

GC-MS Analysis and Antibacterial Assay of Essential Oil Composition from the *Sphaeranthus indicus* L.

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ABSTRACT

Since ancient times, essential oils are recognized for their medicinal value and they are very interesting and powerful natural plant products. In the present study qualitative screening of the chemical constituents of *Sphaeranthus indicus* L. belong to family Asteraceae, commonly known as Gorakmundi showed the presence of various secondary metabolites including flavonoids, terpenoids, alkaloids, glycosides essential oils, etc. In the present investigation of the essential oils by GC-MS of *Sphaeranthus indicus* shown seven additional essential oil (1) Benzene,2-tert-butyle-1-4 dimethoxy, (2) 10 a-Ambros-11(13)-en-12-Oic acid 8 a-hydroxyl-4-oxo-c-lactone, (3) 1H cyclopropa(a)n-naphthalene 1aa 2,3,3a,4,5,6,7ba,octahydro11,3aa 7tetra methyl, (4) 9,12-Octadecadienoic acid(Z Z) (5) Edesm-4(14)en-11-ol, (6) n-Hexadecanoic acid, (7) n-Tetratetracontane. The essential oil composition have an important role in antibacterial activity, to understand the antibacterial activity of the individual compound, we have been studied their activity by bioautography. The terpene has shown a good conclusive evidence of antibacterial activity. The key objective of the present study is to develop a several new essential oils which are very important antibacterial agent.

Keywords: *Sphaeranthus indicus*, essential oils, GC-MS, Antibacterial activity.

INTRODUCTION

Historically plants have served as a basis for development of novel drugs there by contributing to human health and well-being¹. Medicinal plants are very ancient and only true natural medicines useful in several ways for the treatment of different diseases. They can be used directly or in extracted forms for the management of various ailments due to presence of various phytochemicals. For the prevention and treatment of various health ailments, plants and isolated phytochemicals have been used from time immemorial. A large number of phyto drugs prescribed worldwide are derived directly or indirectly from natural sources. A large number of African and Asian populations use traditional medicines for their primary healthcare². India has been endowed with a very rich flora owing to the extreme variations in climate and geographical conditions prevalent in the country. With the advent in science, many of the crude drugs used in traditional system have been investigated scientifically³. *Sphaeranthus indicus* is a medicinal plant widely used in Indian traditional system of medicine for curing various ailments⁴. It grows in rice fields, dry waste places and cultivated lands in tropical parts of India. It is distributed throughout India, Sri Lanka, Africa and Australia from sea level to 1200 m altitude⁵. This plant is used in folk medicine as remedy for Urinary Tract Infections.⁶ The sesquiterpene glycoside and sphaeranthanolide were

isolated from the flowers of *Sphaeranthus indicus* and it was found to be an immune stimulant^{7,8}.

MATERIALS AND METHODS

Experimental Methods

Collection of Plant material and Extraction

Fresh plants and plant parts were collected from the field area of Surat and Valsad Gujarat, India. The taxonomic identities of these plants were confirmed by a Plant Taxonomist Dr. M. H. Parabha former Head of Department of Biosciences, Veer Narmad South Gujarat University, Surat. A voucher specimen has been kept in our research laboratory. Fresh plant materials were washed under running tap water, air dried and homogenized to fine powder and stored in an airtight bottle.

Qualitative Analysis by High Performance Thin Layer Chromatography

Extraction Essential oil : 100 mg Powdered plant material was extracted with 20 ml Petroleum ether for 1 h under reflux and extract was filtered and evaporated to about 2 ml; 20 µl of the filtrate is used for HPTLC investigation. Stationary Phase: TLC Plate Silica gel 60 F 254 (Merck) 20cm x 20cm was cut in to 10cm x 10cm size with the help of cutter and used as stationary phase.

