CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1204485

Available online at: http://www.iajps.com Research Article

PRELIMINARY PHYTOCHEMICAL AND GC MS ANALYSIS OF DIFFERENT EXTRACTS OF *SPHAERANTHUS INDICUS* LEAVES

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Running Title: Phytochemcial and GC MS study of *Sphaeranthus indicus*

Abstarct:
The use of plants as sources of medicines is an age old practice. The present study deals with the phyto-chemical and GC MS analysis of different extracts of one medicinal plant, Sphaeranthus indicus. The results indicated the presence of flavonoids and alkaloids in methanol and water extracts. Triterpenoids and saponins were present in ethanol and water extracts. Tannins were present in methanol and water extracts whereas steroids were present in ethanol and methanol fractions. Amino acids and anthraquinons were present in water and cardiac glycosides in ethanol fractions. The GC MS results of methanol and water extracts indicated the presence of some important biomolecules such as 2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl) (21.41%), 2,2,6-Trimethyl-1-(3-methylbuta-1,3-dienyl)-7-oxabicyclo[4.1.0]heptan-3-ol (10.96%), 3-Hexenoic acid, 2-methyl-, methyl ester (10.55%) etc. which indicated the medicinal values of this plant.

Key words: *Sphaeranthus indicus, GC MS, Phytochemical, Triterpenoid, Steroid, Flavonoid,*

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QR code

Please cite this article in press M Ram Krishna Rao and N. Vijaya Lakshmi., ***Preliminary Phytochemical and GC MS Analysis of Different Extracts of Sphaeranthus Indicus Leaves,*** *Indo Am. J. P. Sci, 2018; 05(03).*

INTRODUCTION:

The role of plants and plant parts as medicines and sources of medicine is immense. The knowledge of the active principles and medicinal compounds in plants is continuously being updated through various biochemical methods. [1-6] The use of medicinal plants for their curative properties is a time tested. Most of the phyto-chemicals like tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids have therapeutic values. [7-9] The knowledge of these phyto-constituents is very helpful in drug discovery and new drug molecule formulation. [10-12] The discovery, development and use of modern medicines have a deep rooted connection with the age old practice of folk and traditional medicinal background of the natives. [13] Thus the ancient wisdom has been the basis of modern medicine and will remain as one of the important sources of future medicine and therapies. [14] The World health Organization also has recognized the importance of traditional medicines and has been active in creating strategies, guidelines and standards for botanical medicines. [15]

Sphaeranthus indicus is known as Mahamundi or Mundi in Ayurveda and as Kottai Karandhai in Tamil. This plant and has been widely studies for its various medicinal roles. Mahajan *et al*, 2015; Varsha *et al*, 2010; Zachariah *et al*, 2010, have extensively reviewed the ethno-botanical use of this plant, the phyto-chemicals present in various parts of this plant and their medicinal roles. [16-18] There are reports of its antivarial (Vimalanathan *et al*, 2009), antibacterial and antifungal (Tambekar and Khante, 2010, Sangeetha *et al*, 2010, Irfan *et al*, 2014, Meher *et al*, 2013), neuro-protective, central nervous depressant and anticonvulsant activities (Nanda *et al*, 2010), fertility enhancing (Sandhya *et al*, 2006), analgesic and antipyretic (Nanda *et al*, 2009), hepato-protective activity (Sundari *et al*, 2013), antidiabetic (Muhammad *et al*, 2011), haemostatic effect and anticancer (Nahata *et al*, 2013), antioxidant activities (Krishna *et al*, 2013). [19-30]

In the present study the phyto-chemical and GC MS analysis of different extracts of the leaves of *Sphaeranthus indicus* was undertaken.

MATERIALS AND METHODS:

Collection of Samples

Fresh leaves of *Sphaeranthus indicus* were collected from Karaikudi, Tamil Nadu, India, where this plant is extensively used for treating various diseases ethnically. The Leaves were thoroughly washed to remove any dust and impurities and shade dried. The dried leaves were ground to fine powder.

Preparation of Extracts

500 grams of dried powder of *Sphaeranthus indicus* was packed in separate round bottom flasks for sample extraction using ethanol, methanol, hexane and distilled water as solvents. The extraction was conducted with 750 ml of the solvent for a period of 72 hours. At the end of the extraction the solvents were concentrated under reduced pressure and the crude extracts were stored in refrigerator.

