

# SCREENING OF BIOACTIVE COMPOUNDS AND EVALUATION OF IN VITRO ANTIOXIDANT ACTIVITY OF HYDRO-ETHANOLIC EXTRACT OF *Sphaeranthus indicus* LEAVES

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## Abstract

Oxidative stress is the outcome of the difference between antioxidants and pro-oxidants in an organism, and it is vital in the pathogenesis of several degenerative disorders. The use of natural antioxidants is effective in delaying the oxidation of biomolecules and thus preventing diseases. The present study aimed to investigate the phytochemical screening and in vitro antioxidant activity of *Sphaeranthus indicus* leaf extract. Phytochemical screening of *Sphaeranthus indicus* leaves showed the presence of tannin, saponin, flavonoids, steroids, terpenoids, triterpenoids, alkaloids, anthraquinones, polyphenol, glycoside, coumarins while emodins and anthocyanins were absent. GC-MS analysis was performed to identify the bioactive chemical constituents present in the extract. Twenty compounds were identified in the leaf extract of *Sphaeranthus indicus* by GC-MS analysis. *Sphaeranthus indicus* leaf extract showed significant antioxidant activity against free radical scavenging by DPPH, total antioxidant, iron chelator, hydroxyl radical scavenging, and nitric oxide scavenging activity. The effects were comparable to those of the standard antioxidant ascorbic acid and these antioxidant properties were concentration dependent. This study recommends the biological assay of extracts against disorders related to oxidative stress for the development of phytomedicine with antioxidant properties. These antioxidant activities could be due to the presence of antioxidant phytochemicals such as flavonoids, phenols, terpenoids and saponins, among others.

**Keywords:** Oxidative stress, *Sphaeranthus indicus*, Phytochemicals, Antioxidants. Free radicals

## INTRODUCTION

Oxidative stress is the result of the disparity between pro-oxidants and antioxidants in an organism, and it is important in the pathogenesis of several degenerative disorders, such as arthritis, Alzheimer's disease, cancer and the diseases cardiovascular. Free radicals can damage biomolecules, such as nucleic acids, lipids, proteins, polyunsaturated fatty acids, carbohydrates, and DNA, leading to mutations. The use of antioxidants is effective in delaying the oxidation of biomolecules. Antioxidants are substances that prevent and stabilize free radical damage by supplying antioxidant electrons to these damaged cells. Antioxidants also convert free radicals into waste by-products, which are eliminated from the body. Consumption of fruits and vegetables fortified with antioxidants is known to reduce the risk of several diseases caused by free radicals (Hamid et al., 2010). Classically, oxidative stress is managed using various synthetic antioxidant compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG). Despite their use, these synthetic antioxidant compounds have been associated with adverse effects (Gryglewski et al., 1987). Therefore, due to the profound consequences of oxidative stress and the drawbacks of synthetic antioxidants, the need for alternative, safer, easily accessible and potent antioxidants is warranted. Given the alternative and complementary strategies available, herbal medicines have a better chance. to provide potent, safer, affordable and easily accessible therapies for diseases related to oxidative stress (Koleva et al., 2002).

Phytochemicals are receiving increasing attention, both scientifically and commercially. As is nowadays usually predictable, numerous plant compounds and colours have effects on animals and humans. There is now a great effort to study and understand at a fundamental level and the significant health effects of these compounds. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern medicines have been isolated from natural resources (Ali, 2014). Traditional medicine is an important source of new compounds potentially useful for the development of chemotherapeutic agents. The essential values and uses of certain plants have been elaborated and published, but many of them remain unexplored to this day. Therefore, there is a need to explore their uses and conduct pharmacognostic and pharmacological studies to discover their medicinal properties (Ankita and Jain, 2012). In modern medicine, plants are used as sources of direct therapeutic agents,

as models for new synthetic compounds and as taxonomic markers for the elaboration of more complex semi-synthetic chemical compounds (Akerele, 1992; Sivaraj et al., 2011). In particular, despite the extensive use of wild plants as medicines in India, there are few reports in prose of the antioxidant activity and chemical composition of these plants. In the present study, we made a systematic record of the relative antioxidant activity in selected medicinal plant species, which are traditionally used (Mantle et al., 2000; Oke and Hamburger, 2002). Bearing in mind, the present study aimed to investigate the phytochemical analysis and antioxidant activity of *Sphaeranthus indicus* leaf extract.