Sample Application: Samples of plant and standard was loaded on the separate pre-activated silica gel TLC plate. Material was applied by using Camag Linomat-5 sample applicator. Linomat-5 was programmed by Win CATS

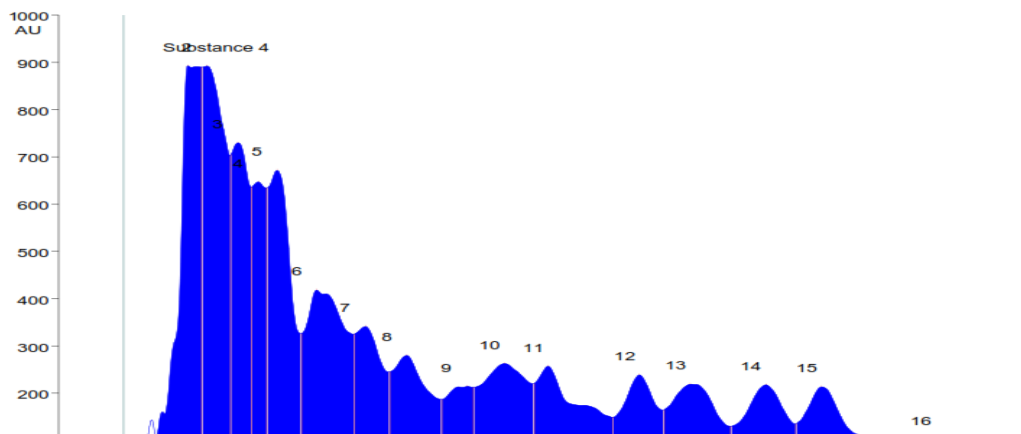


Figure 1: HPTLC Peak fingerprint of *Sphaeranthus indicus* for essential oil 365 nm.



Figure 2: HPTLC Peak fingerprint of Standard α - Pinene for essential oil 365 nm.

Table 1: HPTLC Peak list of the chromatogram of methanol extract *Sphaeranthus indicus* for essential oil 365 nm.

Track	No of Peak	Max. Rf	Max Height	Area %
1	1	0.04	802.3 AU	13.06%
1	2	0.05	802.1 AU	13.89%
1	3	0.09	639.6 AU	8.42%
1	4	0.11	556.7 AU	5.72%
1	5	0.13	581.3 AU	10.15%
1	6	0.18	327.8 AU	10.03%
1	7	0.23	250.9 AU	4.99%
1	8	0.28	189.4 AU	5.06%
1	9	0.35	124.0 AU	2.51%
1	10	0.39	172.2 AU	5.92%
1	11	0.44	166.7 AU	5.42%
1	12	0.55	148.4 AU	3.49%
1	13	0.61	128.7 AU	4.25%
1	14	0.69	127.4 AU	3.58%
1	15	0.76	123.1 AU	3.41%
1	16	0.89	10.7 AU	0.13%

Table 2: Peak list of the chromatogram of standard α -pinene for essential oil 365 nm.

Track	No of Peak	Max Rf	Max Height	Area %
5	1	0.04	660.5AU	100.00%

Detection: (a) UV-254 nm (b) UV-365 nm (c) Blue-Violet with Spray reagents Anisaldehyde-Sulphuric acid.⁹

Isolation of the essential oil by Hydro distillation method
Plant powder of *Sphaeranthus indicus* was subjected to hydrodistillation for 4 h using a Clevenger- type apparatus. The oil was dried over anhydrous Sodium Sulfate and Stored at 2⁰ C in the dark. The yield based on dry weight was 0.1% (W/W).¹⁰ GC-MS analysis of this extract was further carried out.

Isolation of the essential oil extraction by Reflux unit
100 mg Plant powder of *Sphaeranthus indicus* was extracted with 20 ml Petroleum ether for 1 h under reflux and extract was filtered and evaporated to about 2 ml; 20 μ l of the filtrate is subjected to petroleum ether for extraction of essential oil separation. This extract was for further GC-MS study

Gas Chromatography- Mass Spectrum Analysis (GC-MS)
GC-MS technique was used to study the identification of the bioactive components present in the both different essential oil extracts. GC-MS technique was carried out at SAIF IIT laboratory, Mumbai. Aglient 7890 instrument for

Planar Chromatography Manager for loading the samples
Chromatogram Development

Solvent system: Combination of various organic solvent was used for the better resolution of the secondary metabolites is Toluene: Ethyl acetate (97:3)

GC and Joel Accu TOF GCV instrument MS was used. The inert gas helium (99.9%) was used as carrier gas with the flow rate of 1ml/min. HP5 column with specification

length 30 mm, internal diameter 0.32mm, film of 0.25mm and temperature limit-60⁰ C to 325⁰ C was used. The total run time of GC was 35 min. The oven temperature raised

