reducing Antioxidant Power (FRAP) assay

FRAP reagent (1.8 ml) was mixed with 0.2ml of test sample, then incubated at 37°C for 10 minutes in a water bath. The FRAP reagent contains 20mM TPTZ (2, 4, 6-tri(2-pyridyl)-S-triazine) solution 20mM FeCl<sub>3</sub>, 6H<sub>2</sub>O and 0.3M Acetate buffer with pH 3.6. After incubation the absorption was measured immediately at 593nm. The above mixture without the plant extract served as control (Iris *et al.*, 1996). Ethanolic solution of known Fe (II) concentration was used as standards. The values were expressed as mmol Fe (II)/g dry weight of the extract. The assay was carried out in triplicate and the mean values with ± S. D are presented.

### 2.3 ABTS<sup>+</sup> Radical scavenging activity (Giao *et al.*, 2007)

ABTS<sup>+</sup> decolorisation assay involves the generation of the ABTS<sup>+</sup> chromophore by the oxidation of ABTS<sup>+</sup> with potassium persulphate. It is applicable for both hydrophilic and lipophilic compounds. The scavenging activity of the leaf extracts on ABTS<sup>+</sup> radical cation was measured at 734 nm.

The reaction was initiated by the addition of 1.0 ml of diluted ABTS<sup>+</sup> to 10 µl of different concentrations (200 - 1000 µg / ml) of leaf extract and also to 10 µl of ethanol as a control. Ascorbic acid was used as positive control. The absorbance was read at 734 nm after 6 minutes and the percentage inhibitions were calculated. The inhibition was calculated according to the equation,

$$I = A_0 - A_1/A_0 \times 100,$$

Where,

A<sub>0</sub> is absorbance of control reaction,

A<sub>1</sub> is absorbance of test compound.

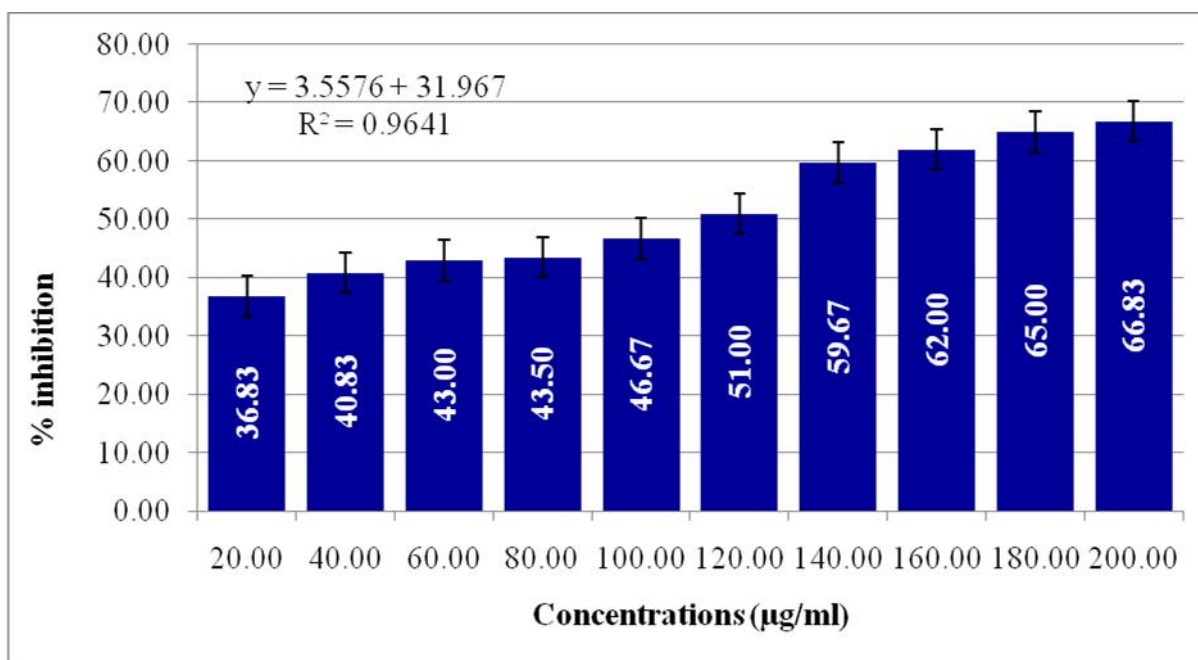
## 3. Results

The antioxidant activity of petroleum ether extract, chloroform extract and ethanol extracts of *B. longiflora* was tested at different concentrations as shown in figure 1-4 and table 1. It was observed that the increase in the concentration of the extract showed increased antioxidant activity in all the three extracts

tested in the present investigation. Furthermore, among the three extracts tested, ethanol extract was found to have significant antioxidant activity when compared with the other two solvents. Besides the maximum antioxidant activity with reference to DPPH assay was noticed at 200 µg/ml concentration of ethanol extract of *B. longiflora* (74.33% inhibition) with the IC<sub>50</sub> value of 72µg/ml. Likewise, the FRAP