## MATERIALS AND METHODS

### Collection of plant materials

The leaves of *Sphaeranthus indicus* were collected in December 2020 from Nemmeli, Chengalpattu district, Tamil Nadu, India.

### Preparation of Hydro-alcoholic extract

10grams of *Sphaeranthus indicus* leaves powder were used for extraction. Extraction was performed with cold extraction using the maceration method into ethanol and water solvent (70:30) for 24 hours using the “intermittent shaking” method to obtain an extract. The extract was filtered using Whatman filter No 1 paper and filtrate was used for phytochemical and in vitro antioxidant assay.

### Qualitative phytochemical analysis

A preliminary phytochemical test was carried out by using standard procedure Sofowara (1993), Trease and Evans (1989) and Harborne (1973, 1984).

### GC MS Analysis

GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument. Software adapted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0 (Srinivasan et al., 2013). The mass spectrum was interpreted with the aid of the database and the unknown component was compared with the spectrum of the known components stored in the NIST08s, WILEY8 and FAME libraries. The name, molecular weight and structure of the components of the test materials were ascertained (Dr. Dukes, 2013).

### In vitro antioxidant studies

The DPPH radical scavenging activity was screened by the of Shimada et al. (1992) method. The total antioxidant activity by the method of Prieto et al. (1999). The chelating activity of the plant extract for Fe<sup>2+</sup> ferrous ions was measured according to the method of Dinis et al., (1994). The hydroxyl radical scavenging activity was measured with the Fenton reaction by the method of Yu et al. (2004). The nitric oxide radical scavenging activity was determined according to the method reported by Garrat (1964).

### Statistical analysis

Tests were carried out in triplicates for 3 separate experiments. The amount of *Sphaeranthus indicus* leaf extract needed to inhibit free radicals' concentration by 50%, IC<sub>50</sub>, was graphically determined by a linear regression method using Ms-Windows-based GraphPad Instat (version 3) software. Results were expressed as graphically/ mean  $\pm$  standard deviation.

## RESULTS AND DISCUSSION

### Qualitative phytochemical analysis

In the present study to investigate the phytochemical analysis of a hydro-alcoholic extract of *Sphaeranthus indicus* leaf powder. Table 1 represents the qualitative phytochemical analysis of a hydroalcoholic extract of *Sphaeranthus indicus* leaf powder. The results of the present study showed that the presence of tannin, saponin, flavonoids, steroids, terpenoids, triterpenoids, alkaloids, anthraquinone, polyphenol, glycoside, coumarins while emodins and anthocyanins were absent. The high intensity of the color indicates the respective high phytochemical concentration present.

Table 1: Qualitative phytochemicals analysis of hydroalcoholic extract of *Sphaeranthus indicus* leaves powder

S. No	Phytochemicals	Hydroalcoholic extract
1	Tannin	++
2	Saponin	++
3	Flavonoids	++
4	Steroids	++
5	Terpenoids	++
6	Triterpenoids	+
7	Alkaloids	+
8	Anthraquinone	+
9	Polyphenol	++
10	Glycoside	+
11	Coumarins	++
12	Emodins	-
13	Anthocyanins	-

(-) Absent, (+) Present and (++) high concentration

Compounds belonging to the respective groups have been reported to impart various medicinal characteristics to plants. Due to the presence of flavonoids, plants possess antioxidant properties as flavonoids are water-soluble antioxidants with free radical scavenging properties along with anticancer activities (Yadav and Agarwala, 2011). Alkaloids were known to possess analgesic and antibacterial properties (Nassar et al., 2010) while terpenoids were well known due to their antibacterial, anti-inflammatory and anticancer properties (Chung et al., 1998). Tannins have anticancer and antibacterial activities (Radhika et al., 2013). Triterpenoids may enhance insulin release by altering glucose metabolism and thus act as an antidiabetic potential (Sabbah et al., 2017). Steroids were well known to have cardio-tonic effects as well as insecticidal and antibacterial effects (Bagrov et al., 2009). Phenolic compounds and phytosterol present in plants are responsible for antimicrobial, antiallergic, antidiabetic, antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic properties (Khan et al., 2015)

