Pharmacogn. Res., 2023; 15(3):492-496.
https://www.phcogres.com

Original Article

Physico-chemical and Phytochemical Analysis of *Sphaeranthus indicus* Linn. (Whole Plant)

Sumedh Joshi[1,*], Megha[2], Bhargav Vijay Bhide[3], Shivani Ghildiyal[3], Tanuja Manoj Nesari[4]

[1]Department of Dravyaguna, RJVS Bhaisaheb Sawant Ayurveda Mahavidyalaya, Sawantwadi, Maharashtra, INDIA.
[2]Research Fellow (Chemistry), CCRH, New Delhi, INDIA.
[3]Department of Dravyaguna, All India Institute of Ayurveda, New Delhi, INDIA.
[4]Director, All India Institute of Ayurveda, New Delhi, INDIA.

ABSTRACT

Introduction: Quality, safety, and efficacy are the three main components of any therapy used for disease cure. Ayurveda encompasses vast knowledge regarding the use of numerous herbal sources for disease management. *Sphaeranthus indicus* Linn. (Known as *Mundi*) is one such drug praised in classical as well as contemporary medicinal science for its therapeutic efficacy. The present study aims at development of quality standards for the *Sphaeranthus indicus* Linn. (Whole plant) sample utilizing physico-chemical and phytochemical analysis. **Objectives:** To perform physico-chemical, phytochemical, HPTLC, FTIR and UV analysis of *Sphaeranthus indicus* Linn (Whole plant) sample. **Materials and Methods:** Physico-chemical analysis was done as per the guidelines mentioned by Ayurvedic Pharmacopoeia of India. Phytochemical analysis, HPTLC, FTIR and UV analysis was done as per the globally accepted standard guidelines. **Observations and Results:** The observed values were then compared to the standard values to make the monograph of *Sphaeranthus indicus* Linn. (Whole Plant).

Keywords: Ayurveda, Dravyaguna, Geraniol, HPTLC, Mundi, *Sphaeranthus indicus*.

Correspondence:
Sumedh Joshi
Assistant Professor, Department of Dravyaguna, RJVS Bhaisaheb Sawant Ayurveda Mahavidyalaya, Sawantwadi, Maharashtra, INDIA.
Email: sumedhjoshi10395@gmail.com

Received: 01-02-2023;
Revised: 02-03-2023;
Accepted: 30-03-2023.

INTRODUCTION

Mundi, a well-described plant in Ayurveda classics, has a botanical source as *Sphaeranthus indicus* Linn., commonly known as East Indian Globe thistle. It is an annual herb belonging to the family Asteraceae. This herb is globally distributed from Africa to Australia. Within India, it is said to be found throughout tropical parts, especially in rice fields, dry waste places, and cultivated lands.[1] In Ayurveda, this herb is described by the name Mundi, and is indicated in management of various disorders like *Atisar* (~ diarrhea), *Ama Vata* (~arthritis), *Apasmar* (~Epilepsy), *Kasa* (~Cough), *Krimi* (~Worm infestation), *Mutrakriccha* (~diseases of urinary tract), *Pandu* (~Anaemia), *Pleeharoga* (~diseases of spleen), *Rakta dhatu vikara* (~Haematological diseases), *Shleepada* (~filariasis), *Unmada* (~Psychosis), *Chhardi* (~Hyperemesis), *Vata rakta* (~Gout and related diseases), *Vidradhi* (~Internal and external abscess), *Vrana* (~Wound) and *Yoniroga* (~Diseases of female genital tract) etc.[2] The whole plant and its anatomical parts have been reported with different types of secondary metabolites which include eudesmanolides, sesquiterpenoids, sesquiterpene lactones, sesquiterpene acids, flavone glycosides, flavonoid C-glycosides, isoflavone glycoside, sterols, sterol glycoside, alkaloid, peptide alkaloids, amino acids and sugars.[3] The whole plants, its isolated secondary metabolites and different anatomical parts have been reported for ovicidal,[4] antifeedant,[5] anthelmintic,[6] antimicrobial,[7] antiviral,[8] analgesic,[9] antipyretic,[10] hepatoprotective,[11] antitussive,[12] wound healing,[13] broncho-dilatory,[14] mast cell stabilizing activity,[15] anxiolytic,[16] neuroleptic,[17] immunomodulatory,[18] anti-diabetic,[19] anti-convulsant[20] and many other activities. In the contemporary research mainly the extracts obtained from leaves and flower heads are studied, while the whole plant is not thoroughly accessed for phytochemical and pharmacological properties. Thus, present study aims at development of quality assurance parameters for the whole plant sample of *Sphaeranthus indicus* Linn.

