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Antioxidant Potential of Leaf Extract of *Orthosiphon thymiflorus* (Roth.) Sleensen

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Abstract: The present study was aimed to investigate the antioxidant activity of Methanolic leaf extract of *Orthosiphon thymiflorus* (Roth.) Sleensen (Labiatae). In evaluation, Methanolic extract of the leaf powder was prepared and screened for *in vitro* antioxidant activities by 1, 1 - diphenyl - 2 - picrylhydrazyl (DPPH) free radical scavenging activity and by reducing power assay method. Both the methods were compared with a natural antioxidant ascorbic acid (vitamin C) as a standard. In DPPH free radical scavenging assay, IC50 values of methanolic leaf extract and Ascorbic acid was found to be 39.0µg/ml and 10.6 µg/ml respectively. The results concluded that the extract has potential source of antioxidants of natural origin.
Key Words: Antioxidant, *Orthosiphon thymiflorus*, DPPH, free radical, reducing power.

Introduction

Free radicals of different forms are constantly generated for specific metabolic requirement and quenched by an efficient antioxidant network in the body. When the generation of these species exceeds the levels of antioxidant mechanism, it leads to oxidative damage of tissues and bio molecules, eventually leading to disease conditions, especially degenerative diseases. [1]Reactive oxygen species (ROS), sometimes called active oxygen species, are various forms of activated oxygen, which include free radicals such as super oxide ions and hydroxyl radicals, as well as non free-radical species such as hydrogen peroxide. [2, 3]However, normally a balance between oxidative events and antioxidative forces maintains the status quo within living cells. When normal balance is upset, either by loss of reducing agents or protective enzymes or by both events simultaneously, the tissue is considered to be under oxidative stress. It can then cause oxidative damage of all major groups of bio molecules (DNA, proteins, lipids and small cellular molecules) leading to pathogenesis of various diseases like cancer, emphysema, cirrhosis, atherosclerosis, arthritis cardiovascular diseases, diabetes, asthma, hepatitis, liver injury, immune deficiency diseases, neurodegenerative diseases and aging. [4-7]Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias. Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties. [8-11]Antioxidants act as a major defence against radical mediated toxicity by protecting the damages caused by free radicals. Antioxidant based drugs or formulations for the prevention and

treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer, have appeared in the last three decades. [12, 13]Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, and also by acting as oxygen scavengers. [14, 15]Antioxidant supplements or foods containing antioxidants have been reported to protect the human body by reducing oxidative damage. [16] There are some synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), commonly used in processed foods. However, the possible toxicity as well as general consumer rejection led to decreasing use of these synthetic antioxidants. [17] In addition, it has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of human disease. [18]Many plant extracts and phytochemicals have been shown to have antioxidant/free radical scavenging properties, and it has been established as one of the mechanisms of their action. [19] Some of the non-nutritive antioxidants of plants are phenolic compounds, flavonoids, coumarins, benzyl isothiocyanate etc. [20] This has attracted a great deal of research interest in natural antioxidants. *Orthosiphon thymiflorus* is a medicinal plant native to South East Asia and some parts of tropical Australia. It is an herbaceous shrub which grows to a height of 1.5 meters. It is a popular garden plant because of its unique flower, which is white and bluish with filaments resembling a cat's whiskers. *Orthosiphon thymiflorus* aqueous extracts have found to be having diuretic, [21] anti-inflammatory and acetylcholine antagonistic action 27[22, 23]Leaf juice has been used by the tribes as a lotion. [24]

Materials and Methods

***Plant material and extraction*:** The leaves of *Orthosiphon thymiflorus* (Roth.) Sleensen (Labiatae), collected from the tribal areas of Attapady, Palakkad district, Kerala state, South India were authenticated by the Botanical survey of India, Coimbatore, Tamilnadu (BSI). A voucher specimen (no.BSI/SRC/5/23/10-11/Tech-936) was deposited in the departmental herbarium. The leaves were cut, air dried and ground into coarse powder. This powder was stored in air tight container and used for extraction. The dried and powdered material was extracted with Methanol using a soxhlet apparatus. The extraction was carried out for 24 h at room temperature with mild shaking. The extract was filtered, concentrated and the weight of the residue was recorded and percent yield calculated.