The presence of saponins in the plant is very important due to their use in the treatment of hypocholesterolemia and hyperglycemia due to anticancer, antifungal, antioxidant, antibacterial and weight loss properties (Lira et al., 2017). Glycosides play a role in anticoagulant activity and antitumor activity (Xiao, 2017). Coumarins are responsible for the antimalarial and plasmoidal properties of ants (Ntie-Kang et al., 2014). Anthocyanin provides plants with anti-obesity, anti-inflammatory, anti-cancer and neuroprotective properties (Chien et al., 2015). Anthraquinones found in plants are responsible for regulating immunity and play a therapeutic role in autoimmune diabetes (Rastogi et al., 2015). Triterpenoids have anticancer properties (Gracelin et al., 2013). Various phytochemicals with antioxidant value present in medicinal plants are responsible for this bioactivity. Qualitative phytochemical profiling of the studied plant extracts showed the presence of flavonoids, phenols and tannins among other antioxidant phytochemicals.

#### Identification of bioactive compounds in *Sphaeranthus indicus* leaves extract by GC MS analysis

Twenty compounds were identified in *Sphaeranthus indicus* leaves extract using GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in Figure 1 and Table 2. The prevailing compounds are 9,12,15-Octadecatrienoic acid methyl ester Octadecanoic acid, Phytol, 9,12-Octadecadienoic acid and 9-Octadecenoic acid were found in this your sample. The presence of various bioactive compounds justifies the use of the plant for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents and subjecting its biological activity (Table 3 will definitely give fruitful results).

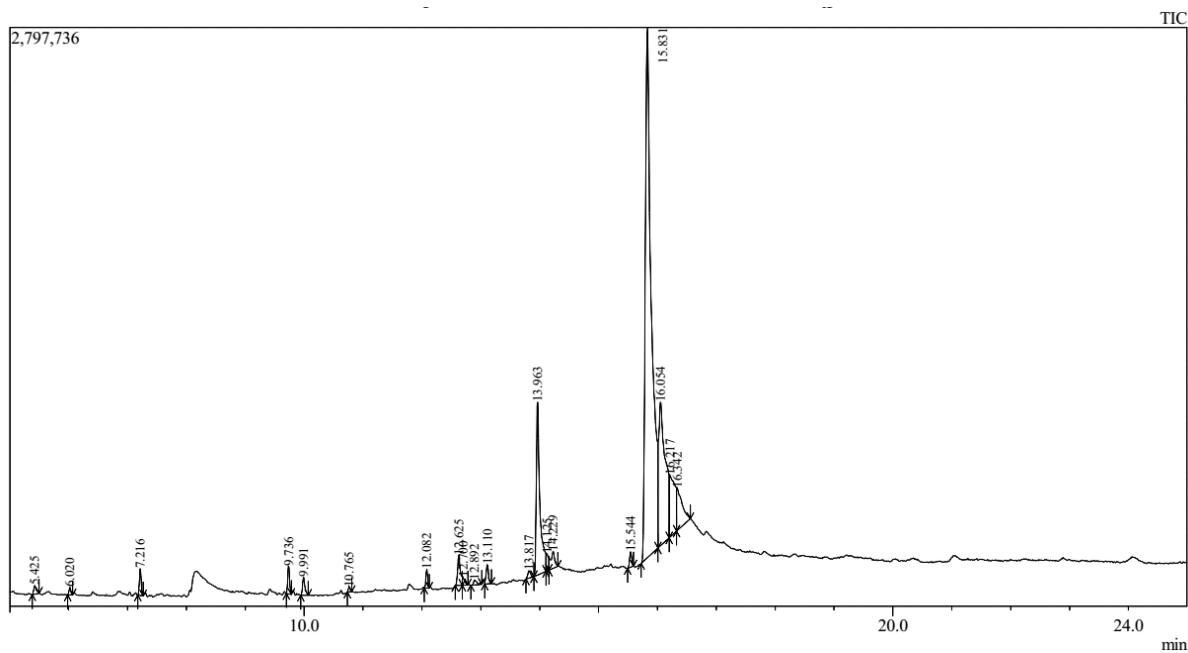


Figure 1: Chromatogram of your sample

Table 2: Identification of active compounds in Sphaeranthus indicus leaves extract using GCMS

