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## Antioxidant activity, total phenolic content and total flavonoid content of *Perilla ocymoides* Linn.

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### ABSTRACT

Recent years have witnessed a global increase in attempts to identify pharmacologically potent and biologically safe antioxidant compounds for use in food industry and preventive medicine. Although traditional systems of medicine utilize several plants for their medicinal properties, however, their use requires scientific validation for wider acceptance. *Perilla ocymoides* Linn. (Lamiaceae) commonly known as sookloti, is a small evergreen shrub. The present study was conducted to evaluate the antioxidant activity of the leaves of *P. ocymoides* and to quantify the total phenolics and flavonoids. The methanol extract of *P. ocymoides* leaves exhibited DPPH radical scavenging activity and recorded considerable amounts of total phenolics and flavonoids. The present work showed that the methanol extract of *P. ocymoides* can be considered as a source of natural antioxidants and hence provides a basis for developing valuable food additives to enhance human nutrition.

**Keywords:** *Perilla ocymoides*, Antioxidant activity, IC<sub>50</sub>, Total Phenolic Content, Total Flavonoid Content

### INTRODUCTION

The recent years have witnessed a global increase in attempts to identify pharmacologically potent and biologically safe antioxidant compounds for use in food industry and preventive medicine. Traditional systems of medicine rely on several plants that have been shown to contain biologically active components with medicinal properties. It has been shown that plants produce significant amounts of antioxidants to counteract the oxidative stress developed by the formation of reactive oxygen species and other free radicals during metabolism. Antioxidants, such as vitamins, phenolics and other phytochemicals, derived from plants and other natural sources have received much attention due to their better biological compatibility. These secondary metabolites are known to function as chemo preventive agents against oxidative damage [1]. Phenolics or polyphenols are known to possess high antioxidant activity and have received considerable attention because of their physiological functions, including antioxidant, antimutagenic and antitumour activities [2]. It is, therefore, imperative to evaluate the antioxidant activity of the commonly consumed medicinal plants and appreciate their use for maintaining human health. The present work aimed at determining the antioxidant activity, total phenolic content and total flavonoid content of *Perilla ocymoides* Linn.

*Perilla ocymoides* Linn. (Lamiaceae) commonly known as sookloti, a small evergreen shrub with aromatic leaves, shoots and inflorescences. It is found in Assam and other parts of North-east India and is used for flavouring curries. Leaves and young shoots are used medicinally for stomach troubles, to induce appetite, and to increase digestive ability [3]. The plant is also regarded useful for women in curing uterus infection and irregular menstruation [4].

### MATERIALS AND METHODS

#### Chemicals and Equipment:

All the chemicals used in the study were of analytical grade. Dimethyl Sulfoxide (DMSO), Ciprofloxacin and 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), and other general purpose laboratory chemicals and reagents were procured

from Merck Specialities Pvt. Ltd, Mumbai, India. Microbiological media were obtained from HiMedia, Mumbai, India.

#### Preparation of Plant Extracts:

The plant leaves were collected from their natural habitats and processed. These were washed with distilled water and shade dried. The material was then ground into fine powder using electric blender. Extract was prepared by following cold maceration method. Dried powder was soaked in methanol (1: 2 w/v) for 48 hours with intermittent shaking, after which the extract was filtered through Whatman No. 1 filter paper into pre-weighed beakers and dried in a rotatory vacuum evaporator (IKA RV 10 Digital) until a constant dry weight was obtained. The extract was stored aseptically at 5°C for further use.

#### In vitro Antioxidant Activity Assay:

The free radical scavenging activity of the extract was measured *in vitro* by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay [5]. The plant extract was dissolved in DMSO to obtain concentrations in the range of 10 µg/ml to 500 µg/ml. 30 mM solution of DPPH was prepared in DMSO and 1 ml of this solution was mixed with 3.0 ml of the different concentrations of the extract. The mixture was incubated at room temperature for 30 min and the absorbance was measured at 517 nm against blank using SHIMADZU UV-1800 Spectrophotometer (SHIMADZU, Japan). A reaction mixture without the plant extract served as the control. The percentage scavenging was calculated by the following equation:

$$\% \text{ inhibition} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Control}}} \times 100$$

IC<sub>50</sub> was defined as the concentration of the extract required to achieve 50% inhibition of DPPH radicals. The experiment was performed in triplicate and IC<sub>50</sub> was expressed as mean ± standard deviation. Ascorbic acid was used as standard antioxidant.

