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Spectral characterisation and assessment of triterpenoids isolated from leaf extract of Glochidion talakonense

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Abstract

Glochidion talakonense belongs to the family Phyllanthaceae, is a tree, up to 7m high, that grows wild throughout Talakona, Seshachalam biosphere reserve in Chittoor district of Andhra Pradesh, India. In traditional medicine, this species has been applied to use for a various biological activity in traditional medicine. However, there is no information about chemical composition and bioactivity of this species. This study focuses on the isolation and identification of compounds from *Glochidion talakonense*. The methanol extract of the *Glochidion talakonense* leaf was separated by liquid-liquid extraction, followed by different chromatographic techniques to purify the chemical composition. The result showed that five terpenoids including lupeol (1), Glochidiol (2), Lupa-1,20(29) diene-3-one (3), Lup-20(29)-ene-3β,16β-diol (4), Lup-20(29) ene-1,3-dione (5) were isolated from the methanol extract. The chemical structures of these terpenoids were elucidated based on IR, NMR and MS spectral analysis, and by comparison with previous references. These compounds have not been previously isolated from *Glochidion talakonense.*

Keywords: *Glochidion talakonense,* Triterpenoid, Lupeol, Glochidiol, Lupa-1,20(29) diene-3-one, Lup-20(29)-ene-3β,16β-diol, Lup-20(29) ene-1,3-dione.

INTRODUCTION

The genus Glochidion contains flowering plants that belong to the family Phyllanthaceae, which was referred to as Cheese trees or Buttonwood in Australia, and Leaf flower trees in various scientific documents. About 300 species were recorded, which were widely distributed in the Pacific area, and tropical Asia (Angiosperm phylogeny). In India, the genus is represented by c. 22 species and 8 varieties, of which 3 species and one variety are reported from Andhra Pradesh (Babu, 1997; Chakrabraty and Gangopadhyay, 1995, 2012). Recently, one new species (*Glochidion talakonense)* was described from the Seshachalam Hills (Rasingam et al., 2014). While exploring the Talakona area of Seshachalam Biosphere Reserve. This tree species, reaching up to 7 m in height, features spreading branches and oblong-elliptic leaves. The axillary inflorescences are 8-many-flowered, with unisexual or bisexual flowers. Male flowers are brownish-pink, while female flowers exhibit a greenish hue with a purple tinge. The fruits are capsular, shallowly lobed, and either glabrous or puberulous. Seeds number 12 and are glabrous (Rao et al., 2016).

Study on chemical constituents of the genus Glochidion species showed that they contained many types of metabolites including triterpenoid (Vu KT et al.,2010, Puapairoj P et al., 2005, Kabir S et al., 2020), steroids (Al-Hasan A et al.,

2012), flavonoid (Tran DT et al., 2011), lignans (Liu M, Xiao HT et l.,2008).
With the purpose of providing more information about the chemical constituents of G. talakonense, this paper describes the isolation and structure elucidation of triterpenoid compounds from the methanolic extract of G. *talakonense* leaf including lupeol (1), Glochidiol (2), Lupa-1,20(29) diene-3-one (3), Lup-20(29)-ene-3β,16β-diol (4), Lup-20(29) ene-1,3-dione (5). These metabolites have not been previously isolated from the G. *talakonense*. Therefore, it can be considered that this plant may possess a lot of medicinal value which may be in one way or the other beneficial for the human well-being. Much attention can be given in complete exploration of the different species of this genus as they have not yet come in the limelight of the researchers.
However, the review of literature clearly suggests that of no pure compound from the leaf of Glochidion talakonense extracts has yet been isolated. Hence, the present study focuses on the systematic evaluation and characterising of Glochidion talakonense.

MATERIALS AND METHODOLOGY

Plant materials

The healthy and disease-free plant was sourced from the forest areas of the Seshachalam hills, which are situated across Chittoor and Kadapa districts in Andhra Pradesh. These hills were designated as a Biosphere Reserve by the Government of India in 2010. Authentication of the plant was conducted by DR. K. N. Sunil Kumar, Research officer/Sci-II and HOD Department of pharmacognosy, and Dr P. Elankani, Research officer (Sidda) Sci-IV/In charge, Sidda Central Research Institute, Chennai. The plant was identified as *Glochidion Talakonense* (Phyllanthaceae) and was certified Forma No. PCOG002-ACF/G26062401T.