MATERIALS AND METHODS

Collection of plant material

The plant of *Sphaeranthus indicus* Linn. was collected from natural habitat (A/P Tal Mogarne, Dist. -Sindhudurg, Maharashtra, India, having coordinates 16.0566° N, 73.5874° E) during the months of October to December. Proper care was taken during the collection and preservation of sample. Herbarium specimens are stored at Department of Dravyaguna, All India Institute of

DOI: 10.5530/pres.15.3.051

Ayurveda, New Delhi. Authentication was done from Botanical Garden of Indian Republic, Botanical Survey of India, Noida. The collected sample was shade dried and stored into air-tight baggage. It was then powdered for further analysis as well as experimental studies.

Physico-chemical analysis

It incorporates calculation of loss on drying, extractive values (aqueous and alcoholic extract) and ash values (total ash and acid-insoluble ash). It was done according to the SOPs given by Ayurvedic Pharmacopoeia of India.[1]

Primary phytochemical analysis

Five solvents viz, 50% alcohol, N-hexane, ethanol, methanol and chloroform were used to prepare extract. 1 gm of the dried powdered drug was mixed with 10 mL solvent, stirred for 6 hr and then kept steady for next 18 hr. Filtered and used to perform chemical tests for primary phytochemical analysis as per the standard guidelines.[21]

Qualitative analysis of heavy metals

The raw drug was heated in muffle furnace to obtain the white ash. The white ash was then subjected to various chemical analyses to determine the presence of heavy metals.[22] Observations of heavy metal analysis are depicted in Table 1.

HPTLC Fingerprinting

Six different extracts were prepared in the ratio (1:10) by cold maceration method, including methanol, ethanol, n-hexane, petroleum ether and acetone. The Merck TLC Silica gel F_{264} plate was used as the stationary phase and Chloroform: Methanol (9:1) was used as the mobile phase.

Table 1: Observation and inference of heavy metal analysis.

Heavy metal tested	Observation	Inference
Cobalt	Blue ppt formed which turned pink on warming	Cobalt is absent
Copper	A green ppt is formed	Copper is absent
Mercury	Red ppt is formed that dissolved in an excess of the reagent.	Mercury is absent
Nickel	A blue ppt is formed which turns green on warming	Nickel is absent
Silver	A yellow ppt. is developed.	Silver is absent
Zinc	A white ppt is formed	Zinc is absent

Table 2: Observations of physico-chemical analysis.

Parameter studied	Observed value	Permissible range as per API
Loss on drying	0.64%	Not more than 1%
Alcohol-soluble extractive	9.20%w/w,	Not less than 2%
Water soluble extractive	9.40%w/w	Not less than 6%
Total ash	18.4%w/w	Not more than 23%
Acid insoluble ash	6.0%w/w	Not more than 9%

HPTLC Standardization

Two samples of *S. indicus* whole plant were taken, one from the local market and another is collected from the natural habitat described earlier. Methanolic extract was prepared in the ratio 1:10, by cold maceration method. The Merck TCL Silica gel F_{264} plate was used as the stationary phase and Toluene: ethyl acetate (93:7) was used as the mobile phase. Geraniol procured from Sigma-Aldrich was used as the standard (As per Vol 13 of Quality standard of Indian medicine, published by Indian Council of Medical Research)

FTIR analysis

FTIR analysis was done by using Universal Attenuated Total Reflectance (UATR) method by using standard guidelines.

UV spectroscopy

Methanolic extract prepared using the cold maceration method was used along with the geraniol (procured from Sigma-Aldrich) as the standard.

RESULTS

Physico-chemical analysis

Physico-chemical analysis of the sample was done in triplet and average is compared with the values given in the Ayurvedic Pharmacopoeia of India.[1] The observations are depicted in Table 2. It was done to evaluate the presence of the concerned phytochemical utilizing the specific chemical test. Observations are depicted in Table 3.

HPTLC fingerprinting

HPTLC fingerprinting has shown presence of 18 different peaks when examined at 366 nm wavelength. The details are depicted in Table 4.

