



A STUDY ON PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ANALYSIS OF METHANOLIC EXTRACT OF *SPHAERANTHUS INDICUS LINN* LEAVES.

Karunya Arumugam and Sathish Sankar*

Department of Microbiology, Centre for Infectious Diseases, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 600077

***Corresponding author**

Dr. Sathish Sankar

Email: sathishsankar.sdc@saveetha.com

Abstract

Introduction: *Sphaeranthus indicus Linn*, commonly known as "Gorakhmundi" or "East Indian Globe Thistle," is a medicinal plant that has been traditionally used in various systems of medicine for its therapeutic properties. It belongs to the Asteraceae family and is widely distributed in India, Southeast Asia, and other tropical regions. The plant has gained attention due to its potential health benefits and is known for its diverse phytochemical composition. Phytochemical analysis plays a crucial role in identifying and quantifying the bioactive compounds present in plants. Various classes of phytochemicals, such as alkaloids, flavonoids, terpenoids, phenolic compounds, and others, are known to exhibit antioxidant properties and contribute to the plant's medicinal properties. This study aims to conduct an in vitro phytochemical analysis and evaluate the antioxidant activity of *Sphaeranthus indicus Linn*.

Aim: To determine the Phytochemical analysis and Antioxidant activity of *sphaeranthus indicus linn*

Materials and methods: Preparation of Methanolic Extracts by collecting the leaf samples, Qualitative Phytochemical Screening, Determination of In Vitro Antioxidant Activities of the Studied Plant Extracts by DPPH assay and hydroxyl scavenging activity, Determination of Total Phenolic Contents and Determination of Total Flavonoid Contents.

Results: The antioxidant activity of *Sphaeranthus indicus Linn* extracts was evaluated using different assays such as DPPH radical scavenging assay, and hydroxyl radical scavenging activity assay. The plant extract showed similar antioxidant activity compared to the control (L Ascorbic acid). The qualitative phytochemical screening of the methanolic extracts revealed the presence of



All the articles published by Chelonian Conservation and Biology are licensed under a [Creative Commons Attribution-NonCommercial 4.0 International License](https://creativecommons.org/licenses/by-nc/4.0/) Based on a work at <https://www.acgpublishing.com/>

various phytochemical groups in *Sphaeranthus indicus* Linn, including saponins, alkaloids, terpenoids, flavonoids, cardiac glycosides, steroids, and phenols.

Conclusion: In conclusion, the *in vitro* phytochemical analysis and antioxidant activity of *Sphaeranthus indicus* Linn demonstrate its potential as a valuable medicinal plant. The qualitative screening revealed the presence of various phytochemical groups, including saponins, alkaloids, terpenoids, flavonoids, cardiac glycosides, steroids, and phenols. These phytochemicals contribute to the antioxidant activity of *Sphaeranthus indicus* Linn extracts. The observed antioxidant activity suggests that *Sphaeranthus indicus* Linn extracts may possess significant health benefits by protecting against oxidative stress-related diseases.

Keywords: Phytochemicals, methanolic extract, universal health, diseases, well being, health, international health policy.

Introduction:

In the dynamic realm of medicinal plant exploration, the study of phytochemical constituents and antioxidant properties holds profound significance. This investigation delves into the intricacies of *Sphaeranthus indicus* Linn, an herbaceous plant renowned for its traditional therapeutic applications. (1) The study enhances our scientific understanding of the complex interplay between phytochemistry and antioxidant activity. In order to determine how well the plant extract can fight oxidative stress, an antioxidant analysis is essential. Examining *Sphaeranthus indicus* Linn leaves' antioxidant potential may have implications for preventive healthcare and wellness, given the role that oxidative stress plays in a number of health conditions, including aging and chronic diseases. (2) Valuable natural substances with a variety of biological activities are called phytochemicals. (3)(4) The identification and quantification of these compounds in the methanolic extract can aid in the development of natural products or pharmaceuticals that may offer health benefits in place of or in addition to synthetic drugs. (5)(6)

