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# Isolation and structural revelation of flavonoid constituents from *Leptadenia reticulata Linn*

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**ABSTRACT**

*The aim of the present study was to isolate and reveal the active components present in the whole plant of Leptadenia reticulata. The plant was extracted with ethanol. The phytochemical results show the presence of Carbohydrates, glycosides, saponins, phenolic compounds, Taninns, flavonoids and terpenes. The ethanol extract of Leptadenia reticulata was undergone coloumn chromatography with different solvent fractions. Two compounds were isolated from ethanolic extract. Compound 1 was eluted with benzene: Chloroform 40:60 v/v and it has been named as 6-amino-3-hydroxy-4-(4'-methylphenyl)-2H-chromen-2-on(C16H13NO3). and compound 2 was eluted with ethyl acetate: ethanol, 50:50, v/v named as Squalene ($C_{30}H_{50}$)*

**Keywords:** *Leptadenia reticulata*, chromatography, Isolation,solvent fractions.

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## INTRODUCTION

*Leptadenia reticulata* (Retz) or jivanti is a much branched twining shrub belongs to Asclepiadaceae family. Jivanti grows throughout India, flower are greenish yellow, in many flowered cymes or subaxillary cymes, the follicles are sub woody and turgid Stem is cylindrical and bent occasionally at places. It is 5 to 10 cm long, 0.5 to 2.5 mm in diameter. The surface is rough, longitudinally ridged, Wrinkled and furrowed, transversely cracked and with vertically elongated lenticels at places [1].*Leptadenia reticulate* belonging to family Asclepiadaceae, well known for its tonic, restorative and stimulant property in the Indian system of medicine. This plant is distributed in the southern parts of India. The main constituents reported are stigma sterol, beta-sitosterol, flavonoids, pregnane glycosides and proteins [2]. Jivanti is jeevana tonic that boosts energy level of the body as per according to ayurveda. It is beneficial for the patient for the persons who suffer from weak debility or a lack of energy. It also increases longevity, memory enhancement, immune-modulation and adoption [3]. Lactogenic, anabolic and galactogogue effect was also observed in it [4-6]. This valuable medicinal plant has been also used for the treatment of various ailments such as hematopoesis, emaciation, dyspnoea, night blindness, fever, burning sensation, inflammation and cancer [7, 8, 9].

## MATERIALS AND METHODS

### Plant material

The whole plants of *Leptadenia reticulata* (Linn), were collected from Tirunelveli District of Tamil Nadu, India. The plant was identified and authentificated by Dr.V.Chelladurai, Reseach officer, Botany, Central council for research in Ayurveda and siddha, Govt of India, Thirunelvi. The whole plant of *Leptadenia reticulata* (Linn), were dried under shade , segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

**Extraction**
The powdered plant materials were successively extracted with ethanol (60-800C) by hot continuous percolation method in Soxhlet apparatus [10] for 24 hours. The extract was concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained. The ethanolic extract was stored in screw cap vial at 40C until further use.

**Preliminary phytochemical screening**
The extract was subjected to preliminary phytochemical screening for the detection of various plant constituents present. The ethanolic extract of *Saccharum spontaneum* was subjected to the following chemical tests such as tests for alkaloids[11], test for carbohydrates[11], tests of glycosides[11], tests for phytosterol[12], test for coumarins[12], test for flavonoids[13,14], test for tannins and phenolic compounds[15], tests for proteins and amino acids[11], test for saponins[11], test for fixed oils[11].

**TLC characterization of ethanolic extract of *Leptadenia reticulata***
The principle of separation is either partition or adsorption. The constituent which is having more affinity for mobile phase moves with it, while the constituent which is having more affinity for stationary phase gets adsorbed on it. This way various compounds appear as a band on the TLC plate, having different Rf values. The ethanolic extract of *Leptadenia reticulata* was subjected to thin layer and high performance thin layer chromatographic studies for the separation and identification of their components.