Geraniol was used as the standard and it was observed that the R_f value is 0.19. Derivatization was done using 10% methanolic sulphuric acid and plate was scanned at 540 nm wavelength.

Both the market as well as collected sample showed presence of Geraniol in the proportion of 665.0 mcg/100 mg and 669.5 mcg/100 mg respectively. Details of calibration curve are shown in Figure 1.

Table 3: Qualitative phytochemical assessment of *S. indicus* whole plant extracts.

Type of extract	Proteins	Alkaloids	Flavonoids	Saponins	Tannins
Hydroalcoholic	Present	Absent	Present	Present	Absent
N-hexane	Absent	Present	Absent	Present	Absent
Methanol	Present	Absent	Present	Absent	Present
Ethanol	Present	Absent	Present	Absent	Present
Chloroform	Absent	Absent	Absent	Present	Absent

Table 4: Peaks obtained after HPTLC fingerprinting.

Track	R_f	X (mm)	Y (mm)
1.	0.619	20.0	46.4
2.	0.602	29.4	45.3
3.	0.602	38.8	45.3
4.	0.606	48.2	45.6
5.	0.608	57.6	45.7
6.	0.610	67.0	45.8
7.	0.603	76.4	45.4
8.	0.598	85.8	45.1
9.	0.595	95.2	44.9
10.	0.589	104.6	44.5
11.	0.587	114.0	44.4
12.	0.590	123.4	44.6
13.	0.590	132.8	44.6
14.	0.597	142.2	45.0
15.	0.605	151.6	45.5
16.	0.610	161.0	45.8
17.	0.623	170.4	46.6
18.	0.634	179.8	47.3

FTIR analysis done using UATR method revealed presence of 8 different peaks. The data obtained from FTIR was then compared with the IR spectrum library, publish by Sigma-Aldrich,[23] to draw the inference. Details of FTIR analysis are depicted in Table 5.

UV spectrophotometry

The observations of UV analysis of *S. indicus* Whole plant, as compared with the standard Geraniol, reveals same absorption peaks indicating presence of geraniol in the methanolic extract of *S. indicus* whole plant. The graphs of both are depicted in Figure 2 and Figure 3.

DISCUSSION

Quality, safety and efficacy are three major components of evaluation of any herbal drug used in Ayurveda. Quality of

Table 5: Observations and inference of FTIR analysis.

Peak wavelength	Appearance	Group	Compound class
3416.81	Strong, broad	O-H stretching	Alcohol
2925.44	Strong, sharp	C-H stretching	alkyne
1734.79	Medium	C-H stretching	alkane
1631.04	Strong	C=O stretching	esters
1383.86	Strong	C=C stretching	α, β-unsaturated ketone
1320.64	Medium	C-H bending	aldehyde
1261.41	Strong	C-N stretching	aromatic amine
1155.43	Strong	C-O stretching	aromatic ester

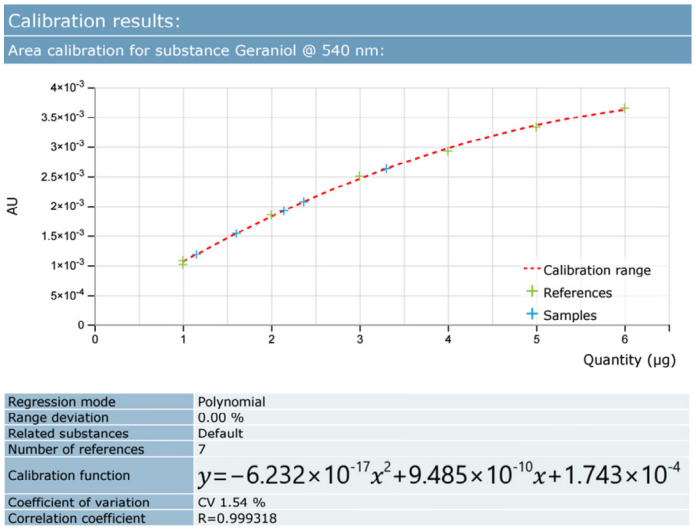

Figure 1: Calibration curve for the HPTLC with geraniol as the standard.

