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Isolation and characterization of Lupeol from stems of *Girardinia diversifolia*

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Abstract: *Girardinia diversifolia*, growing abundantly in the Middle Himalayas, belongs to the family *Urticaceae* and commonly known as '*Dans kandali'* in Uttarakhand. A phytochemical investigation of the plant was carried out to ascertain the biochemical setup of the plants. Lupeol, a bioactive triterpenoid, was isolated from the stem extract of *Girardinia diversifolia* for the first time by extraction and isolation through spectroscopic studies. Present paper discusses the extraction techniques, characterization, chemical and biological properties of the Lupeol and its industrial potential which can be used for taking up future research and development activities for strengthening the existing pharmacological sciences besides, refining and utilizing traditional knowledge for the welfare of the society.

Key words: *Girardinia diversifolia*, Lupeol, spectroscopic studies.

Introduction

Girardinia diversifolia, commonly known as '*Himalayan Nettle'* in Uttarakhand belongs to the family *Urticaceae*. It is abundantly distributed in the Himalayas ranging from Kashmir to Kumaun between altitudinal ranges of 2,100 to 3,200m [1]. It can be seen growing extensively as an underutilized biomass in the forest areas situated in outskirts of the villages. The whole plant (leaf and stem) is used as cattle fodder to improve milk production. The stem portion of the plant yields valuable fiber, which is traditionally used by the tribal for making rope which is used for making variety of articles for packing grains and transportation purposes. After extracting the fiber from the stem, residue of the bark portion is used as fuel wood. Traditionally, bark powder is also used as a bandage material for faster healing of wounds and setting of broken bones. Taking cognizance of the fact that *Girardinia diversifolia* has a remarkable association with the local communities, owing to its unique properties and variety

of uses, present study has been undertaken for chemical examination of the species involving isolation and characterization of chemical compounds present in stem.

Material and methods

Plant material

The stems of plant material were collected in the Middle Himalayas in Mussoorie and Dhanaulti areas of Dehradun District of Uttarakhand, India. Plant species were identified in consultation with taxonomist, Botany Division, Forest Research Institute, Dehradun.

Chemicals and materials

Melting point was determined on a melting point apparatus in a sealed glass capillary tube. MS were run on HP-5890 mass spectrometer. IR was studied in Shimadzu-408 spectrophotometer. ^{1}HNMR and ^{13}CNMR were recorded on Bruker WM-400 (500 MHz) spectrometer using DMSO-d6 using TMS as an internal standard at room temperature. All the reagents were purchased from MERCK. TLC Plates coated with silica gel G to a thickness of 0.25 mm were used. SD-fine silica gel (100-200 mesh) was used for column chromatography.

Extraction and isolation

The stems (500g) of *Girardinia diversifolia* were chopped and powdered after air drying and were sequentially extracted with the solvents of increasing polarity [petroleum ether (60-80^{o}C), chloroform and methanol]. Solvent were subsequently, removed under vacuum to obtain extracts of respective solvents. Extracts with higher percentage of yield were used for further analysis and solvents with relatively lower yield were discarded.

Identification of Lupeol isolated from Girardinia diversifolia

Identification of lupeol was carried out by using different spectroscopic techniques and comparing the results to previously reported literature that elucidated the structure of this compound.

Results and Discussion

Extraction and isolation

Removal of the solvents (petroleum ether, chloroform and methanol) under vacuum yielded three respective extracts. The yield of the chloroform extract (0.07%) and methanol extract (0.09%) was poor and not considered for chemical examination while yield of petroleum ether extract (4.16%) was quite considerable and therefore, examined for further analysis. Per cent yield of petroleum ether, chloroform and methanol extracts has been presented in the Figure -2. Petroleum ether extract was column chromatographed over silica gel and eluted with ethyl acetate in petroleum ether, one pure compound was isolated which was designated as GHSHA. GHSHA was obtained as white crystals (11mg, 0.002 % yield and m.p. 215-216^{o}C) on elution with petroleum ether-ethyl acetate (88:12). It gave Liebarmann Burchard test for triterpenoids and steroids. It was characterized by using spectral data (GC-MS, IR, ^{1}H-NMR and ^{13}C-NMR) which were found to be in good agreement with those reported for lupeol.