Phyto-chemical Analysis

The extracts prepared were analyzed for the presence of alkaloids, saponins, tannins, steroids, flavonoids, anthraquinones, triple sugars, amino acids, proteins, glycosides and reducing sugars as per standard protocols [31-33].

Test for Alkaloids

About 0.5 g of the prepared residue was dissolved in 2 N Hydrochloric acids. The mixture was filtered and the filtrate was divided into two equal portions. One portion was treated with a few drops of Mayer's reagent and the other was treated with equal amount of Dragendorff's reagent respectively. The appearance of creamish precipitate and orange precipitate respectively, indicated the presence of alkaloids.

Test for Saponins

About 0.5 g of the leaf extract was vigorously shaken with water in a test tube and then heated to boil. Frothing was observed which was taken as evidence for the presence of the Saponins.

Test for Tannins

About 0.5 g of leaf extract was added in 10 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration.

Test for Steroids

2 ml of acetic anhydride was added to 2 ml of leaf extract along with 2 ml Conc. sulphuric acid. The color change from violet to blue or green is observed for the presence of steroids.

Test for Flavonoids

2 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution few drops of conc. Hydrochloric acid was added and the red color was observed for flavonoids and orange color for flavones.

Test for Anthraquinones
About 0.5 g of extract was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% of ammonia solution. A pink violet or red color in the ammonical layer was observed for the presence of anthraquinones.

Test for Cardiac Glycosides
0.2 g of extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of cardiac glycosides.

Test for Amino acids
To 2ml of sample was added to 2 ml of Ninhydrin reagent and kept in water bath for 20 minutes. Appearance of purple color indicated the presence of amino acids in the sample.

Test for Proteins
To 2 ml of the extract solution, 1ml of 40% NaOH solution and 1 to 2 drops of 1% $CuSO_4$ solution was added. A violet color indicated the presence of peptide linkage of the molecule.

Test for Tri-Terpenoids
5 ml of each extract was added to 2 ml of chloroform and 3 ml of con. H_2SO_4 to form a monolayer of reddish brown coloration of the interface indicated positive result for the tri-terpenoids.
Test for Triple Sugar

To 2 ml of extract 2 drops of Molisch's reagent was added and shaken well. 2ml of con. H_2SO_4 was added on the side of the test tube. A reddish violet ring appeared at the junction of two layers immediately, indicated the presence of triple sugars.

The GC MS process is mentioned hereunder: Instrument: Gas chromatography (Agilent:GC:(G3440A) 7890A. MS MS:7000 Triple Quad GCMS,) was equipped with Mass spectrometry detector .

GC-MS Principle: In gas chromatography Helium gas is used as stationary phase. The principle of separation in GLC is partition. For GLC the compound should be volatile. They have to be heated to higher temperature and converted in to vapors in injector portion and mixed with gaseous mobile phase then the components are separated according to their partition co-efficient. In GLC the Mass spectrometer is used as detector. Mass Spectrometer is used for the determination of molecular weight of the compound and also for their structure elucidation. The compounds are identified by GC-MS Library (NIST & WILEY).

Sample Preparation: 100 microlitre sample Dissolved in 1 ml of suitable solvents. The solution stirred vigorously using vortex stirrer for 10 seconds. The clear extract was determined using gas-chromatography for analysis.

GC-MS protocol: Column DB5 MS (30mm×0.25mm ID ×0.25 µm , composed of 5% phenyl 95% methyl poly siloxane), Electron impact mode at 70 eV; Helium (99.999%) was used as carrier gas at a Constant flow of 1ml/min Injector temperature 280 °C; Auxilary Temperature : 290°C Ion-source temperature 280 °C. The oven Temperature was programmed from 50 °C (isothermal for 1.0 min), with an increase of 40°C/min, to 170°C C (isothermal for 4.0 min), then 10°C/min to 310°C (isothermal for 10min) fragments from 45 to 450 Da. Total GC running time is 32.02 min.

RESULTS AND DISCUSSION:

The phytochemical analysis results are indicated in Table1. The GC MS reports of Methanol extract and water extract of *S. indicus* are shown in Figure 1, Table 2, Figure 2, Table 3, respectively. The results obtained were tabulated and the medicinal values of the important compounds present were collected from various sources such as Dr. Duke's Phytochemical and Ethnobotanical data base, NIST Chemical Library and available literature.

