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Research Article

ISOLATION AND IDENTIFICATION OF NON-POLAR CHEMICAL ENTITY FROM *LEPTADENIA RETICULATA* AERIAL PARTS

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ABSTRACT

Objective: *Leptadenia reticulata* is the medicinal plant having many biological activities. It is necessary to find out which types of phytochemical constituents are present in the plant. The objective of this investigation was to isolate and identify the non-polar chemical entity of the areal parts of *L. reticulata* a plant used for medicinal purpose in folklore.

Methods: Petroleum ether extract of the stem bark was used for this study. Non-polar solvent was used to extract non-polar chemical entity from areal parts of *leptadeniareticulata*. Through the saponification process, saponifiable and unsaponifiable matter was separated. Phytochemical constituents were separated using column chromatography. Separated fractions were analyzed on gas chromatography and mass spectrometry (GC-MS) and nuclear magnetic resonance spectroscopy (NMR).

Results: Hentriacontane compound was isolated and confirmed from GC-MS and NMR whereas phytol, Lupeol, β-amyrin, Campesterol, Stigmasterol, gamma-sitosterol identified through GC and mass spectroscopy.

Conclusion: The present study showed that *L. reticulata* areal parts three sterols chemical entity Campesterol, Stigmasterol, gamma-sitosterol and phytol (diterpene alcohol), lupeol (triterpenoid), beta-amyrin (triterpene), and hentriacontane (alkane hydrocarbon). Core determination of the experiment was the development efficient method to isolate or identify the non-polar chemical entity through chromatographic technique.

Keywords: *Leptadenia reticulata*, Jivanti, Hentriacontane, Triterpenoid, Sterol.

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INTRODUCTION

Leptadenia reticulata (Robert Wight and George Arnott Walker Arnott) is known for their biological activity of roots, leaves, and tender stalks. It belongs to family Asclepiadaceae. Other names of the plant are Jivanti, SwarnJivanti or Dori [1,2]. It is used to cure many diseases such as fever, eye diseases, cough, and night-blindness [3].

L. reticulata plant has many biological activities such as lactogenic effect, antibacterial activity, antifungal activity, antioxidant activity, anti-implantation activity, anti-asthmatic activity, modulating effect, hepatoprotective activity, antidiabetic activity, anti-inflammatory activity, and anticancer activity against Dalton's ascitic lymphoma [4].

The therapeutic potential of this *L. reticulata* plant is due to the presence of varied bioactive compounds such as a triterpene alcohol simiarenol, apigenin, ferulic acid, β-sitosterol, hentricontanol, rutin, α-amyrin, β-amyrin, deniculatin, luteolin, diosmetin, stigmasterol, reticulin, and leptaculatin [5].

For the identification of non-polar component from plant material, first, it is necessary to separate lipids from plant for that purpose many classical methods and references are available for the extraction and identification of steroids and terpenoids from plant material [6-10]. even though It is necessary to establish specific method to isolate of these molecules or fractionsand they should be standardized.

Preliminary phytochemical analysis of *L. reticulata* areal parts indicated the presence of steroids, terpenoids, flavonoids, and glycosides. Hence, the present investigation was undertaken to determine the easy, rapid isolation, and detection method for non-polar chemical moiety from unsaponifiable matter in *L. reticulata* areal parts.

METHODS

Chemicals and reagents used in research work are Petroleum ether, Methanol, KOH, Anhydrous Na_2SO_4, *n*-hexane, ethyl acetate (EA), diethyl ether, glacial acetic acid (GAA), and thin-layer chromatography (TLC) F254 Plate were purchased from Merck (India) Ltd. without further purification.

Extraction

L. reticulata plant (areal part) was purchased from Vivan life science Ltd., Ahmedabad. The plant was dried and grinded. 446.426 g powder was extracted with petroleum ether for 24 h at 60°C to obtained extract 16.777 g.

