ISSN 2583– 0260 (online) *ISSN 2394 -7837 (print)*

PHYTOCHEMICAL EVALUATION AND DEVELOPMENT OF QUALITY CONTROL PARAMETERS IN *ELEPHANTOPUS SCABER* L.

Neethu S. and Pillai Lakshmi Sreekumar

Received: 7/6/2022 **Revised: 25/6/2022** **Accepted:28/62022**

Abstract

In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing as well as in developed countries owing to its natural origin and lesser side effects. Now, medicines are being manufactured on the large scale in pharmaceutical units, where manufactures come across many problems such as availability of material, authentification of material, proper standardization methodology of single drugs and formulation and quality control parameters. Standardization involves organoleptic, physical, chemical, biological and botanical assessments. The plant *Elephantopus scaber* belonging to family Asteraceae is selected for present study. This plant is native to tropical Africa, but it is found almost all regions. The whole plant was shade dried and ground to fine powder followed by organoleptic, fluorescent and physicochemical analysis. The plant powder was subjected tosoxhlet extraction using methanol. Phytochemical screening of the plant extract was done according to the standard biochemical procedures. Antioxidant activity of the plant extract was tested using DPPH assay. Organoleptic analysis revealed colour, odour, taste and texture of *Elephantopus scaber*. In fluorescence analysis, on treatment with different solvents, colour change could be noticed in the plant powder. Phytochemical analysis revealed a number of phytochemicals such as tannins, phenolics, coumarins, lignin, terpenoids etc. in the plant extract. The plant possessed a good antioxidant potential which was comparable to standard BHT. Determination of the natural antioxidants from plant extracts will help to develop new drug candidates for antioxidant therapy. As the use of synthetic antioxidants is being restricted by food regulation agencies such as FAO, the specific antioxidant compounds from the plant could be exploited as nutritional supplements. However, further studies are needed to clarify the *in vivo* potential of *Elephantopus scaber* in the management of human diseases.

Keywords: Elephantopus, Pharmacognosy, Fluorescence, DPPH, Atioxidant

Introduction

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. Medicinal plants are considered as a rich resource of ingredients which can be used in drug development either as pharmacopoeial, non-pharmacopoeial or synthetic drugs. Apart from that, these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants are considered as important source of nutrition and as a result of that they are recommended for their therapeutic values (Hina, 2016). 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 (Prasad, 2002). 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 snakes bite and jaundice treatments (Pushpangadan, 2006).The plant selected for present investigation is *Elephantopus scaber* which is an erect herb up to 80 cm tall. The plant is a native to Tropical Africa, Eastern Asia

Department of Botany, N.S.S. College Pandalam
Pathanamthitta, Kerala, India, 689501

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 (Rupali et al., 2015). The whole plant of *Elephantopus 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 (Peer, 1980 and Tsai, 1999). The root decotion of *Elephantopus scaber* is widely used to treat diarrhoea, dysentery, stomach troubles and blood vomiting in tuberculosis in Nepal (Ahamed *et al.,* 2009 and Ho *et al.,* 2009). Sesequiterpenes lactones, 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 (Raj *et al.,* 2002). Sesquiterpene lactones such as deoxyelephantopin, isodeoxyelephantopin, scabertopin and isoscabertopin have been found to be prominent anticancer constituents (Farha *et al.,* 2013).

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. 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. *Elephantopus scaber* is one such plant used in the folklore herbal medicine practices in the villages of South Kerala. The current study attempts to investigate the phytochemical profile of the plant, through the evaluation of its biological activity.

Materials and Methods

Plant material

The plant selected for study, *Elephantopus scaber* was collected from Kottarakkara, Kollam. The aerial parts of the plant were used for present study.

I. Powder analysis

Fresh plant of *Elephantopus scaber* was collected in polythene bag. Dirt was removed from the collected material. It was shade dried and then powdered in an electric grinder and sieved with fine mesh sieve. The powder was then used for the organoleptic study and solvent extraction.

A. Organoleptic study

Organoleptic (literally "impression on the organs") refers to the evaluation by means of the organs of sense and includes the macroscopic appearance of the plant material, its colour, odour, and taste, occasionally the sound of 'snap' of its fracture and the 'feel' of the powder to the touch (Wozniak *et al*., 1997). The plant powder characteristics like the colour, odour, taste and nature were evaluated.

B. Fluorescence analysis

The crude drug powder was treated as such with eight different reagents. The solvents used were water, hydrochloric acid, sulphuric acid, nitric acid, sodium hydroxide, acetic anhydride, methanol and acetone. Each solution was loaded on an activated thin gel layer slide and the fluorescence under normal light, short UV (256nm) and long UV (365nm) was observed (Chase and Pratt, 1949).

C. Physicochemical characterization

Different physicochemical parameters were determined according to the official methods and guidelines on quality control for medicinal plant materials.