#### Determination of Total Phenolic Content (TPC):

The total phenolic content of the plant extracts was determined by the Folin-Ciocalteu method [6]. A 0.5 ml aliquot of the diluted extract (0.25 mg/ml) was mixed with 2.5 ml of 10% Folin-Ciocalteu reagent. The mixture was vortexed and after 2 minutes, 2 ml of 7.5% Na<sub>2</sub>CO<sub>3</sub> was added. The mixture was incubated for one hour at room temperature and absorbance was measured at 765 nm against blank. Blank was concomitantly prepared using 0.5 ml distilled water, 2.5 ml 10% Folin-Ciocalteu reagent and 2 ml of 7.5% of Na<sub>2</sub>CO<sub>3</sub>. Gallic acid was used as standard for preparing the calibration curve. The total phenolic content in extract was expressed in terms of Gallic acid equivalent (mg of GA/g of extract).

#### Determination of Total Flavonoid Content (TFC):

The total flavonoid content was determined using the Dowd method [7]. 5 ml of 2 % aluminium trichloride (AlCl<sub>3</sub>) in methanol was mixed with the same volume of the extract solution (0.05 mg/ml) and incubated for 10 minutes. Absorbance was recorded at 415 nm using spectrophotometer against blank. The total flavonoid content was calculated from the calibration curve of rutin and was expressed as rutin equivalent (mg RE/g of extract).

#### Statistical Analysis

Statistical analysis was performed using SigmaStat 3.5 and the results were expressed as the mean of the three replicates ± standard deviation.

## RESULTS

The methanol extract of the leaves of *P. ocimoides* exhibited DPPH radical scavenging activity. IC<sub>50</sub> for the extract was obtained to be 734.17 ± 2.21 µg/ml and for the standard (ascorbic acid) was observed to be 4.19 ± 0.01 µg/ml (Figure 1; Table 1). The total phenolic content (TPC) of the methanol extract of *P. ocymoides* was calculated to be 25.95 ± 0.78 mg GAE/g and the total flavonoid content (TFC) of the methanol extract of leaves of *P. ocymoides* was determined to be 18.34 ± 0.25 mg RE/g extract (Table 1).

DPPH Radical Scavenging Activity of  
*P. ocymoides* Leaves

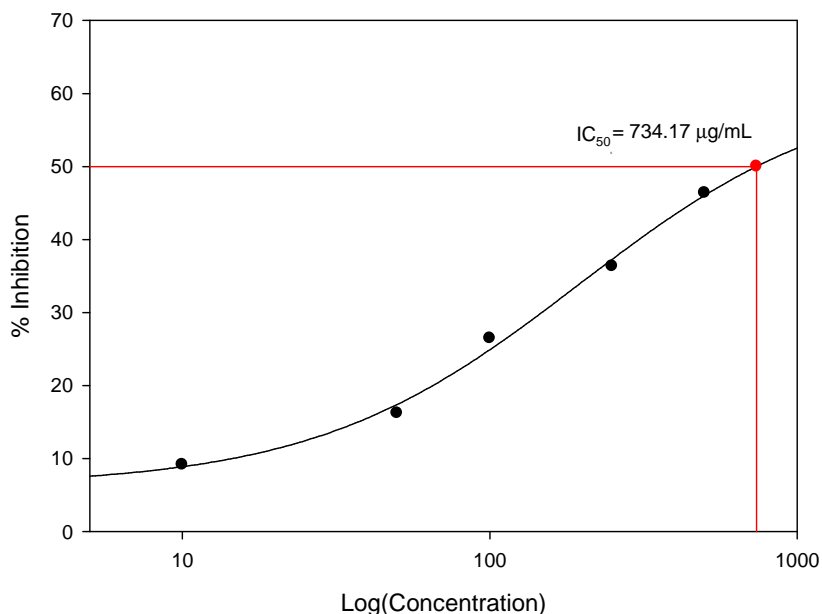


Figure 1: DPPH Radical Scavenging Activity of *P. ocymoides* Leaves

Table 1: TPC and TFC of *P. ocymoides* leaves

Extract	TPC (mg GAE/g Plant extract)	TFC (mg RE/g Plant extract)
Methanol	25.95 ± 0.78	18.34 ± 0.25

### DISCUSSION

The antioxidants in human diet are of great interest as possible protective agents to counter-balance the oxidative damage caused by the reactive oxygen species produced during metabolism of carbohydrates, proteins and fats. DPPH is a stable free radical and is often used to evaluate the antioxidant activity of several natural compounds [8, 9, 10]. Antioxidants on interaction with DPPH, transfer either electrons or hydrogen atom to DPPH, and neutralize it. The methanol extract of *P. ocymoides* leaves showed considerable antioxidant activity by neutralizing the DPPH free radical. The use of plant-based antioxidants is often related to the reduced risk of coronary heart diseases, neurological disorders and certain type of cancers [11, 12]. The plant phenolics are reported to possess the ability to donate hydrogen atoms from the hydroxyl groups on their ring structures [13]. The presence of considerable amounts of phenolics and flavonoids in the methanol extract of *P. ocymoides* may contribute to its antioxidant activity.

### CONCLUSION

The methanol extract of *P. ocymoides* can be considered as a source of natural antioxidants. Our findings provide a basis for developing valuable food additives to enhance human nutrition.

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