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Trace elemental analysis and antimicrobial activities of *Elephantopus scaber* L.

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Abstract
Screening of various Indian medicinal plants has various degrees of antimicrobial activities against pathogenic and opportunistic microorganisms. Since the number of effective exogenous antibiotics is decreasing, concerted efforts are to be made to identify antimicrobial materials from natural products and traditional medicines. In the present study, we are reporting the antimicrobial activity of the traditionally used Ayurvedic medicinal plant *Elephantopus scaber* and its elemental composition analysis. The antimicrobial activities are found to be higher in leaf extracts of *Elephantopus scaber* whereas no inhibition was found for root extracts. The elements such as Si, Ca, Cl, Mg, S, K and P are more abundant in the leaf than the root whereas heavy metals are higher in roots than in leaves. In the root, presence of Al, Fe, Ti, Sr, and V is more when compared to leaves whereas Zn, Cu, As, Rb and Sr are less available and are equally present in roots as well as leaf. The elements Cr, Co, Ni, Se, Br and Pb are not found in both leaves and roots.

Keywords: Trace elemental analysis, antimicrobial activities*, Elephantopus scaber,* Medicinal plant

INTRODUCTION

Human life is deeply associated with plants either as food or drug. Primitive man tried to cure diseases from plants growing abundantly around him. The trial and error experience of such usage helps to learn the medicinal characters of different plants. Among the traditional practices, Siddha, Unani and Ayurveda are very well recognised. India and Nepal are practicing Ayurvedic system of Medicine for more than 2000 years. Many European countries and organizations like National Institute of Health (NIH), USA, and World Health Organization (WHO) have recognized Ayurveda as an alternative and complementary medicine. Around 8,000 species of plants are used as medicine all over the world for the treatment of various diseases and around approximately 30% of the population in developing countries rely chiefly on this traditional treatment. As per the traditional Ayurvedic practice, irrespective of its kind some medicinal characters were found in each and every plant found in the plant kingdom. But quantity of the drug intake decides whether it is drug or poison. The Herbal drugs are considered less toxic and free from side effects than synthetic drugs [1].

Modern science has deep knowledge of physical and biological structure of human body, whereas traditional knowledge systems have a holistic knowledge of the physical and spiritual fields that pervade nature. While accepting the truth behind the physical body, the role of mind and consciousness in health and disease states are emphasized by Ayurveda. Ayurveda is distinctly different from biomedicine, which relates physiology structure of human body. But to explore the scientific truth behind Ayurveda one has to follow the biomedical procedures.

It was emphasized that the concept in Ayurvedic medicine is one's thoughts create health or illness and the quantum physics concept are that thoughts create the physical reality. There is a need to explain the fundamental principles of Ayurvedic medicines in modern context. In order to know the real value of plant genetic resources, screening of plants for their activity is very essential and is an urgent need [2]. The collection and identification of medicinally important plants led to the development of the branch of Pharmacognosy, to the present state in which pharmaceutically important plant products are identified.

The importance of medicinal plants lies in their biological active principles, which are the real healers in the process of medication. There are two types of plant chemicals, primary metabolites such as common sugars, carbohydrates, proteins, amino acids and chlorophyll. These are universally present in all kinds of plants whether medicinal or non medicinal. The other types of chemicals called secondary metabolites include alkaloids, terpenoids and phenolics etc. which do not have an essential role in plant metabolism and vary in their distribution from plant to plant. Secondary metabolites are mostly accumulated in plant cells in smaller quantities than primary metabolites. They are synthesized in specialized cells at particular development stages, making their extraction and purification difficult. These secondary metabolites exert a profound physiological effect on the mammalian system are

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known as the active principles of that plant. The physiological effect of these active principles is used for curing ailments. The crude drugs of plant origin are used in the Indian system of medicine (Ayurveda) and large number of drugs of plant origin is used in Western medicine.

Very small amount of elements present in any biological material are called trace elements which are measured in ppm (µg/ml) in solid substances and ppm (ng/ml) in liquid substances. They have wide implications in different disciplines ranging from biomedical sciences, Geology, Material science, Archaeology etc. With the advent of various instrumental techniques and sophisticated instruments, the possibility of detection of trace elements to very low limits have become possible now a days and which in fact led to detection and study of the role of trace elements in various fields. Being the integral parts of many important biomolecules they perform crucial functions in biological systems. Marginal or severe trace element imbalances can be considered risk factors for several diseases of public health importance.