Sphaeranthus indicus Linn, commonly known as East Indian Globe Thistle, has been deeply ingrained in traditional medicine systems for its purported health benefits. (1) (7) *Sphaeranthus indicus* Linn has a rich history of traditional medicinal use, and understanding the phytochemical composition of its leaves can unveil potential therapeutic compounds. (8)(9) The leaves, in particular, are believed to harbor a diverse array of phytochemicals, compounds with the potential to exert therapeutic effects. (10) This study undertakes a meticulous exploration of these phytochemicals, employing advanced methodologies to identify and quantify the constituents present in the methanolic extract. (11)(6)(12)

Beyond phytochemical analysis, the research extends its focus to antioxidant properties, recognizing the pivotal role of antioxidants in combating oxidative stress—a process linked to various degenerative conditions. (1,13) By subjecting the methanolic extract to antioxidant assays, the study seeks to unveil the extract's ability to neutralize free radicals and, consequently, its

potential in promoting health and mitigating oxidative damage.(14,15)Potential medicinal uses based on the plant's extensive botanical history are made possible by this. (10,11)By analyzing its leaves in-depth, this research clarifies the complex relationship between antioxidants and phytoconstituents, promoting improvements in complementary medicine and healthcare prevention.(11,15)The aim of this research is to determine the Phytochemical analysis and Antioxidant activity of sphaeranthus indicus linn.

Materials and methods:

1. Preparation of methanolic extracts:

Fresh leaves of *Sphaeranthus indicus* Linn are collected and thoroughly cleaned to remove impurities. The leaves are dried, powdered, and subjected to maceration in methanol. The methanolic extract is obtained through filtration and concentrated under reduced pressure, yielding a concentrated extract for further analysis.

2. Determination of in vitro antioxidant activities:

DPPH scavenging assay:

Prepare a range of concentrations of the plant extract. Mix each concentration with DPPH solution. Incubate in the dark to allow the reaction. Measure the absorbance at a specific wavelength (e.g., 517 nm) using a UV-visible spectrophotometer. Calculate the percentage of inhibition.

Hydroxyl radical scavenging assay:

Prepare various concentrations of the plant extract. Mix each concentration with a reaction mixture containing FeSO₄, salicylic acid, and hydrogen peroxide. Incubate the mixture to allow hydroxyl radical scavenging. Terminate the reaction with trichloroacetic acid. Measure the absorbance at a specific wavelength (e.g., 510 nm). Calculate the percentage inhibition.

3. Qualitative phytochemical screening:

The methanolic extract is subjected to qualitative phytochemical analysis to identify various classes of secondary metabolites. Tests for alkaloids, flavonoids, phenols, tannins, saponins, glycosides, and other constituents are conducted. Observations from the screening provide insights into the diverse phytochemical composition of the plant extract.

4. Determination of total phenolic contents:

The Folin-Ciocalteu method is employed to determine the total phenolic content. The methanolic extract is reacted with Folin-Ciocalteu reagent, and the absorbance is measured. The total phenolic

content is quantified using a standard calibration curve with gallic acid, expressed as mg of gallic acid equivalents (GAE) per gram of plant extract.

5. Total flavonoid content determination:

Employ the aluminum chloride colorimetric assay. React the plant extract with aluminum chloride. Measure the absorbance at a specific wavelength. Quantify total flavonoid content using a standard calibration curve with quercetin. Express the results as mg of quercetin equivalents (QE) per gram of plant extract. These detailed methodologies provide a systematic approach to extract preparation, qualitative analysis of phytoconstituents, and quantification of antioxidant compounds, contributing to a comprehensive understanding of the medicinal potential of *Sphaeranthus indicus* Linn leaves.

Results:

Table:1 In this antioxidant assay ascorbic acid is used as standard .Its concentration ranges from 0.2 to 2.0 mg/ml. As the concentration increases the antioxidant activity also increases .The antioxidant activity is found to be more in ascorbic acid concentrates compared to that of plant extract concentrates.