General experimental procedures

Nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (LC-MS), infrared spectroscopy (IR) was used to determine the chemical structures of the isolated compounds.

Instruments used

NMR spectra were measured using a AVANCE II 500 MHz (AVANCE NEO 500MHz FT-NMR SPECTEROMETER, Bruker, Switzerland), Mass spectra were performed on an Alliance 2795 Q-TOF Micromass Mass Spectrometer (Waters Corporation, UK) at the SAIF & Central instrumentation laboratory, Panjab University, Chandigarh. The IR spectrum was obtained, as KBr discs, on a Hitachi 270-30 type spectrometer. Optical rotation was measured with a Jasco DIP-1000 KUY polarimeter. The melting point (m.p.) was recorded on a Digital melting apparatus (Electrothermal IA 9100, UK).

Chemicals and reagents

All the chemicals and reagents like acetone (99.5%), dichloromethane (99.5%), chloroform (99.0%), ethyl acetate (99.5%), n-hexane (96%), methanol (99.5%) and distilled water. DMSO Chromatographic techniques were performed to purify and isolate compounds. Column chromatography (CC) was realized on silica gel 60 (0.040 – 0.063 mm, Merck, Germany). Thin layer chromatography (TLC) was conducted on pre-coated silica gel 60 F254 (Merck, Germany). Visualization of TLC plates was carried out under UV light (254 and 365 nm), and then the plates were dipped in a 5% vanillin/H2SO4 or 10% H2SO4 solution and heated at 120°C for 5 min.

Extraction and isolation

The dried leaf of *Glochidion Talakonense* (1.25 kg) of the powder is subjected for successive extractions by using solvent methanol for 17 hrs respectively. The combined solution was evaporated under reduced pressure at 45°C to obtain crude methanol extract. The crude extract was suspended in 1L of water and then partitioned sequentially with n-hexane and ethyl acetate to obtain the corresponding fractions: n-hexane (7.8 g, HF), EtOAc (10.0 g, EF), and water layer (1L, WF). The n-hexane fraction (7.8 g) was subjected to a silica gel VLC (10 cm x 20 cm) eluted step by step with a gradient of n-hexane – acetone (100:0 to 0:100, v/v) to afford 9 sub-fractions (HF1 to HF9). Sub-fraction HF5 (1.26 g) was separated

by silica gel CC (n-hexane – dichloromethane, 100:0 to 0:100, v/v) to yield compound 3 (64 mg) and compound 5 (25 mg). Sub-fraction HF 7 (560 mg) was subjected on silica gel CC eluted with an isocratic solvent of n-hexane – acetone (8:2, v/v) to obtain compound 1 (43 mg). Sub-fraction HF 3 (104 mg) was purified on silica gel CC (n-hexane – dichloromethane, 9:1-8:2, v/v) to afford compound 2 (85 mg). Sub-fraction HF 8 (112 mg) was recrystallized in n-hexane-acetone mixture (2:1, v/v) to give compound 3 (39 mg). Sub-fraction HF9 (250 mg) was purified by another silica gel CC eluted with an isocratic solvent of 100% CH_2Cl_2 to yield compound 4 (20 mg).

Results and Discussion

The n-hexane fraction from the leaf of Glochidion talakonense was separated and purified by silica gel VLC and CC many times to afford five compounds. The chemical structures of these compounds (Figure 1) were elucidated by analysis of spectroscopic data including IR, NMR, LC-MS, as well as comparison with previous publications. All of these metabolites were identified as triterpenoid derivatives possessed a skeleton of lup-20(29)-ene by the characteristic signals of an isopropylene group at about δC 145, 109 and 38 ppm in the 13C-NMR spectrum, combined with the observation of two olefin protons at about δH 5.8 ppm in the 1H-NMR spectrum.