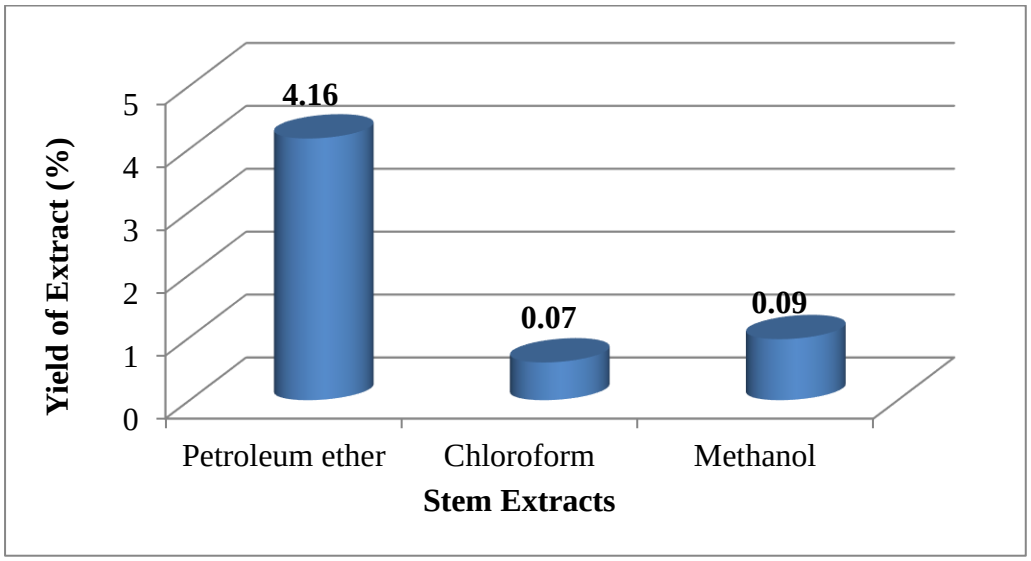

Figure-2: Percentage Yield of *Girardinia diversifolia* Stem Extracts in Different Solvent

Identification of Lupeol isolated from Girardinia diversifolia

Identification of lupeol was carried out by using different spectroscopic techniques and comparing the results to previously reported literature that elucidated the structure of this compound. The detailed properties are given below:-

MS (m/z); 426 [M^+]

IR ν cm^{-1}(KBr); 3385, 3082, 1635, 886

^{1}H NMR (500MHz, DMSO-d_6) of Lupeol isolated from *Girardinia diversifolia* comparing to those reported [2] given in Table-1.

Table-1: ^{1}H-NMR of Lupeol Isolated from *Girardinia diversifolia*

***Burn et al., (2000)* [2]**	**Lupeol from *Girardinia diversifolia***	**Position**
0.90s	0.90s	1a
1.67	1.67s	e
1.60	1.85d	2a
1.56	1.95m	e
3.19	3.17dd (11.2; 4.9 Hz)	3
-	-	4
0.68	0.68d	5
1.51s	1.50m	6a
1.39	1.40m	e
1.39	1.34m	7a
-	1.40m	e
-	-	8
1.27s	1.23m	9
-	-	10

1.41s	1.40m	11a
1.23	1.22m	e
1.07	1.0d	12a
1.67	1.61m	e
1.66	1.66m	13
-	-	14
1.00s	0.99t	15a
1.68	-	e
1.37	1.34d	16
-	-	17
1.36	1.36dd	18
2.38	2.38dt	19
-	-	20
1.32s	1.32m	21a
1.92	1.92m	e
1.19	1.19m	22a
0.96	0.74s	23
0.76	0.76s	24
0.83	0.81s	25
1.03	1.02s	26
0.94	0.94s	27
0.78	0.77s	28
4.56bs	4.58m	29a
4.69t	4.62m	e
1.68	1.65s	30

^{13}C NMR (500MHz, DMSO-$d_{6)}$ of Lupeol isolated from *Girardinia diversifolia* comparing to those reported [2,3] given in Table-2.

