EXTRACTION, ISOLATION AND PURIFICATION OF PURE COMPOUNDS 3-METHYL-HENEICOSANE FROM MESOSPHAERUM SUAVEOLENS (LANCHAK) OF LAMIACEAE FAMILY

Abstract

Authors

A naturally occurring hydrocarbon 3-methyl-Heinecosane was isolated from the petroleum ether extract of the dry plant Mesosphaerum suaveolens (Lanchak) and characterised by UV-Visible was Spectroscopy, Fourier transforms infra red (FT-IR) spectroscopy, Nuclear Magnetic Resonance (NMR) and **GC-Mass** spectroscopy. The fragmentation ions with their m/z of compound 1, 3-methyl-Heineicosane were studied and all of the relative abundance found in the GC-MS spectra is in agreement.

Keywords: 3-methyl-Heinecosane; Mesosphaerum suaveolens; Lanchak; m/z; relative abundance Khomdram Utarani Chanu

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The mesosphairon word comes from the Greek and Latin mesosphaerum, meaning a type of tuberose with medium-sized leaves and its specific epithet suaveolens, means with a sweet fragrance due to the aroma of essential oils exhaled by the trichomes present on its leaves^{1,2}.

I. PLANT IDENTIFICATION

Kingdom: Plantae Class: Angiosperms Family: Lamiaceae Genus: <u>Mesosphaerum</u> Species: M. Suaveolens



Mesosphaerum Suaveolens locally known 'Lanchak' in Manipur belongs to the Lamiaceae family. It is a branching shrub, native to tropical parts of Asia, Africa and Australia, mostly grown on hilly regions, wastelands and open forests. In Manipur, it is mostly distributed in dry hilly area and roadside. It is 3.5-6.9 ft. tall, stems are hairy, leaves are oppositely arranged in oval shape, tips are broadly pointed, flowers are pink or purple arranging in clusters form in the upper leaf axils^{3,4}. In prehistoric time, people being used the medicinal plants for both treatments of diseases as well as for food^{5,6}. Ancient people of Kangleipak used the seed of Lanchak as a source of food during war as minimal amount of these seeds were sufficient to sustain the energy and quench the hunger for longer period of time, hence the Meitei word "Lanchak" was name thereafter. Practically, the Lamiaceae family has ethno medicinal value and medicinal applications⁷⁻¹².

In Northeast people of Manipur, traditionally the plant species Mesosphaerum Suaveolens used to treat numerous ailments such as anti-inflammatory, anti-diabetic, respiratory, gastrointestinal, wounds, cold, fever, infections and various skin complaints. In literature reported that the plant species used as therapeutic resources in Brail¹³. Leaves of this species are mainly used to treat respiratory diseases (bronchitis, asthma, flu and colds). M. Suaveolens contains a high-rise biotechnological potentiality, importantly in its essential oil¹⁴.

Based on the place of occurrence, in Manipur locally this species is known as "Lanchak", in India as "pignut" in Brail as "bamburral" or "alfaema-brava"¹⁵. Mesosphaerum Suaveolens has many synonyms Hyptis congesta Leonard, Hyptis Suaveolens, Ballota

Suaveolens L., Bystropogon Suaveolens (L.). In literature, there are some compounds have been characterised their structures are reported (Table 1). Its stem is photosynthetic quadrangular and hairy with closely spaced branches and nodes. It leaves has oval, pilose limb, serrate or cordate margin, acute apex, and obtuse base with opposite crossed phyllotaxis. The petioles are short, canaliculate, as are its stems. Its inflorescences made up of up to twenty flowers pinpoint around the nodes and nearby the leaf axils. The flowers are pedunculate with a persistent, calyx is tubular, and sepals are five pointed. The corolla is also tubular with 5 lilac petals with evident lobes. The fruits of Mesosphaerum Suaveolens are dry, indehiscent and uniseminated originating from a bicarpellate gynoecium. The dimorphic seeds two part per fruit. Such diasporas seeds of Mesosphaerum Suaveolens are elongated with dorsoventral flattening, longitudinal median ridge, starting near the hilum and expands to the top of the seed with retusa boundary with black coloration⁴⁰⁻⁴². Geographically distribution of M. suaveolens is native to tropical America and it is ruderal, it wind up invading natural ecosystems in tropical and subtropical regions of the globe. Because of this widespread occurrence, the Mesosphaerum Suaveolens species is considered a pantropicalruderal species⁴³⁻⁴⁶.