Lupeol (1): white powder, m.p. 210°C. TLC: Rf values 0.25 (n-hexane: CHCl3 = 70:30), 0.46 (n-hexane: Ethyl acetate = 95:5), and 0.58 (n-hexane: acetone = 85:15) ESI-MS m/z = 427.1 [M+H]+ (molecular formula $C_{30}H_{50}O$). 1H-NMR (500 MHz, DMSO) δH (ppm). 13C-NMR (500 MHz, DMSO) δC (ppm).

Glochidiol (2): white powder, m.p. 213-214°C. TLC: Rf values 0.12 (CHCl3 : Ethyl acetate = 95:5), 0.6 (CHCl3 : acetone = 90:10), and 0.89 (CHCl3 : MeOH = 90:10). ESI-MS: m/z = 442.7 [M+H]+ (molecular formula $C_{30}H_{50}O_2$). 1H-NMR (500 MHz, DMSO) δH (ppm).13C-NMR (500 MHz, DMSO) δC (ppm).

Lupa-1,20(29) diene-3-one (3): white powder, m.p. 163-164°C. TLC: Rf values 0.19 (n-hexane: CHCl3 = 90:10), 0.44 (n-hexane: ethyl acetate = 95:5), and 0.67 (n-hexane: acetone = 95:5). ESI-MS: m/z = 423.1 [M+H]+ (molecular formula $C_{30}H_{46}O$). 1H-NMR (500 MHz, DMSO) δH (ppm). 13C-NMR (500 MHz, DMSO) δC (ppm).

Lup-20(29)-ene-3β,16β-diol (4): white powder, m.p. 212-213°C. TLC: Rf values 0.24 (CHCl3: Ethyl acetate = 95:5), 0.15 (CHCl3: acetone = 90:10), and 0.71 (CHCl3: MeOH = 90:10). ESI-MS: m/z = 443.3 [M+H]+ (molecular formula $C_{30}H_{50}O_2$). 1H-NMR (500 MHz, DMSO) δH (ppm). 13C-NMR (500 MHz, DMSO) δC (ppm).

Lup-20(29) ene-1,3-dione (5): white powder, m.p. 166-167°C. TLC: Rf values 0.15 (n-hexane: CHCl3 = 90:10), 0.34 (n-hexane: ethyl acetate = 95:5), and 0.34 (n-hexane: acetone = 95:5). ESI-MS: m/z = 423.1 [M+H]+ (molecular formula $C_{30}H_{47}O_2$). 1H-NMR (500 MHz, DMSO) δH (ppm). 13C-NMR (500 MHz, DMSO) δC (ppm).

Figure 1: *Glochidion talakonense* Leaf

Compound 1: White powder; m.p. 210°C. IR vmax (film) cm−1 : 3380, 1650, 1730, 292, 2789, 1600; 1 H-NMR (500 MHz, DMSO) δ: 4.68 (1H, d, J = 2.4 Hz), 4.58 (1H, m), 1.68 (3H, s), the presence of six other methyl groups was

observed by the six singlets (three protons for each signal) at 1H 1.03, 0.96, 0.95, 0.83, 0.79 and 0.76. 3β-hydroxyl group identified by the doublet of doublets at 3.19 (1H, dd, J = 11.4, 4.8 Hz), The 13C-NMR spectrum showed 30 carbons for the triterpenoid of lup- 20(29)-ene skeleton which was characteristic by the observation of two olefinic carbons of isopropylene functional group at 13C 151.0 and 109.3, and seven methyl carbons at 13C 28.0, 15.4, 16.1, 16.0, 14.6, 18.0, 19.3 (Table). The presence of one hydroxyl group at position 3 was determined by an oxygenated carbon signal at 13C 79.0; ESI-MS m/z = 427.1 [M+H]+ (molecular formula $C_{30}H_{50}O$).

Compound 2: white powder, m.p. 213-214°C; IR νmax (film) cm−1 : 3363, 2925, 2260, 1700, 1614, 1076, 1036; 1 H-NMR (500 MHz, DMSO) δ : 3.42 (1H, dd, J = 11.4, 4.8 Hz, H-1) (Table); The 13C-NMR data of 4 confirmed this analysis by the observation of an oxygenated methine carbon at 13C 79.0 ppm (Table); ESI-MS: m/z = 442.7 [M+H]+ (molecular formula $C_{30}H_{50}O_2$).