Table-2: ^{13}C-NMR of Lupeol Isolated from *Girardinia diversifolia*

***Burn et al., (2000)*[2]**	***Fotie et al. (2006)*[3]**	**Lupeol from *Girardinia diversifolia***	**Position**
38.7	38.6	38.0	1
27.4	27.3	27.4	2
79.0	78.9	79.0	3
38.8	38.4	38.7	4
55.3	55.2	55.3	5
18.3	18.2	18.3	6
34.2	34.2	34.3	7
40.8	40.7	40.0	8
50.3	50.3	50.5	9
37.1	37.1	37.5	10
20.9	20.9	20.9	11
25.1	25.0	25.5	12
38.0	38.0	38.0	13
42.8	42.9	43.0	14
27.4	27.4	27.4	15
35.6	35.5	35.8	16
43.0	42.9	42.9	17
48.3	48.2	48.3	18
48.0	47.9	47.9	19
150.9	150.8	150.4	20
29.8	29.8	29.8	21
40.7	39.9	40.0	22
28.0	27.9	28.0	23
15.3	15.3	15.3	24

16.1	16.1	16.1	25
15.9	15.9	15.9	26
14.5	14.5	14.5	27
18.0	17.9	18.0	28
109.3	109.3	109.3	29
19.3	19.2	19.3	30

Discussion

The Mass spectra shows the molecular ion at m/z 426 [Figure-3] corresponding to the formula $C_{30}H_{50}O$. The cleavage is seen between C-8/C-14 and C-12/C-13 bonds with H transfer, expected to a lupane or hopane skeleton for compound GHSHA, compared with the published literature [4,5].

The presence of OH group is supported by IR absorption band at 3385 cm^{-1} together with the existence of an oxymethine signal in the ^{1}H NMR, which was displayed as a double doublet (11.2, 4.9Hz) indicative of its axial orientation. The existence of exocyclic double bond, by IR values at 3082, 1656, 886 cm^{-1} and corroborated by two singlet assigned to the exocyclic methylene protons at δ 4.58 and 4.62, furthermore the vinyl methyl signal at δ 1.65 indicated the presence of an isopropenyl group. The ^{1}H NMR spectrum displayed signals for tertiary methyl groups at δ 0.74, 0.76, 0.81, 0.94, 0.77 and 1.02. The ^{13}C NMR and DEPT NMR shows 30 carbon resonance (6 CH_3, 11 methylene (one sp^2 at δc 109.3), 7 methines (one oxygenated at δc 79.0) and 6 quaternary carbons (one olefinic at δc 150.4). Based on the above described spectral characteristics of GHSHA, it was identified as lupeol. The spectral data were found to be in the agreement with reported literature for lupeol [2,3]. This is the first report of isolation of Lupeol [Figure-4] in the plant *Girardinia diversifolia* from Uttarakhand (India).

Many biological activities on lupeol has been reported including, the stimulation of programmed cell death in human leukaemia cell line (HL-60) [6]. Lupeol shows antiarthritic activity [7]. It inhibits the growth of highly aggressive human metastatic melanoma cells [8]. It works as potential antidiabetic constituent [9]. This compound is reported to be antiangiogenic [10], antioxidant and anti-inflammatory [11,12]. It inhibits early responses of tumour growth induced by benzoyl peroxide [13], also plays very important role in normalization of lipid profile [14]. It showed wound healing activity [15], protective effect in hypercholesterolemia associated with renal damage [16] and suppression of immune factors [17].