One of the major applications of trace elements is in the field of biomedical sciences. Starting from the middle of last century there is a growing recognition of trace elements as an important factor in nutrition and disease pathology. Elements are now recognized as a key factor in the pathogenesis of various critical diseases like cancer, diabetes, cardiovascular diseases etc. and also hold a key for their remedies. Deficiencies or overload of minerals or their impaired metabolic pathways lead to abnormal physiological functioning, causing diseases. The significance of these micronutrients arises largely from the fact that they interact and influence the functioning of enzymes and also form the integral part of many enzymes and hormones [3]. The metabolic pathway of all the elements and their interactions with other bimolecules and also their connection with disease pathology is not fully known yet and this offers a wide area of research. Complete understanding of the subject can lead to solve the secrets of many diseases and their cure. Study of medicinal plants in terms of their elemental constitutions also comes in the realm. Plants with known curative properties can throw the light on the efficacy of the minerals in correcting the disorders. Generally accepted definition of essentiality of an element is that dietary deficiency of the element must consistently and adversely change a biological function from optimal, and this change is preventable or reversible by supplementing physiological amounts of the element [4, 5]. The elemental imbalance, an abrupt shift from the normal levels, indicates the wrong functioning of the physiological system irrespective of its reason.

Screening of various Indian medicinal plants has various degree of antimicrobial activity against various pathogenic and opportunistic microorganisms [6–8] and such bioactive plant extracts may also provide some alternative compounds. Antimicrobial activity of medicinal plants against drug resistant bacteria have been reported for *Camellia sinensis, Rosa canina, Scutellaria amoena, Arctostaphylos uva-ursi* and many other plants from various parts of the world [9-10]. The antimicrobial activities in traditionally used Indian medicinal plants are less exposed. The medicinal plant extracts (drug) prepared from various preparations is used for the treatment of wounds, skin diseases, dysentery, cough, cold, jaundice, leprosy etc [11].

The phytochemical constituents of *Elephantopus scaber* L. have been reported as sesquiterpene lactones, elephantopin and scabertopin [12], epofriedelinol, lupeol and stigomasterol [13, 14]. The leaves of the plant were known to be used for diuretic, bronchitis, small pox, diarrhoea and as a brain tonic [15], anti inflammatory and antitumor activity [16.] Many sesquiterpene lactones isolated from the *Asteraceae* members possess antibacterial properties and were used for treating ulcer's and wounds. The roots of *Elephantopus scaber* were broadly used as an antipyretic, cardio tonic and diuretic. Terpenoids and 2,6,23 –trienolide compounds extracted from *Elephantopus scaber* is a potential candidate for diabetes [17].Multi-drug resistance in Gram positive (*Staphylococcus aureus*, *Streptococcus pneumoniae*), Gram negative (*Escherichia coli, Shigella sp, Haemophilus influenzae*) and other bacteria like *Mycobacterium tuberculosis* has been reported from all over the world [18 – 20].

Methicillin resistant *Staphylococcus aureus* (MRSA) has gained much attention in the last decade, as the MRSA is a major cause of hospital acquired infections. β- lactam antibiotics are the preferred drugs against *S. aureus* infections. Since the number of effective exogenous antibiotics is decreasing, concerted efforts are to be made to identify antimicrobial materials from natural products and traditional medicines. In this paper we are reporting the antimicrobial activity of the traditionally used Ayurvedic medicinal plant *Elephantopus scaber* and its elemental composition analysis as a preliminary study of drug analysis.

MATERIALS AND METHODS

Fresh *Elephantopus scaber* L. are collected from Kanyakumari District, the southernmost part of India (8.29302 Latitude and 77.21828 Longitude) washed well in fresh water and shadow dried. The leaves and root are separated and made into fine powder using Mortar and pestle. The petroleum ether extract of the leaf and root are extracted separately by saxhlet setup for 72 hours. The bacterial species namely *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* are subjected to the experiment for antimicrobial activity against pet-ether extract of the said plant leaf and root separately and the inhibition effect observed are tabulated in Table-1, in comparison with the observation of Avani and Neeta [21]. Similar method was followed for Chloroform extract and is tested with the organisms *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*, which is given in Table-2.

