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Pharmacognostical, phytochemical and heavy metal determination of Indian globe thistle plant-*Sphaeranthus indicus* L

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Abstract
Standardization of leaf components is the main objective of the present study and was validated by organoleptic features, physico - chemical parameters and elemental analysis. The results of physico - chemical study showed 6.82 ± 0.6724% of moisture content, 13.1±1.0897 % of total ash, 1.166 ± 0.2517% of acid insoluble ash and 5.55 ± 0.2500% of water soluble ash. The extractive percentages were noticed as 4.0867±0.2501% (Hexane), 7.7400±0.1562 % (Chloroform), 8.6600±0.6400 % (Acetone), 9.613±0.5587 % (Ethanol), 12.346±0.6886 % (Methanol) and 11.553±0.1665% (Water) in all the tested extracts. The preliminary phytochemical screening reveals the presence of phytocompounds alkaloids, coumarins, flavonoids, glycosides, phenols, saponins, steroids, tannins and terpenoids. The elements copper, zinc, manganese, cadmium, lead and mercury were present within the permissible limits of the regulatory authorities WHO and ICH.

Keywords: *Sphaeranthus indicus* L. physico-chemical analysis, phytochemical studies, plant metabolites and elemental analysis

Introduction
Standardization and quality measurement of raw materials and herbal medicines are important parameters in drug development. In recent days, use of herbal drugs in human recovery is increasing worldwide particularly in the developed nations. The importance and uses of medicinal plants for public health has been appreciated by WHO and has evolved guidelines to support and standardize their efficacy (WHO, 2002) [1]. In parallel with increasing dependence on herbal products for their therapeutic benefits, there has been an increasing concern over their safety and toxicity (Ernst, 2002) [2]. In general, internal factors of geographic conditions, geochemical composition, water, air and external factors like storage and transport can imparts significant changes in the properties, qualities of medicinal plants and their formulations (Saad *et al*., 2006) [4]. According to ICH guidelines (ICH, 2017) [3], any drug proposed to be used as medicine should have to undergone risk assessment for elements under class 1 category (As, Cd, Hg, and Pb).The assessment of herbals and their formulations are can be achieved by stepwise assessment such as identity of sample, organoleptic, pharmacognosy, physicochemical, phytochemical screening and test for bio activity. Among these, phytochemical profiling gets major credits since it directly correlates with the biological activity of the plant (Sulaiman and Indira, 2017) [5].
Sphaeranthus indicus L. is an Indian aromatic herb belongs to the family Asteraceae and commonly known as Mundi and East Indian Thistle Globe. The plant grows (20 – 30 cm) well in paddy fields, waste lands of tropical and subtropical area in India, Sri Lanka, Africa and Australia from sea level to 1200 m altitude (Chatterjee and Pakrashi, 2003) [6].
In accordance with ethanobotanical review, the plant *S. indicus* repeatedly used in the treatment of epileptic convulsions, mental illnesses, hemicranias and skin diseases (Kirtikar and Basu, 1987) [7]. In Ayurvedic literature, the various parts of this plant used in the treatment of hemicranias, jaundice, hepatopathy, cough, gastropathy, hernia, hemorrhoids, helminthiasis, dyspepsia, and skin diseases. Externally, the plant paste used to treat pruritus and edema, arthritis, filariasis, gout and cervical adenopathy. It also employed in the treatment of piles and hepatitis (Paranjape, 2001) [8]. From the literature, it is confirmed that, the plant *S. indicus is* a multipurpose medicinal plant, with potential to employ and cure many kinds of common ailments. In order to explore it efficacy, standardization of its raw material is very important. This study was confirmed the safety and authenticity of medicinal plant. So that the therapeutic effects of the plant may be used properly and scientifically to reaches the larger populations of the world. Hence, the present research work was carried out to standardize and to prove the therapeutic efficiency of the medicinal plant *Sphaeranthus indicus* L. as safe and potential source for medications.