Saponification of petroleum ether extract [11-13]

Petroleum ether extract (16.777 g) was taken, and 60 ml of 20% Methanolic KOH was added. After that mixture was refluxed for 7.5 h 20 ml of distilled water added then extracted with diethyl ether. The combined ether extract was washed with distilled water until it became neutral with litmus paper and dried over anhydrous sodium sulfate. Ether was evaporated to obtain unsaponifiable fraction. The aqueous portion left after ether extraction was named as a saponifiable portion. In an unsaponifiable fraction, anhydrous Na_2SO_4 was added to remove moisture and then filtered. After evaporation of diethyl ether, 9.512 g unsaponifiable matter was obtained. Best separation of unsaponifiable matter on TLC was found in Hexane: EA: GAA (8:2:0.125) and investigated using Liebermann–Burchard reagent.

Isolation was carried out using a column chromatography technique. Silica gel 60–120 mesh was used. n-hexane was used as a solvent than increased polarity with EA. First 11 fractions were eluted using hexane then remaining mixture from the column was eluted using a gradient

Table 1: Identified compounds by GC-MS

No.	Sample name	Compound name	RT	Molecular formula	Molecular weight	Peak area %
1	DMJ-1	Hentriacontane	51.331	$C_{31}H_{64}$	436.84	100.0
2	DMJ-6	Phytol	33.023	$C_{20}H_{40}O$	296.53	27.55
		Beta-amyrin	54.446	$C_{30}H_{50}O$	426.73	58.95
		Lupeol	55.066	$C_{30}H_{50}O$	426.72	13.5
3	DMJ-8-2	Campesterol	52.741	$C_{28}H_{48}O$	400.68	22.54
		Stigmasterol	53.151	$C_{29}H_{48}O$	412.69	16.09
		Gamma-sitosterol	53.983	$C_{29}H_{50}O$	414.71	61.37

GC-MS: Gas chromatography-mass spectrometry

Table 2: Interpretation of NMR

Chemical Shift	No. of Proton	Multiplicity	Interference
1.19	54H	Singlet	^{1}H
1.50	4H	Multiplate	^{2}H
0.83–0.80	6H	Multiplate	^{3}H

NMR: Nuclear magnetic resonance

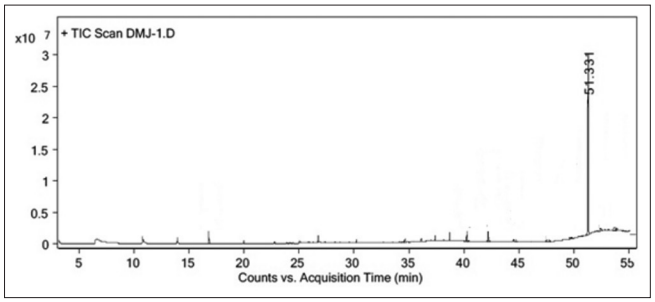


Fig. 1: Gas chromatography graph of hentriacontane

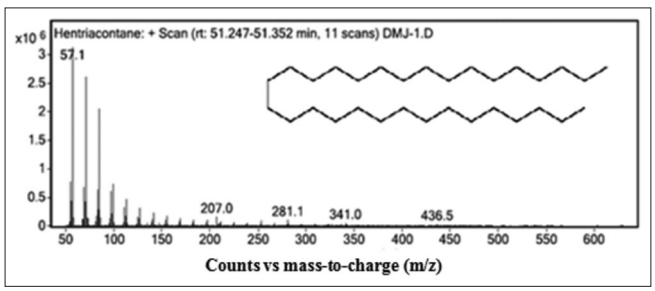
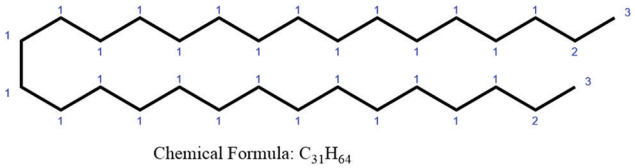