1. Loss on drying (Indian Pharmacopoeia, 1992)
2. Foaming index (WHO, 1992)
3. Swelling index (WHO, 1992)
4. Foreign matter (Indian Pharmacopoeia, 1996)
5. P^H (Iqbal et al., 2010)

II. Phytochemical Screening

Preparation and yield of extract (Indian Pharmacopoeia , 1996)

About 15g of the powdered plant material was subjected to extraction by Soxhlet apparatus using 100ml methanol. The extract was concentrated under reduced pressure and preserved in refrigerator until further use. The percentage of the crude extract was determined using the following equation.

Percentage yield (%) =

$$\frac{Weight\ of\ the\ crude\ extract}{Weight\ of\ the\ sample} \times 100$$

B. Qualitative analysis (Harborne, 1973)

1. Tannins
2. Saponins
3. Flavonoids
4. Alkaloids
5. Terpenoids
6. Phlobatannins
7. Glycosides
8. Simple phenolics
9. Coumarins
10. Quinones
11. Acids
12. Flavonols
13. Lignin
14. Steroids
15. Gums and Mucilage

C. Quantitative analysis

1. Determination of alkaloids (Harborne, 1973)
2. Determination of phenolics (Spanos and Wrolstad, 1990)

Bioactivity study - Antioxidant activity DPPH (2-2- Diphenyl-1-Picrylhydrazyl) assay (Brand-Williams *et al*., 1999)

About 1ml of 0.135mm DPPH prepared in methanol was mixed with 1ml of methanol extract ranging from 20-80µg/ml. The reaction mixture was vortexed thoroughly and left in dark at room temperature for 30 minutes. The absorbance was measured spectrophotometrically at 517nm. BHT (Butylated Hydroxy Toluene) was used as standard.

The scavenging ability of the plant extract was calculated using the equation:

Percent of inhibition (%) =

$$\frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

Results and Discussion

A. Organoleptic Study

Colour : Light green
Odour : Pleasant
Taste : Tasteless
Texture : Granular powder

Organoleptic evaluation is a qualitative method wherein the pharmacognist uses his sense organs to study the characteristic feature of crude drug, especially the crude drugs of plant origin such as colour, texture, odour, taste and so on (Selvam, 2010)

B. Fluorescence analysis

Fluorescence is the phenomenon exhibited by various chemical constistuents present in the plant material under UV light (Pandya, 2011). Fluorescence analysis is used to characterize the crude drugs. It is also one of the pharmacognostic procedures useful in the identification. The dry powder was subjected to fluorescence analysis with different reagents in normal light, short UV and long UV. The colour changes are summarized (Table 1).

C, Physicochemical characterization

The physicochemical analysis of plant drugs is important for detecting adulterations or improper handling of drugs (Raad, 2014). A total of five physicochemical parameters were evaluated in *Elephantopus scaber* (Table 2). The plant moisture content was reported in low amounts. Forming index was more than 100ml. The pH was found to be 6.6. Foreign matter and swelling index were not observed.

II. Phytochemical Screening

Phytochemical screening is a method of bioactive compound identification that is unknown in plant extracts through qualitative analysis.

A. Yield Extract

The methanol extract was prepared by Soxhlet extraction. The yield of the methanol extract was 4.1%. The major step involved in phytochemical screening is extraction. Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent.

B. Qualitative Analysis

The plant *Elephantopus scaber* contains phytochemicals have antioxidant which help in fighting against many diseases. A total of 15 phytochemicals were qualitatively analysed in methanol extract of the plant. Most of the compounds were present in the extract. Phlobatannins, steroids, gums and mucilage were absent in the plant extract. The phytochemical screening tests are provided in table 3.

C. Quantitative analysis

The quantitative analysis of two phytochemicals was done in the methanol extract of *Elephantopus scaber* (Table 4) by standard procedures. The amount of alkaloid was lesser than the amount of phenols.

D. Bioactivity study - Antioxidant activity

1. DPPH [2,2-Diphenyl-1-Picrylhydrazyl] Assay (Brand-Williams *et al.,*1999)

The scavenging activity of the extract was compared with that of BHT standard and percentage inhibition was calculated (Graph 1). The percentage inhibition of the DPPH radical by the methanol extract of the plant increased with increase in concentration. In this assay the methanol extract of *Elephantopus scaber* had good DPPH scavenging activity with IC_{50} value of 38.2µg/ml. The IC_{50} value of BHT was 35.8µg/ml. The results are shown in Table 5.

A rapid, simple and inexpensive method to measure the antioxidant capacity involves the use of the free radical, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) which is used to test the ability of compound to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity (Kirtikar *et al.,* 2006). The DPPH assay method is based on the DPPH, a stable free radical. When antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor and is reduced to the DPPHH and as consequence the absorbance decreased from the DPPH (Harborn, 1998).