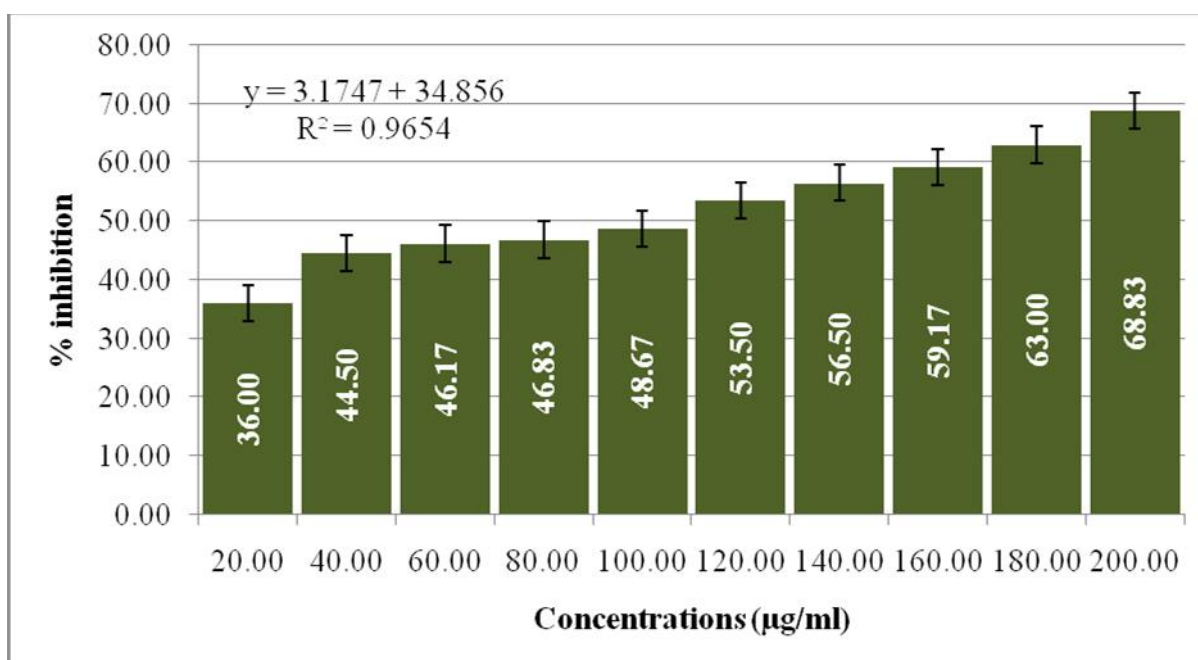
assay also showed the maximum antioxidant activity in the ethanol extract with the IC<sub>50</sub> inhibition value of 38µg/ml. In the same way, the ABTS+ assay clearly showed that at 1000µg/ml envisaged 65.66, 57.73 and 47.33% inhibitory activity in the petroleum ether extract, chloroform extract and ethanol extracts of *B. longiflora* (Table 1)

**Figure 1: Antioxidant activity (DPPH assay) of petroleum ether extract of *Barleria longiflora* at different concentrations**