Materials and Methods

Chemicals used

Chemicals and reagents used were of analytical grade which are included Mayer's reagent, glacial acetic acid, FeCl3, NaoH, 1% solution of gelatin containing 10% ferric chloride solution, lead acetate, dilute hydrochloric acid, Conc. H_2SO_4, HNO_3, H_2O_2 and double distilled water.

Plant collection and Authentication

Fresh leaves of *Sphaeranthus indicus* was collected from the paddy fields at Thanjavur district in the month of January to March 2021.The plant specimen was identified and authenticated as *Sphaeranthus indicus* L of Asteraceae family by the Taxonomist, Dr. Soosairaj, Assistant professor, St. Joseph's college, Tiruchirappalli. Voucher specimen (Accession No. 2992) was deposited at the respected department for further reference.

Preparation of Plant extracts

The active principles of collected plants were extracted using organic solvents varying from low to higher polarities. The freshly collected plant leaves were washed with clean water and dried in shade for about 10 -15 days. The complete air dried parts of *S. indicus* were milled into homogenized powder using a electric grinder. 70 g of each powder was extracted successively with n-Hexane (to extract fatty substance), Acetone, Ethanol, Methanol and Water respectively on soxhlet apparatus. Finally the organic extracts were concentrated in vacuum using a rotary evaporator, while aqueous extract was dried using water bath to get sticky mass. The extractive value has been calculated and the extracts were refrigerator at 4˚C.

Morphological studies

Morphological assessment was carried out by physical observation. The organoleptic characters – structure, shape, texture, colour, odour and taste were assessed according to WHO guidelines (WHO, 1992) [9].

Physico-chemical Evaluation

Physicochemical parameters such as moisture content, ash values and extractive values of *Sphaeranthus indicus* were determined following the guidelines of World Health Organization (WHO, 1998).

Moisture content

Moisture content of the dried leaf of *S. indicus* was determined based on the test of loss on drying. Two gram of fresh leaves weighed and was dried completely in an oven at 105°C and allows it for cooling. The final weight of dried leaf was taken and the experiment repeated thrice. The result was calculated as loss of weight in percentage (w/w).

Determination of Total ash

Two gram of dried leaf powder of *S. indicus* was taken in pre-weighed crucible and ignited by gradually increasing the temperature to 600 °C until it became white. The crucible was cooled in desiccators and weighed. The experiment was repeated thrice and the total ash content was calculated in percentage (w/w).

Determination of Acid insoluble ash

Twenty-five milliliters of HCl was added to the total ash in crucible and covered with a watch-glass and boiled for 5 min. The insoluble matter was collected in an ashless filter paper and washed in hot water until the filtrate was neutral. The filtrate was transferred to pre-weighed crucible and ignited. The crucible was cooled in desiccators and weighed. The experiment was repeated thrice and the acid insoluble ash content was calculated in percentage (w/w).

Determination of water soluble ash

Twenty-five milliliters of water was added to the crucible containing total ash and boiled for 5 min. Insoluble matter was filtered using an ashless filter paper. The filter paper was washed with hot water and ignited again in a crucible for 15 min at a temperature not exceeding 450 °C. The crucible was then weighed. Water soluble ash was calculated by subtracting the residue weight from the weight of total ash in percentage (w/w) and the experiment was repeated thrice.

Determination of extractive value

Five gram of dried leaf powder of *S. indicus* was extracted with different solvents system (n-Hexane, Chloroform, Acetone, Ethanol, Methanol and water). They were kept in shaker for 1 hour followed by followed by gentle boiling for few hours and then it was cooled and filtered. Twenty five ml of each filtrate was transferred to a pre-weighed flat bottomed dish and evaporated to dryness on a water bath. Then the dish was dried at 105 °C for 6 h and cooled in desiccators and weighed. The process was repeated thrice and extractive percentages were calculated (w/w).

Preliminary Phytochemical Analysis

The different extracts of *Sphaeranthus indicus was* subjected to qualitative test using standard procedures to identify the phytoconstituents alkaloids, cardiac glycosides, coumarins, flavonoids, glycosides, phenols, saponins, steroids, tannins and terpenoids as described by Harborne (1973) [11], Trease and Evans (1989) [12].