Peak#	R. Time	Area%	Height%	M. weight (g/mol)	Molecular formula	Molecular Name
1	5.425	0.37	0.72	168	C <sub>12</sub> H <sub>24</sub>	Cyclohexane, hexyl
2	6.020	0.22	0.63	226	C <sub>15</sub> H <sub>30</sub> O	2-Pentadecanone
3	7.216	0.82	2.25	224	C <sub>16</sub> H <sub>32</sub>	3-Hexadecene,
4	9.736	0.88	2.35	252	C <sub>18</sub> H <sub>36</sub>	9-Octadecene, (E)
5	9.991	0.72	1.52	330	C <sub>20</sub> H <sub>26</sub> O <sub>4</sub>	1,2-Benzoldicarbonsaeure, DI-(HEX-1-EN-5-YL-ESTER)
6	10.765	0.15	0.39	100	C <sub>6</sub> H <sub>12</sub> O	1-Penten-3-ol, 4-methyl
7	12.082	0.54	1.59	280	C <sub>20</sub> H <sub>40</sub>	3-Eicosene
8	12.625	1.41	2.53	278	C <sub>20</sub> H <sub>38</sub>	9-Eicosyne
9	12.700	0.30	0.68	112	C <sub>7</sub> H <sub>12</sub> O	3-Methoxy-1-Cyclohexene
10	12.892	0.31	0.42	222	C <sub>16</sub> H <sub>30</sub>	1-Hexadecyne
11	13.110	0.77	1.63	336	C <sub>22</sub> H <sub>40</sub> O <sub>2</sub>	19,19-Dimethyl-8,11-Icosadienoic acid
12	13.817	0.37	0.63	292	C <sub>19</sub> H <sub>32</sub> O <sub>2</sub>	9,12,15-Octadecatrienoic acid, methyl ester
13	13.963	10.54	14.64	284	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	Octadecanoic acid
14	14.125	0.63	1.45	232	C <sub>12</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub>	2-([(1-Cyano-1-Methylethyl) Amino] Carbonyl) Benzoic acid
15	14.229	1.14	1.46	587	C <sub>36</sub> H <sub>75</sub> O <sub>3</sub> P	Phosphonic acid, dioctadecyl ester
16	15.544	0.55	1.30	296	C <sub>20</sub> H <sub>40</sub> O	Phytol
17	15.831	54.80	44.88	280	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	9,12-Octadecadienoic acid
18	16.054	16.12	12.13	282	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	9-Octadecenoic acid
19	16.217	5.64	5.23	238	C <sub>15</sub> H <sub>26</sub> O <sub>2</sub>	N-Terpinenyl ester of n-pentanoic acid
20	16.342	3.73	3.56	110	C <sub>8</sub> H <sub>14</sub>	2-methylmethylenecyclohexane

Table 3: Biological activity of compounds identified in *Sphaeranthus indicus* leaves extract using GCMS

S. No	Molecular Name	Biological activity*
1	3-Hexadecene	Kidney function stimulant, Cognition disorders treatment, Antianginal
2	9-Octadecene	<b>Anticancer, antioxidant</b> and antimicrobial activities, Fucosterol-epoxide lyase inhibitor, Chymosin inhibitor
3	3-Eicosene, 9-Eicosyne	Antimicrobial activity
4	9,12,15-Octadecatrienoic acid, methyl ester	Hypocholesterolemic, <b>cancer preventive</b> , nematicide, hepatoprotective, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronal properties,
5	Octadecanoic acid	Antiviral, antiinflammatory, 5- $\alpha$ -reductase inhibitor, hypocholesterolemic, Suppository.
6	Phytol	Antimicrobial, <b>Anti-cancer</b> , Anti-inflammatory
7	9,12-Octadecadienoic acid	Antiinflammatory, Nematicide, Insectifuge, Hypocholesterolemic, <b>Cancer preventive</b> , Hepatoprotective, Antihistaminic, Antiacne, Antiarthritic, Antieczemic, 5-Alpha reductase inhibitor, Antiandrogenic, Anticoronal.
8	9-Octadecenoic acid	Antiinflammatory, Antiandrogenic, <b>Cancer preventive</b> , Dermatogenic, Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiogenic, Insectifuge,.

\*Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database].

