QUANTIFICATION OF SELECTED PHYTOMETABOLITES AND ANTIOXIDANT ACTIVITY OF Elephantopus scaber L

Moumita Chatterjee

Institute of Wood Science and Technology, 18th cross Malleswaram, Bangalore-560003

DOI: http://dx.doi.org/10.24327/ijrsr.2020.1104.5235

ABSTRACT

The present study focused on evaluation of selected phytometabolites and in vitro antioxidant activity of Elephantopus scaber L. While quantifying the phytometabolites it is observed that the plant is good source of phytometabolites such as Chlorophyll content (0.171 mg/g), Ascorbic acid concentration (53.16 ppm), Phenol content (168.16 µg/ml), Flavonoids content of (220.6 µg/ml), Tannin content (1060 µg/ml), Lycopene (0.11307 mg/100mg) and β-Carotene (1.04508 mg/100mg) etc. Antioxidant activity of extract was expressed as percentage of DPPH radical inhibition. Further studies of this plant species should be directed to carry out in vivo studies of its phytometabolites in order to prepare potent natural pharmaceutical products.

INTRODUCTION

Understanding medicinal potential of a plant in the surroundings of man has been the subject addressed by him almost since the dawn of his consciousness as a part of his struggle for existence. The direct ‘man-plant’ relationship formed the basis for discovery of different types of species serving as source of food, medicine, fodder, fuel, fibre, floss, timber and other phytoresources essential for sustenance of life. Concomitantly with socio-cultural advancement science and technology started exploring the chemical weapons produced by plants to survive and reveal their pharmacological effects and bioactivities. While doing so, series of secondary metabolite were identified belonging to such categories as alkaloids, phenols, flavonoids, tannins, terpenoids and glycosides etc. that can thrill, kill and heal man(Borneo,et al., 2008,Katalinić, Miloš, Kulišić&Jukić, 2004,Mulabagal&Tsay, 2004). Scientific endeavours started establishing their pharmacological effects that paved the pathway for isolation, attenuation, characterization, therapeutic proving and hence resulted in discovery of active principles constituting medicines suitable for use against certain ailments/diseases. Elephantopus scaber by virtue of its inbuilt medicinal attributes has adhered to the concern of traditional herbal practitioners as well as phytochemists and modern medical scientists. The plant is of Indian origin that has been tested and appreciated by men of ethnomedicine for use against certain common as well as refractory diseases. Elephantopus scaber L. belongs to the most advanced dicot familyie Asteraceae is a reputed medicinal plant for being used extensively by tribal communities almost all over India. A review of literature reveals a wide range of phytochemicals with considerable therapeutic properties (Das & Mukherjee, 2015, Das & Mukherjee, 2014). It is traditionally used by the herbal healers which have presently been drawing the attention of scientists and researchers. Intake of antioxidant substances largely prevents the detrimental effects of disturbed antioxidant-prooxidant balance. (Ghosh, et al., 2008, Ogjanović, et al., 2008). Sheeba, et al., (2012) has investigated in vitro antioxidant activity by determining superoxide scavenging, hydroxyl scavenging and Fe2+-ascorbate induced lipid peroxidation inhibiting activity of methanolic extract of E. scaber root, which was found to be a scavenger of superoxide with an IC50 of 48±5 μg mL−1 and inhibited hydroxyl radicals generated by Fe3+/ascorbate/EDTA/H2O2 system with an IC50 of 72±12 μg mL−1. There was lipid peroxidation inhibiting activity was with an IC50 of 103±18 μg mL−1. In vivo experiments showed that administration of methanolic extract of E. scaber root significantly (p = 0.05) restored the activities of the antioxidant enzymes SOD, catalase and peroxidases and the level of glutathione to near normal compared with the corresponding
CCl4 intoxicated group. Koppula & Ammani, (2011) have studied the total phenolic content and antioxidant activity of methanol extract of several concentrations of E. scaber ranging from (100-500 μg mL−1). The extracts showed significant antioxidant activity. The antioxidant activity increased with increasing concentration of extract. Ganga, Venkateswara, Pavanı&Dasari,(2012) investigated the phenolic content in hydro-alcoholic, hexane, ethyl acetate and methanolic fractions of leaves and it was found to be 4.49, 3.39, 8.76 and 3.34 mg g−1, respectively. Among the selected fractions ethyl acetate fraction showed high phenolic content. The four extracts also possessed concentration dependent inhibition using DPPH, superoxide and hydroxyl radicals scavenging activity. It was found that the ethanolic extract of E. scaber showed high DPPH scavenging activity with SC50=12.4 μg mL−1. Furthermore, the extract also strongly inhibited xanthine oxidase activity with IC50 value of 93.1 μg mL−1, since XOD catalyses the oxidation of hypoxanthine and xanthine to uric acid which play a crucial role of gout (Pongpiriyadacha, Nuansrithong & Sirintharaweche, 2009).The present author felt the necessity to undertake a study based on quantification the few selected phytometabolites which has medicinal properties and antioxidant activity.

