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A Study on Quantitative Analysis of Phytochemicals and Evaluation of *In-vitro* Antioxidant Activity of Leaf Extracts of *Elephantopus scaber*

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Abstract

Dietary phytochemicals are considered as an effective tool to cure various human physiological disorders. Several epidemiological studies have indicated that high intake of natural products is associated with reduced risk of a number of chronic diseases. During recent years consumers have been more concerned about the addition of synthetic additives to food. Therefore, an interest is growing for the search of natural phytoactives with biological activities. *Elephantopus scaber* has tremendous reputation in indigenous traditional system of medicine in India by virtue of which it has drawn attention and concern of scientists for validation of its medicinal properties through phytochemical and pharmacological evaluation. The present study was conducted with the main purpose of quantitative analysis of phytochemicals and evaluation of *in-vitro* antioxidant activity of leaf parts of *E. scaber*. Leaves of *E. scaber* was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with methanol. Results revealed that methanolic leaf extract of *E. scaber* exhibited percentage inhibition of 33.42%, 49.09%, 74.96%, 81.07%, and 93.09% at the concentration of 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, and 100µg/ml respectively in DPPH radical-scavenging activity assay with an IC_{50} value of 58 µg/ml which was at par with the standard BHT with an IC_{50} value of 66 µg/ml. Furthermore, quantitative estimation of phytochemicals in methanolic leaf extract of *E. scaber* revealed the presence of high quantity

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of polyphenolic compounds (28.46 GAE) compared with flavonoids (11.53 GAE). In conclusion, methanolic leaf extract of *E. scaber* has potential to exhibit antioxidant properties mainly due to the presence of secondary metabolites *viz.* polyphenols and flavonoids present in it. Hence, methanolic leaf extract of *E. scaber* could be explored for the development of natural antioxidant agents.

Keywords: *Elephantopus scaber*, Antioxidant agents, Leaf extracts, Polyphenols, Flavonoids

Introduction

Most of the traditional systems of India including Ayurveda have their roots in folk medicine. Traditional system of medicine in India functions through two major streams the local health tradition and the classical scientific system of tradition. The carriers of local health care system are millions of people who cure diseases at home as a birth attendant and practitioners of snake bite and jaundice treatments.[1] Medicinal plant based traditional system of medicines are playing an important role in providing health care to large section of population, especially in developing countries. It is a well-known fact that the traditional system of medicines always played an important role in meeting the global health care needs. India has the unique distinction of having six recognized system of medicine in this category. They are Ayurveda, Siddha, Unani and Yoga, Naturopathy and Homeopathy.[2]

Elephantopus scaber which is an erect herb up to 80 cm tall (Figure 1). The plant is a native to Tropical Africa, Eastern Asia, Indian Subcontinent, Southeast Asia and Northern Australia. Its natural habitat is subtropical or tropical moist montane forest. It is a perennial herb found as under growth in shady places. The whole plant of *E. scaber* is well known as herb of Chinese folk medicine which is widely used in the treatment of nephritis, edema, dampness, pain in the chest, fever and cough of pneumonia, scabies and arthralgia due to wounding.[3] The root decotion of *E. scaber* is widely used to treat diarrhoea, dysentery, stomach troubles and blood vomiting in tuberculosis in Nepal.[4,5] Sesequiterpenes lactones,

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triterpenoids, steroids, flavonoids and essential oil constituents have been reported from various part of the plant. The plant has been extensively screened for anticancer activity.[6] Thus, *E. scaber* has tremendous reputation in indigenous traditional system of medicine in India by virtue of which it has drawn attention and concern of scientists for validation of its medicinal properties through phytochemical and pharmacological evaluation. Most of the plants used in herbal medicine practices, used by plant healers of remote villages and primitive aborigines have not yet been completely investigated for their phytochemical constituents and pharmacological activities.

Figure 1: Showing *Elephantopus scaber* plant

Antioxidant compounds in food play an important role in human health, which delays or inhibits oxidative damage of the biomolecules. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl.[7] Herbal plants considered as a good antioxidant since ancient times. A great number of aromatic and other medicinal plants contain chemical compounds exhibit antioxidant properties. Sources of

natural antioxidants are primarily the plant phenolics that may occur in all parts of plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks.[8] With these viewpoints, the presented study was conducted with the main purpose of quantitative analysis of phytochemicals and evaluation of *in-vitro* antioxidant activity of leaf extract of *E. Scaber*.