9,12-Octadecadienoic acid is an unsaturated fatty acid ester that has anti-inflammatory, nematicidal, insecticidal, cholesterol-lowering, cancer-preventive, hepatoprotective, antihistamine, anti-acne, anti-arthritic, anti-eczema, 5- alpha reductase, antiandrogens and anticoronaries. (Dhayabaran and Thangarathinam, 2016) while 9-octadecenoic acid which is a steroid has anti-inflammatory, anti-androgenic, cancer preventive, dermatogenic, hypocholesterolemic, 5-alpha reductase inhibitor, anemiogenic and insecticidal properties (Kuppuswamy et al. 2013). According to Akpuaka et al. (2013) identified the hexadecane is a hydrocarbon which has antifungal, antioxidant and antibacterial activities, eicosanoid which is a fatty acid has antibacterial activity.

Phytol is said to have antioxidant, antiallergic, antinociceptive, and anti-inflammatory activities (Santos et al., 2013). Recent studies have revealed that phytol is an excellent immunostimulant. It is superior to several commercial adjuvants in inducing long-term memory and activating innate and acquired immunity. Phytol also showed antimicrobial activity against *Mycobacterium tuberculosis* and *Staphylococcus aureus* (Saikia et al. 2010). Similarly, Sridharan et al. (2011) in the leaves of *Mimosa pudica*. A similar result was also observed in *Lantana camara* leaves (Sathish kumar et al., 2013; Inoue et al., 2005).

#### In vitro antioxidant activity

Oxidative stress plays an important role in the development and pathophysiology of many diseases (Adwas et al., 2019). For the assessment of the antioxidant potential of endogenous compounds, a single assay method is not sufficient. In the present study different antioxidant models as DPPH, total antioxidant, iron chelating, hydroxyl radical scavenging and nitric oxide scavenging activity were used to assess the antioxidant properties of *Sphaeranthus indicus* extract.

#### DPPH radical scavenging activity

The DPPH method is one of the most popular procedures for testing the antioxidant potential of a plant extract. DPPH is a relatively stable radical that acts as an antioxidant or free radical scavenger by donating hydrogen ions to compounds in the oxidized state (Soare et al., 1997). The effect of antioxidants on DPPH is thought to be due to their ability to donate hydrogen

(Baumann, 1979). Radical scavenging activities are very important in preventing the deleterious role of free radicals in various diseases, including cancer. DPPH free radical scavenging is an accepted mechanism for screening the antioxidant activity of plant extracts. In the DPPH test, the violet-colored DPPH solution is reduced to a yellow-colored product, diphenylpicryl hydrazine, by the addition of *Sphaeranthus indicus* leaf extract in a concentration-dependent manner. This method has been widely used to predict antioxidant activities due to the relatively short time required for analysis.

Figure 1 represents the DPPH radical scavenging activity of *Sphaeranthus indicus* leaf extract and compared with Ascorbic acid. The DPPH radical scavenging activity was directly proportional to the concentration of *Sphaeranthus indicus* leaf extract. The half-maximum inhibition concentration (IC<sub>50</sub>) of Ascorbic acid (53.90 µg/ml) and *Sphaeranthus indicus* leaf extract (62.44 µg/ml) were and respectively (Figure 1a). The overall present study was % of inhibition based on the concentration of extract depended ( $R^2 = 0.991$ ). The results obtained in this study suggest that all the extracts from leaf extract showed radical scavenging activity by their electron transfer or hydrogen donating ability. Flavonoids and related phenolic compounds content and radical scavenging antioxidant activity are highly correlated (Huang and Prior, 2005).

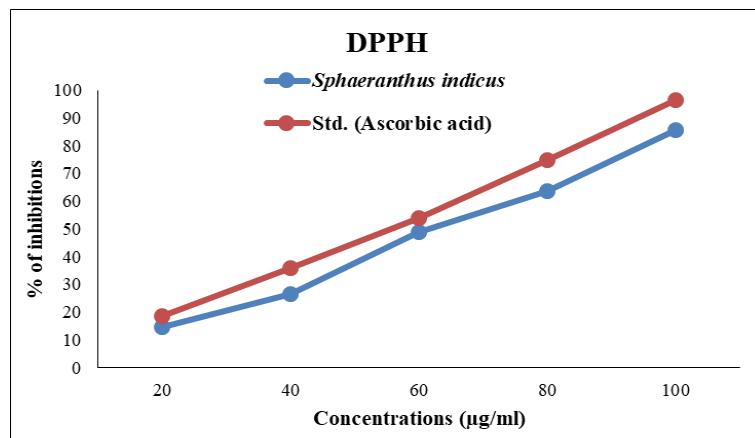


Figure 1a: DPPH radical scavenging activity of *Sphaeranthus indicus* leaf extract and compared with Ascorbic acid