Table 1: Shows the preliminary phytochemical constituents of ethanolic, methanolic, hexane and water extract of *Sphaeranthus indicus*

+ = Present; - = Absent

SL. NO	PHYTOCHEMICALS	ETHANOL	METHANOL	HEXANE	WATER
1	FLAVONOIDS	-	+	+	-
2	ALKALOIDS	-	+	+	-
3	TRI TERPENOIDS	+	-	-	+
4	SAPONINS	+	-	-	+
5	TANINS	-	+	-	+
6	TRIPLE SUGAR	-	-	-	-
7	AMINO ACID	-	-	-	+
8	ANTHROQUINONES	-	-	-	+
9	STEROIDS	+	+	-	-
10	PROTEINS	-	-	-	+
11	CARDIAC GLYCOSIDES	+	-	-	+

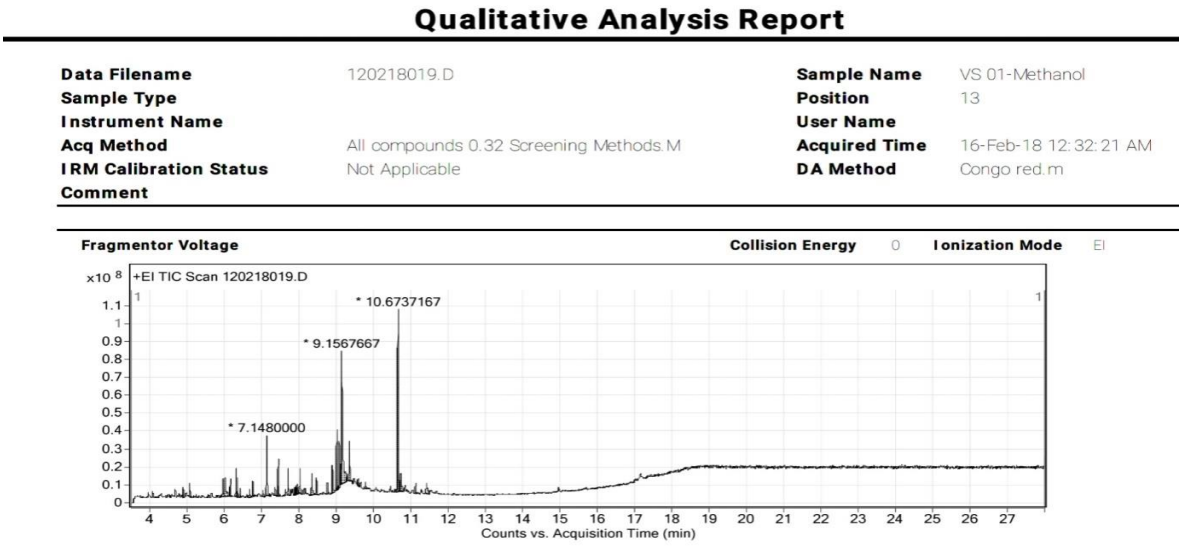

Fig. 1: The GC MS report of Methanol extract of *S. indicus* (VS 01).

Table 2: Indicating the Retention time, name of the compounds, molecular formula and weights, percentage peak values and possible medicinal roles of *Sphaeranthus indicus* Methanol extract as shown by the GC MS analysis.