Materials and Methods

Collection Leaves of *E. scaber*

The leaves of *E. scaber* were purchased from local vegetable market in Chikkaballapur, Karnataka, India. The leaves were gently and thoroughly washed with running tap water to remove the dirt particles and wiped off, and sprayed with ethanol, and then shade dried. The dried leaves were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.

Extraction

Approximately 50 g of dried and coarsely powdered leaves of *E. scaber* were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 500 mL of methanol. The extract was concentrated by distilling the solvent in a rotary flash evaporator and dried at 40ºC. The extract was preserved in airtight containers and stored at room temperature until further use.

Quantitative Estimation of Phytochemicals

Total phenolics

The concentration of total phenolics in the methanolic leaf extract of *E. scaber* was determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, and its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium.[9] The phenolic content of the extract was determined from calibration curve which was made by preparing

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gallic acid solution (0-0.8 mg/ml) in distilled water and was expressed in mg gallic acid equivalent/g of extract powder.

Total flavonoids

Aluminum chloride colorimetric method was used for flavonoids determination in methanolic leaf extract of *E. scaber*.[10] The flavonoid content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoid was expressed in terms of mg gallic acid equivalent/g of extract powder.

Antioxidant Assay

The modified literature protocol of Blois was used for antioxidant assay.[11] Briefly 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution (1mL;1mM) was prepared in methanol and mixed with methanolic leaf extract of *E. scaber/standard* butylated hydroxytoluene (BHT) (3mL, containing 20-100ug) in double distilled water. The control was also run which contains only double distilled water. The hydrogen atom or electron donation abilities of extract and standards were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl1-picrylhydrazyl (DPPH). The absorbance was measured at 517 nm after 30 min incubation. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. Radical scavenging activity was expressed as the inhibition percentage of free radical by the samples/standard and was calculated using the following formula; Inhibition% = (Control abs – Extract abs / Control) × 100. The IC_{50} value was determined by using linear regression equation *i.e.*, Y = Mx + C; Here, Y = 50, M and C values were derived from the linear graph trendline.

Results

Quantitative estimation of phytochemicals

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Quantitative estimation of phytochemicals in methanolic leaf extract of *E. scaber* was represented in Table 1 and plotted in Figure 2. Results revealed that total polyphenols quantity was found to be highest (28.46 GAE) in methanolic leaf extract of *E. scaber* when compared with total flavonoid quantities (11.53 GAE).

Table 1: Quantitative analysis of phytochemicals present in methanolic leaf extract of *E. scaber*

Phytochemical Components	**Methanolic Leaf extract of *E. scaber***
Total flavonoids	11.53 GAE
Total phenolics	28.46 GAE

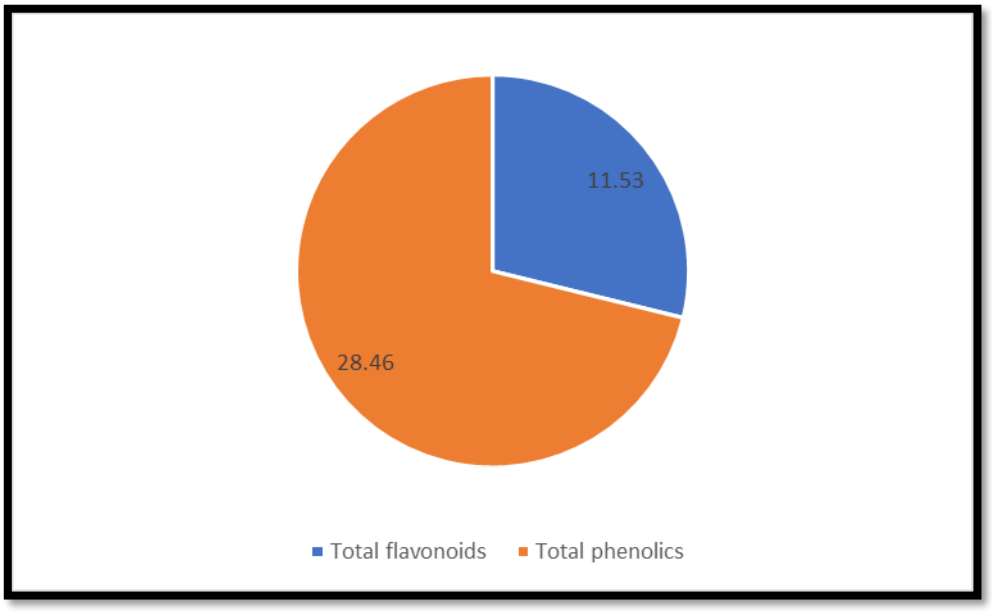

Figure 2: Quantitative analysis of phytochemicals present in methanolic leaf extract of *E. scaber*