To our interest, the traditional healing effect through Ayurvedic practice, in one way, it is based on the presence of trace elements in the human body. The imbalance of trace elements, either excess or less quantity, causes diseases and which can be treated by substituting the required elements. Finding such element in Ayurvedic medicinal plants can be possible only through x-radiation analysis or PIXE analysis. An EDXRF analysis is conducted on the *Elephantopus scaber* leaf and root separately at UGC, DAE, CSR Kolkata. The target was prepared using KBr press of 130 kg/cm^2 for 120 seconds. Four targets of same samples are prepared and subjected to the radiation in order to get an accurate result. The trace elements ranging from Mg to Pb are identified with their presence in the sample at ppm level. The concentrations of elements composition in the leaf and roots of *Elephantopus scaber* observed are given in Table-3.

RESULTS AND DISCUSSION

Antimicrobial analysis

Finding the microbial activity of medicinal plants took an important role in predicting the medicinal characters. To know the

antimicrobial activities of the plant extract, the pathogenic organisms such as *Escherichia coli* and *Pseudomonas aeruginosa*, *Salmonella typhi* have been grown in a separate nutrient agar plate and then 50 µg petroleum ether extract of *Elephantopus scaber* bearing disc has been placed on surface of the media of the 3 plates, incubated at 37°C for 24 hrs. The zone of inhibition from all the plates has been observed, that the extract has antimicrobial activities on the test pathogenic bacteria. The extract concentration of 1 mg/ml, and 2 mg/ml did not show any inhibition effect. The inhibition effect is found to be maximum for *Elephantopus scaber* leaf extract of 4mg/ml (Plate 1) whereas no inhibition was found for Root extract on all the three test bacteria. According to the results of Avani and Neeta [21], no Inhibition was reported for the petroleum ether extract of whole plant of *Elephantopus scaber* on the said bacteria. In comparison with this, present study shows that the compounds responsible for antimicrobial activities are higher in the leaves (Table-1). A zone of inhibition was also found in all the plates of *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* against Chloroform extract at 4mg/ml concentration (Table-2). The experimented plates are photographed and are given in Plate 2. The results obtained are compared with Avani and Neeta [21] and is given in Table-1&2.

Table 1. The antimicrobial activity of pet-ether extract of *Elephantopus scaber* leaf and root in comparison with Avani and Neeta (2005).

Microorganism	**Work Ref.**	***Escherichia Coli***			***Pseudomonas aeruginosa***			***Salmonella typhi***		
		Pet-ether Extract Con. (mg/ml)			**Pet-ether Extract Con. (mg/ml)**			**Pet-ether Extract Con. (mg/ml)**		
Plant part		1	2	4	1	2	4	1	2	4
Leaf	A	---	---	---	---	--	---	---	---	---
	B	---	---	+++	---	---	+++	---	---	+++
Root	A	---	---	---	---	---	---	---	---	---
	B	---	---	---	---	--	---	---	---	---

A - Avani & Neeta (2005), B - the current paper

Table 2. Antimicrobial activity of Chloroform extract of the *Elephantopus scaber*.

Microorganism	***Bacillus subtilis***			***Staphylococcus aureus***			***Escherichia coli***		
	ChloroformExtract Con. (mg/ml)			**Chloroform Extract Con. (mg/ml)**			**Chloroform Extract Con. (mg/ml)**		
Plant parts	1	2	4	1	2	4	1	2	4
Leaf	--	-- -	+++	--	-- -	+++	---	- --	+++
Root	--	-- -	---	--	-- -	---	---	- --	---

*(--- No inhibition , +++ Inhibition)

The inhibition effect is observed in all microorganisms studied only on leaf and at 4mg/ml concentration.

Table 3. Trace elemental composition of leaf and root of *Elephantopus scaber* observed from the EDXRF experiments.

Element	**Leaf (ppm)**	**Root (ppm)**
Magnesium	2579	1042
Aluminium	2178	7725
Silicon	36481	10500
Phosphorus	1534	580
Sulphur	2159	435
Chlorine	8411	1223
Potassium	1534	580
Calcium	10161	6688
Titanium	257	849
Vanadium	8	25
Chromium	ND	ND
Manganese	299	146
Iron	1404	6934
Cobalt	ND	1
Nickel	ND	ND
Copper	17	17
Zinc	44	43
Arsenic	8	10
Selenium	ND	1
Bromine	11	3
Rubidium	261	148
Strontium	142	172
Lead	1	16

*ND – Not Detected.