***Preparation of Orthosiphon thymiflorus stock solution*:** Methanolic extract of *Orthosiphon thymiflorus* was prepared at the concentration of 1000 µg/ml in methanol. From the stock solution, different concentration viz. 10, 20, 40, 60, 80and 100 µg/ml were prepared in methanol and used for antioxidant studies.

***Preparation of standard stock solution*:** Ascorbic acid was used as standard for the study and its stock solution was prepared in the concentration of 1000 µg/ml in methanol. It was prepared freshly and used immediately for the study. From the stock solution, different concentration viz.10, 20, 40, 60, 80, & 100 µg/ml were prepared in methanol and used for antioxidant studies.

***Determination of total antioxidant activity*:**
DPPH Radical Scavenging Activity [25]: The free radical scavenging capacity of the methanolic leaf extract of *Orthosiphon thymiflorus* was determined using DPPH. [26, 27] DPPH scavenging activity was measured by spectrophotometric method at 517 nm. Methanolic extract of the roots of *Orthosiphon thymiflorus* (0.05 ml each) and standard compound, ascorbic acid were added in different concentrations (50-1000 µg/ml) to the methanolic solution of DPPH (100 µM, 2.95 ml). After 30 min, absorbance was measured in triplicate. [28] Percentage scavenging of the DPPH free radical was measured using the following equation:
% DPPH radical-scavenging = [(Absorbance of control - Absorbance of test Sample) / (Absorbance of control)] x 100.

***Reducing power assay*:** Leaf Extract (25-300 µg) in 1 ml of distilled water was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide [K3Fe(CN)6] (1%), and incubated at 50°C for 30 min. Then, 2.5 ml of trichloroacetic acid (10% v/v) was added to the mixture and then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of upper layer solution was mixed with 2.5 ml of distilled water and 0.5 ml FeCl3 (0.1%) and the absorbance was measured at 700 nm. [29-30]Ascorbic acid was used as reference.

Results and Discussion

DPPH Free radical scavenging activity: In free radical scavenging activity, DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become stable diamagnetic molecule. The reduction capability of DPPH radical was determined by the decrease in its absorbance at 517 nm, which is induced by different antioxidants (Table 1).

Table 1: ***Percentage inhibition of standard and methanol extract at various concentrations (µg/ml) in DPPH scavenging model***

Concentration	DPPH radical inhibition (%)	
	Ascorbic acid	Methanol extract
10	47.16±0.002	17.62±0.002
20	54.22±0.120	26.66±0.335
40	60.12±0.002	51.12±0.005
60	70.14±0.024	59.33±0.004
80	90.14±0.004	82.15±0.006
100	99.56±0.221	88.15±0.001