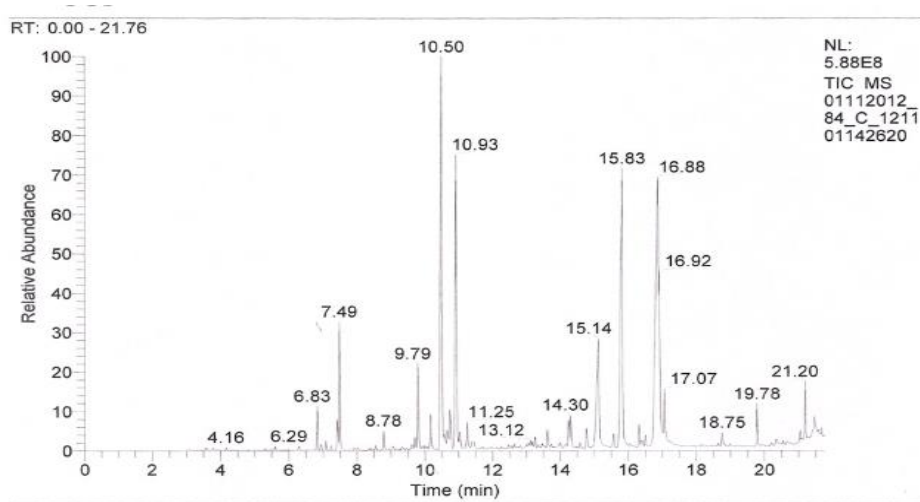


Figure 3: Gas chromatogram of petroleum ether extract *Spheranthus indicus*.