Alkaloids: Plant of extracts of *S. indicus* was treated with few drops of 1 ml 2N HCl, filtered and mixed with few drops of Mayer's reagent. Formation of white or pale precipitate showed the presence of alkaloids.

Cardiac glycosides: 2 ml of *S. indicus* extract, 1 ml of glacial acetic acid, $FeCl_3$ and Conc. H_2SO_4 were added. Formation of blue precipitate indicates the presence of cardiac glycosides.

Coumarins: 2 ml of *S. indicus* extract was added with 3ml of 10% NaoH. Formation of yellow colour indicates the presence of coumarins.

Flavonoids: In a test tube containing 0.5 ml of *S. indicus* extract, 5-10 drops of dilute hydrochloric acid and a small piece of Mg were added. The solution was boiled for few minutes. In the presence of flavonoids, the reddish pink or dirty brown color was produced.

Glycosides: 2 ml of sample (*S. indicus*) extracts was dissolved in 1.0 ml of water and then aqueous solution of sodium hydroxide was added. Formation of a yellow color indicates the presence of glycosides.

Saponins: The *S. indicus* extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. One cm length of foam was formed indicating the presence of saponins.

Steroids: *S. indicus* extracts (0.5ml) was dissolved in 1.0ml of chloroform and filtered. To the filtrate on ice, 1.0ml of acetic acid was added and then a few drops of conc. Sulphuric acid were run down the side of the test tube. The appearance of blue, bluish-green or a rapid change from pink to blue colour indicates the presence of steroids.

Phenols: The extracts of *S. indicus* were dissolved in 5.0ml of distilled water and 2.0ml of 1% solution of gelatin containing 10% ferric chloride solution were added. White precipitate indicates the presence of phenolic compounds.

Tannins: In a test tube containing 5.0 ml of *S. indicus* extract, a few drops of 10% solution of lead acetate was added. Formation of white precipitate indicates the presence of tannins.

Terpenoids: To the 1.0ml of *S. indicus* extract solution, 2.0ml of chloroform and 1.0ml of Conc. Sulphuric acid was added. Formation of reddish brown color indicates the presence of terpenoids.

Heavy Metal Analysis

Sample preparation and Digestion

The leaf samples of *Sphaeranthus indicus* L were dried in an oven at 70˚C for 15 days. The digestion process was as follows for the estimation of heavy metal accumulations. 0.1g of dried plant leaves of *S. indicus* was added in a conical digestion flask, where 6ml of Nitric acid (HNO_3) was added and kept overnight at 25˚C. Digestion flask was placed on the hot plate at 150˚C until the acid gets evaporate. When fumes appeared, 1ml hydrogen peroxide (H_2O_2) was added to each digestion flask. Then sample flask was left to cool down and the colorless liquid solution was diluted to 50 ml using water. The filtered solution was used to measure heavy metals and minerals (Cu, Zn, Mn, Cd, Hg and Pb) accumulation in Atomic Absorption Spectrophotometer (AAS, Perkin Elmer) (Gong *et al*., 2020) [13].

Result and Discussion

Standardization is an integral part of establishing the identity of crude drug. Keeping in view the medicinal plant *Sphaeranthus indicus* (leaf) was completely investigated for its morphological, physicochemical characters and its major active phyto constituents.

Morphological assessment of *Sphaeranthus indicus* leaves:

Morphological evaluation is helpful in authentication of crude drug by evaluating the external appearance. The plant, *S. indicus* belongs to the family Asteraceae, is a diffuse, aromatic annual herb present with toothed wings. The leaves were alternate, oblanceolate, apex obtuse, decurrent to stem, dentate and serrate. The size of the leaf varied from 3-4×1-2 cm. The upper surface of the leaf was observed with green in colour and lower surface dull green colour and the leaf blade was very thin. Leaves of *S. indicus* exhibits characteristic odour and pungent taste.