**Preparation of plates**
100g of silica gel G was weighed and made into a homogenous suspension with 200 mL of distilled water to form slurry. The slurry was poured into a TLC applicator, which was adjusted to 0.25 mm thickness on flat glass plate of different dimensions (10X2, 10X5, 30X5, 20X10 cm etc.). The coated plates were allowed to dry in air, followed by heating at 100-105°C for 1 hour, cooled and protected from moisture. Before using, the plates were activated at 110°C for 10 minutes.

**Identification method**
The ethanolic extract of *Leptadenia reticulata* was dissolved in ethanol separately and spotted using a capillary tube on TLC plates 2 cm above from the bottom of the plate. The selection of solvent systems were based on increasing the order of polarity. The different spots developed in each system were detected by means of iodine staining.

**Isolation of ethanolic extract of *Leptadenia reticulata* by using column chromatography**
The 20gms of ethanolic extract of *Leptadenia reticulata* was admixed with 20gms silica gel (60/120 meshes) to get uniform mixing. 200gms of silica gel (70/325 meshes) was taken in a suitable column and packed very carefully without air bubbles using petroleum ether as filling solvent. The column was kept aside for 1 hour and allowed for close packing. Admixture was then added at the top of the stationary phase and started separation of compounds by the eluting with various solvent mixtures with increasing order of polarity. All the column fractions were collected separately and concentrated under reduced pressure. Finally the column was washed with hexane, ethyl acetate and ethanol.

**Characterization of isolated Compounds**
**FT-IR**
IR spectra of the compounds isolated from the ethanolic extract of *Leptadenia reticulata* were recorded using a Nicolet 170SX. The spectral resolution for the Nicolet 170SX was 0.25$cm^{-1}$, and the spectral data were stored in the database at intervals of 0.5 $cm^{-1}$ at 4000-2000 $cm^{-1}$, and of 0.25 $cm^{-1}$ at 2000-400 $cm^{-1}$.Liquid samples were measured with liquid film The solid samples were measured by using KBr disc methods.

**$^1$HNMR**
$^1$HNMR spectra of the compounds isolated from the ethanolic extract of *Leptadenia reticulata* were recorded using a JEOL AL-400 (399.65 MHz). The measuring conditions for the most of the spectra were as follows: flip angle of 22.5-30.0 degrees, pulse repetition time of 30s. The long pulse repetition time and small flip angle was used to ensure precise relative intensities. The $^1$HNMR chemical shifts were referred to TMS in organic solvents and TSP in $D_2O$.

**$^{13}$C NMR**

$^{13}$C NMR spectra of the compounds isolated from the ethanolic extract of *Leptadenia reticulata* were recorded with a Bruker AC-200 (50.323 MHz). The measuring conditions for the most of the spectra were as follows: a pulse flips angle of 22.45-45 degrees, a pulse repetition time of 4-7 seconds, and a resolution of 0.025-0.045 ppm. The spectra whose spectral codes started with "CDS" were reconstructed from peak positions, intensities, and line widths by assuming all resonance peaks were Lorenz lines. The chemical shift was referred to a TMS for all solvents.

**Mass Spectrum**

Mass spectra of the compounds isolated from the ethanolic extract of *Leptadenia reticulata* was recorded with JEOL JMS-700 by the electron impact method where an electron is accelerating voltage 75eV and an ion accelerating voltage of 8-10nV. The reservoir inlet systems were used. The dynamic range for the peak intensities were 3 digits and the accuracy of the mass number was 0.5.