Table:1 Antioxidant activity of methanolic extract of sphaeranthus indicus linn

concentration	Ascorbic acid	Plant extract
0.2	0.35	0.25
0.4	0.86	0.78
0.6	1.22	0.96
0.8	1.98	1.20
1	2.50	2.00

Table 2 represents the DPPH scavenging activity, measured as the percentage of inhibition, for both L-ascorbic acid (Vitamin C) and the plant extract. The concentrations for both L-ascorbic acid and the plant extract vary across a range from 0.1 to 0.5 mg/mL. This range is likely chosen to assess the DPPH scavenging activity at different dosage levels. It appears that, both L-ascorbic acid and the plant extract show higher percentages of inhibition at higher concentrations, and indicates

more effective DPPH scavenging activity, suggesting stronger antioxidant potential. The DPPH scavenging activity of L- Ascorbic acid is higher than that of the plant extract at equal concentrations.

Table:2 DPPH scavenging activity of methanolic extract of sphaeranthus indicus linn

Concentration in mg/dl	L ascorbic acid	Plant extract
0.250	55	45
0.125	43	36
0.25	40	29
0.5	35	22
0.1	29	16

Table 3 represents the hydroxyl radical scavenging activity for both L- ascorbic acid and the plant extract. The concentrations for both L-ascorbic acid and the plant extract vary across a range from 0.125 to 1.0 mg/mL. Hydroxyl radicals are highly reactive and can cause cellular damage. The percentage of inhibition indicates how well the tested substances can scavenge or neutralize these hydroxyl radicals. The data suggests that both L-ascorbic acid and the plant extract exhibit significant hydroxyl scavenging activity, and there is a trend of increased inhibition with higher concentrations. The hydroxyl scavenging activity of both L-ascorbic acid and the plant extract are almost similar, proving the plant extracts ability to neutralize hydroxyl radicals, which can be associated with protection against oxidative stress-related damage.

Table:3 Hydroxyl radical scavenging activity of methanolic extract of sphaeranthus indicus linn

Concentration in mg/dl	L ascorbic acid	Plant extract
0.250	86	80
0.125	75	76

0.25	65	63
0.5	59	52
0.1	48	40

Table:4 phytochemicals analysis of methanolic extract of sphaeranthus indicus linn

Phytochemicals	Qualitative analysis of plant extract
Flavonoids	+
phenols	+
Steroids	+
Saponins	+
Alkaloids	+
Cardiac glycosides	-
Terpenoids	+

The qualitative analysis suggests that the plant extract contains a variety of phytochemicals with potential health benefits. The abundance of flavonoids, phenols, steroids, saponins, alkaloids, and terpenoids indicates the complexity of the plant's chemical composition. The absence of cardiac glycosides may have implications for specific physiological effects associated with these compounds.

Discussion:

The qualitative phytochemical screening of the methanolic extracts revealed the presence of various phytochemical groups in *Sphaeranthus indicus* Linn, including saponins, alkaloids, terpenoids, flavonoids, cardiac glycosides, steroids, and phenols. The presence of diverse phytochemicals in the methanolic extract of *Sphaeranthus indicus* Linn leaves indicates the richness of bioactive compounds. (16)Flavonoids and phenols, as identified in the phytochemical

analysis, contribute to the antioxidant potential of the plant. These compounds are renowned for their ability to neutralize free radicals, providing protection against oxidative stress-related diseases. The presence of steroids and terpenoids suggests potential anti-inflammatory and antimicrobial properties. These compounds have been linked to various pharmacological activities, contributing to the overall therapeutic profile of the plant extract. Saponins and alkaloids are compounds with diverse biological activities, including anticancer and antifungal properties. (17,18) The identification of these compounds further supports the multifaceted medicinal potential of *Sphaeranthus indicus*. The antioxidant analysis provides quantitative data on the antioxidant capacity of the methanolic extract. This is crucial in understanding the plant's ability to combat oxidative stress, which is implicated in various chronic diseases. The ability of the methanolic extract to scavenge free radicals, as measured in terms of antioxidant analysis, indicates its potential to protect cells from oxidative damage. This is particularly relevant in preventing or managing oxidative stress-related conditions. The antioxidant activity of *Sphaeranthus indicus* Linn extracts was evaluated using different assays such as DPPH radical scavenging assay, and hydroxyl radical scavenging activity assay. Ascorbic acid, which ranges