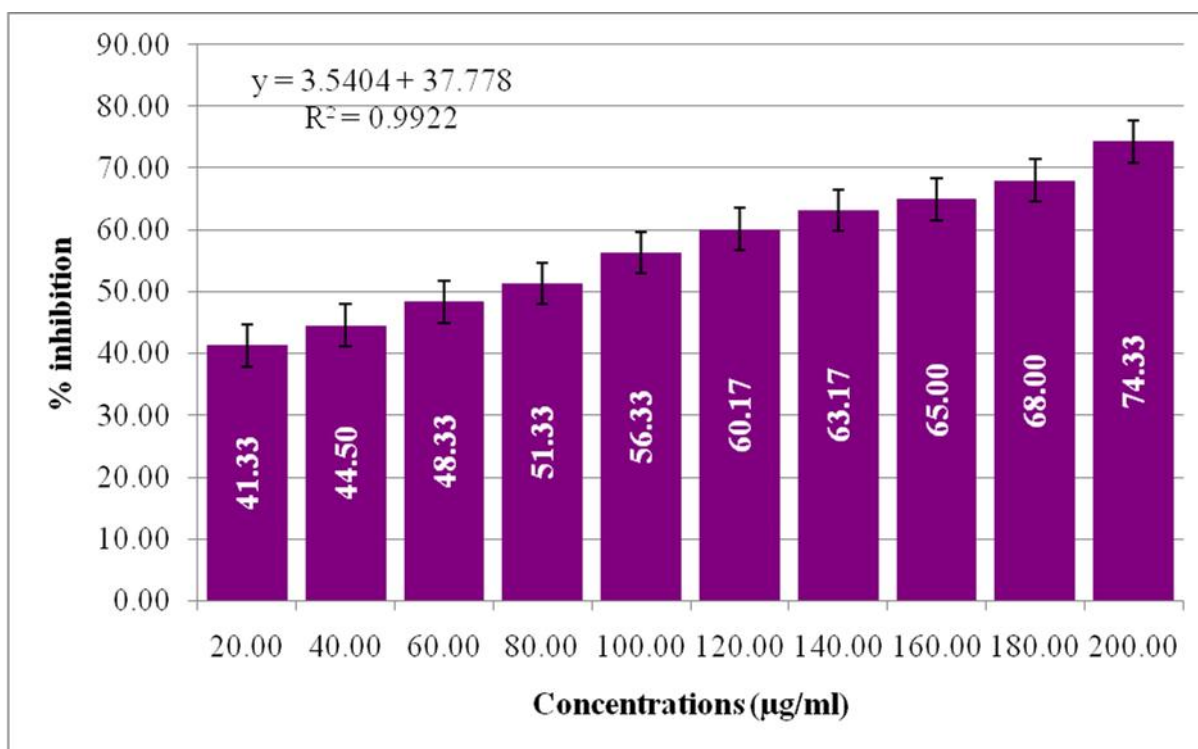
IC<sub>50</sub> =116µg/ml

**Figure 2: Antioxidant activity (DPPH assay) of chloroform extract of *Barleria longiflora* at different concentrations**