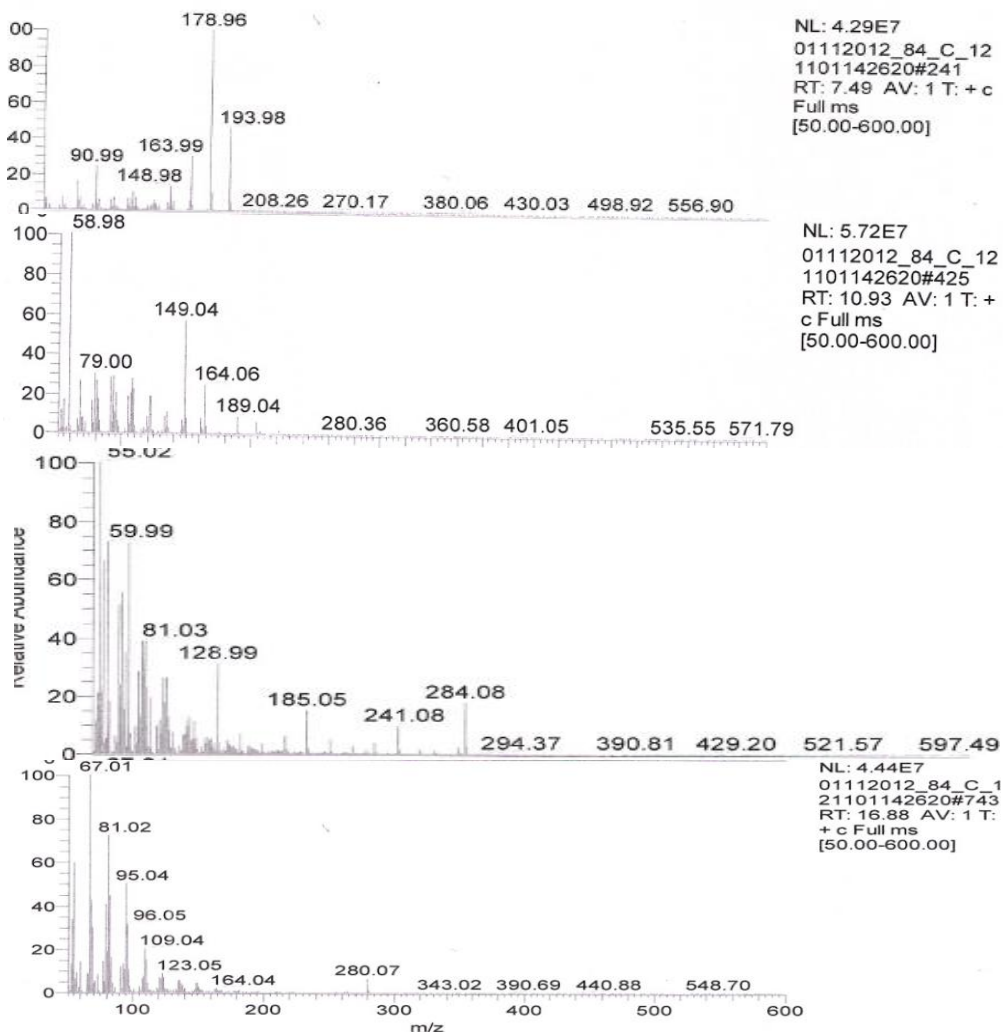


Figure 4: MS of petroleum ether extract *Spheranthus indicus*.

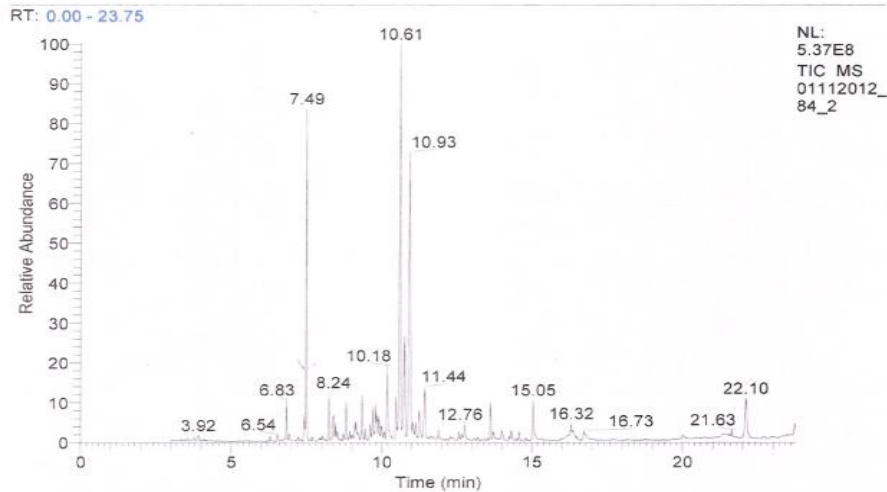


Figure 4: Gas chromatogram of *Spheranthus indicus* essential oil collected by Hydro distillation method.