Physico-chemical evaluation

Physico-chemcial evaluations are the important pharmacognostic parameters to assure quality, safety and purity of medicinal plants. Its quantitative determination is helpful in setting the standards of crude drugs.

Moisture content and Ash content of *S. indicus* leaves:

In the present study, moisture content of *S. indicus* was determined as 6.82 ± 0.6724 % based on fresh weight of the leaves. Moisture content is an inexorable component of crude drugs, which must be eliminated as far as practicable. The insufficient drying of crude drugs leads to the enzymatic destruction of active principles by molds and bacterial growth (Mukherjee, 2002; Trivedi, 2006) [14, 15]. Ash values are the purity as well as identity index of the drug. Foreign inorganic substances (salts and silica) can be determined with reference to total ash values (Musa *et al*., 2006) [16]. In the present investigation, the ash values were found out by three different methods which measured the amount of total ash as 13.1±1.0897, acid-insoluble ash as 1.166 ± 0.2517and water soluble ash as 5.55 ± 0.2500 (Table – 1). High ash values state the presence of adulteration, substitution or contamination. Total ash content of *S. indicus*, observed lesser than 14% (which is the maximum acceptable limit recommended by European Pharmacopoeia) (Vaikosen *et al*, 2011) [17]. Acid-insoluble ash is a part of total ash and measures the amount of silica, oxalates, carbonates, phosphates, oxides and silicates respectively. Water soluble ash is the positive indicators of water-soluble portion of the total ash (Abdu *et al*., 2015) [18]. The amount of water-soluble ash is higher than the acid-insoluble ash, whereas the amount of total ash is double the quantity of water-soluble ash. From the present investigation it is inferred that, the higher amount of ash values proves the quality and purity of the leaf powder. Hence, the plant leaf could be used as a raw material in the preparation of drug.

Table 1: Moisture content and Ash values of *Sphaeranthus indicus* leaf

S. No	Type of Ash	Ash Values (%)
1.	Moisture Content	6.82 ± 0.6724
2.	Total ash	13.1±1.0897
3.	Acid insoluble ash	1.166 ± 0.2517
4.	Water soluble ash	5.55 ± 0.2500

Data were presented as mean ±SD, as triplicates

Extractive value of *S. indicus* leaves

Extractive percentages of solvent extracts play an important role in evaluation of the drugs and it varies depends on the polarity of the solvents. From the present investigation, it was inferred that, the highest extractive percentages observed in polar solvents such as methanol, water and ethanol followed by acetone, chloroform and hexane (Table 2). Solublity of drug constituents (extractive value) may vary according to the polarity of the solvent (Kokate *et al*., 2009) [19]. From this study it is concluded that, the polar solvents such as methanol, water and ethanol were the potent solvent for phenolic compounds. Similar report was stated by Anwar and Przybylski (2012) [20] in *Linum usitatissimum* L. that the polar solvents are highly suitable for the isolation of phenolic compounds.

Table 2: Extractive value of *Sphaeranthus indicus* leaf

S. No	Name of the Extract	Extractive value (%)
1.	Hexane	4.0867±0.2501
2.	Chloroform	7.7400±0.1562
3.	Acetone	8.6600±0.6400
4.	Ethanol	9.613±0.5587
5.	Methanol	12.346±0.6886
6.	Water	11.553±0.1665

Data were presented as mean ±SD, as triplicates

Phytochemical analysis of *S. indicus* leaf

Active biocompounds of *S. indicus* leaf extracts were analyze qualitatively and the study reveals the presence of alkaloids, cardiac glycosides, coumarins, flavonoids, glycosides, phenols, saponins, steroids, tannins and terpenoids (Table 3). The resulted biocompounds might be the key components for the therapeutic potential of *Sphaeranthus indicus.* The phytocompounds like coumarins, phenols were presents in all the tested extracts and cardiac glycosides, flavonoids, glycosides observed in all the solvent extracts except hexane. Saponins present in acetone, ethanol, methanol and water extracts while, methanol and water extracts showed strong foam contents (saponins) and tannins. Alkaloids were observed in methanol and terpenoids observed in water extracts, whereas steroids were not observed in the present study. This shows that, the components of plants may vary according to the environmental condition. In the present study, the presence of phenolic compounds like coumarins, flavonoids and phenols in polar solvents indicates its suitability as drug and are known to have curative

properties against several ailments and pathogens. The above results clearly indicate that, polar solvents are more suitable for the extraction of phenolic group of compounds.

