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Research Article

ISOLATION AND QUANTIFICATION OF LUPEOL IN *Strobilanthes ciliatus Nees* BY HPTLC METHOD

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ABSTRACT

Strobilanthes ciliatus Nees of family Acanthaceae was used in the treatment of rheumatalgia, leucoderma, skin diseases, dropsy, and leprosy in Ayurvedic system. Lupeol was isolated from the petroleum ether extract of aerial parts of *Strobilanthes ciliatus Nees* by column chromatography and identified by IR, NMR, and MS spectral data. It was quantified in the petroleum ether extract by HPTLC method, and found to be 0.16±0.02% w/w.

Keywords: *Strobilanthes ciliatus*, Acanthaceae, Lupeol, HPTLC.

INTRODUCTION

Strobilanthes ciliatus of Acanthaceae family is a highly potential medicinal plant in ayurveda. The roots are useful in the treatment of rhematalgia, lumbago, sciatica, limping, chest congestion, strangury, fever, leucoderma, skin diseases, inflammation, and general debility [1]. Its stem is widely used for whooping cough, bronchitis, dropsy, leprosy, and pruritus [2]. Very few reports are available in the literature and the plant is reported to contain lupeol, betulin, stigmasterol, and stigmasterol glucopyranoside [3]. This prompted as to investigate the phytoconstituents of this plant and to quantify one of the biomarker compounds.

Nowadays, HPTLC has become a routine analytical technique due to its advantages of reliability in the quantification of analyte at micro and even in nanogram levels [4, 5, 6]. Qualitative and quantitative standardization of lupeol was performed using HPTLC [7]. Lupeol has been identified as a biomarker and was quantified by HPTLC in *Clerodendrum phlomidis* [8], *Bergia suffruticosa* [9], *Grewia tiliaefolia* [10], and *Mimosoups elengi* [11]. Till now no biomarker has been identified from *S.ciliatus*. So, in the present study, isolation and quantification of compound lupeol from *S.ciliatus* by HPTLC method was carried out.

MATERIALS AND METHODS

General: All chemicals used were of analytical grade, Lupeol was purchased from m/s Sigma-Aldrich, Bangalore, India. IR spectra were recorded on a Perkin- Elmer model 1650 FT-IR spectrometer. ^{1}H NMR spectra were measured at 400 MHz on a Bruker- 400 spectrometer using TMS as internal standard and $CDCl_3$ as a solvent. MS spectra were obtained on a Shimadzu LCMS instrument.

Preparation of plant extract

S. ciliatus was collected from Chittur, Palakad district, Kerala and was identified by Botanical Survey of India, Coimbatore. Air dried pieces of aerial parts of *S.ciliatus* (1 kg) was thoroughly cold percolated and extracted with petroleum ether (60-80° C) for 72 hours. The extract was filtered and concentrated to yield the residue 1.8g (0.18%).

Isolation of compound

The residue from the petroleum ether extract of *S.ciliatus* was dissolved in minimum quantity of chloroform, it was subjected to column chromatography over a column of silicagel and eluted with PE: EA and further eluted with increasing amount of ethylacetate. Elute of 20ml were collected and the solvent distilled. The homogeneity of the fractions was examined by TLC on silicagel-G plates. The spots were developed and visualized under iodine vapour. Similar fractions were combined. Fractions 10-16 yielded compound-1 (80mg)

Characterization of compound

The isolated compound-1 was characterized by IR, ^{1}H-NMR, ^{13}C-NMR and MS methods. HPTLC analyses of petroleum ether extract of *S.ciliatus* for lupeol

Procedure

Sample solution preparation

The given petroleum ether extract 50mg was weighed accurately in an electronic balance(Afcoset) and dissolved in 1ml of methanol and centrifuged at 3000 rpm for 3min and this solution was used as the test solution for HPTLC analysis.

Standard solution preparation

The given standard lupeol 1mg was weighed in an electronic balance and dissolved in 1ml methanol and this solution was used as a standard solution for the HPTLC method.

Sample application

1µl of test solution and 2µl of standard solutions were loaded as 5mm band length in the 4 x 10 Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

Spot development

The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with 8:2 petroleum ether and ethylacetate as mobile phase and the plate was developed in the same mobile phase up to 90mm.

Photo-documentation

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254nm and UV366nm.

Derivatization

The developed plate was sprayed with the spray reagent (Anisaldehyde-Sulpuricacid reagent/Terpenoid) and dried at 100° C in Hot air oven. The plate was photo-documented in Day light mode and UV 366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber.