Compound 3: white powder, m.p. 163-164°C; IR νmax (film) cm−1 : 3332, 2959, 2267, 1726, 1605, 1013; 1 H-NMR (500 MHz, DMSO) δ: , the presence of two olefin protons at 1H 7.10 (1H, d, J = 10.2 Hz, H-1) and 5.79 (1H, d, J = 10.2 Hz, H-2) in the 1H-NMR spectrum, 1H 3.90 (1H, m, H-1) ;13C-NMR (500 MHz, DMSO): signals at13C 159.8 (C-1) and 125.2 (C- 2), combined with the absence of the signal of hydroxyl carbon at 13C 79.6 ppm as present Table; ESI-MS: m/z = 423.1 [M+H]+ (molecular formula $C_{30}H_{46}O$).

Compound 4: white powder, m.p. 212-213°C.IR νmax (film) cm−1 : 3363, 2927, 1605, 1587, 1517, 1171, 1073, 1020; 1 H-NMR (500 MHz, DMSO) δ: 3.90. m,3.00. dd, 14.4, 7.8, 2.22, dd, 14.4, 3.6, 2.38, dt, 6.0, 10.8, 1.06. s, 1.06. s, 0.84. s, 1.04. s, 0.98. s, 0.80. s, 4.68. d. 2.4, 4.57. m, 1.68, s; 13C-NMR (500 MHz, DMSO): (Table 3), indicated the existence of a hydroxyl group in its structure; ESI-MS: m/z = 443.3 [M+H]+ (molecular formula $C_{30}H_{50}O_2$).

Compound 5: white powder, m.p. 166-167°C; IR νmax (film) cm−1 : 3367, 2878, 1726, 1587, 1227, 1075, 1014; 1 H-NMR (500 MHz, DMSO) δ: The absence of an oxygenated methine proton signal at □H 3.19 in the ^{1}H-NMR spectrum of **5** (Table 2 **)** indicated that this compound did not have a hydroxyl group at position 3. In addition, the observation of carbonyl carbon at 13C 218.2 ppm instead of the oxygenated methine carbon at 13C 79.0 ppm in the ^{13}C-NMR spectrum of **5** (Table 3**)** revealed that the 3*β*-hydroxyl group has been supplanted by a ketone group at position 3 in compound **5**. ESI-MS: m/z = 423.1 [M+H]+ (molecular formula $C_{30}H_{47}O_2$).

These triterpenoids possess a wide range of biological activities. Previous studies have shown that lupeol has antibacterial (Bello IA et al., 2018), anti-inflammatory (Saleem M et al., 2009), and anti-cancer activities (Sharma D etal., 2022). This study also concluded that glochidiol could be a promising compound for the treatment of lung cancer (Chen H, et al.,2021). Moreover, glochidone also possessed antidiabetic effects (Verma A et al., 2021). These important data may orientate for further studies on the biological activity and phytochemical of *G. talakonense*.

Table 1: The important ^{1}H-NMR data (δppm) for **1** (500 MHz, DMSO)

No	**1**
3	3.19. dd. 11.4. 4.8
19	2.39. dt. 6.0. 10.8
23	0.96. s
24	0.76. s
25	0.83. s
26	1.03. s
27	0.95. s
28	0.79. s
29	4.68. d. 2.4 4.58. dd. 2.4. 1.2

30	1.68. s	

Table 2: The important ^{1}H-NMR data (δppm) for **2** to **5** (500 MHz, DMSO)

No	2	3	4	5
1	3.42. dd. 11.4. 4.8	7.10. d. 10.2	3.90. m	
2		5.79. d. 10.2	3.00. dd. 14.4. 7.8	2.48, ddd. 7.8, 9.6, 15.6
			2.22. dd. 14.4. 3.6	2.41, ddd. 4.2, 7.8, 15.6
3	3.24. brd. 12.0			
19	2.37. dt. 6.0. 10.8	2.39. dt. 6.0. 10.8	2.38 dt. 6.0. 10.8	2.38, dt. 6.0. 10.8
23	0.75. s	1.08. s	1.06. s	1.03. s
24	0.95. s	1.13. s	1.06. s	1.07. s
25	0.90. s	1.07. s	0.84. s	0.93. s
26	1.04. s	1.11. s	1.04. s	1.07. s
27	0.95. s	0.96. s	0.98. s	0.96. s
28	0.79. s	0.81. s	0.80. s	0.80. s
29	4.68. d. 2.4	4.71. d. 2.4	4.68. d. 2.4	4.69. d. 2.4
	4.55. dq. 2.4. 1.2	4.59. dq. 2.4. 1.2	4.57. m	4.57. m
30	1.67. s	1.69. s	1.68. s	1.68. s