#### Total antioxidant capacity

The antioxidant potential of *Sphaeranthus indicus* leaf extract was estimated from their ability to reduce the reduction of Mo (VI) to Mo(V) by the antioxidant-enriched fractions and the subsequent formation of a green phosphate complex. /Mo (V) at acidic pH. The antioxidant activity depends on the presence of its bioactive compounds, mainly polyphenols, flavonoids, carotenoids and vitamins E and C (Oktay et al., 2003). Figure 2 noted the total antioxidant activity of *Sphaeranthus indicus* leaf extract and compared it to ascorbic acid. Total antioxidant activity increased with increasing concentration of *Sphaeranthus indicus* leaf extract. The half-maximal inhibitory concentration (IC<sub>50</sub>) of ascorbic acid (54.78 µg/ml) and *Sphaeranthus indicus* leaf extract (65.49 µg/ml) were and respectively (Figure 2). This suggests that the concentration of bioactive compounds present in the extract is important in showing antioxidant activity. Thus, the higher concentration of extracts shows higher antioxidant activity. The present overall study was % inhibition based on extract concentration depended ( $R^2 = 0.996$ ). Our results are consistent with data published elsewhere (Oktay et al., 2003) and suggest that antioxidant capacity can be attributed to the chemical composition and polyphenol content of the extract.

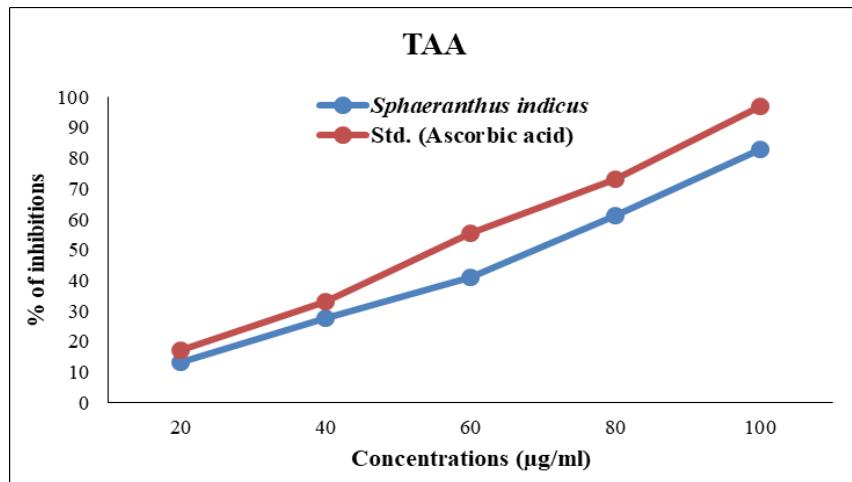


Figure 2: Total antioxidant activity of *Sphaeranthus indicus* leaf extract and compared with Ascorbic acid

#### Iron chelating activity

As excess free iron has been implicated in the induction and formation of free radicals in biological systems, we have tested our herbal extracts in a metal chelation assay. Chelation therapy reduces iron-related complications in humans and thus improves quality of life and overall survival in certain diseases such as thalassemia major (Hebbel et al., 1990). Moreover, cerebral dysregulation of iron and its association with the formation of amyloid precursor protein plaques is implicated in the pathology of Alzheimer's disease (AD) and iron chelation could therefore be considered as a rational therapeutic strategy for the MA (Ebrahimzadeh et al., 2009). Foods are often contaminated with transition metal ions which may be introduced by processing methods. The transition metal, iron, is capable of generating free radicals from peroxides by Fenton reactions and may be implicated in human cardiovascular disease (Ebrahimzadeh et al., 2009). Because Fe<sup>2+</sup> also has been shown to cause the production of oxyradicals and lipid peroxidation, minimizing Fe<sup>2+</sup> concentration in Fenton reactions affords protection against oxidative damage. The chelating of ferrous ions by the extract was estimated by the method of Dinis et al., (1994).