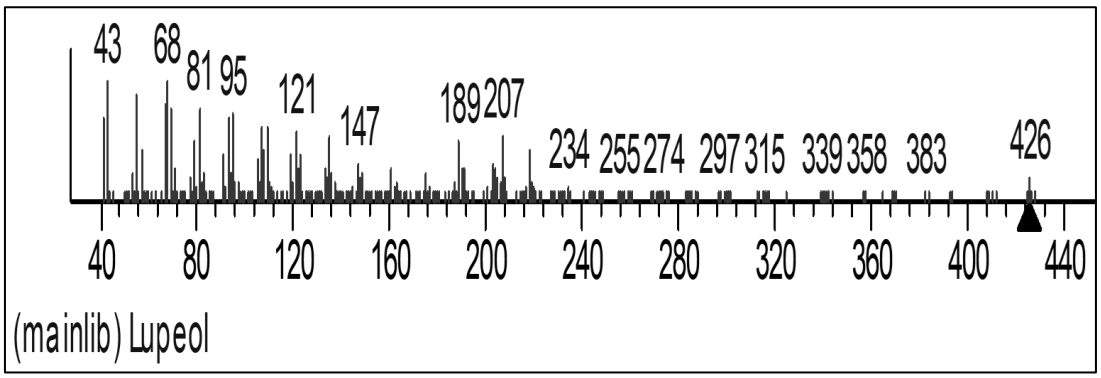

Figure-3: Mass Spectra of Lupeol

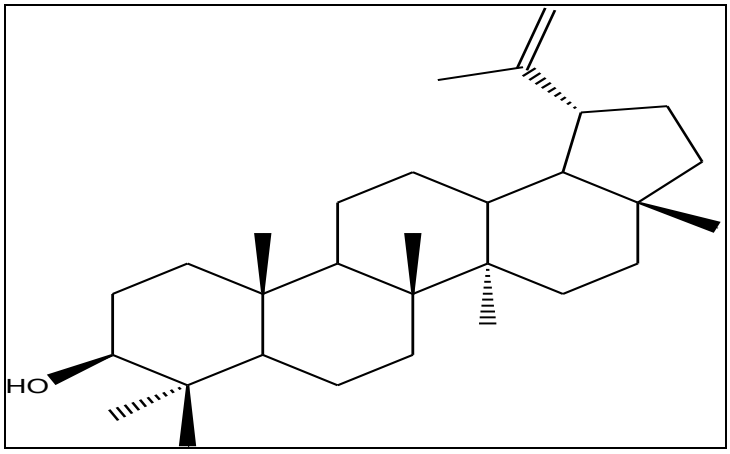

Figure-4: Structure of Compound GHSHA (Lupeol)

Conclusion

In this study the presence of lupeol in *Girardinia diversifolia* has been reported for the first time. It has been shown to possess many biological activities like anti-arthritic, anti-diabetic, anti-inflammatory etc. Present study provides an avenue for taking up future research and development activities for strengthening the existing pharmacological sciences besides, refining and utilizing traditional knowledge for the welfare of the society. Findings of the present study may be utilized to develop resource based local livelihood practices by screening of the economically viable and commercially important chemical compounds from the all plants inhabiting predominantly in the hilly areas.