Table 3: ^{13}C-NMR data (δppm) for **1** to **5** (500 MHz, DMSO)

No	1	2	3	4	5
1	38.7	79.0	159.8	35.5	207.2
2	27.4	38.1	125.2	45.1	39.6
3	79.0	75.8	205.5	79.6	218.2
4	38.9	38.9	44.7	47.1	47.3
5	55.3	53.2	53.5	51.4	55.0
6	18.3	18.0	19.0	19.6	19.7
7	34.3	34.1	33.8	33.0	33.6
8	40.9	42.9	41.8	43.0	40.8
9	50.5	51.5	44.5	50.7	49.8
10	37.2	43.6	39.6	42.9	36.9
11	21.0	23.9	21.3	23.1	21.5
12	25.2	25.1	25.1	25.2	25.2
13	38.1	37.6	38.3	38.0	38.2
14	42.9	41.4	43.0	41.2	43.0
15	27.5	27.5	27.4	27.5	27.5
16	35.6	35.6	35.5	79.6	35.5
17	43.0	42.9	43.1	43.0	42.9
18	48.3	48.4	48.2	48.3	48.3
19	48.0	48.0	47.9	47.9	48.0
20	151.0	150.8	150.8	150.7	150.9
21	29.9	29.8	29.8	29.8	29.9

22	40.0	40.0	40.0	40.0	40.0
23	28.0	27.9	27.8	27.9	26.7
24	15.4	14.9	21.4	19.9	21.0
25	16.1	11.9	19.3	11.8	16.0
26	16.0	16.2	16.5	16.0	15.8
27	14.6	14.5	14.4	14.5	14.5
28	18.0	18.0	18.1	18.0	18.0
29	109.3	109.4	109.5	109.5	109.4
30	19.3	19.2	19.2	19.3	19.3