FTIR analysis

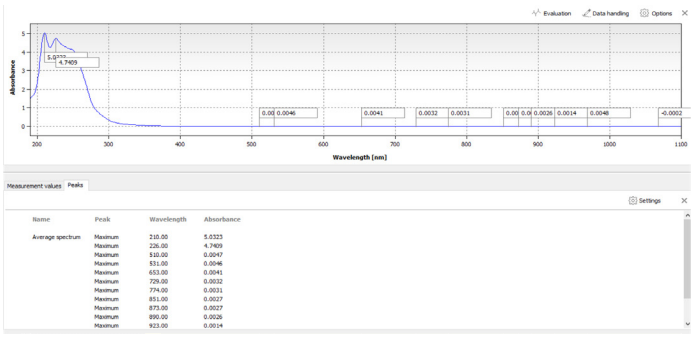

Figure 2: UV analysis of Geraniol.

Figure 3: UV analysis of *S. indicus* (Whole plant).

raw drugs can be assessed by various analytical methods mentioned in the principles of conventional phytochemistry. Analysis of the herb *Sphaeranthus indicus* Linn. was done as per guidelines mentioned in API and Quality standards of Indian Medicine (Database Published by Indian Council of Medical Research). Analytical study incorporated following parts viz, pharmacognostic, physico-chemical study, phytochemical study and sophisticated analysis like HPTLC, UV and FTIR techniques. Ash value of any herb is indicative of the percentage of inorganic parts present in the sample. It was calculated as per the methodology given by the Ayurvedic Pharmacopeia of India. Ash value was obtained within permissible limits, as the drug was collected from the natural habitat and is free of adulterants.

Extractive values are indicative of quality and purity of the drug. According to the guidelines of API, water and alcohol extractive values are to be calculated for the assurance of drug quality. Ethanol is used to calculate alcohol extractive value.

According to the concepts of conventional sciences, the factors responsible for the medicinal activities are the secondary metabolites present the plant original products. Secondary metabolites include alkaloids, flavonoids, steroids, phenols, tannins and much more. Thus, it is important to evaluate the drug for presence of various phytoconstituents. Being, chemical entities, various tests are designed to estimate presence of specific phytoconstituent in the extract. Some phytoconstituents get extracted in specific solvents only. Thus, it is important to study various extracts for presence of different phytoconstituents. In the present study, five types of extracts were evaluated for presence of proteins, alkaloids, saponins, tannins and flavonoids. Standardized tests were conducted under controlled environment in phytochemistry lab. Proteins, flavonoids and saponins are present in most of the extracts (Hydroalcoholic, methanol and ethanolic extracts) while tannins were found in methanolic and ethanolic extract and alkaloids are found in N-hexane extract as they are lipid soluble under normal conditions. Anxiolytic, anti-inflammatory, antimicrobial and ani-epileptic potential of *Mundi* can be attributed to presence of flavonoids and tannins present in it.[24] Though, there are various phytoconstituents present in the extracts of *Mundi*, there is a paucity of scientific data regarding their anxiolytic activity. Therefore, we cannot assign anxiolytic activity to the specific constituent.

High performance thin layer chromatography, is a sophisticated method based on the principle of the chromatographic separation technique. It is characterized by significantly shorter development times, lower solvent consumption and improved resolution. Highly reproducible results and traceable records are obtained through standardized methodology and the use of appropriate instruments (usually software controlled) for all steps of the analysis. HPTLC methods can be used for quality assurance of the plant, as well as to find out the variety of phytoconstituents present in the sample. In the present study geraniol is used as standard as mentioned in Quality standards of Indian medicine (Vol 13). *S. indicus*, first from its natural habitat (denoted by vial ID SA) was analyzed in comparison to geraniol. After quantification, it was seen that market sample (denoted by vial ID SA 2) contains 665 micrograms of geraniol in 100 mg of herb, while the collected sample contains 669.5 mcg. Geraniol possesses antimicrobial, anti-inflammatory, antioxidant, anti-cancer, and neuroprotective activities.[25] HPTLC fingerprinting is the representation of the phytochemical composition of a plant extract or plant formulation in the form of bands at 254 nm, 366 nm and white light. It is a sequence of peaks or zones of a specific chromatogram for a sample. The United States Pharmacopeia defines the HPTLC fingerprint as follows: "It is the electronic image of the visual HPTLC chromatogram." The HPTLC fingerprint is scored based on R*f*, the color, and the relative intensity of the bands in the electronic image. The HPTLC technique can be used for plant fingerprinting. Each plant has a different fingerprint pattern. When the plant extract is processed into an appropriate mobile phase, the observed pattern of separation is the imprint of the plant. HPTLC is a preliminary step to identify phytochemical compounds and secondary metabolites of a plant. Fingerprints play an important role in the quality control of herbal medicinal products. It can be used for raw material authentication and detection of adulterant or spurious material. Six extracts of *Mundi* including methanol, ethanol, n-hexane, petroleum ether, chloroform and acetone, were analyzed in mobile phase of chloroform and methanol (9:1). Total 18 peaks were obtained