Study Site
Golapbagan is the alternative name of the city, which remains in use since the British period which is located at 23° 15’08.82” N and 87° 50’51.03” E. Golapbag Campus of Burdwan University Located at It has an average elevation of 40 meters above sea level (131 ft). The city is situated 1100 km from New Delhi and a little less than 100 km north-west of Kolkata on the Grand Trunk Road (NH-2) and Eastern Railway. The Campus is rich in phytoresearches as therepresent numerous individual trees of Barringtonia acetangula, Polyalthia longifolia, Swietenia mahagogo ni, Drypetes Roxburghii, Saraca asoca, Markhamia stipulata, Manilkara hexasandra, Aphananthera polystachia, Albizia saman, Naringia renulata, Pongamia pinnata and several others in the garden.

Materials and Methods

Sample Preparation

Mature disease free leaves of Elephantopus scaber (Asteraceae) was collected from Golapbag campus of Burdwan University. The plant is identified by Prof. Ambarish Mukherjee and voucher specimen was kept in the herbarium of Burdwan University (BURD) for future reference. The mature leaves were washed thoroughly under running tap water and distilled water, blotted dry and then dried by keeping inside hot air oven at 50°C temperature used for immediate extraction using HPLC grade methanol.

Estimation of Total chlorophyll content

Total chlorophyll content (TCh) The chlorophyll content was determined by the colorimetric method (Arnon, 1949). Firstly 3g of fresh leaves were blended in a mortar and pestle and then extracted with 20 ml of 80% acetone and left for 15 minutes. The liquid portion was centrifuged at 2,500 rpm for 3 minutes. The supernatant was separated from the pellet and absorbance was taken at 645 nm and 663nm for Chlorophyll a and Chlorophyll b using a spectrophotometer. Calculations were done using the formulae given below: Chlorophyll a = \( \frac{[(12.7 \times O.D645 – 2.69 \times O.D663) \times V]}{(1000 \times W)} \) mg/g Chlorophyll b = \( \frac{[(22.9 \times O.D645 – 4.68 \times O.D663) \times V]}{(1000 \times W)} \) mg/g Total chlorophyll content (TCh) = Chlorophyll a + Chlorophyll b (mg/g) where, V = Total volume of the chlorophyll solution (ml) and W = Weight of the tissue extracted (g).

Leaf pH

In order to measure the pH of the leaf extract, 1g of the fresh leaves was homogenized in 10ml of deionised water and filtered. The filtrate was added to the pH meter with buffer solution of pH 4 and 9 (Singh & Rao, 1983).

Estimation of Ascorbic acid content

Ascorbic acid content (AA) In order to determine the ascorbic acid content, colorimetric method was followed (Roe, 1961). Firstly, 10 ml of 6% trichloroacetic acid (TCA) was added to 1g of the fresh foliage leaves in mortar and pestle to homogenize and then centrifuged at 5000 rpm for 5 minutes. The supernatant was separated and to it, a pinch of activated charcoal was added and filtered. The volume of the filtrate was made up to 100 ml by adding distilled water. 5 ml of diluted supernatant was taken in a test tube and mixed with 3 ml of 2% 2, 4 - DNPH in 9 (N) H2SO4, to which 1-2 drops of 10% thiourea solution in 70% ethanol was added and was boiled for 15 minutes in water bath and cooled at room temperature. To each sample, 5 ml of 80% H2SO4 was added at 0°C. The absorbance was measured at 530nm with a colorimeter after 30 minutes. The concentration of unknown samples was extrapolated from a standard ascorbic acid solution of 50ppm using the formula: Concentration of unknown solution = (Concentration of standard solution x O.D530 of unknown) / O.D530 of standard solution.