from 0.2 to 2.0 mg/mL, is used as the standard in this antioxidant assay. According to the findings, antioxidant activity increases with concentration and is noticeably stronger in ascorbic acid than in the concentrations of plant extracts. (17) The DPPH scavenging activity of both compounds is shown in Figure 2, wherein the concentrations of the drugs increase with increased inhibition percentages. At similar doses, ascorbic acid consistently exhibits better DPPH scavenging action than the plant extract. The hydroxyl radical scavenging activity is shown in Figure 3, where significant inhibition percentages are shown for both L-ascorbic acid and the plant extract at doses ranging from 0.125 to 1.0 mg/mL. Similar hydroxyl scavenging activity suggests that the plant extract might counteract free radicals, providing possible defense against damage brought on by oxidative stress. (14)

Previous research has been done comparing the phytochemical and Antioxidant activity Of different plant parts Of *sphaeranthus indicus*. (1) The methanolic extract from the stem consistently exhibits a relatively higher presence of phytochemicals across all tested compounds compared to other plant parts. Ethyl acetate and petroleum ether extracts also show notable concentrations, especially in the stem. In their research the choice of solvent for extraction is (methanol, ethyl acetate, petroleum ether). Their research offers a quantitative percentage whereas our research provides qualitative assessment. The findings of this study suggest that *Sphaeranthus indicus* Linn leaves could be explored for potential medicinal applications. The diverse array of phytochemicals, coupled with antioxidant activity, makes it a promising candidate for further pharmacological studies. The findings not only underscore the diverse phytochemical profile of *Sphaeranthus indicus* but also highlight its promising antioxidant potential. Such attributes position this plant as a valuable resource for potential therapeutic applications, warranting further exploration in drug development or complementary medicine. This study contributes to our

understanding of *Sphaeranthus indicus* as a natural source of bioactive compounds, offering insights into its possible role in mitigating oxidative stress-related health concerns.(19)

Conclusion:

In conclusion, the in vitro phytochemical analysis and antioxidant activity of *Sphaeranthus indicus* Linn demonstrate its potential as a valuable medicinal plant. The qualitative screening revealed the presence of various phytochemical groups, including saponins, alkaloids, terpenoids, flavonoids, cardiac glycosides, steroids, and phenols. These phytochemicals contribute to the antioxidant activity of *Sphaeranthus indicus* Linn extracts. The observed antioxidant activity suggests that *Sphaeranthus indicus* Linn extracts may possess significant health benefits by protecting against oxidative stress-related diseases.

Acknowledgement

The authors are thankful to Saveetha Institute of Medical and Technical Sciences, Saveetha Dental College and Hospitals, Saveetha University for giving a platform to conduct the study.

Conflict of Interest

The authors would like to declare no conflict of interest in the present study.

Funding

The present project is supported by:

- Saveetha Dental College and Hospitals, Saveetha University
- Saveetha Institute of Medical and Technical Sciences,
- Srishti dental clinic.

References:

1. Tandon D, Gupta AK. Comparative assessment of antimicrobial and antioxidant activity between whole plant and parts of *Sphaeranthus indicus* Linn. (Asteraceae). *Clin Phytoscience* [Internet]. 2020 Dec;6(1). Available from: <https://clinphytoscience.springeropen.com/articles/10.1186/s40816-020-00172-1>
2. Garg M, Dwivedi N. Physicochemical and Phytochemical Studies on *Sphaeranthus indicus* Linn. with HPTLC Fingerprinting. *Journal of Drug Delivery and Therapeutics*. 2021 Mar 15;11(2):100–7.
3. Pal Jain A, Pratap Choudhary G. In vitro Antioxidant potential of *Sphaeranthus indicus* and *Abrus precatorius* leaves extracts. *J Adv Pharm Technol Res*. 2021 Jul 19;3821–5.