Antioxidant assay

The results of effect of methanolic leaf extract of *E. scaber* on DPPH radical-scavenging activity was represented in Table 2 and plotted in Figure 3. Results depicted that methanolic leaf extract of *E. scaber* exhibited inhibition percentage of 33.42%, 49.09%, 74.96%,

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81.07%, and 93.09% at a concentration range of 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, and 100µg/ml, respectively with an IC_{50} value of 58 µg/ml in comparison with the standard BHT with an IC_{50} value of 66 µg/ml.

Table 2: Effect of methanolic leaf extract of *E. scaber* and BHT on DPPH radical-scavenging activity

Conc. of Methanolic Leaf extract of *E. scaber* (µg/ml)	**Inhibition (%)**	**Conc. of BHT (µg/ml)**	**Inhibition (%)**
20	33.42 ± 0.15	20	36.25 ± 0.13
40	49.09 ± 0.09	40	54.11 ± 0.18
60	74.96 ± 0.19	60	81.40 ± 0.24
80	81.07 ± 0.23	80	88.83 ± 0.34
100	93.09 ± 0.41	100	94.40 ± 0.44
IC_{50} (ug/mL) =58.00		IC_{50} (ug/mL) = 66.00	

Values were expressed as Mean ± SD; n=3

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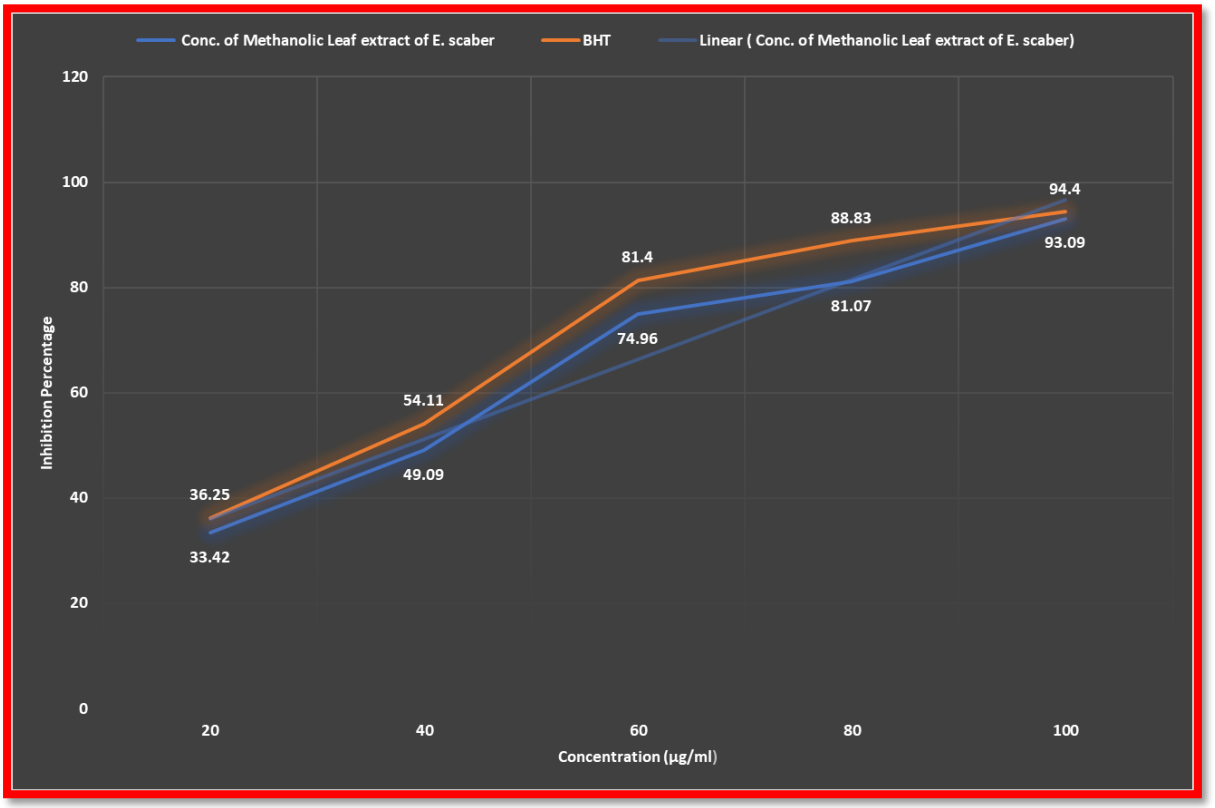