Estimation of Total Phenol

The total phenolic content was determined spectrophotometrically by the Folin Ciocalteu method described by Singleton, Orthofer&Lamuela-Raventos, 1999
with some modifications. Briefly, 1ml of sample was mixed with 9ml of distilled water. Then 1ml of 1:10 Folin-Ciocalteu reagent is added and shaken gently. After 5 min 10ml of 7% Na₂CO₃ solution was added to the mixture and volume made as 25ml. After 90 minutes of incubation in the dark at room temperature, the absorbance of the reaction mixture was measured at 760 nm. The total phenolic content was expressed as µg/ml gallic acid using calibration curve.

**Estimation of Total Flavonoids**

Total flavonoid content was determined by using aluminium chloride colorimetric assay1 with some modifications. 1ml of extracts was added to the 4ml of distilled water. Then, 0.3ml of 5% NaNO₂ was added to the mixture and allowed to stand for 5 minutes. Then 0.3ml of 10% AlCl₃ was added and again allowed to stand for 5 minutes. Then, 2ml of 1M NaOH solution was added to the mixture and the mixture dialuted to 10ml with distilled water. The mixture was incubated at room temperature (25°C) for 5 minutes. The absorbance was measured at 510 nm. Gallic acid was used as the standard and the absorbance values were expressed as µg/ml Gallic acid equivalents. All the measurements were taken in triplicate and then, the mean values were calculated.

**Estimation of Total Tannins**

The tannins were determined by Folin-Ciocaltue method. About 0.1ml of sample extract was added to a volumetric flask (10ml) containing 7.5ml of distilled water and 0.5ml of Folin-Ciocaltuephenol reagent, 1ml of 35% Na₂CO₃ solution and dilute to 10ml with distilled water. The mixture was shaken well and kept at room temperature for 30 minutes. A set of reference standard solutions of gallic acid (20, 40, 60, 80, 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725nm with an UV/Vis. Spectrophotometer. The tannin content was expressed in terms of µg of GAE / ml of extract.

**Total phenolic content**

The total phenolic content evaluated by the Folin-Ciocaltue method which has been expressed as Gallic acid equivalent concentrations µg/mL. The Gallic acid calibration curve (y = 0.003x + 0.068, R² = 0.939) was plotted by taking the absorbance readings for Gallic acid from 20 µg/ml to 100 µg/ml.

**Total flavonoid content**

The results of the evaluation of the total flavonoid content of the examined plant extract which has been expressed as a Quercetin equivalent concentration µg/ml by plotting the Quercetin calibration curve (y = 0.003x – 0.008, R² = 0.983).

**Total tannin content**

The results of the evaluation of the total tannin content of the examined plant extracts have been presented in Table 4. The total flavonoid content has been expressed as a gallic acid equivalent concentration µg/ml by plotting the gallic acid calibration curve (y = 0.001x – 0.020, R² = 0.958).

**β-carotene and lycopene content**

The dried extract was vigorously shaken with 10ml acetone-hexane mixture (4:6) for 1 minute and was filtered through Whatman No.4 filter paper. The absorbance was measured at 453nm, 505nm, 663nm (Chandra,Balamurugan, Thiripura&Rekha,2012). Contents of β-carotene and lycopene were calculated according to the following equations:

\[\text{Lycopene (mg/100mg)} = -0.0458 \times A_{663} + 0.372A_{505} - 0.0806 A_{453}\]

\[\text{β-Caratene(mg/100mg)} = 0.216 A_{663} - 0.304 A_{505} + 0.452A_{453}\]

**Estimation of Antioxidant activity by DPPH method**

Determination of the free radical scavenging activity of the extract was carried out using the assay described by Tekao, Watanabe, Yagi & Sakata, 1994 and Kumarasamy, et al., 2007 with some modifications.

10gm dried leaf powder extracted in 100 ml of 80% HPLC grade methanol, defatting was done using hexane. Finally the extract was dried and made ready for analysis. The crude extract of methanol concentrations of 1.0 mg/ml was mixed with 2 ml of the 2, 2-DPPH in methanol. The solution was incubated for 30 min at room temperature in dark before reading the absorbance (A) at 517 nm. As a positive control, 1 ml of methanol solution was used with the 2, 2-DPPH in methanol. The radical solution (2 ml; 0.004 g per 100 ml methanol) was added to a test tube and 1 ml of the dissolved extract was added prior to the measurement. In its radical form, the 2, 2-DPPH absorbs light at 517 nm but upon reduction by an antioxidant or radical species, the absorption decreases. The decrease in absorbance was then, converted to percentage antioxidant activity using the formula shown below:

\[\% \text{DPPH scavenging} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\]

**RESULTS AND DISCUSSION**

During this quantification Studies we have quantified ten different phytochemicals. Mature leaves possess Chlorophyll a and Chlorophyll b was 0.061 mg/gand 0.110 mg/g respectively. Totalchlorophyll content (0.171 mg/g) is moderate. Those plant species having low chlorophyll content that means they experienced high air pollution while concentration of NO₂induces the chlorophyll production (Pandey&Agrawal, 1994).