Table 3: Qualitative Phytochemical Screening of *S. indicus* leaf

S. No	Name of the test	H	Ch	Ace	E	M	W
1.	Alkaloids	-	-	-	-	+	-
2.	Cardiac Glycosides	-	+	+	+	+	-
3.	Coumarins	+	+	+	++	++	++
4.	Flavonoids	-	+	+	++	++	++
5.	Glycosides	-	+	+	+	+	-
6.	Phenols	+	+	+	+	++	++
7.	Saponins	-	-	+	+	++	++
8.	Steroids	-	-	-	-	-	-
9.	Tannins	-	-	-	+	++	++
10.	Terpenoids	-	-	-	-	-	+

'+' indicates presence; '++' indicates strong presence; '-'indicates absence; H- Hexane: Ch- Chloroform: A- Acetone: E- Ethanol: M- Methanol: W- Water

Heavy Metal Analysis of *S. indicus* leaf

Elemental determination and its quantification are the crucial parameter for herbal drugs. Quantification of elements in atomic absorption spectroscopy shows 0.820 ± 0.0030 ppm of copper, 0.339 ± 0.0021ppm of zinc and 1.631 ± 0.0051 ppm of manganese, 0.220 ± 0.0070 ppm of cadmium, 0.090 ± 0.0012 ppm of lead and 0.002 ± 0.0000 ppm of mercury. The elemental concentrations of *S. indicus* (Table 4) leaves were in the permissible limit of WHO and shows its resources and as a results of that the plant can recommended for therapeutic uses. Exposure of elements beyond the permissible limits leads to severe impacts in human health. The increased amount of copper causes dermatitis, irritation of the upper respiratory tract, abdominal pain, nausea, diarrhea, vomiting, and liver damage (Ulla *et al*., 2012) [21], higher levels of lead or its long term usage leads to toxicity, characterized by colic, anaemia, chronic, nephritis headache, brain damage and central nervous disorders (Klaassen CD, 2001) [22]. The increased amount of Hg results in permanent brain damage and impairs kidney functions (AYUSH 2011) [23]. In accordance with ICH regulation (ICH, 2017) [3], minerals like Mn and Zn are biological metals serve either as cofactors and activator of enzyme, exerts catalytic properties and acts as regulators of nerve transmission, muscle contraction, osmotic pressure and salt-water balance. The result of present investigation reveals that, *S. indicus* leaf with more number of ash values, phytocompounds, higher amount of minerals and lesser amount of heavy metals are responsible of its therapeutic properties.

Table 4: Elemental concentration of *Sphaeranthus indicus* leaf

S. No	Name of the elements	Elemental concentration (ppm)	Permissible limits as per WHO (ppm)
1.	Copper	0.820 ± 0.0030	40
2.	Zinc	0.339 ± 0.0021	100
3.	Manganese	1.631 ± 0.0051	11
4.	Cadmium	0.220 ± 0.0070	0.3
5.	Lead	0.090 ± 0.0012	10
6.	Mercury	0.002 ± 0.0000	1

Data were present in Mean ± SD, as triplicates.

Conclusion

The phytochemicals alkaloids, cardiac glycosides, coumarins, flavonoids, glycosides, phenols, saponins, steroids, tannins and terpenoids which are present in *Sphaeranthus indicus* provides scientific evidence for its therapeutic efficiency. The high quantities of essential trace elements and lesser amount of toxic elements assure a better possibility to use *Sphaeranthus indicus* as effective medicine. Hence the present study proved the purity of the plant as a potent drug in the formulation studies. However, in-depth investigations need to be carried out to isolate and identify the active components of the plants and to explore its mechanisms of action as well as their potential role in biological activity and antioxidant activities as well.