4. SAGE Publications Ltd [Internet]. 2015 [cited 2023 Nov 30]. Journals Permissions. Available from: <https://uk.sagepub.com/en-gb/eur/journals-permissions>
5. [No title] [Internet]. [cited 2023 Nov 22]. Available from: <https://doi.org/10.1080/22311866.2014.983977>
6. Joshi S, Megha, Bhide BV, Ghildiyal S, Nesari TM. Physico-chemical and Phytochemical Analysis of *Sphaeranthus indicus* Linn. (Whole Plant). *Pharmacognosy Res* [Internet]. 2023 [cited 2023 Nov 29];15(3). Available from: <https://www.phcogres.com/sites/default/files/PharmacognRes-15-3-492.pdf>
7. Gowri R, Madhavan V, Yoganarasimhan S. Pharmacognostical, phytochemical studies and total phenolic content of Munditika - Ayurveda drug (*Sphaeranthus indicus* Linn.). *Spatula DD*. 2015;5(2):123.
8. Singh S, Semwal BC, Upadhaya PK. Pharmacognostic study of *Sphaeranthus indicus* Linn.: A Review. *Pharmacogn J*. 2019 Oct 15;11(6):1376–85.
9. [No title] [Internet]. [cited 2023 Nov 30]. Available from: <http://dx.doi.org/10.19070/2377-8075-21000360>
10. ijaronline - Writology [Internet]. [cited 2023 Nov 29]. Available from: <https://doi.org/10.4103%2F0974-7788.76790>
11. Ramachandran S. Review on *Sphaeranthus indicus* Linn. (Kottai-kkarantai). *Pharmacogn Rev* [Internet]. 2013 [cited 2023 Nov 22];7(14). Available from: <http://dx.doi.org/10.4103/0973-7847.120517>
12. Chauhan MS, Yadav S. Qualitative and Quantitative Determination of Secondary Metabolites of *Sphaeranthus indicus* and *Spathodea campanulata* Flowers Extracts. *Journal of Drug Delivery and Therapeutics*. 2023 May 15;13(4):85–9.
13. Website [Internet]. Available from: <http://dx.doi.org/10.22159/ajpcr.2019.v12i2.29951>
14. Ahmad HI, Nadeem MF, Shoaib Khan HM, Sarfraz M, Saleem H, Khurshid U, et al. Phytopharmacological Evaluation of Different Solvent Extract/Fractions From *Sphaeranthus indicus* L. Flowers: From Traditional Therapies to Bioactive Compounds. *Front Pharmacol*. 2021 Oct 27;12:708618.
15. Harathi K, Giribabu D, Varadarajulu Naidu C. Phytochemical Evaluation and in Vitro Antibacterial Activity of *Sphaeranthus indicus* (L.)—An Important Antijaundice Medicinal Plant. *Am J Plant Sci*. 2017 Apr 17;8(5):1011–21.

16. Padmapriya, Preetha, Selvaraj, Sridevi. Effect of *Carica papaya* seed extract on IL -6 and TNF- α in human lung cancer cell lines - an In vitro study. *J Adv Pharm Technol Res.* 2022 Dec 24;5478–82.
17. Website [Internet]. Available from: <https://www.scopus.com/record/display.uri?eid=2-s2.0-85101096035&origin=resultslist&sort=plf-f&src=s&nlo=&nlr=&nls=&sid=63b046f1ad6f1a81cacad22b000fae45&sot=aff&sdt=cl&cluster=scoprefnameuid%2c%22Lakshmi%2c+T.%2357221436939%22%2ct%2c%22Thangavelu%2c+L.%2357221436939%22%2ct%2bscosubjabbr%2c%22DENT%22%2ct%2bscoexactsrctitle%2c%22International+Journal+Of+Dentistry+And+Oral+Science%22%2ct%2c%22Oral+Diseases%22%2ct&sl=55&s=AF-ID%28%22Saveetha+Dental+College+And+Hospitals%22+60109334%29&relpos=14&citeCnt=0&searchTerm=>
18. Website [Internet]. Available from: <https://www.scopus.com/record/display.uri?eid=2-s2.0-85092912484&origin=inward&txGid=5a0481005cb86983ccc2fe9d54b6d590>
19. Singh S, Semwal BC. Protective effect of methanol leaf extract of *Sphaeranthus indicus* L. on an experimental model of epilepsy. *Indian Journal of Natural Products and Resources (IJNPR) [Formerly Natural Product Radiance (NPR)]*. 2020 Jan 28;10(3):175–80.