Sl. NO	Retention Time	Compound	Mol. Formula	Mol. Wt	% Peak Value	Medicinal Role
1	5.974	3-Hydroxy-.beta.-damascone	C13H20O2	208	1.25	17-beta-hydroxysteroid dehydrogenase-inhibitor, Aryl-Hydrocarbon Hydroxylase inhibitor, Testosterone Hydroxylase Inducer, Beta 2 Receptor agonist, Beta adrenergic Receptor Blocker, Beta adrenergic agent, Beta Glucuronidase Inhibitor, Anti-inflammatory, Chemoprotective [34]
2	6.022	4,6,10,10-Tetramethyl-5-oxatricyclo[4.4.0.0(1,4)]dec-2-en-7-ol	C13H22O	194	2.59	5-alpha reductase inhibitor, alpha agonist, alpha amylase inhibitor, Testosterone 5 alpha reductase inhibitor, anti-amyloid beta, beta blocker, ER beta binder, beta adrenergic receptor blocker
3	6.171	2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-, [R-[R*,R*-(E)]]-	C13H20O2	208	1.17	Beta adrenargic receptor blocker, dopamine receptor blocker, endothelial derived relaxing factor promoter, fertility regulator, testosterone 5 alpha reductase inhibitor, 17 beta hydroxysteroid dehydrogenase inhibitor, testosterone hydroxylase inducer
4	6.324	5,6,6-Trimethyl-5-(3-oxobut-1-enyl)-1-oxaspiro[2.5]octan-4-one	C14H20O3	236	2.19	Not Known
5	6.364	2,4,7,9-Tetramethyl-5-decyn-4,7-diol	C14H26O2	226	1.44	Not Known
6	6.769	2,2,6-Trimethyl-1-(3-methylbuta-1,3-dienyl)-7-oxabicyclo[4.1.0]heptan-3-ol	C14H22O2	222	1.7	Oligosaccharide provider
7	7.148	5,5,8a-Trimethyl-3,5,6,7,8,8a-hexahydro-2H-chromene	C12H20O	180	5.65	HIF1 alpha inhibitor, suppress HMG Co A reductase activity, 5 alpha reductase inhibitor
8	7.457	2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	C15H26O	222	3.22	A neuromuscular blocker mostly in diabetic muscles
9	7.718	10,12-Tricosadiynoic acid, methyl ester	C24H40O2	360	2.42	Catechol O methyl transferase Inhibitor, methyl Donor, Arachidonic acid inhibitor, Urine acidifier, Inhibit Uric acid production

10	8.482	2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H-chromen-4a-yl hydroperoxide	C14H22O3	238	1.56	Anti 5 HT, Antidote (heavy metals), ACE Inhibitor, HIF 1 alpha Inhibitor, Tyrosine Hydroxylase Activator
11	8.774	4-(1,5-Dihydroxy-2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-one	C13H20O3	224	1.33	A neuromuscular blocker mostly in diabetic muscles
12	8.909	Ethyl 6,9,12,15-octadecatetraenoate	C20H34O2	306	2.91	Not Known
13	9.028	3H-Naphtho[2,3-b]furan-2-one, 4-hydroxy-4a,5-dimethyl-3-methylene-3a,4,4a,5,6,7,9,9a-octahydro-			5.32	Not Known
14	9.090	Ethyl 6,9,12,15-octadecatetraenoate	C20H34O2	306	4.03	Not Known
15	9.156	2-(4a,8-Dimethyl-6-oxo-1,2,3,4,4a,5,6,8a-octahydro-naphthalen-2-yl)-propionaldehyde	C15H22O2	234	20.48	Not Known
16	9.365	1,4-Hexadien-3-one, 5-methyl-1-[2,6,6-trimethyl-2,4-cyclohexadien-1-yl]-	C16H22O	230	3.82	Catechol o transferase inhibitor
17	10.67	Cyclobuta[1,2:3,4]dicyclooctene-1,7(2H,6bH)-dione, dodecahydro-, (6a.alpha.,6b.alpha.,12a.alpha.,12b.beta.)-			20.03	Not Known
18	10.73	1-Heptatriacotanol	C37H76O	536	3.28	Enzyme inhibitor, anti-hypercholesterolemic effects [35]
19	11.13	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	C23H32O	324	1.04	Not Known