Fig. 2: Mass spectra of hentriacontane



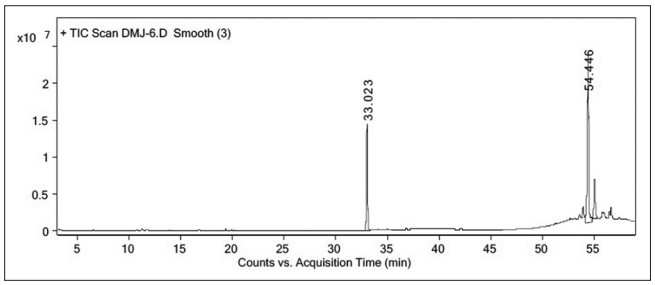


Fig. 3: Gas chromatography graph of phytol, β-amyrin, and lupeol

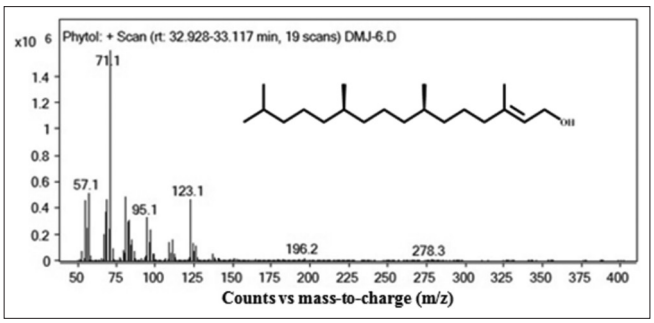


Fig. 4: Mass spectra of phytol

system of hexane and EA. Fractions 3 and 4 were found single spot. Hence, fractions 3 and 4 were mixed and named as DMJ-1. Remaining all fractions found mixture on TLC. Hence, all the fractions were mixed and further chromatography on column with silica gel 60–120 mesh and eluted with 99.5:0.5, 99:1, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45, 50:50, 45:55, 40:60, 35:65, and 0:100 systems with hexane: EA. 130 fractions were eluted with a volume of about 10 ml. TLC analysis was using system hexane: EA (8:2). Selected fractions were mixed, and gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) were performed. From all the fractions F81 to F85 were shown same rf value spot so, it was mixed and named as DMJ-6 and F101 to F105 was shown same Rf value spot so, it was mixed and name DMJ-8. To DMJ-8 fraction wash of hexane was given named as DMJ- 8-2.

GC-MS Protocol [14]

The GC-MS analysis was carried out using Agilent Technologies (AT; CA, USA) Model 5977B MS occupied to an AT 7820A GC equipped with an AT-5 fused silica capillary column (30 m×0.32 mm i.d.; 0.325 µm film thickness). The column temperature was initially held at 40°C for 5 min, then increased at 10°C/min to 300°C and then held at 300°C for 15 min; helium flow rate was 1 ml/min. The ion source of the MS was operated at 260°C and the transfer line at 280°C. Electron impact ionization was carried out at 965 Volt, and quantitative determination was based on the total ion current corrected for the detector response of each compound. For analysis, compounds were dissolved in diethyl ether (2 ml) and 1 µl aliquots by auto-injector. The mass range from 50 to 550 Amu was scanned at a rate of 1562 (n = 2] unit/s. Compounds were identified by direct comparison of their MS and retention times (R_t) with those of authentic samples and with data from the NIST 14 Library having >250,000 patterns.

RESULTS

Unsaponifiable Fraction (Table 1)

Compound-1(DMJ-1): Hentriacontane, M.F.- $C_{31}H_{64}$, M.W. - 436.84, RT-51.331.

GC-MS fragment: The peak at 51.331 min had a mass [M^+]436. The daughter ion spectra of these compounds (inserts) revealed the characteristic fragments *m/z*57.1, 207.0, 281.1, 341.0, and 405.0 (Figs. 1 and 2).