The significance source of Mesosphaerum suaveolens are essential oils, triterpenes, alkaloids, phenols, flavonoids, saponins and sterols^{48,49}. The essential oil obtained exclusively from its leaves of this species has already been chemically characterized in many studies. Due to the high level of genetic polymorphism Mesosphaerum Suaveolens species allows high variability in the composition and content of the major constituents and adaptation to changes in environmental characteristics has been found⁵⁰. From the M. suaveolens extracts, terpenoids had a great predominance mainly mono, di, tri, and sesquiterpenes class of terpenoids was reported. The diterpenes, suaveolic acid be highlighted with recognized antimicrobial and allelopathic action⁵¹. Moreover, phenolic acids; phenylpropanoids, flavonoids^{13, 26} and fatty acids^{38, 39} were also identified in different parts of M. suaveolens.

This study aimed at extraction, isolation, identification and characterisation of new pure compounds was isolated from the aerial parts of Mesosphaerum Suaveolens and to studies the biological activity of the compounds.

Compound, the Citation	Structure of Compound	Method of Identification	Plant Part
Sabinene, ¹⁴⁻¹⁸	H ₃ C H ₃ C	GC-MS	Leaves
Suaveolol, ¹⁹⁻²³		¹ H-NMR and ¹³ C-NMR	Leaves

Table 1: Identified Constituents in Mesosphaerum Suaveolens (L.) Kuntze (Lamiaceae)

Oleanolic acid, ²⁴	HO HO HO HO	¹ H-NMR and ¹³ C-NMR	Leaves
Betulinic acid, ²⁵		¹ H-NMR and ¹³ C-NMR	Roots
Ursolic acid, ^{25,26}	HO HO H	¹ H-NMR and ¹³ C-NMR	Roots, Leaves
Lupeol, ²⁶		¹ H-NMR and ¹³ C-NMR	Leaves
Gallic acid, ²⁷⁻ 28	НО ОН НО ОН	HPLC-DAD and HPTLC	Leaves and stems
Ellagic acid, ¹¹	но он но он но он	HPLC-DAD	Leaves and aerial part
Ferulic acid, ²⁸	ОН	HPTLC	Leaves and stem

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Catechin, ^{10,13}	HO OH OH OH OH OH	HPLC-DAD	Leaves
Eucalyptol, ²⁹⁻ 31	H ₃ C CH ₃	GC-Mass Spectroscopy	Leaves (essentia l oil)
Suaveolol, ³²⁻³⁴		¹ H-NMR and ¹³ C-NMR	Leaves
β- Caryophyllene	H_2C H_3 H_2C H_1 H_3 CH_3 H_3	GC-Mass Spectroscopy	Leaves
Germacrene D ³⁶		Essential oil	Leaves
Quercetin, ^{10,13,} 26,37	HO OH OH OH OH OH OH	HPLC-DAD and UPLC- Mass Spectroscopy	Leaves and stem
Ethyl caffeate, ³⁷	HOHO	UPLC-Mass Spectroscopy	Leaves
Syringic acid, ³⁷	О ОН НО ОН	UPLC-Mass Spectroscopy	Leaves
Oleic acid, ³⁸	ОН	GC-Mass Spectroscopy	Seeds

Stearic acid, ³⁸	O O O O O O O O O O O O O O O O O O O	GC-Mass Spectroscopy	Seeds
Palmitoleic acid, ³⁸	O O O O O O O O O O O O O O O O O O O	GC-Mass Spectroscopy	Seeds
Palmitic acid, ³⁹	ОН	GC-Mass Spectroscopy	Seeds and leaves
Undecanoic acid, ³⁹	ОН	GC-Mass Spectroscopy	Leaves

- 1. Other Biological Activities: Additionally to the aforesaid activities, the M. suaveolens herbaceous species present bioactive compounds against other biological organisms. Among these were call attentiom to parasitic organisms of human beings, as reported,⁵² the evaluation of the trypanocidal action (Trypanosomabruceibrucei) in vivo of gold nanoparticles from M. suaveolens was displayed. After seven days of infection, M. suaveolens species can cause a total clearance of the parasite. Furthermore in insecticidal measures against malaria vectors "Anopheles spp.", M. suaveolens shows antiplasmodial activity "Plasmodium falciparum 3D7"53-55.
- 2. Antiulcer Activity: The leaves of Mesosphaerum suaveolens are generally used for the treatment of gastric ulcers but no active ingredient had been identified and is Vera-Arvaze et al firstly done by evaluating such an effect.21 Vera-Arvaze isolated the diterpenesuaveolol from the leaves and analyse it against an induced experimental model. The outcome hand over that diterpenesuaveolol had a gastroprotective effect of more than 70%. After one year of publication of the mentioned study, make use of the ethnopharmacological approach of M. suaveolens evaluated its antiulcer potential across the ethanolic extract and its fractions was reported.56 The results for all products had high significance, p > 0.001. At a dose of 500 mg/kg, the fraction obtained from the hexane being the most effective with 74% inhibition of induced gastric ulcer.