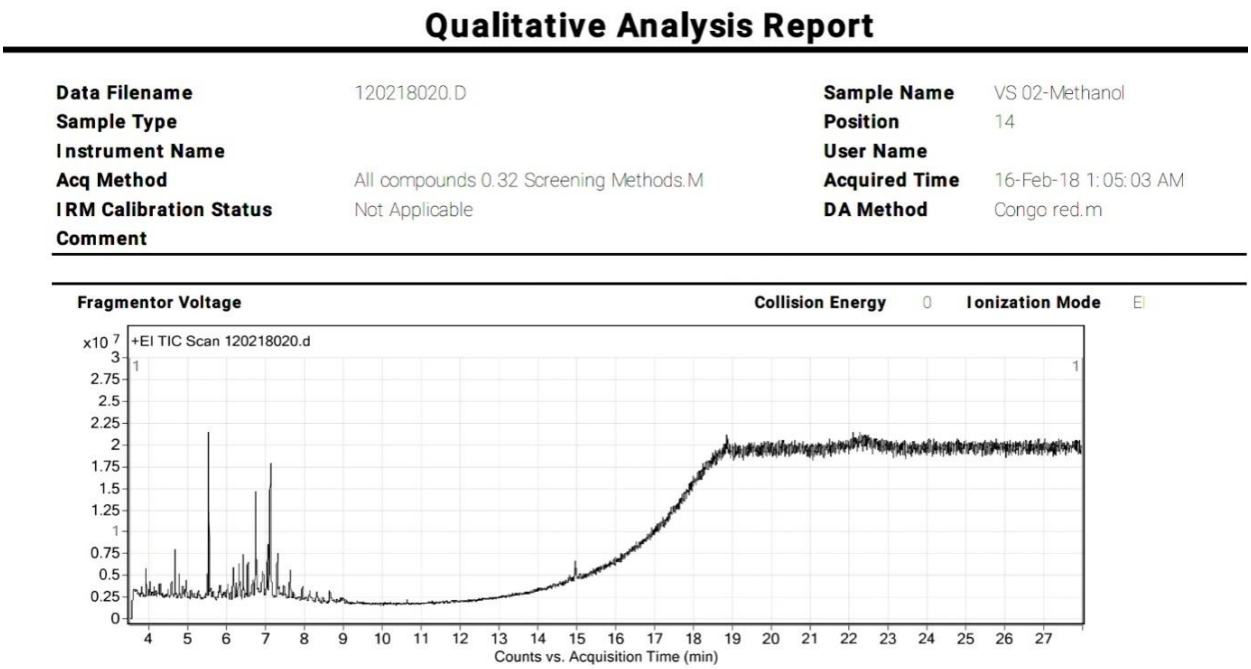

Table 3: Indicating the Retention time, name of the compounds, molecular formula and weights, percentage peak values and possible medicinal roles of *Sphaeranthus indicus* water extract as shown by the GC MS analysis.

Sl No	Retention Time	Compound	Mol. Formula	Mol. Wt.	%Peak Value	Medicinal Role
1.	3.94435	Paromomycin	C25H47N5O14	615	1.54	Paromomycin is an antibiotic used to treat a number of infections including amebiasis, giardiasis, leishmaniasis, and tapeworm infection. It is a first line treatment for amebiasis or giardiasis during pregnancy.
2.	4.96015	Benzeneethanol, 4-hydroxy-	C8H10O2	138	1.6	Not Known
3.	5.5029167	Homovanillyl alcohol	C9H12O3	168	1.64	Alchohol dehydrogenase inhibitor, Alcohol detoxicant, A metabolite of Dopamine, Cardioprotective [36]
4.	5.5500333	3-Hexenoic acid, 2-methyl-, methyl ester	C7H12O2	128	10.5	Catechol O methyl Transferase Inhibitor, methyl Donor, Arachidonic acid inhibitor, Urine acidifier, Inhibit Uric acid production
5.	5.84025	Dihydroxanthin	C17H24O5	308	1.99	Not Known

Continue……

6.	6.0400167	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	C21H34O2	318	1.81	Catechol O methyl Transferase Inhibitor, methyl Donor, Arachidonic acid inhibitor, Urine acidifier, Inhibit Uric acid production
7.	6.1832333	5,6,6-Trimethyl-5-(3-oxobut-1-enyl)-1-oxaspiro[2.5]octan-4-one	C14H20O3	236	3.85	Not Known
8.	6.3226833	cis-Z-.alpha.-Bisabolene epoxide	C15H24O	220	3.85	Increase Zinc bioavailability, 5 alpha reductase inhibitor, HIF1 Alpha Inhibitor, I Kappa Beta alpha Phosphorylation inhibitor, Increase Alpha mannosidase activity, Interlukin 1 alpha inhibitor, Testosterone 5 alpha reductase inhibitor, TNF alpha inhibitor
9.	6.4376333	4,6,10,10-Tetramethyl-5-oxatricyclo[4.4.0.0(1,4)]dec-2-en-7-ol			3.63	Decrease endothelial leucocyte and platelet adhesion, decongestatnt, decrease epinephrine/ nor epinephrine production, fibrinolysis enzyme activator, fertility enhancer, enterorelaxant, endocrine tonic, endothelial derived relaxing factor promoter.
10.	6.7523333	2,2,6-Trimethyl-1-(3-methylbuta-1,3-dienyl)-7-oxabicyclo[4.1.0]heptan-3-ol	C14H22O2	222	10.96	Oligosaccharide provider
11.	7.0670333	10-Methyl-8-tetradecen-1-ol acetate	C17H32O2	268	5.95	Oligosaccharide provider, Catechol O methyl transferase Inhibitor, methyl guanidine inhibitor,
12.	7.1424167	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	C13H18O2	222	21.41	17-beta hydroxysteroid dehydrogenase inhibitor, Aryl hydrocarbon hydoxylase inhibitor, Testosterone hydroxylase inducer,