## RESULTS AND DISCUSSION

The ethanolic extract of *Leptadenia reticulata* (Linn.) was subjected to screening for its phytochemical constituents. The phytochemical screening results are shown in Table 1. The ethanolic extracts containing alkaloids, carbohydrates and glycosides, phenolic compounds, saponins, tannins, protein and amino acids, coumarins &flavonoids.

| S.NO | TEST | ETHANOLIC EXTRACT |
|---|---|---|
| 1. | ALKALOIDS | - |
| 2. | CARBOHYDRATES AND GLYCOSIDES | + |
| 3. | PHYTOSTEROLS | - |
| 4. | FIXED OILS AND FATS | - |
| 5. | SAPONINS | + |
| 6. | PHENOLIC COMPOUNDS AND TANNINS | + |
| 7. | PROTEINS AND AMINOACIDS | - |
| 8. | FLAVONOIDS | + |
| 9. | LIGNIN | - |
| 10. | TERPENES | + |

*Positive* + *Negative* –

The ethanolic extract of ***Leptadenia reticulata*** was subjected to the TLC chromatographic profile and column chromatographic separation. The ethanolic extract of ***Leptadenia reticulata*** dissolved in their mother solvent was taken in a capillary tube and spotted on TLC plates 2cm above its bottom. Most of the sample for application were between 0.1 – 1%. The applied spots were of equal size as far as possible and diameter ranging from 2-3mm. The solvent system for ethanolic extracts was developed by trial and error method using various solvents which were differing in polarities.

**TLC Profiles**

| S.NO | SOLVENT SYSTEM | NO OF SPOT | Rf VALUE |
|---|---|---|---|
| 1. | Benzene:choloroform(40:60) | 2 | 0.50, 0.50 |
| 2. | Benzene:choloroform (20:80) | 2 | 0.60, 0.60 |
| 3. | Ethyl acetate: Methanol (50:50) | 2 | 0.30, 0.50 |
| 4. | Ethyl acetate:Methanol (10:90) | 2 | 0.40, 0.50 |

The ethanolic extract of *Leptadenia reticulata* was subjected to column chromatographic separation using normal phase silica gel column. The dark brown solid (20 g ethanolic extract of *Leptadenia reticulata*) was adsorbed on silica gel (20 g) and transferred to a column of silica gel (200g equilibrated with benzene). Elution was performed with benzene (100%), benzene: chloroform (90:10), benzene: chloroform (70:30), benzene: chloroform (50:50), benzene: chloroform (30:70), chloroform (100), chloroform: ethyl acetate (70:30), chloroform: ethyl acetate (50:50), chloroform: ethyl acetate, (30:70), ethyl acetate (100), ethyl acetate: ethanol (80:20), ethyl acetate: ethanol (70:30) ethyl acetate: ethanol (50:50) , ethyl acetate: ethanol (30:70)and ethanol(100).

Fractions of 100ml were collected every time, distilled off the solvent and the homogeneity of the resulting residues was examined on TLC by using different solvent systems and similar fractions, identified by their TLC behaviour, were mixed together. Fractions 23-38 (eluted with benzene: Chloroform 40:60) and fractions 48-78 (eluted with ethyl acetate:ethanol, 50:50 v/v). Fractions 23-38 (eluted with benzene: Chloroform 40:60) gave a solid designated

as compound 1 (210 mg) and 48-78 (eluted with ethyl acetate: ethanol, 50:50, v/v) gave another solid designated as compound 2 (115mg).

## DISCUSSION

### Characterization Of Isolated Compound 1

The spectral data IR, $^{1}$HNMR & $^{13}$CNMR and Mass of the compound 1 are good in agreement with the structure proposed for the compound.

### IR Spectrum:

The IR spectrum of the compound 1 was analysed from the IR data. The presence of –NH group known from the absorption at 3320cm$^{-1}$. Absorption at 2920 cm$^{-1}$ shows the presence of –C-H (aromatic) group. A strong band at 1664 cm$^{-1}$ is due to the presence of –C=O group. The presence of –C=C (aromatic) indicates in the absorption at 1611 cm$^{-1}$.

### $^{1}$H & $^{13}$CNMR Spectral data:

The $^{1}$H & $^{13}$CNMR spectral data of compound 1 are analyzed. Based on the $^{1}$HNMR chemical shift values and $^{13}$CNMR chemical shift values of the compound 1 are found to be 4-phenylcoumarin derivative (Fig ).