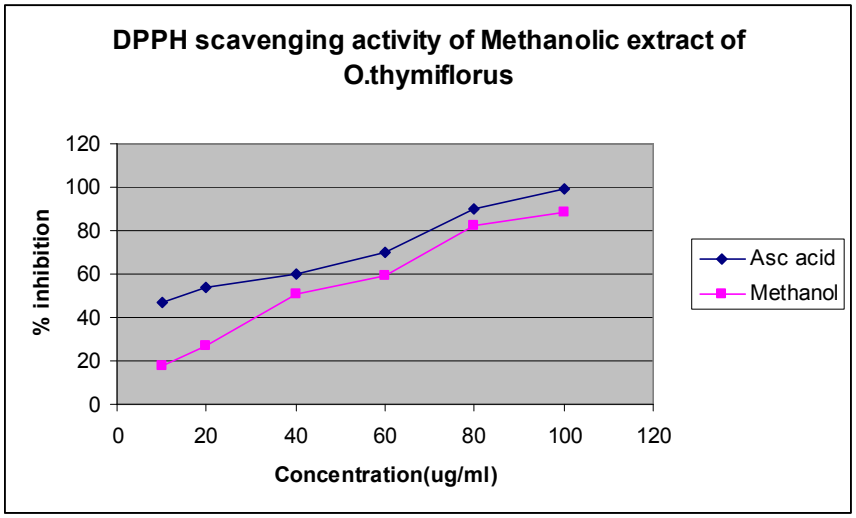

Figure 1: ***DPPH scavenging activity of methanol extract of Orthosiphon thymiflorus and standard ascorbic acid.***

Reducing power assay: Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. The reducing power of the methanolic extract increases with the increase in amount of sample (Table 2).

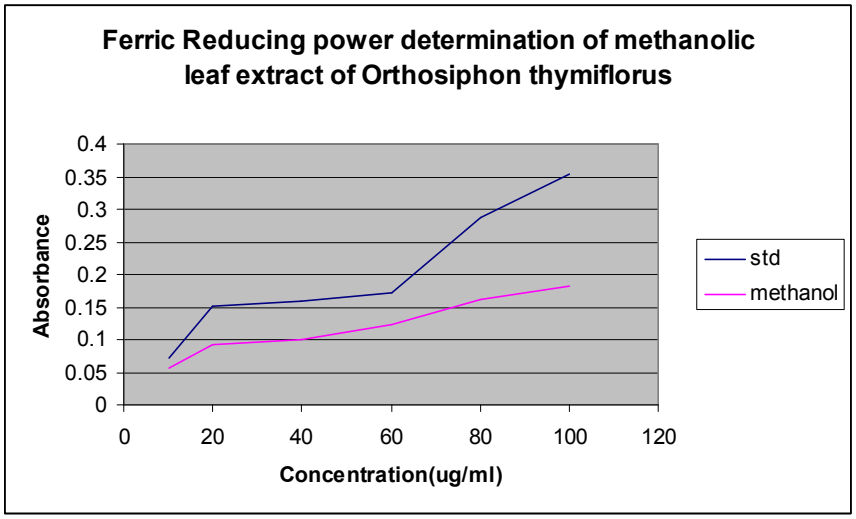

Figure 2: ***Ferric reducing power determination of methanolic extract of Orthosiphon thymiflorus and standard ascorbic acid.***

Table 2: ***Absorbance of Standard and methanol extract at various concentrations (µg/ml) in ferric reducing power determination model***

Concentration(µg/ml)	Absorbance	
	Standard	Methanol
10	0.072± 0.022	0.056± 0.003
20	0.152± 0.001	0.092± 0.032
40	0.160± 0.002	0.099± 0.033
60	0.173± 0.004	0.124± 0.001
80	0.286± 0.021	0.162± 0.001
100	0.355± 0.022	0.182± 0.003

Both the methods have proven the effectiveness of various extracts compared to the standard antioxidant, ascorbic acid. The methanolic leaf extract of *Orthosiphon thymiflorus* exhibited a significant dose dependent inhibition of DPPH activity. The IC50 value was found to be 39.2 µg/ml. The presence of reductants (i.e. antioxidants) in *Orthosiphon thymiflorus* leaf extract causes the reduction of the Fe3+/ferricyanide complex to the ferrous form. Therefore, the Fe 2+ can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. The reducing power of *Orthosiphon thymiflorus* leaf extract was very potent and the power of the extract increased with quantity of sample.

Conclusion

It is well known that free radicals are one of the causes of several diseases. The result from the two in - vitro antioxidant model reveals that the leaf powder extract of *Orthosiphon thymiflorus* had significant antioxidant activity. The activity may be due to the presence of tannins and flavonoids found in preliminary phytochemical analysis. Further studies are in progress for the isolation of active constituents responsible for antioxidant activity.

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