Figure 3: Effect of methanolic leaf extract of *E. scaber* and BHT on DPPH radical-scavenging activity

Discussion

Due to the biologically advantageous effects of plant-based products resulting from the antioxidant activities of phenolic phytochemicals, active research on plant-based products has been driven in recent years. Because they have no harmful effects on people, and hence plant products are preferred to synthetic compounds in the treatment of diseases. In India, there are many different traditional medical systems that rely heavily on local plant species for their raw drug materials. As a result, it's important to consider traditional medicines as potential new therapeutic agents. Moreover, among ancient civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. *E. scaber* has been traditionally used as

medicine to treat rheumatism, diarrhoea, gout, eczema, gum infections, toothaches, spider and snake bites. The extracts or compounds from *E. scaber* have been shown to have antibiosis, antivirus, and cytotoxicity actions in previous bioactivity studies. With this scenario, the present study was conducted with the main purpose of quantitative analysis of phytochemicals and evaluation of *in-vitro* antioxidant activity of methanolic leaf extract of *E. Scaber.*

DPPH is a stable free radical, which has been widely accepted as a tool for estimating free radical scavenging activities of plant extracts. One of the reasons is that this method is simple and highly sensitive. The antioxidant effect is proportional to the disappearance of DPPH in test sample. DPPH accepts an electron or hydrogen radical to become a stable diamagnetic molecule. This reaction is stoichiometric with respect to the number of hydrogen atoms absorbed, or to the number of electrons captured. Then, colour changing from purple to yellow is the consequence of the reducing ability of antioxidant toward DPPH stable free radical.[12] The reduction capability of DPPH radical is determined by the decrease in absorbance at 517 nm by the antioxidants present in the extracts. Our study results on effect of methanolic leaf extract of *E. scaber* on DPPH radical-scavenging activity exhibited percentage inhibition of 33.42%, 49.09%, 74.96%, 81.07%, and 93.09% at the concentration of 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, and 100µg/ml respectively with an IC_{50} value of 58 µg/ml which was at par with the standard BHT with an IC_{50} value of 66 µg/ml. Furthermore, quantitative estimation of phytochemicals in methanolic leaf extract of *E. scaber* revealed the presence of high quantity of polyphenolic compounds (28.46 GAE) compared with flavonoids (11.53 GAE).

Literature reports evidenced that phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals.[13] Vinson et al., conclusively shown close relationship between total phenolic content and antioxidative activity of the fruits and vegetables.[14]

Flavonoids are versatile bioactive secondary metabolites present in almost all plant species. Most representative family members include flavones, flavanes, flavonols, catechins, and anthocyanidins. Their antioxidant potential toward ROS depends on structural characteristics such as the number and substitution pattern of hydroxyl groups and the extent at which these groups are glycosylated.[15] In accordance with the literature findings, the antioxidant activities of methanolic leaf extract of *E. scaber* could be ascribed to the presence of total phenolic and flavonoid compounds.

The results obtained in the present study are encouraging as this study evidenced that the methanolic leaf extract of *E. scaber* contains abundant quantities of secondary metabolites *viz.* polyphenols and flavonoids. Moreover, methanolic leaf extract of *E. scaber* exhibited considerable antioxidant properties. Hence this study supplies as evidence-based study for methanolic leaf extract of *E. scaber* could be exploited in the management of various human ailments related to oxidative stress.

Conclusion

In conclusion, methanolic leaf extract of *E. scaber* has potential to exhibit antioxidant properties. The antioxidant properties of *E. scaber* could be ascribed to secondary metabolites mainly polyphenols and flavonoids present in it. Hence, the methanolic leaf extract of *E. scaber* could be explored for the development of natural antioxidant agents. However, further *in-vivo* studies are recommended to confirm the safety, efficacy and possible mechanism of action antioxidant activities of *E. scaber*.

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