We still required hunting the biologically important active compound, so we choose this plant Mesosphaerum suaveolens for extraction, isolation and purification of pure compound for the hunt of biologically important active compound. We purified and characterised and confirmed compound 1 from spectroscopic data as 3-methyl-Heneicosane.



Figure 1: Compound 1, 3-methyl-Heneicosane

II. MATERIAL AND METHOD

The fresh plants of Mesosphaerum Suaveolens were collected from Wangu Tejpur (Near Ibudhou Santhong Apanba). The arial parts of the plants are cleaned with water to

remove physical impurities, air-dried, keep for 6 days in the shadow, and grind to a fine powder by using grinder. Then the air-dried powder were weighed on an analytical balance, get 700g weighed.

III. EXTRACTION AND ISOLATION OF PLANT MATERIALS

The weighted powders of *Mesosphaerum Suaveolens* (700g) were subjected to extraction in the Soxhlet apparatus with petroleum ether at 70° C continuously for conjugative 3 weeks as shown in **Figure 3:** After filtration, each extraction solvent was then evaporated using rota evaporator under reduce pressure, the crude product were found. Before packing in an open column, crude products are dried with the silica gel for 2 days in the shade. Wet packing of column was performed by mixing with minimum amount of Silica gel and the solvent petroleum ether. Then, it was loaded to a column packed with silica gel and elution started with 100% of petroleum ether and ready to purify the compound by using column chromatography with increasing ratio of Ethyl Acetate with Petroleum Ether gradually.TLC plates were prepared manually. TLC plates are used for checking the presence of compounds and purity of the compounds.

IV. OPEN COLUMN CHROMATOGRAPHY

Column glass column
Adsorbent silica gel
Solvent petroleum ether
Sample loading vet packing
Detection Fraction were examined by TLC technique.

Wet packing method was used for the packing of column using the slurry of 60-120 silica gel mesh for column chromatography for the separation of pure compound from the crude products. The column elution was started with 100% petroleum ether and polarity was increased gradually.

V. PURIFICATION OF COMPOUNDS

When the column was eluted with petroleum ether (100%) gave fraction (1-60) as a mixture of two compound (showing two spot) in one dimension ascending, which were concentrated by evaporation The mixture of compound was dissolved in petroleum ether and the compound 1, 3-methyl-Heneicosane was precipitate out as white solid by putting the methanol. The process of precipitation was repeated for three times until all the oily yellow liquid was completely removed and is crystallised as white crystal from dichloromethane. After characterisation from the UV-visible, IR, NMR, GC-MS, the structure of the compound 1, 3-methyl-Heneicosane is shown in Figure 1. The compound 1, 3-methyl-Heneicosane is soluble in n-hexane, petroleum ether, Dichloromethane and chloroform. But it is not soluble in methanol, acetone and acetonitrile.

The running of column chromatography method is further carried out with 3% ethyl acetate with 97% petroleum ether and it was collected as compound **2** and characterisation by different spectroscopic method is undergoing. Moreover many compounds are also collected

Eluent polarity percent and fractions isolated from the column chromatography are given in table 2.

Table 2:	Fractionation –	Extract

Eluent % (Column Polarity)	Fraction No.	Inference
PE 100%	1-60	Compound 1, 3-methyl-Heneicosane
PE:EA, 97:3 v/v	61-299	Orange Single spot

Analytical Thin-Layer Chromatography (TLC)

- 1. Technique : One dimension, ascending
- 2. Adsorbant : silica gel
- 3. Layer thickness : 0.2 mm
- 4. Distance : 2 by 4 cm
- 5. Temperature : Laboratory temperature(28-35°C)
- 6. Locating reagent : Iodine granules

VI. CHARACTERIZATION OF COMPOUNDS

The UV-visible spectra does not shows any characteristic absorption spectra indicating there is no $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transition. The IR spectrum of compound 1, 3-methyl-Heneicosane shows some characteristic stretching peak. It shows stretching frequencies at 2849 cm⁻¹ and 2918 cm⁻¹ corresponding to stretching frequencies of C-H bonds. The stretching frequency at 1474 cm⁻¹ corresponds to C-H scissoring. From the IR spectrum, it is also confirm that there are no aromatic groups as well as any other functional group. That is the compound is aliphatic compound without any other functional groups as shown in **Figure 2**.