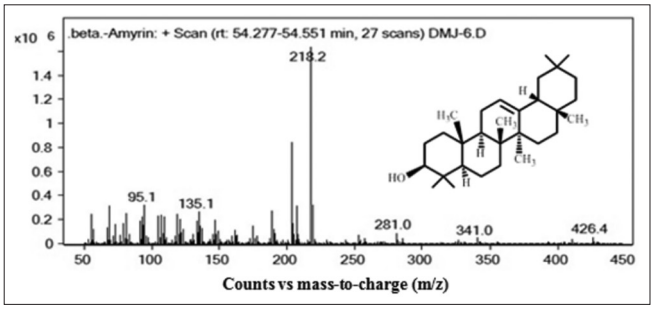


Fig. 5: Mass spectra of β-amyrin

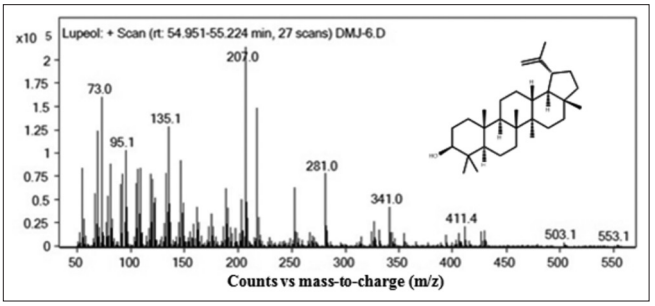


Fig. 6: Mass spectra of lupeol

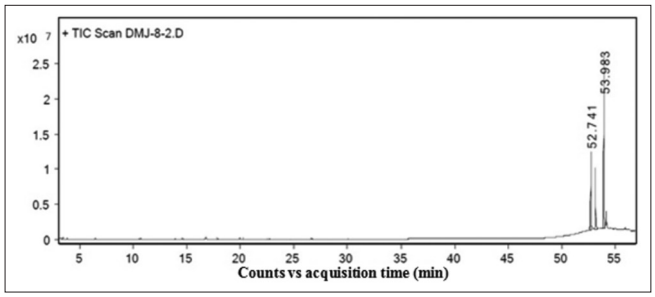


Fig. 7: Gas chromatography graph of campesterol, stigmasterol, and gamma-sitosterol

NMR Data:($CDCl_3$, 400MHz) δppm (Table 2)

Compound-2(DMJ-6): Phytol, M.F. -$C_{20}H_{40}O$, M.W. - 296.53, RT-33.023

GC-MS fragment: The peak at 33.023 min had a mass [M^+]296. The daughter ion spectra of these compounds (inserts) revealed the characteristic fragments *m/z* 57.1, 71.1, 95.1, 123.1, 196.2, and 278.3 (Figs. 3 and 4).

Compound-3(DMJ-6): β-amyrin, M.F. - $C_{30}H_{50}O$, M.W. - 426.73, RT-54.446

GC-MS fragment: The peak at 54.446 min had a mass [M^+]426. The daughter ion spectra of these compounds (inserts) revealed the characteristic fragments *m/z* 95.1, 135.1, 218.2, 281.0, 341.0, and 426.4 (Figs. 3 and 5).

Compound-4(DMJ-6): Lupeol, M.F. - $C_{30}H_{50}O$, M.W. - 426.72, RT-55.066

GC-MS fragment: The peak at 55.066 min had a mass [M^+]426. The daughter ion spectra of these compounds (inserts) revealed the characteristic fragments *m/z* 73.0, 95.1, 207.0, 281.0, 341.0, and 411.4 (Figs. 3 and 6).

Compound-5(DMJ-8-2): Campesterol, M.F.- $C_{28}H_{48}O$, M.W. - 400.68, RT-52.741.

GC-MS fragment: The peak at 52.741 min had a mass [M^+]400.4. The daughter ion spectra of these compounds (inserts) revealed the characteristic fragments *m/z* 73.0, 145.1, 207.0, 255.2, 289.3, 341.0, and 400.4 (Figs. 7 and 8).