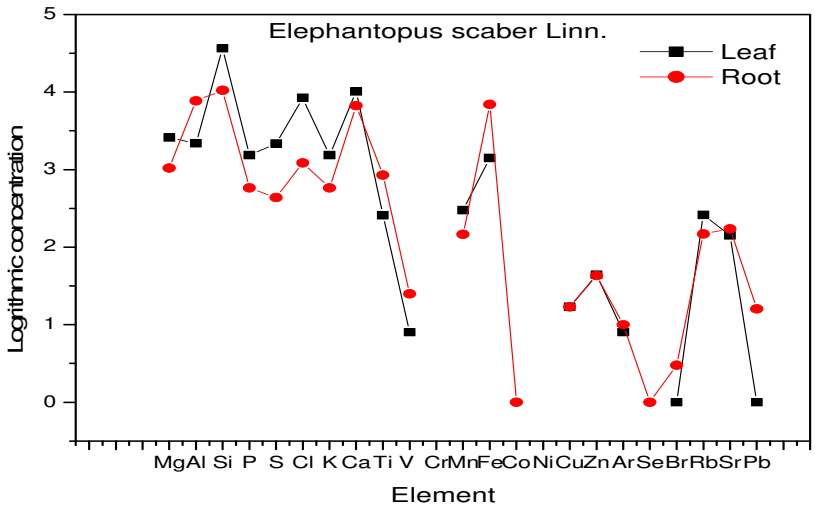

Fig 1. Elemental constitution (logarithmic value) of *Elephantopus scaber* Leaf (square shape) and root (sphere shape) recorded using EDXRF.

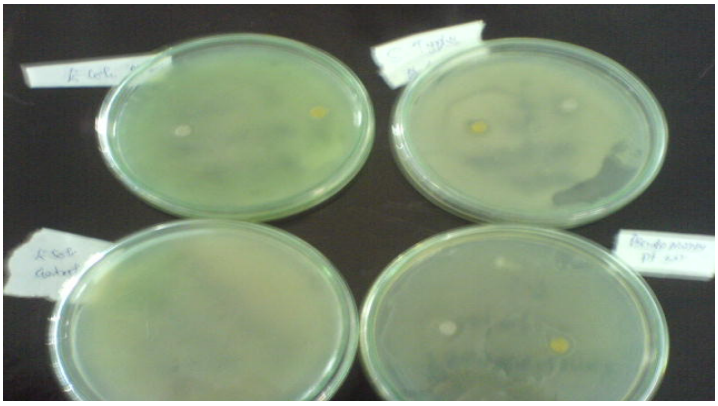

Plate 1. Photograph of the zone of inhibition observed on the plates (a) *Escherichia coli* (b) *Salmonella typhi* (c) *Pseudomonas aeruginosa* and (d) controlled of the Pet-ether extract of the leaf of *Elephantopus scaber*.

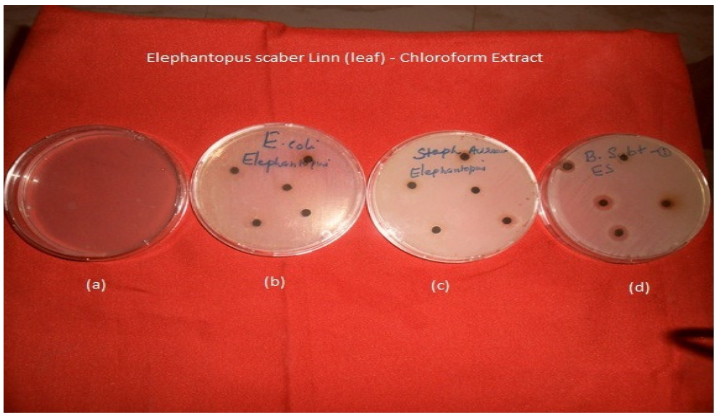

Plate 2. Photograph of the zone of inhibition observed on the plates (a) control (b) *Escherichia coli* (c) *Staphylococcus aureus* and (d) *Bacillus subtilis* of The Chloroform extract of the leaf *of Elephantopus scaber*.