after scanning at 254 and 366 nm wavelength. This fingerprint can be further used as quality assurance of *Sphaeranthus indicus* Linn. sample.

Ultraviolet spectroscopy works on the principle of absorbance of UV light and different wavelength from 400 to 1000 nm wavelength. Geraniol is used as a standard to compare (as given in 13th vol of Quality Standard of Indian Medicine). Maximum absorbance of observed at 210 nm wavelength in both geraniol as well as methanolic extract of *S. indicus*. This observation is in accordance with the previous works[1] and databases available.

FTIR analysis revealed presence of 8 distinct peaks indicative of presence of various functional groups like Alcohol, alkyne, alkane, esters, α, β-unsaturated ketone, aldehyde, aromatic amine and aromatic ester. This data may be useful for quality assurance and standardization of samples of *S. indicus* whole plant.

CONCLUSION

Data obtained from the present study can be used as the reference parameters for the quality assurance of *Sphaeranthus indicus* Linn. (whole plant) sample in further studies.

ACKNOWLEDGEMENT

Authors would like to acknowledge, VD. Aishwarya Joglekar (Assistant Professor, Department of Samhita Siddhant, D.Y. Patil college of Ayurveda, Pune), Arun Kumar (SRF Taxonomy, RRDR, AIIA, New Delhi) for their valuable support in the present study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FTIR: Fourier transform infrared spectroscopy; **HPTLC:** High Performance thin layer chromatography; **TLC:** Thin Layer Chromatography; **UVS:** Ultra Violet spectroscopy.