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References

1. World Health Organization (WHO). Quality Control Methods for Medicinal Plant Materials. AITBS Publishers, Delhi, 2002, 46-51.

2. Ernst E. "Toxic heavy metals and undeclared drugs in Asian herbal medicines," *Trends in Pharmacological Sciences*, 2002:23(3):136-139.
3. ICH guidelines Quality, Safety, Efficacy and Multidisciplinary, http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html [cited 23rd June, 2017]
4. Saad B, Azaizeh H, Abu-Hijleh G, Said O. "Safety of traditional Arab herbal medicine," *Evidence-Based Complementary and Alternative Medicine*, 2006:3(4):433-439.
5. Sulaiman CT, Indira Balachanran. Chapter 10. Phytochemical standardization of Botanical drug, 2017.
6. Chatterjee A and Pakrashi SC, National Institute of Science communication and information resources; 1st ed, 2003, 5.
7. Kirtikar KR, Basu BD. Indian medicinal plants. 2nd ed. Dehradun: International Book Distributors, 1987.
8. Paranjape P. Indian medicinal plants. In: Forgotten healer: A guide to Ayurvedic herbal medicine. Delhi: Chaukhamba Sanskrit Pratisthan, 2001, 148-9.
9. WHO. Quality control methods for medicinal plant material. Geneva: WHO, 1992, 22-34.
10. World health organization. organization mondiale De La santé, Quality control methods for plant materials, 559. Rev. 1 orginal English, 1998, 08-67.
11. Harborne JB. Phytochemical Methods 3rd Edn. Chapman and Hall Ltd. publishers, London, 1973, 135-203.
12. Trease and Evans. Pharmacognosy 13th Edition. Bailliere Tindale publisher, London, 1989, 832.
13. Gong L, Qiu D, Yao X, Yang G. Determination of Heavy Metals in the Plant Sample Pretreatment MethodsIOP Publishing, 2020, 022008.
14. Mukherjee PK, Wahil A. Integrated approaches towards drug development from Ayurveda and other systems of medicine. *Journal of Ethnopharmacology*, 2002:103:25-35.
15. Trivedi PC. Herbal medicine: traditional practices. Aavishkar Publishers, Jaipur, 2006, 322.
16. Musa KY, Katsayal AU, Ahmed A, Mohammed Z and Danmalam UH. Pharmacognostic investigation of the leaves of *Gisekia pharmacioides*. *Afr J Biotechnol*, 2006:5:956-7.
17. Vaikosen EN, Alade GO. Evaluation of pharmacognostical parameters and heavy metals in some locally manufactured herbal drugs. J. Chem. Pharm. Res, 2011:3(2):88-97.
18. Abdu BA, Adamu U, Sani SM, Joshua O. Physical and Phytochemical Study of Some Local Herbal Remedies. Journal of Pharmacy and Biological Sciences, 2015:10(4):05-10. DOI: 10.9790/3008-10440510
19. Kokate.– Kokate CK, Purohit AP, Gokhule SB. Pharmacognosy. Nirali Prakashan; 38th edition, Pune, 2007-2009.
20. Farooq Anwar, Roman Przybylski. Effect of solvents extraction on total phenolics and antioxidant activity of extracts from flaxseed (*Linum usitatissimum* L.) *Acta Sci Pol Technol Aliment*, 2012:11(3):293-301.
21. Ulla R, Khader JA, Hussain I, AbdElsalam NM, Talha M and Khan N "Investigation of macro and micro-nutrients in selected medicinal plants," *African Journal of Pharmacy and Pharmacology*,2012:6(25):1829-1832.
22. Klaassen CD. Casarett and Doull's toxicology 6th ed. New York: McGraw-Hill, 2001, 827-34.
23. AYUSH. Permissible levels of contaminants for AYUSH standard mark, section III annex C version 1, voluntary certification scheme for AYUSH products, certification. India: Ayush, 2011.