Ferrozine can quantitatively form complexes with Fe<sup>2+</sup>. Figure 3 noticed the iron chelating activity of *Sphaeranthus indicus* leaf extract and compared with ascorbic acid. The iron chelating activity was directly proportional to the concentration of *Sphaeranthus indicus* leaf extract. The half-maximal inhibitory concentration (IC<sub>50</sub>) of ascorbic acid (54.11 μg/ml) and *Sphaeranthus indicus* leaf extract (63.36 μg/ml) were and respectively (Figure 3). The present overall study was % inhibition based on extract concentration dependent ( $R^2 = 0.998$ ). In the presence of other chelating agents, the formation of complexes is disturbed with the result that the color of the complexes decreases. In this assay, the extract and EDTA interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ions before ferrozine. The absorbance of the Fe<sup>2+</sup>-ferrozine complex decreased dose-dependently with increasing concentration. The metal chelating capacity was important since the extract reduced the concentration of the transition metal catalyzing lipid peroxidation<sup>28</sup>. Chelating agents have been reported to be effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Ebrahimzadeh et al., 2010). *Sphaeranthus indicus* leaf extract showed good Fe<sup>2+</sup> chelating ability.

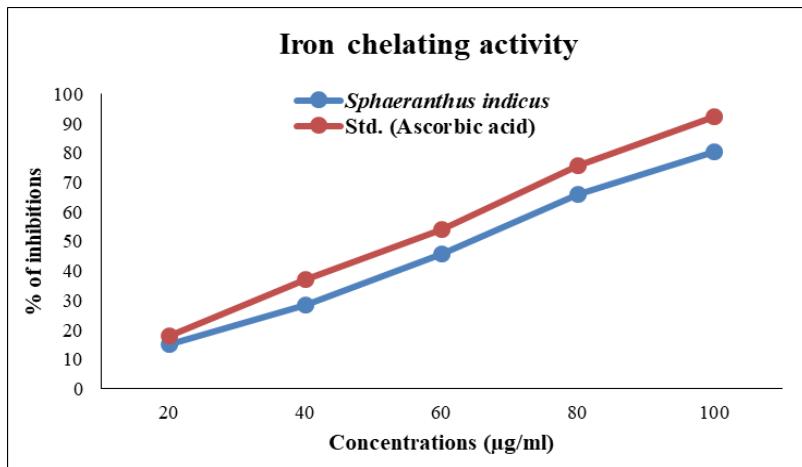


Figure 3: Iron chelating activity of *Sphaeranthus indicus* leaf extract and compared with Ascorbic acid

#### Hydroxyl radical scavenging activity

The mutagenic capacity of free radicals is due to the direct interactions of hydroxyl radicals with DNA, leading to DNA degradation and therefore playing an important role in cancer formation (Liu et al., 2009). The hydroxyl radicals are formed by incubating an Fe<sup>+3</sup> - EDTA premix with ascorbic acid and H<sub>2</sub>O<sub>2</sub> at pH 7.4, causing the degradation of 2-deoxy-ribose and generating a malondialdehyde-like product (MDA). Adding the hydroethanolic extract of *Sphaeranthus indicus* leaves to the reaction mixture scavenges hydroxyl radicals and prevents further damage. Figure 4 noted the activity of *Sphaeranthus indicus* leaf extract and compared it to ascorbic acid. The hydroxyl radical scavenging activity was directly proportional to the concentration of *Sphaeranthus indicus* leaf extract. The half-maximal inhibitory concentration (IC<sub>50</sub>) of ascorbic acid (53.01 µg/ml) and *Sphaeranthus indicus* leaf extract (63.19 µg/ml) were and respectively (Figure 4). The present overall study was % inhibition based on extract concentration dependent ( $R^2 = 0.998$ ). The extracts showed appreciable hydroxyl radical scavenging activity compared to the standard antioxidant ascorbic acid and may serve as anticancer agents by inhibiting the interaction of hydroxyl radicals with DNA. The ability of extractives to quench hydroxyl radicals could be directly related to the prevention of lipid peroxidation.