### Mass Spectrum:

The mass spectrum of the isolated compound 1 is presented in the fig. the m/z value of isolated compound of the molecular ion is found as 267 which includes the isotopes of corresponding atoms.Based on the spectral data the tentative structure of compound 1 to proposed as 6-amino-3-hydroxy-4-(4'-methylphenyl)-2*H*-chromen-2-one are given below (Fig). The Molecular Formula of the compound 1 was deduced as $C_{16}H_{13}NO_3$.

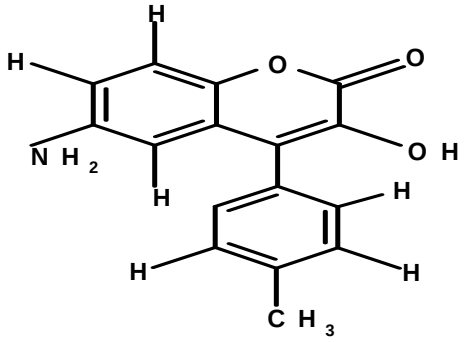


**Fig 1: Tentative Structure of Compound 1**

**(**6-amino-3-hydroxy-4-(4'-methylphenyl)-2*H*-chromen-2-one)

**$^{1}$H NMR Spectrum**

| S.No | Chemical shift value | Functional group |
|---|---|---|
| 1 | δ 12.49 | s, 2H, 6-$NH_2$ |
| 2 | δ 9.40 | broad hump, 1H, 3-OH |
| 3 | δ 7.67-7.66 | d, 1H,5-H |
| 4 | δ 7.55-7.52 | d, 1H, 7-H |
| 5 | δ 6.91-6.89 | d, 1H, 8-H |
| 6 | δ 6.40 | d, 1H, 2'-H |
| 7 | δ 6.18 | d, 1H, 3'-H |
| 8 | δ 2.50 | s, 3H, 4'-$CH_3$ |

**$^{13}$C NMR Spectrum:**

| S.No | Chemical shift value (ppm) | Functional group |
|---|---|---|
| 1 | δ 175 | C-2 |
| 2 | δ 163 | C-9 |
| 3 | δ 160 | C-6 |
| 4 | δ 156 | C-3 |
| 5 | δ 147 | C-7 |
| 6 | δ 146 | C-4 |
| 7 | δ 145 | C-10 |
| 8 | δ 135 | C-8 |
| 9 | δ 121 | C-5 |
| 10 | δ 121 | C-5' |
| 11 | δ 119 | C-4' |
| 12 | δ 115 | C-2' |
| 13 | δ 114 | C-3' |
| 14 | δ 93 | 4'-$CH_3$ |

**STRUCTURE AND IDENTIFICATION OF COMPOUND 2**

The spectral data IR, $^1$HNMR & $^{13}$CNMR and Mass of the compound 2 are good in agreement with the structure proposed for the compound.

**IR Spectrum**:

The IR spectrum of the compound 2 was analysed from the IR data. The spectrum shows the absorption bands at 2966cm$^{-1}$ due to the presence of alkyl group and 2917cm$^{-1}$ due to the presence of –C-H (aliphatic) group. The presence of –C=C indicates in the absorption at 1443 and 1377cm$^{-1}$.

**$^1$H & $^{13}$CNMR Spectral data:**

The $^1$H & $^{13}$CNMR spectral data of compound 2 are analyzed. Based on the $^1$HNMR chemical shift values and $^{13}$CNMR chemical shift values of the compound 2 are found to be 4-phenylcoumarine derivative

**Mass Spectrum:**

The mass spectrum of the isolated compound 2 is presented in the fig. the m/z value of isolated compound of the molecular ion is found as 410($M^+$) which includes the isotopes of corresponding atoms. Based on the spectral data the tentative structure of compound 1 to proposed as squalene are given below (Fig ). The parent molecular ion peak (m/z) appear at the mass 410($M^+$), which corresponds to the molecular formula $C_{30}H_{50}$.