REFERENCES

1. Anonymous. Ayurvedic pharmacopoeia. part 1, Vol 4, Page no 70-72. New Delhi: Government of India, Ministry of Health and Family Welfare, Department of Indian System of Medicine and Homeopathy; 2001.
2. Saraswathidevi HN, Mahesh CD, Pradeep S. A Classical Review on Mundi (*Sphaeranthus indicus* Linn.). Journal of Ayurveda and Integrated Medical Sciences. 2019;4(4):300-10.
3. Galani VJ, Patel BG, Rana DG. *Sphaeranthus indicus* Linn.: A phytopharmacological review. International Journal of Ayurveda Research. 2010;1(4):247.
4. Sharma M, Saxena RC. *Sphaeranthus indicus* as a mosquito larvicidae. J Appl Zool Res. 1996;7(1):87-8.
5. Lalla PM, Deshpande AR, Musaddip M, Shahare NH, Ukesh CS. Antimicrobial activity of medicinal plant *Sphaeranthus indicus*. In: International Conference on Modern Trends in Plant Sciences with Special Reference to the Role of Biodiversity in Conservation. Maharashtra, Amravati. 2005;96.
6. Sonia S, Bhupesh S, Shruti T, Piyali R. Anthelminitic activity of the whole plant of *Sphaeranthus indius* Linn. Journal of Pharmacy Research. 2011;4(11):4254-5.
7. Duraipandiyan V, Kannan P, Ignacimuthu S. Antimicrobial activity of *Sphaeranthus indicus* L. Ethnobotanical leaflets. 2009;2009(3):5.
8. Tiwari A, Saxena RC. Repellent and feeding deterrent activity of *Sphaeranthus indicus* against *Tribolium castaneum* (Herbst). Bio Sci Res Bull. 1993;9(1-2):57-9.
9. Zachariah SM, Pappachen LK, Aneesh TP, Alex L, Sumith G, John MS, Praseetha MC, Nagalekshmi R. Phytochemical and pharmacological screening of *Sphaeranthus indicus* Linn. for antimicrobial activity. International Journal of Pharmaceutical Sciences and Research. 2010;1(10):169.
10. Nanda BK, Jena J, Rath B, Behera B. Anticonvulsant Activity of whole parts of *Sphaeranthus indicus* Linn. Extract in Experimental Mice. Drug Invention Today. 2010;2(3).
11. Sundari K, Karthik D, Ilavenil S, Kaleeswaran B, Srigopalram S, Ravikumar S. Hepatoprotective and proteomic mechanism of *Sphaeranthus indicus* in paracetamol induced hepatotoxicity in wistar rats. Food Bioscience. 2013;1:57-65.
12. SK, Saroj KM, Tripathi VJ, Singh AK, Singh RH. An antimicrobial principle from *Sphaeranthus indicus* L.(Family Compositae). International Journal of Crude Drug Research. 1988;26(4):235-9.
13. Chopda MZ, Patole SS, Mahajan RT 2010. Wound healing activity of *Sphaeranthus indicus* (Linn) in albino rats. Bioresources for rural livelihood. Narendra publishing house I. Delhi, 239-44.
14. Sarpate RV, Deore TK, Tupkari SV. Bronchodilatory effect of *Sphaeranthus indicus* Linn against allergen induced bronchospasm in guinea pigs. Pharmacogn Mag. 2009;5(19):74.
15. JE, Srinivasan KK, Dinakaran V, Joseph A. Mast cell stabilizing effects of *Sphaeranthus indicus*. Journal of ethnopharmacology. 2009;122(2):394-6.
16. Galani VJ, Patel BG. Effect of hydroalcoholic extract of *Sphaeranthus indicus* against experimentally induced anxiety, depression and convulsions in rodents. Int J Ayurveda Res. 2010;1(2):87-92. doi: 10.4103/0974-7788.64412, PMID 20814521.
17. Mhetre NA, Ambavade SD, Bodhankar SL. Neuroleptic activity of extract of *Sphaeranthus indicus* in mice. Indian J Nat Prod. 2006;22(2):24-7.
18. Anarthe SJ, Malavika E, Pravalika A, Ganga MR. Screening of immunomodulatory activity of *Sphaeranthus indicus* Linn. whole plant. IOSR JPBS. 2015;10(6):91-9.
19. Ramachandran S, Asokkumar K, Maheswari MU, Ravi TK, Sivashanmugam AT, Saravanan S, Rajasekaran A, Dharman J. Investigation of antidiabetic, antihyperlipidemic, and *in vivo* antioxidant properties of *Sphaeranthus indicus* Linn. in type 1 diabetic rats: an identification of possible biomarkers. Evidence-Based Complementary and Alternative Medicine. 2011:1-8.
20. Ambikar DB, Mohanta GP. Evaluation of neuropharmacological activity of petroleum ether, methanolic and aqueous extracts of flower heads of *Sphaeranthus indicus* in mice. J Appl Pharm Sci. 2014;4(4):112-8.
21. Kokate CK. Practical pharmacognosy. 4th ed, , Vallabh Prakashan. New Delhi; 1994;4:29.
22. Xu Z, Zhang Q, Li X, Huang X. A critical review on chemical analysis of heavy metal complexes in water/wastewater and the mechanism of treatment methods. Chemical Engineering Journal. 2022;429:131688.
23. [cited 10-1-2023] Available from: https://www.sigmaaldrich.com/IN/en/technical-documents/technical-article/analytical-chemistry/photometry-and-reflectometry/ir-spectrum-table.
24. Ahmad HI, Nadeem MF, Shoaib Khan HM, Sarfraz M, Saleem H, Khurshid U, Locatelli M, Ashraf M, Akhtar N, Zainal Abidin SA, Alghamdi A. Phytopharmacological evaluation of different solvent extract/fractions from *Sphaeranthus indicus* L. flowers: From traditional therapies to bioactive compounds. Frontiers in Pharmacology. 2021;12:708618.
25. Rekha KR, Selvakumar GP, Sethupathy S, Santha K, Sivakamasundari RI. Geraniol ameliorates the motor behavior and neurotrophic factors inadequacy in MPTP-induced mice model of Parkinson's disease. Journal of Molecular Neuroscience. 2013;51:851-62.

Cite this article: Joshi S, Megha, Bhide BV, Ghildiyal S, Nesari TM. Physico-chemical and Phytochemical Analysis of *Sphaeranthus indicus* Linn. (Whole Plant). Pharmacog Res. 2023;15(3):492-6.