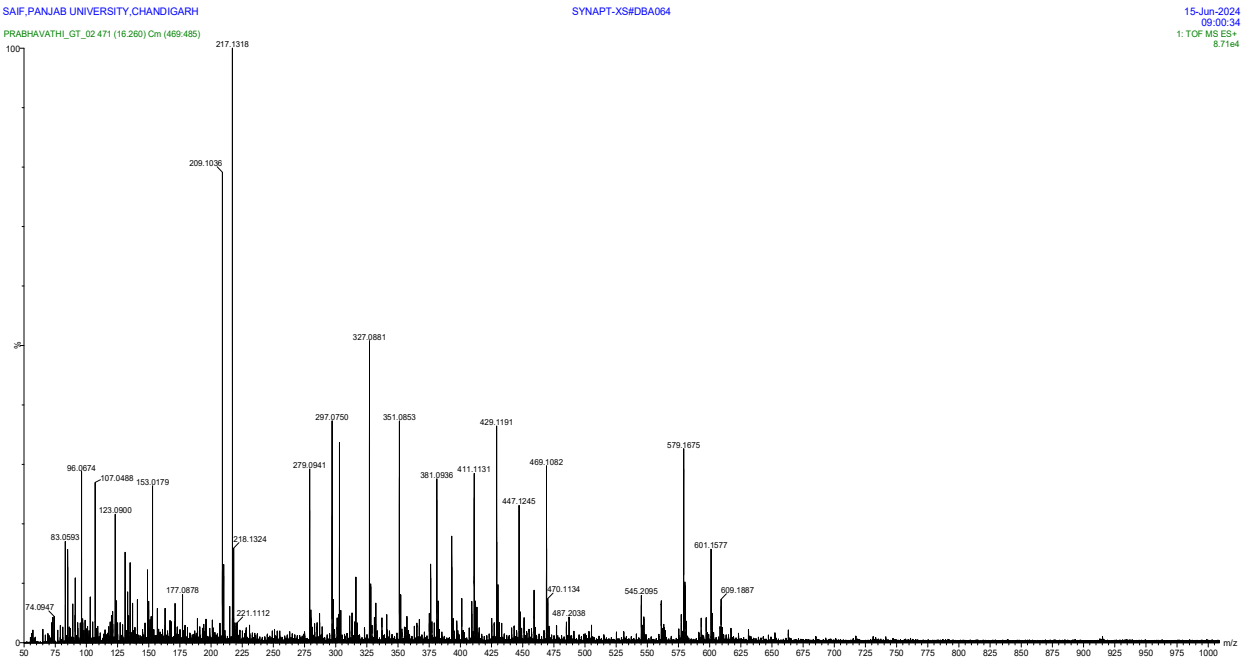


Figure 2: LC-MS spectrum of *Glochidion talakonense* Leaf

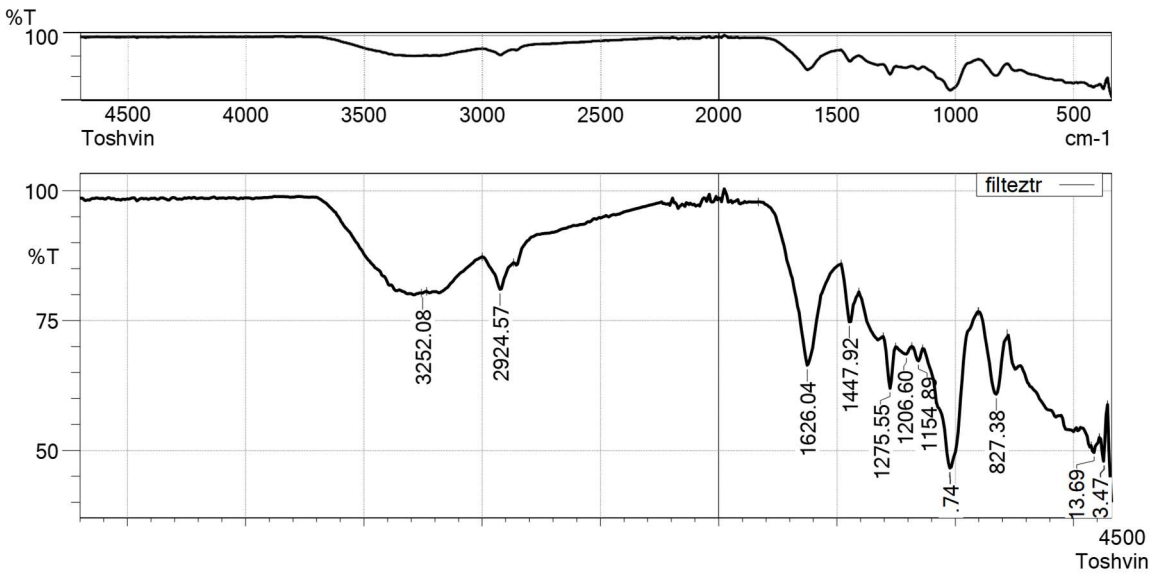


Figure 3: IR spectrum of *Glochidion talakonense* Leaf

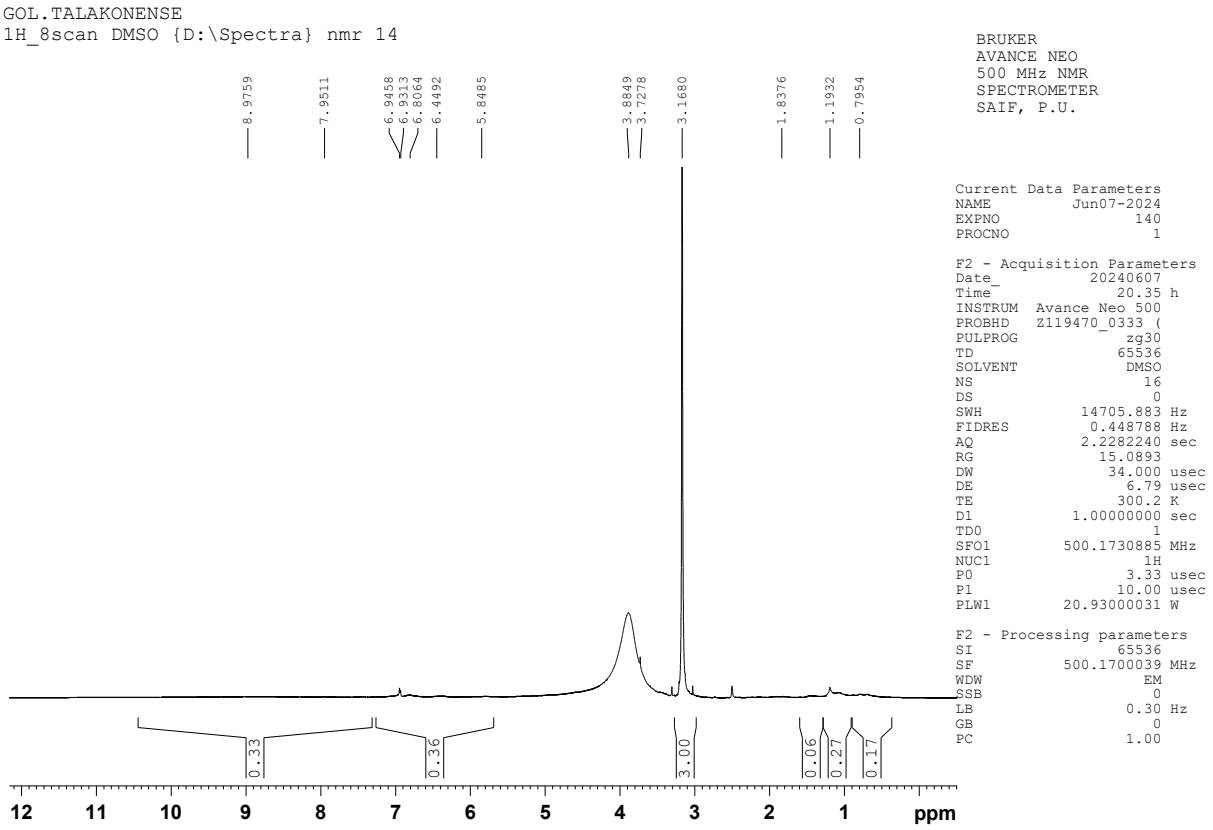


Figure 4: 1H NMR spectrum of *Glochidion talakonense* Leaf

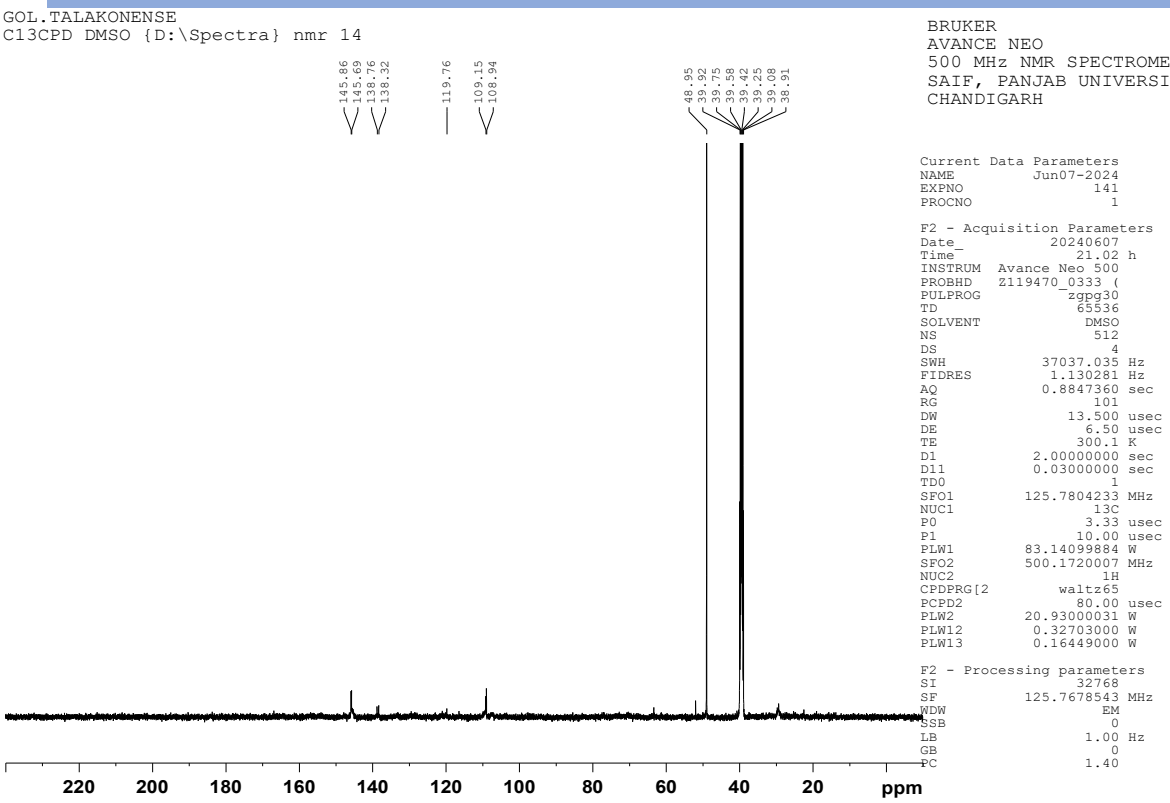
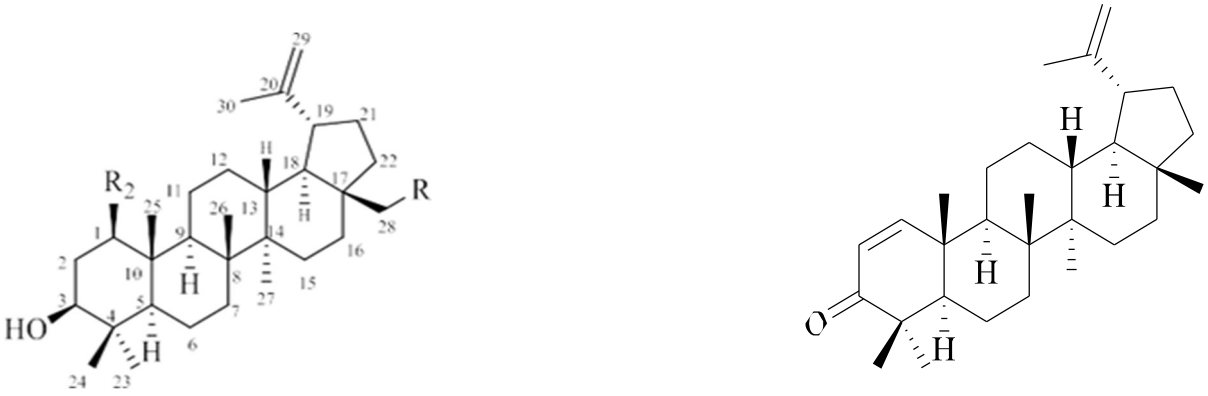


Figure 5: C13 NMR spectrum of *Glochidion talakonense* Leaf



Compound-1 R1=H; R2=H
Compound-2 R1=H; R2=OH
Compound-4 R1=H; at C-16 R2=OH
Compound-3 at C-3=O
Compound-5 at C-1=O

Figure 6: Structure of the isolated compounds from *Glochidion talakonense* Leaf

Conclusion

Five terpenoids were isolated from the n-hexane fraction of *Glochidion talakonense* including lupeol (1), Glochidiol (2), Lupa-1,20(29) diene-3-one (3), Lup-20(29)-ene-3β,16β-diol (4), Lup-20(29) ene-1,3-dione (5). The chemical structures of these triterpenoids were elucidated based on IR, NMR and LC-MS spectral analysis. These terpenoids have not been previously reported from *Glochidion talakonense* collected in Talakona. The isolated triterpenoids compounds from *Glochidion talakonense* could be seen as characteristic metabolites of genus Glochidion. Further studies on the biological activities and chemical composition of *Glochidion talakonense* are being conducted to provide scientific information on

its use in traditional medicine.

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Conflict Of Interest
The authors declared no conflict of interest

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