Leaf pH is 7.55 i.e basic in nature; high pH increases the efficiency of conversion of a hexose sugar to ascorbic acid (Escobedo, Wagner & Nowak,2008).

The Ascorbic acid concentration of the leaf is 53.16 ppm. Ascorbic Acid i.e Vitamin C is potent antioxidant compound which increases the tolerance of the plants to oxidative stresses aswell as it affects the physiological activities of the plants such as cell wall synthesis, photosynthetic carbon fixation, defense and cell division [Seyyednjad,Majdian, Koochak&Niknejad,2011].

Phenol content of the leaves is 169.2µg/ml(Fig 3 and Table 1).
Fig 3 Standard curve of Phenol

Table 1 Total Phenol Content

<table>
<thead>
<tr>
<th>Sample</th>
<th>Value (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf extract</td>
<td>169.2</td>
</tr>
</tbody>
</table>

Total Flavonoids content of the leaves i.e1233.2µg/ml. (Fig 4 and Table 2)

Fig 4 Standard curve of Flavonoid

Table 2 Total Flavonoid content

<table>
<thead>
<tr>
<th>Sample</th>
<th>Value (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf extract</td>
<td>1233.2</td>
</tr>
</tbody>
</table>

Total Tannins content of the leaves i.e3210 µg/ml. (Fig 5 and Table 3)

Fig 5 Standard curve of Tannins

Table 3 Total Tannins content

<table>
<thead>
<tr>
<th>Extract</th>
<th>Value (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf extract</td>
<td>3210</td>
</tr>
</tbody>
</table>

Leaf possess Lycopene and β-Carotene0.002597mg/100mg and 0.045738mg/100mg respectively.

Antioxidant activity: Stable radical DPPH has been widely used in the determination of the antioxidant activity of plant extracts. A reduction of the 2, 2-DPPH radicals can be observed by a decrease in the absorbance at 517 nm. Percentage inhibition values were calculated by considering methanol as the control (Table 4). Based on Percentage values of 2, 2-DPPH radical scavenging activity it can be conclude that plant extract has promising antioxidant property.

Table 4 Percentage of 2, 2-DPPH radical scavenging activity

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic Leaf Extract</td>
<td>84.17 ± 0.002</td>
</tr>
</tbody>
</table>

High concentrations of phenols from phytoextract can be obtained using polar solvents (Čanadanović-Brunet, et al., 2008). Phenols (Tosun, et al., 2009) and Flavonoids (Sharififar, Nudeh-Dehghn&Mirtajaldini, 2008) are class of secondary plant metabolites with significant antioxidant activity. Moreover potentiality depends on the structure and substitution pattern of hydroxyl groups of flavonoid compound. Methanolic extracts from Elephantopusscaber have high concentration of total phenols (Table 1) and flavonoids (Table 2), with potent antioxidant activity (Table 4) of the extract.

CONCLUSION

Now-a-days, plant based drug discovery has gained a great attention globally to develop new pharmaceuticals, findings of the present work speaks the great value of Elephantopusscaber. Based on this information, it could be concluded that this plant is natural sources of antioxidant substances of high importance. The leaves of Elephantopusscaber, are a very good source of phytochemicals such as phenol, Flavonoids, tannins, Ascorbic acid, Lycopene and β – Carotenes. High concentration of phenolic compounds in the extract was obtained using solvents of high polarity; the methanolic extract manifested greater power of extraction for phenolic compounds from the studied plant. Ascorbic acid possess antioxidant activity also it inhibits the sunburn. Further studies of this plant species should be directed to carry out in vivo studies of its phyto metabolites in order to prepare potent natural pharmaceutical products.

Acknowledgement

I hereby acknowledge The Head, UGC-CAS (phase II) Department of Botany, University of Burdwan, West Bengal for providing me infrastructural support.

Reference


********

How to cite this article:

38078 | P a g e