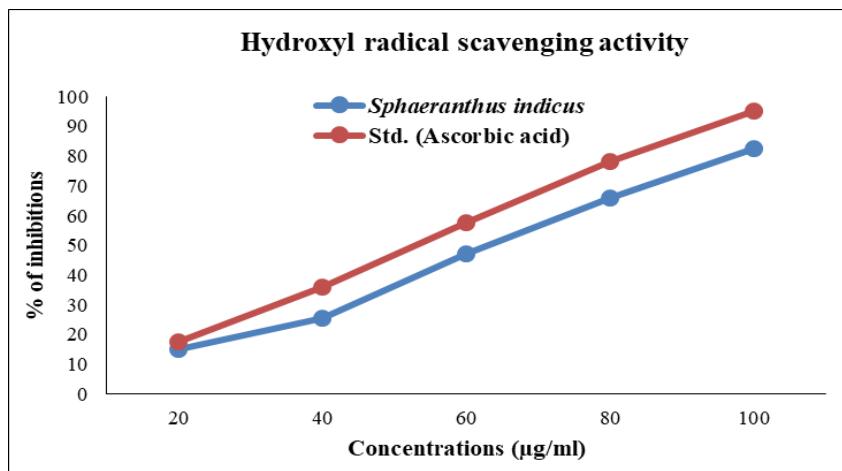


Figure 4: Hydroxyl radical scavenging activity of *Sphaeranthus indicus* leaf extract and compared with Ascorbic acid

#### Nitric oxide scavenging activity

Nitric oxide is a free radical produced in mammalian cells involved in the regulation of various physiological processes. However, excessive nitric oxide production is associated with several diseases such as carcinomas, juvenile diabetes, multiple sclerosis, arthritis, and ulcerative colitis. The development of substances to prevent the overproduction of nitric oxide has

become a new research target for the treatment of chronic inflammatory diseases (Hazra., et al., 2008). To neutralize this radical, no endogenous enzymatic scavenging pathway is present inside the body and most often it is neutralized by several endogenous molecules such as glutathione, melatonin and antioxidants supplemented by diet (Garratt , 2012). Nitric oxide (NO), which is an important bioactive molecule, has several physiological functions (Marcocci et al., 1994). Nitric oxide was generated from sodium nitroprusside and was measured by Griess' reagent. Sodium nitroprusside in an aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitric ions which can be estimated using Griess's reagent. Nitric oxide scavengers compete with oxygen, which reduces nitric oxide production (Marcocci et al., 1994). Figure 5 noted the nitric oxide scavenging activity of *Sphaeranthus indicus* leaf extract and compared it to standard ascorbic acid. The nitric oxide scavenging activity was directly proportional to the concentration of *Sphaeranthus indicus* leaf extract. The half-maximal inhibitory concentration (IC50) of ascorbic acid (54.11  $\mu$ g/ml) and *Sphaeranthus indicus* extract (63.36  $\mu$ g/ml) were and respectively (Figure 5). The present overall study was % inhibition based on extract concentration dependent ( $R^2 = 0.998$ ).

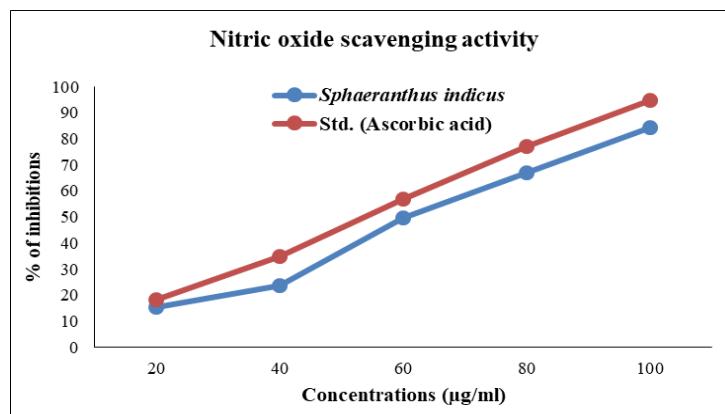


Figure 5: Nitric oxide scavenging activity of *Sphaeranthus indicus* leaf extract and compared with Ascorbic acid

The *Sphaeranthus indicus* extract showed good activities in DPPH radical scavenging, total antioxidant, iron chelating, hydroxyl and nitric oxide radical activity supported by Mallek-Ayadi et al., (2017).

## CONCLUSION

Overall, it can be concluded that *Sphaeranthus indicus* leaves contain a rich source of phytochemicals and potential antioxidant activity confirmed by various in vitro models. Antioxidant activity was directly proportional to leaf extract concentration. The antioxidant activity of leaf extract could be due to the presence of phytochemicals such as phenols, flavonoids, alkaloids, terpenoids, etc. Further studies aimed at isolating and characterizing pure phytoactive principles for improvement are recommended.

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