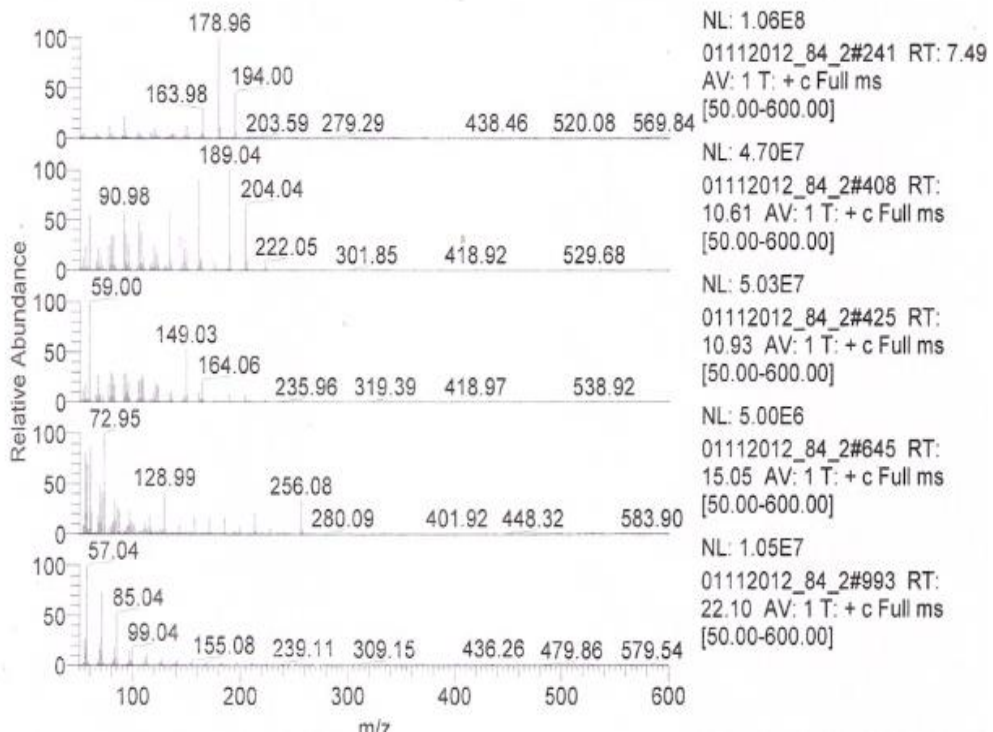


Figure 6: MS of essential oil of *Spheranthus indicus*.

Table 3: Isolation and identification of the essential oil chemical constituents from the petroleum ether extract of *Spheranthus indicus* by GC-MS.

No	Name	RT	Molecular Formula	Molecular Weight
1	Benzene,2-tert-butyle-1-4dimethoxy	7.49	C ₁₂ H ₁₈ O ₂	194
2	Edesm-4(14)en-11-ol.	10.93	C ₁₅ H ₂₆ O	222
3	10 a-Ambros-11(13)-en-12-Oic acid 8 a- Hydroxyl-4-oxo-c- lactone	15.83	C ₁₅ H ₂ O ₃	248
4	9,12-Octadecadienoic acid(Z,Z)	16.88	C ₁₈ H ₃₂ O ₂	280

from 70⁰ C up to 280⁰ C with the rate of 8⁰ C per min rise in temperature. The sample size of 4µl was injected through the injector. The MS was taken at 70eV.

Identification of components

The spectrum of the unknown component was compared with the spectrum of the known components stored in their library.

Bioautography assay

Bioautography assay was carried out for the *Sphaeranthus indicus* methanol extract sample according to method