Elemental Analysis

From the last few decades many branches of biology and health science have achieved a remarkable progress due to the improved analytical technology and discovery of trace elemental contribution in public health either deficiency or toxicity. Knowledge of biochemical dysfunctions from trace elements is essential to identify their crucial role. In humans, only ten trace elements (Fe, Zn, F, Cu, I, Se, Mn, Mo, Cr and Co) are considered to be essential [22]. These inorganic micronutrients invariably have a catalytic function

and are found in the metabolic pathways controlling the assimilation and utilization of other nutrients, in the synthesis of new tissues and in the use of energy. It has been estimated that 600 million people suffer from iron deficiency and 200 million people from iodine deficiency and these deficiencies have an impact on neurological development and so having a socio-economic importance. It has been reported that many children have been rehabilitated from malnutrition by giving Zn and Cu supplements as part of their treatment [23].The different trace elements may act as a drug for the different diseases [24, 25] and the availability of trace elements in the plant studied are given in Table-3.

The sample under the present study refluxed a high level of ferrous and very low copper concentrations. Cobalt and nickel are not detected in the present study in both leaves and roots. This data is similar to the observation of Nielsen *et al.* [26] where it was documented that in an environment in significant level of ferrous and copper might exhibit antagonistic interaction between essential trace and ultra trace elements in a particular biological system on environment.

The elements Si, Ca, Cl, Mg, S, K and P (Table-3) are more abundant in the leaf than the root and comparatively heavy metals are less in leaf. The pharmacological properties of the leaf extract were evaluated for diuretics, anti inflammatory, hepato properties, antibacterial, and used for treating wounds and ulcers, bronchitis, small pox, diarrhea and as a brain tonic by earlier workers [15, 16]. These pharmacological properties may mainly due to the presence of such trace elements in the plant as per the concept of Ayurvedic practice.

Silicon is found in the highest concentration in the leaf and the concentration of Ca and Cl are almost equal that of Mg, Al, S, K, P and Fe levels. Results of the present study also found Mn, Rb and Ti lies between 380 μg/g to 250 μg/g. Sr is known to have the property of replacing Ca, and both of which belongs to the same group 2(11A) in the periodic table. Thus replacement of Ca, which is the main building block of renal calculi, by Sr might make the stone fragile which subsequently comes out of the bladder with urine. However the concentration of Ca is 10161μg/g in the leaf and detailed study required to identify the correct proposition. Manganese content of this herb is correlated with the use of this plant in the treatment of *Raktapitta* in Ayurveda, which is the Sanskrit description of the ailment characterized by bleeding from different parts of the body [27]. Mn is known to enhance the process of aggregation of platelets and thus help in the coagulation of blood, which is essential for the arrest of bleeding [28]. Selenium is not detected in the plant leaf and hence it cannot have any antioxidant property. But in the root a very minute amount (1μg/g) is detected. It is possible that with higher exposure time and by using a thick filter selenium can be detected in the root as a high amount. As characteristic X-ray intensities emanating from elements at these small concentrations are often too weak to be separated from the background. The presence of valuable mineral selenium is quite important information which might have more future implication in the discipline of medicine.

In the root, presence of Al, Fe, Ti, Sr, and V is more when compared to leaves. *Elephantopus scaber* roots were used as an antipyretic, Cardio tonic and diuretic. It is observed from the experiment that Zn, Cu, As, Rb and Sr are less available and are more or less equally present in roots as well as leaf. The elements Cr, Co, Ni, Se, Br and Pb are almost not found in both leaf and root of this plant.

Since Zinc content is less than 100μg/g in these plant parts this cannot be used for the treatment of eye diseases and also compared to lead concentration (1μg/g-leaf; 16μg/g–root) a high amount Zinc (44μg/g-leaf; 43μg/g-root) was detected in the sample and hence it can be concluded that this plant has no lead induced toxicity. The presence of elements in ppm level is plotted in **Fig.1** for both leaf and root of the plant studied. In general, the elements of low atomic number are available more than comparatively high atomic number elements. Hence usage of this plant as a drug in Alternate system of medicine has more curative effects than toxic one.

Identification of the exact nature of trace elemental contribution in the active compounds of plant extracts is quite difficult and the work is in progress. And also to determine the active principle and its mechanism of inorganic mineral drugs or trace elemental drugs have to be explored. Based on the data, the trace elemental compounds present in the *Elephantopus scaber* and their pharmacological properties in the leaf and root are the future research options, necessary for development of new medicine.

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