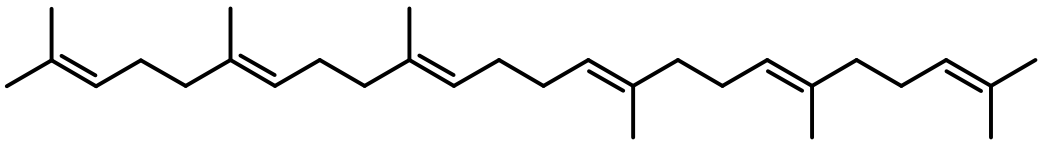

**Fig 5 : Tentative Structure of Compound 2 Squalene**

**$^1$H NMR Spectrum**

| S.No | Chemical shift value | Functional group |
|---|---|---|
| 1 | δ 5.33-5..32 | d, 1H, 11-H |
| 2 | δ 4.6-4.5 | d, 1H, 7-H |
| 3 | δ 3.79-3.74 | d, 1H, 3-H |
| 4 | δ 3.73-3.70 | d, 2H, 12- $CH_2$ |
| 5 | δ 3.68-3.64 | d, 2H, 8- $CH_2$ |
| 6 | δ 3.5-3.48 | d, 2H, 4- $CH_2$ |
| 7 | δ 3.32-3.30 | d, 2H, 9- $CH_2$ |
| 8 | δ 3.29-3.27 | d, 2H, 5- $CH_2$ |
| 9 | δ 3.17-3.01 | d, 3H, 10- $CH_3$ |
| 10 | δ 2.25-2.42 | d, 3H, 6- $CH_3$ |
| 11 | δ 2.19-2.16 | d, 3H, 2- $CH_3$ |
| 12 | δ 2.08-2.01 | d, 3H, 1- $CH_3$ |

**$^{13}$C NMR Spectrum:**

| S.No | Chemical shift value (ppm) | Functional group |
|---|---|---|
| 1 | δ 145 | C-3 |
| 2 | δ 138 | C-7 |
| 3 | δ 128 | C-11 |
| 4 | δ 109 | C-14 |
| 5 | δ 84 | C-18 |
| 6 | δ 77 | C-22 |
| 7 | δ 73 | C-4 |
| 8 | δ 72.91 | C-8 |
| 9 | δ 72.85 | C-12 |
| 10 | δ 72 | C-16 |
| 11 | δ 71 | C-20 |
| 12 | δ 70 | C-5 |
| 13 | δ 63 | C-9 |
| 14 | δ 60 | C-13 |
| 15 | δ 56 | C-17 |
| 16 | δ 34 | C-21 |
| 17 | δ 33 | C-2 |
| 18 | δ 31.72 | C-6 |
| 19 | δ 31.60 | C-10 |
| 20 | δ 31.12 | C-15 |
| 21 | δ 30.30 | C-19 |
| 22 | δ 30.01 | C-23 |
| 23 | δ 29.45 | C-24 |
| 24 | δ 29.12 | C-1 |
| 25 | δ 28 | $C_2$- $CH_3$ |
| 26 | δ 27 | $C_6$- $CH_3$ |
| 27 | δ 25 | $C_{10}$- $CH_3$ |
| 28 | δ 24 | $C_{15}$- $CH_3$ |
| 29 | δ 22 | $C_{19}$- $CH_3$ |
| 30 | δ 14 | $C_{23}$- $CH_3$ |

## CONCLUSION

Thus the present study shows compound 1 was characterized as 6-amino-3-hydroxy-4-(4'-methylphenyl)-2*H*-chromen-2-on(C16H13NO3). and compound 2 was characterized as Squalene ($C_{30}H_{50}$).the compound 1 was a novel compound isolated in this plant ,it is under the category of flavonoids.In future further detailed investigations is to be needed

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