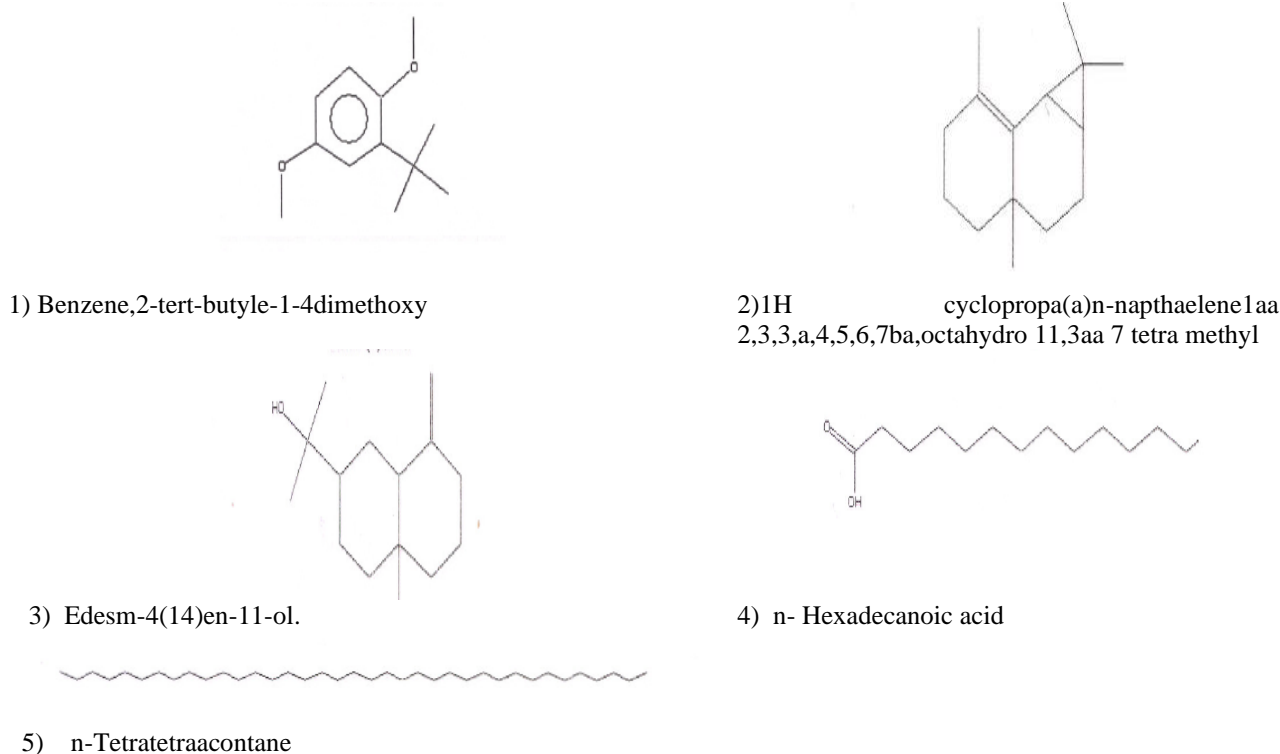
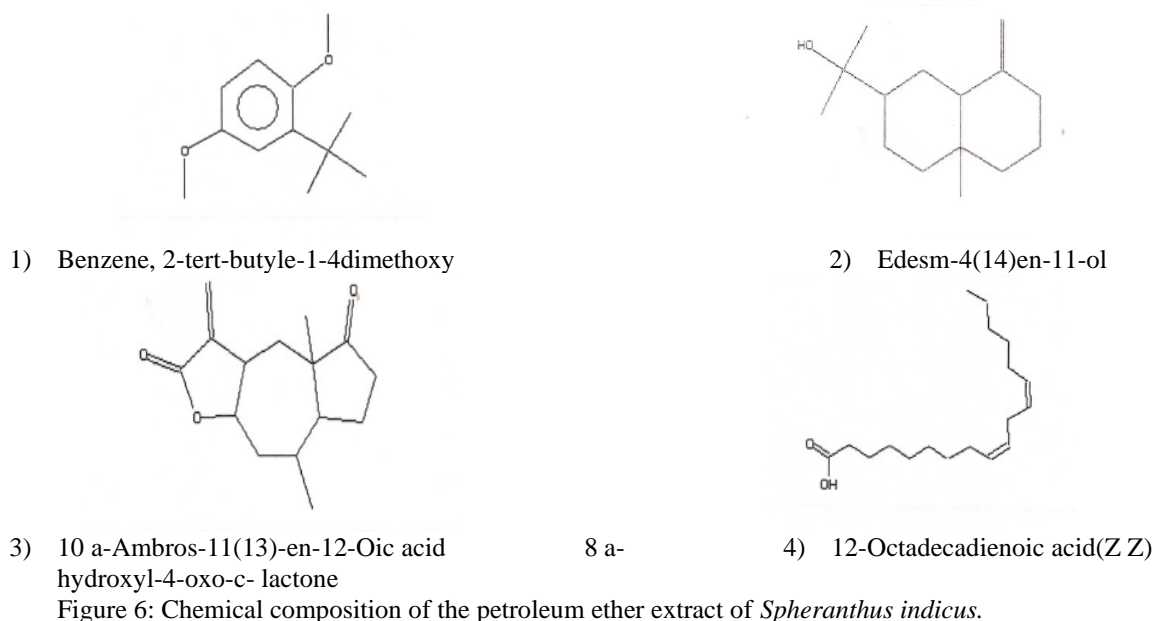


Figure 7: Gas chromatogram of *Spheranthus indicus* essential oil collected by Hydro distillation.

described by Mukherjee. Thin layer chromatographic (TLC) plates, composed of Merck silica gel, received 50 µl of extract placed at a distance from 1 cm of the lower edge of the plate with help of Linomat-5 the mobile phase was Toluene: Ethylacetate (93:7). Bio-autography was carried out after airing the TLC plates for over 8 hours. The plates were covered with 20 ml of sterile Nutrient broth agar at 45°C inoculated with the microorganism *E-coli* which was isolated from UTI's Patient of civil hospital in Surat and then incubated for 24 hours at 37°C. After this period each plate was covered with 5cm³ of a 1% aqueous solution of

2,3,5 triphenyl tetrazolium chloride and incubated for up to 24 hours at 37°C. Inhibition zones were visualized and recorded as clear areas against a red coloured background¹⁰.

RESULT AND DISCUSSION

Medicinal plants contain a range of organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids^{11,12}. Different phytochemicals have been found

Table 4: Isolation of Chemical composition of the essential oil by hydro distillation method *Spheranthus indicus*.