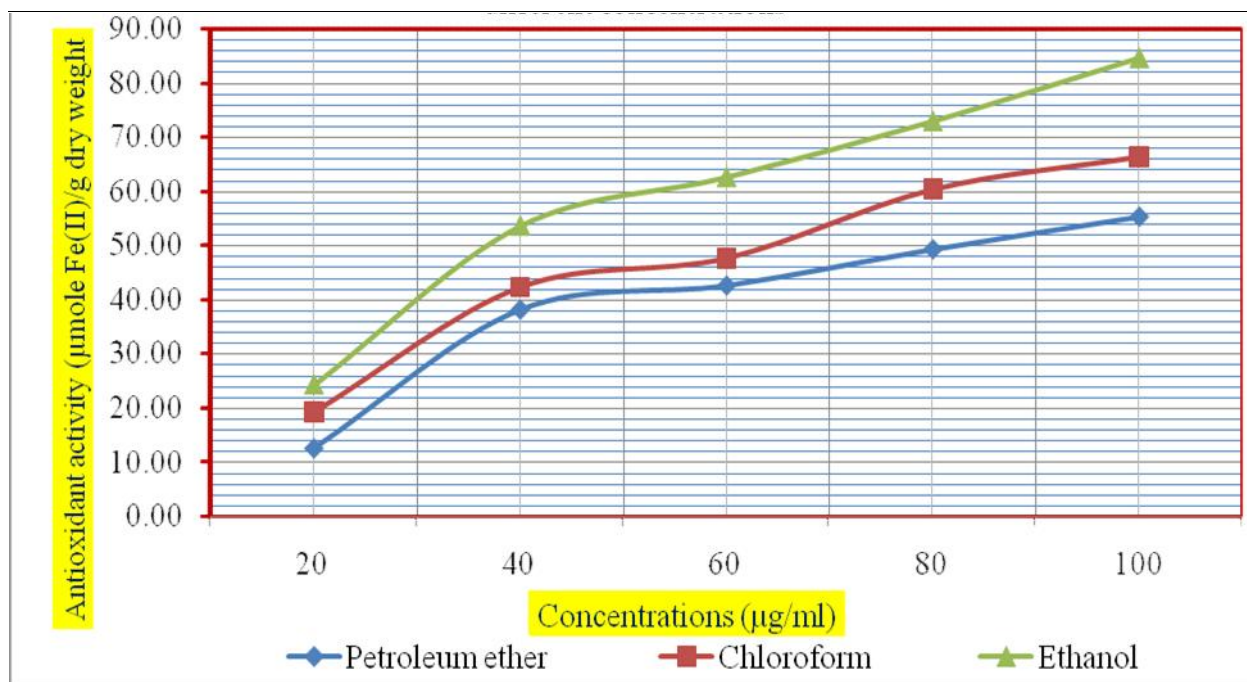
IC<sub>50</sub> =106µg/ml

**Figure 3: Antioxidant activity (DPPH assay) of ethanol extract of *Barleria longiflora* at different concentrations**



IC<sub>50</sub> = 72µg/ml

**Figure 4: Antioxidant activity (FRAP assay) of different extracts of *Barleria longiflora* at different concentrations**



Ethanol extract IC<sub>50</sub> = 38µg/ml

**Table 1: Antioxidant activity (ABTS<sup>+</sup> scavenging assay) of different extracts of *Barleria longiflora***

Extracts tested	% inhibition				
	200 (µg/ml)	400 (µg/ml)	600 (µg/ml)	800 (µg/ml)	1000 (µg/ml)
Petroleum ether	12.67 ± 0.33 <sup>a</sup>	20.33 ± 0.66 <sup>a</sup>	33.66 ± 0.99 <sup>a</sup>	40.99 ± 0.33 <sup>a</sup>	47.33 ± 0.66 <sup>a</sup>
Chloroform	19.33 ± 0.66 <sup>b</sup>	27.33 ± 0.33 <sup>b</sup>	42.66 ± 0.33 <sup>b</sup>	54.99 ± 0.66 <sup>b</sup>	57.73 ± 0.66 <sup>b</sup>
Ethanol extract	25.66 ± 0.99 <sup>c</sup>	37.66 ± 0.56 <sup>c</sup>	46.33 ± 0.33 <sup>b</sup>	57.33 ± 0.99 <sup>b</sup>	65.66 ± 0.33 <sup>c</sup>
Ascorbic acid	35.00 ± 0.00 <sup>d</sup>	49.00 ± 0.00 <sup>d</sup>	64.00 ± 0.00 <sup>c</sup>	72.00 ± 0.00 <sup>c</sup>	84.00 ± 0.00 <sup>d</sup>

Values represent mean ± S.D of three replications. Different alphabet in the column differs statistically at  $p < 0.005\%$  (DMRT).

#### 4. Discussion

There is an inverse relationship between the nutritional intake of antioxidant rich food and the frequency of the human disease Rice-Evans *et al.* (1997). The petroleum ether, chloroform and ethanol extracts of *B. longiflora* had shown the gradual increase in the antioxidant activity studied in the present investigation and found as a dose dependent manner in terms of percentage inhibition depicted in fig 1 to 4 and table 1. The presence of terpenoids has been detected via phytochemical analysis (unpublished data). The Terpenoids have been found to possess antioxidant properties in various studies (Liu *et al.*, 2009; Singh and Singh, 2003; Sauerwein *et al.*, 1990; Maneemegalai *et al.*, 2010; Ajaiyeoba, 2002; Patil *et al.*, 2010; Naveen Prasad *et al.*, 2008; Ayoola *et al.*, 2008; Prosper-Cabral *et al.*, 2007; Anyasor *et al.*, 2010). The present investigation has shown that ethanol extract has active phytochemicals (terpenoids, flavonoids, coumarins and phenols) which are able to inhibit the pathogenic bacteria. However, the presence of phenolic contents was traditionally speculated with the environmental stress experienced by the plant during the time of sampling. Evaluation of antioxidant properties of plants cannot be carried out accurately by a single universal method, (Zengin, *et al.*, 2011) instead a set of assays could be able to provide a clue about the activity. Total antioxidant capacity was reported as ascorbic acid equivalents and the method is mainly used for the spectrophotometric quantification of total antioxidant capacity that employs cost effective reagents. (Prieto, *et al.*, 1999). The strong antioxidant activity was confirmed in ethanol extract. The antioxidant activity may be due to the strong occurrence of polyphenolic compound such as terpenoids and phenols. These findings

provide scientific evidence to support old-style uses and indicate a promising potential for the development of an antimicrobial and antioxidant drug from *B. longiflora* plant.

#### 5. Conclusion

The replacement of synthetic with natural antioxidants (because of implications for human health) may be advantageous. In the present study analysis of free radical scavenging activity and total phenolic and flavonoid content showed that mainly the free radical scavenging activity (RSA) [reactions with 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), Nitric oxide scavenging assay and UV absorption plant of *B. longiflora* can be the potent source of natural antioxidants.

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