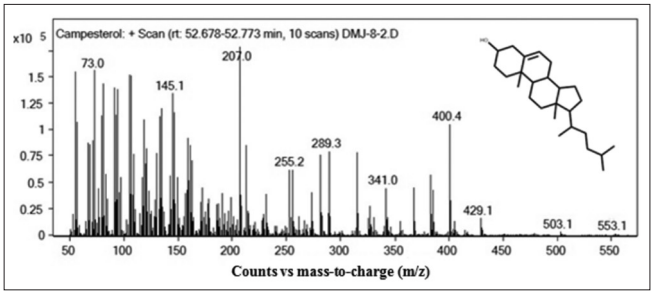


Fig. 8: Mass spectra of campesterol

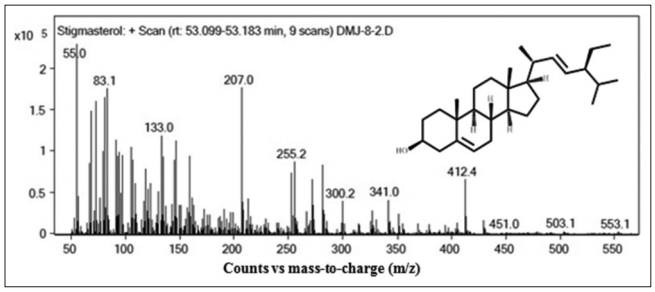


Fig. 9: Mass spectra of stigmasterol

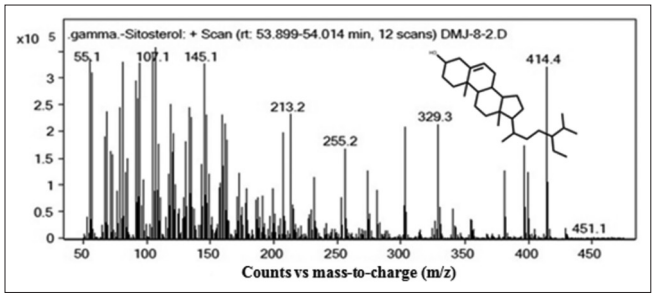


Fig. 10: Mass spectra of γ- stigmasterol

Compound-6(DMJ-8-2): Stigmasterol, M.F. - $C_{29}H_{48}O$, M.W. - 412.69, RT-53.151.

GC-MS fragment: The peak at 53.151 min had a mass [M^+]412.69. The daughter ion spectra of these compounds (inserts) revealed the characteristic fragments *m/z* 55.0, 83.1, 133.0, 207.0, 255.2, 300.2, 341.0, and 412.4 (Figs. 7 and 9).

Compound-7(DMJ-8-2): γ- sitosterol, M.F. - $C_{29}H_{50}O$, M.W. - 414.71, RT-53.983.

GC-MS fragment: The peak at 53.983 min had a mass [M^+]414.71. The daughter ion spectra of these compounds (inserts) revealed the characteristic fragments *m/z* 55.1, 107.1, 145.1, 213.2, 255.2, 329.3, and 414.4 (Figs. 7 and 10).

DISCUSSION

Gas Chromatographic-Flame Ionization Detection (GC-FID) is widely used in the analysis of phytosterols because of easy handling, low cost and good sensitivity. Therefore, GC-mass spectrometry (GC-MS) is a valuable aid for identifying unknown sterol peaks as well as for confirming the identification and purity of identified phytosterols [15]. In present work, petroleum ether extract is used for the isolation of non polar moiety. From unsaponification portion subjected to simple column chromatography and isolated seven different non polar fractions and characterized

by spectroscopic method. The identification was accomplished using computer searches by NIST98 Wiley MS Data library. From the GCMS result tentatively identified six compounds. The structure of these compounds was confirmed by comparison with published data in GCMS library while rest of one compound, hentriacontane compound found highly pure and so, we able to confirm it by NMR. Based on these acceptable results of fractionation, the method that we described in this study could be executed in the analysis of seven phytoconstituant in plant. Comparison with Earlier isolation method for ferulic acid, β-sitosterol, diosmetin, stigmasterol, rutin, β-amyrin, α-amyrin, simiarenol, hentriacontanol [5], we can determine phytosterols and get sample pretreatment without derivatisation.

CONCLUSION

In present work, we have isolated seven non polar chemical entities from *Leptadenia reticulata*. The compounds were identified by comparing their retention time and covate indexes with that of literature and by interpretation of mass spectra and NMR. isolated comopounds hentriacontane, Phytol, Lupeol, gamma sitosterol and beta amyrin are identified which are first time reported in the medicinal plant. The majority of them have reported biological activity, which support the traditional use of the plant. The results obtained with the phytochemical analysis, extraction techniques, chromatographic and spectroscopy methods indicated their potential as rapid and simple tools in the isolation and analysis of various natural products. It is, however, evident that further experiments are still needed in the studied areas especially with activity guided isolation in order to reach their full potential in natural product chemistry.

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AUTHORS' CONTRIBUTION

All Research lab work done by Jayesh Dhalani, GC-MS by Dr. Gaurang Dubal, Interpretationof NMR by Dr. Anilkumar Patel and guided by Dr. Pankajkumar Nariya.

CONFLICTS OF INTEREST

There are no conflicts of interest in this manuscript.

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