Figure 2: Infra Red Spectrum of Compound 1

The ¹NMR spectrum of compound **1**, 3-methyl-Heneicosane shows only two signal in aliphatic region at δ 0.87 ppm for one proton corresponding for five protons ethyl and δ 1.28 ppm for 8.9 protons (1:8.9 ratio) which is equivalent to remaining 43 protons of the compound **1**, 3-methyl-Heneicosane as shown in **Figure 3**.



Figure 3: The ¹NMR Spectrum of Compound 1, 3-methyl-Heneicosane

The compound 1, 3-methyl-Heneicosane has molecular formula of $C_{22}H_{48}$ and it molecular formula weight is 310.46 and it shows a signal with a low relative abundance value, m/z=310 for the molecular ion peak in GC-MS spectra is shown in **Figure 4**.



Figure 4: GC- Mass Spectra of the Compound 1, 3-methyl- Heneicosane

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In the GC-MS spectra, it shows a base peat at relative abundance of 100% at mass to charge ratio, m/z value of 57 which is corresponds to the most stable fragmentation ion butyl carbocation, e.i the secondary carbocation of the compound 1, and this m/z value is also available in another fragmentation of compound 1, 3-methyl-Heneicosane. So m/z value at 57 is the base peak. It also shows peak at relative abundance of m/z value at 43 which is the characteristic value for propyl carbocation. It also shows a peak at relative abundance at m/z value of 295 which indicate the loss of the branching methyl group from the molecular ion at carbon number 3. The m/z value at 71 indicates for the fragment ion of 2-methyl propyl carbocation. All of the relative abundance found in the GC-MS spectra is agreement with the fragmentation of the compound 1, 3-methyl-Heneicosane and it follows the fragmentation of hydrocarbon molecule. Some of the important fragment ions with their m/z values are shown in scheme 1.



Scheme 1: The possible fragmentation ions with their m/z of compound 1, 3-methyl-Heineicosane.

VII. EXPERIMENTAL AND CHARACTERIZATIONS

Physical Measurement: The characterization techniques of the compounds were done by using the spectroscopic technique such as UV-Visible Spectroscopy, Fourier transforms

infrared (FT-IR) spectroscopy, Nuclear Magnetic Resonance (NMR) and GC-Mass spectroscopy.

- 1. UV-Visible Spectroscopy: UV-Visible Spectroscopy is an instrument used to measure the intensity of light passing through a sample, and compare it to the intensity of light before it passes through the sample. The absorption spectra were recorded using Shimazu UV-3600 double monochromator spectrophotometer.
- 2. Fourier transforms infra red (FT-IR) spectroscopy: Fourier transforms infra red (FT-IR) spectroscopy is a technique used to obtain the infrared spectrum of the absorption or emission of a solid, liquid, or gas. This spectroscopic technique is used to determine the functional groups and molecular structures and to identify unknown compounds. The FT-IR spectra of the sample were obtained from Perkin Elmer Spectrum two Spectrophotometer, having spectral resolution 0.5cm⁻¹ with a range of wavelength 400-4000cm⁻¹ at room temperature.
- **3.** Nuclear Magnetic Resonance Spectroscopy: Nuclear magnetic resonance is a technique used to determine the molecular structure at the atomic level. The NMR spectra were note down in a Bruker 400MHz Spectrometer. The chemical shift in the NMR spectra is all given in ppm and TMS as the internal standard.
- Spectroscopic data of compound 1, 3methyl-Heneicosane: IR Data(cm⁻¹): 2918cm⁻¹(C-H, s), 2849cm⁻¹(CH₂, s), 1474cm⁻¹(C-N, s), 1462cm⁻¹(CH₃, s), 720 cm⁻¹(C-H, s). NMR Data (400MHz, : δ 1.28 ppm (8.9 protons (1.8.9)), δ 0.87 ppm (1H). GC-mass Data: m/z=310, m/z=295, m/z=267, m/z=113, m/z=99, m/z=85, m/z=71, m/z=57, m/z=43.

VIII. CONCLUSION

In conclusion, the extraction, isolation and purification of pure compound from the traditionally important plant <u>Mesosphaerum Suaveolen</u> was performed. We have isolated a pure compound and characterised it as 3-methyl-Heneicosane from the spectroscopic technique such as UV-visible, IR, NMR and GC-MS spectroscopy.

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