13.	7.64935	Formic acid, 3,7,11-trimethyl-1,6,10- dodecatrien-3-yl ester	C16H26O2	250	3.37	Acidifier, Arachidonic acid inhibitor, increase aromatic amino acid decarboxylase activity
14.	7.9546333	1-Heptatriacotanol	C37H76O	536	1.19	Enzyme inhibitor, has anti-hypercholesterolemic effects.
15.	8.1449667	6,9,12,15-Docosatetraenoic acid, methyl ester	C23H38O2	346	1.24	Catechol O methyl Transferase Inhibitor, methyl Donor, Arachidonic acid inhibitor, Urine acidifier, Inhibit Uric acid production
16	8.6575333	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, trans-	C28H44O4	444	1.09	Catechol O methyl Transferase Inhibitor, methyl Donor, Arachidonic acid inhibitor, Urine acidifier, Inhibit Uric acid production

The results of phytochemical analysis indicated the presence of flavonoids and alkaloids in methanol and water extracts. Triterpenoids and saponins were present in ethanol and water extracts. Tannins were present in methanol and water whereas steroids were present in ethanol and methanol fractions. Amino acids and anthraquinons were present in water and cardiac glycosides in ethanol fractions. It is interesting to observe from the Tables 2 and 3 that some molecules present in methanol and water fractions such as 3-Hydroxy-.beta.-damascone , (1.25%), 4,6,10,10-Tetramethyl-5-oxatricyclo[4.4.0.0(1,4)]dec-2-en-7-ol, (2.59%), 2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-, [R-[R*,R*-(E)]]- (1.17%), 5, 5, 8a-Trimethyl-3,5,6,7,8,8a-hexahydro-2H-chromene: (5.65%), 2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene- (3.22%), [2R-(2.alpha.,4a.alpha.,8a.beta.)]- (3.22%), 10,12-Tricosadiynoic acid, methyl ester, (2.42%) , 2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H-chromen-4a-yl hydroperoxide: (1.56%), 4-(1,5-Dihydroxy-2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-one (1.33%), 1,4-Hexadien-3-one, 5-methyl-1-[2,6,6-trimethyl-2,4-cyclohexadien-1-yl]-(3.82%), 1-Heptatriacotanol (3.28%), Paromomycin (1.54%), Homovanillyl alcohol (1.64%), 3-Hexenoic acid, 2-methyl-, methyl ester (10.5%), 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)- (1.81%), cis-Z-.alpha.-Bisabolene epoxide (3.85%), 4,6,10,10-Tetramethyl-5-oxatricyclo[4.4.0.0(1,4)]dec-2-en-7-ol (3.63%), 2,2,6-Trimethyl-1-(3-methylbuta-1,3-dienyl)-7-oxabicyclo[4.1.0]heptan-3-ol (10.96%), 10-Methyl-8-tetradecen-1-ol acetate (5.95%), 2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl) (21.41%) , Formic acid, 3,7,11-trimethyl-1,6,10- dodecatrien-3-yl ester (3.37%), 1-Heptatriacotanol (1.19%), 6,9,12,15-Docosatetraenoic acid, methyl ester (1.24%), 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, trans (1.09%), had very important medicinal values as shown. The presence of these biomolecules clearly indicates the potential of this plant towards its multifarious medicinal roles.

CONCLUSION:

Thus it was observed that the ethnobotanical use of *Sphaeranthus indicus* augurs well with the above scientific data.

COMPETING INTERESTS

This is to inform that no conflict of interest exist among the author.

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