No	Name	RT	Molecular Formula	Molecular Weight
1	Benzene,2-tert-butyle-1-4dimethoxy.	7.49	C ₁₂ H ₁₈ O ₂	194
2	1H cyclopropa(a)n-napthaelene 2,3,3,a,4,5,6,7ba,octahydro 11,3aa 7 tetra methyl	1aa 10.61	C ₁₅ H ₂₄	204
3	Edesm-4(14)en-11-ol.	10.93	C ₁₅ H ₂₆ O	222
4	n- Hexadecanoic acid	15.06	C ₁₆ H ₃₂ O ₂	256
5	n-Tetratetracontane	22.1	C ₄₄ H ₉₀	618

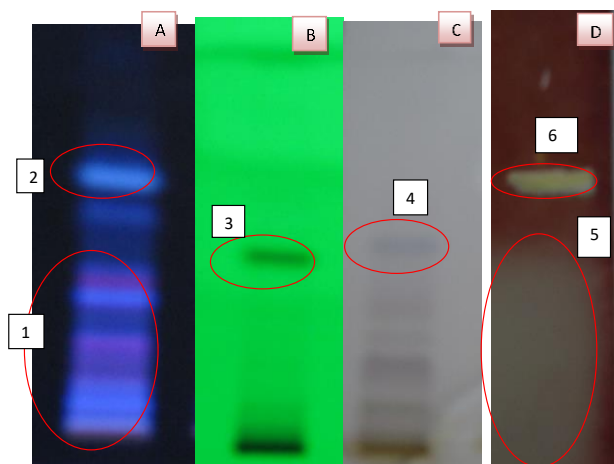
Fig. 8 Bioautogram of *Spheranthus indicus* methanol extract

Fig A : Chromatogram in UV light at 365 nm first circle show more than five band and one band in second circle

Fig B : Chromatogram in UV light at 254 nm third circle show one band

Fig C : Chromatogram at visible light after derivatization violet colour at 0.5 Rf of terpene compound show in fourth circle

Fig D : Chromatogram after Bioautography show zone of inhibition at lower region of 0.5 Rf in fifth and small inhibition zone at 0.5 Rf of terpene compound in six

to possess a wide range of activities, which may help in protection against chronic diseases. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganism's *in vitro* study¹³.

In present study the *Spheranthus indicus* also revealed the presence of α -Pinene when comparing their Rf value with standard α -Pinene by HPTLC method which was supported by Kaul *et al.*,¹⁴. The hydro distilled essential oil of *Spheranthus indicus* was analyzed by GC-MS. Thirty-eight compounds were identified from oil included α -Pinene. In present study the terpene also identify at 0.5 Rf value in visible light after Anisaldehyde-sulphuric acid treatment.⁹ this compound also possess antibacterial activity.(Fig 1&2 and Table1&2)

In the present investigation of the essential oils by GC-MS of *Spheranthus indicus* we are reporting seven additional essential oil (1)Benzene,2-terta-butyle-1-4 dimethoxy, (2) 10 a-Ambros-11(13)-en-12-Oic acid 8 a-hydroxyl-4-oxo-

c- lactone, (3)1H cyclopropa(a)n-napthaelene1aa 2,3,3,a,4,5,6,7ba,octahydro11,3aa 7tetra methyl, (4) 9,12-Octadecadienoic acid(Z Z) (5) Edesm-4(14)en-11-ol, (6) n- Hexadecanoic acid (7) n-Tetratetracontane.

To understand the antibacterial activity of the individual compound we have demonstrated their activity by bioautography. The terpenes have shown a good conclusive evidence of antibacterial activity. Many of the essential oil in the lower region of 0.5 Rf have also shown antibacterial activity, since the isolation was not done and Rf values being very close we are not in the position to specify the exact activity of each band.

CONCLUSION

The results revealed the presence of medicinally important constituents in the plants studied. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Several studies confirmed the presence of these phytochemicals contribute to medicinal as well as physiological properties to the *Spheranthus indicus* studied in the treatment of different ailments. Though the pin pointing of the role of specific isolated compounds is yet to be confirmed, however the extracts from these plants could be seen as a good source for useful phytochemicals which has several pharmacological properties and one amongst them is antibacterial activity which is confirmed by this study.

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