Table 1. Fluorescence analysis of *Elephantopus scaber*

Powder + Reagent	**Visible (400-800nm)**	**UV short (256nm)**	**UV long (365nm)**
Powder as such	Yellowish green	Brownish green	Golden yellow
Powder + H_2O	Green	Dark green	Blackish green
Powder + Conc. HCl	Purple	Dark green	Purple
Powder + Conc. H_2SO_4	Pale green	Bluish green	Bluish green
Powder + Conc. HNO_3	Green	Green	Blackish green
Powder + NaOH	Brownish green	Brownish green	Blackish green
Powder + Acetic anhydride	Greenish black	Dark green	Black
Powder + MeOH	Green	Dark green	Black
Powder + Acetone	Dark green	Green	Green

Table 2. Physicochemical characters of *Elephantopus scaber*

Parameters	**Values**
Loss on drying	11.30% ± 0.16
Foaming index	>100ml
Swelling index	NIL
Foreign matter	NIL
pH	6.6

Table 3. Phytochemicals tested in *Elephantopus scaber*

Sl. Number	**Phytochemicals**	**Present / Absent**
1	Tannins	+
2	Saponins	+
3	Flavonoids	+
4	Alkaloids	+
5	Terpenoids	+
6	Phlobatannins	-
7	Glycosides	+
8	Simple phenolics	+
9	Coumarins	+
10	Quinones	+
11	Acids	+
12	Flavanols	+
13	Lignin	+
14	Steroids	-
15	Gums and mucilage	-

Table 4. Quantitative estimation of *Elephantopus scaber*

Sl. Number	Phytochemicals	Amount (mg/g)
	Alkaloids	0.55
	Phenols	36.4

Table 5. DPPH antioxidant assay of *Elephantopus scaber* extract

Sl. Number.	Concentration of extract (µg/ml)	BHT (percentage of inhibition) (%)	Plant extract (percentage of inhibition) (%)
	20	40	36
	40	58	52
	60	85	78
	80	99	96

Acknowledgement

I thank Dr. Venugopal S, the principal, N.S.S. College Pandalam for providing facilities to complete the project. I express sincere gratitude to Dr. Sandhya P, the head of the department, Post Graduate Department of Botany, N.S.S. College Pandalam for providing all necessary facilities and support to carry out this.

References

Harborne JB. 1973. Phytochemical methods. Edn 2. London: Chapman and Hall.

Hina Z. 2016. Phytochemical review on *Vitex agnus-castus*: A potential medicinal plant. *Chinese Herbal Medicines* Vol 8, Issue 1, 24-29.

Indian Pharmacopoeia. 1992. Volume II. Part I. The Controller of Publications, CSIR, New Delhi.

Indian Pharmacopoeia. 1996. Volume II. Part II. The Controller of Publications, CSIR, New Delhi, pp. 53-54.

Iqbal D, Pawar RK and Sharma RK. 2010. Physicochemical standardization of *Butea monosperma* (Lam.) Kuntze (Palasha): An Ayurvedic drug. *International Journal of Pharmaceutical Quality Assurance* 2(1): 49-51.

Kirtikar KR and Basu BD. 1993. Medicinal plants, Vol 2, International book publisher: Dehradun.

Pandya K, Maniar K, Soni H, Bhatt S, Patel P, Solanki B and Gurav N. 2011. Standardization of anti-diabetic ayurvedic herbomineral formulation. *International Journal of Pharmaceutical Sciences Review and Research* 10 (1), 2011, 174-186.

Peer LM and Metzger J. 1980. Medicinal plants of east and southeast asia: Attributed properties and uses. The MIT Press. London.

Prasad LV. 2002. Indian system of medicine and homeopathy traditional medicine in Asia. Chaudhury RR, Rafei UM., editors. New Delhi: WHO- Regional office for South East Asia. pp. 283-286.

Pushpangadan P. 2006. Resurgence of traditional medicine systems with special reference to Indian system of medicine and modern scientific developments. *Journal of Traditional Medicine Clinical Nature* 7(3).

Raad AK. 2014. Physico-chemical parameters, phytochemical screening and antioxidant activity of seeds of *Peganum harmala* collected from Iraq. *Asian Journal of Biomedical and Pharmaceutical Sciences* 4(28): 20-24.

Rupali DR, Shahadat H, Mohammed R. 2015. A review of anticancer potential of *Elephantopus scaber* and its phytoconstituents. Department of Pharmacy, University of Development Alternative, Lalmatia, Dhaka-1207, Bangladesh. Vol 4. Issue 10.

Selvam ABD. 2010. Can the term phytosensology be preferred over the term organoleptic? Additional article information. *Pharmacognosy Research* 2(4): 271-272.

Spanos GA and Wrolstad RE. 1990. Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. *Journal of Agricultural and Food Chemistry* 38: 1565-1571.

WHO. 2004. Guidelines on good agricultural and collection practices for medicinal plants. World Health Organization, Geneva.