References

1. Annonymous, (1989); A dictionary of Indian Raw Materials, Wealth of India Publication CSIR, New Delhi, 419.
2. Burns, D., Reynolds, W. F., Buchanan, G., Reese, P. B. and Enriquez, R. G. (2000); Assignment of ^{1}H and ^{13}C spectra and investigation of hindered side chain rotation in Lupeol derivatives, Magn. Reson. Chem., 38, 488.
3. Fotie, J., Bohle, D. S., Leimanis, M. L., Georges, E. R., G. and Nkengfack, A.E. (2006); Lupeol long chain fatty acid esters with antimalarial activity from *Holarhena floribunda*, J. Nat. Prod., 69, 62.
4. Ogunkoya, L. (1981); Application of mass spectrometry in structural problems in triterpenes, Phytochem., 29, 121.
5. Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963); Mass spectrometry in structural and stereochemical problems, Part XXXII, Pentacyclic triterpenes, J. American. Chem. Soc., 85, 3688.
6. Arantanechemuge, Y., Hisbasami, H., Sanpin, K., Katsuzaki, H., Imai, K. and Komiya, T. (2004); Induction of apoptosis by Lupeol isolated from mokumen (*Gossampinus malabarica* L. Merr.) in human promyelotic leukemia HL-60, Oncology Reports, 11, 289.
7. Agarwal, R. B. and Rangari, V. D.(2003); Antiinflammatory and antiarthritic activities of Lupeol and 19a-H lupeol isolated from *Stribilanthus callosus* and *Strobilanthus ixiocephala* roots, Indian Jour. of Pharmacol., 35, 387.

8. Saleem, M., Maddodi, N., Zaid, M. A., Khan, N., Hafeez, B. B., Asim, M., Suh, Y., Yun, J. M., Setaluri, V. and Mukhtar, H. (2008); Lupeol inhibits growth of highly aggressive human metastatic meloma cells *in vitro* and *in vivo* by inducing apoptosis, Clin. Cancer Res., 14 (7), 2119.
9. Gupta, R., Sharma, A. K., Sharma, M. C., Dobhal, M. P. and Gupta, R. S. (2012); Evaluation of antidiabetic and antioxidant potential of lupeol in experimental hyperglycaemia, Nat. Prod. Res., 26 (12), 1125.
10. You, Y., Nguyen-Hai, N., Yong, K., Ki-Hwan, B. and Byung-Zun, A., (2003); Antiangiogenic activity of lupeol from *Bombax ceiba*, Phytother. Res., 17, 341.
11. Nguemfo, E. L., Dimo, T., Dongmo, A. B., Azebaze, A. G. B., Alaoui, K., Asongalem, A. E., Cherrah, Y. and Kamtchouing, P. (2009). Anti-oxidative and anti-inflammatory activities of some isolated constituents from the stem bark of *Allanblackia monticola* Staner L. C (*Guttiferae*), Inflamm. Pharm., 17, 37.
12. Sudhahar, V., Kumar, S. A. and Varalakshmi, P. (2006); Role of lupeol and lupeol linoleate on lipemic-oxidative stress in experimental hypercholesterolemia, Life Sci., 78, 1329.
13. Saleem, M. (2009); A novel anti-inflammatory and anti-cancer dietary triterpene, Cancer Lett., 285, 109.
14. Sudhahar, V., Kumar, S. A., Mythili, Y. and Varalakshmi, P. (2007); Remedial effect of lupeol and its ester derivative on hypercholesterolemia-induced oxidative and inflammatory stresses, Nutr. Res., 27, 778.
15. Harish, B. G., Krishna, V., Kumar, H. S. S., Ahamed, B. M. K., Sharath, R., Swamy, K. H. M. (2008); Wound healing activity and docking of glycogen-synthase-kinase-3-rotein with isolated triterpenoid lupeol in rats, Phytomed., 15, 763.
16. Sudhahar, V., Kumar, S. A., Varalakshmi, P. and Sujatha, V., (2008); Protective effect of lupeol and lupeol linoleate in hypercholesterolemia associated renal damage, Mol. Cell. Biochem., 317, 11.
17. Bani, S., Kaul, A., Khan, B., Ahmad, S. F., Suri, K. A., Gupta, B. D., Satti, N. K. and Qazi, G. N. (2006); Suppression of T-lymphocyte activity by lupeol isolated from *Crataeva religiosa*, Phytother. Res., 20, 279.