Scanning

After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV light 366nm. The Peak table, Peak display and Peak densitogram (Fig1&2 respectively) were noted.

RESULT AND DISCUSSION

Isolation of compound from *S.ciliatus*

The petroleum ether extract of the aerial parts of *S.ciliatus* was subjected to column chromatography. Fractions 10-16 eluted with petroleum ether: ethylacetate (80:20) was homogenous by TLC to yield the compound compound-1.

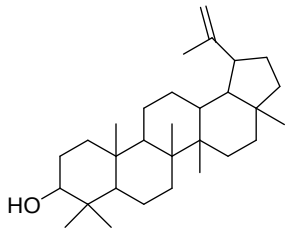
The mass spectrum for compound-1 showed the molecular ion peak at m/z 426, was corresponding to $C_{30}H_{50}O$.The IR spectrum of compound-1 showed characteristic absorption bands for hydroxyl group at 3325 & 1033 cm^{-1} and exomethylene group at1640 & 886 cm^{-1}. In the ^{1}H-NMR spectrum, it exhibited six singlets at δ 0.761, 0.790, 0.842, 0.943, 0.970, 1.04 each integrating for three protons indicating the presence of six tertiary methyl groups. A doublet of doublet at δ 3.20 is due to the proton attached to the secondary carbinol. Two broad singlets were seen at δ 4.57 and 4.69 due to the two exomethylene protons attached at C-29. One singlet at δ 1.64 integrating for three protons indicates the presence of methyl group at C-30. The remaining protons appeared as complex multiplets in between 1.0 to 2.5.

The ^{13}C-NMR signals (Table.1) were in agreement with the reported values of lupeol.

Table 1: ^{13}C-NMR spectral data of the compound-1

Carbon	Multiplicity*	Signal(δ)	Carbon	Multiplicity*	Signal(δ)
1	CH_2	37.17	16	CH_2	35.58
2	CH_2	29.85	17	C	48.30
3	CH	79.04	18	CH	47.92
4	C	38.69	19	CH	48.05
5	CH	55.23	20	C	150.98
6	CH_2	18.07	21	CH_2	28.02
7	CH_2	34.28	22	CH_2	35.86
8	C	40.00	23	CH_3	27.42
9	CH	50.44	24	CH_3	14.24
10	C	42.83	25	CH_3	16.17
11	CH_2	20.93	26	CH_3	15.68
12	CH_2	25.14	27	CH_3	14.20
13	CH	38.69	28	CH_3	18.07
14	C	42.83	29	CH_3	109.31
15	CH_2	27.42	30	CH_3	18.32

*Multiplicity was determined using DEPT-135 and DEPT-90 spectral data.



Structure of compound-1

HPTLC analysis

Chromatographic fingerprint analysis has shown to be a rational and feasible approach for the quality assessment and species authentication of traditional medicine [12, 13]. The developed fingerprint pattern of components can be used to determine the presence of marker compounds.

The chromatographic conditions (solvent system- 8:2 petroleum ether and ethyl acetate) used for *S.ciliatus* extract showed good separation for lupeol and no decomposition of lupeol was observed during the analysis.

HPTLC is an effective tool for quality evaluation of herbal drugs due to its simplicity, low cost and it has been utilized to develop the chromatographic fingerprint for botanical drugs [14, 15]. It also provides adequate information and parameters for comprehensive identification and differentiation of the two closely related herbal medicine.

TLC profile of PE extract of *S.ciliatus*

Table 2: Fingerprinting profile of *S.ciliatus* under UV 366nm

S. No.	Rf value	Colour of the band (UV 366nm)
1	0.67(standard lupeol)	Pink
2	0.02	Green
3	0.05	Green
4	0.07	Green
5	0.12	Green
6	0.17	Blue
7	0.22	Blue
8	0.29	Green
9	0.53	Pink
10	0.67	Pink
11	0.76	Green
12	0.82	Pink
13	0.91	Pink

The fingerprinting profile of S.ciliatus under UV at 366nm was shown in Table.2. It indicates the presence of 12 compounds in the extract. Lupeol appeared as a pink band at an R_f value of 0.67. Fig-I shows the TLC profile of PE extract of *S.ciliatus* after derivatization. The HPTLC chromatogram Fig.2 also shows the presence of lupeol in the PE extract.

TLC densitometric quantification lupeol using HPTLC

Lupeol was resolved well at R_f 0.67, in the sample solution of petroleum ether extract of *S.ciliatus* when the plate was developed in solvent system and derivatized as mentioned above.

The lupeol content was quantified using TLC densitometric methods and was found to be 0.16±0.02% w/w.

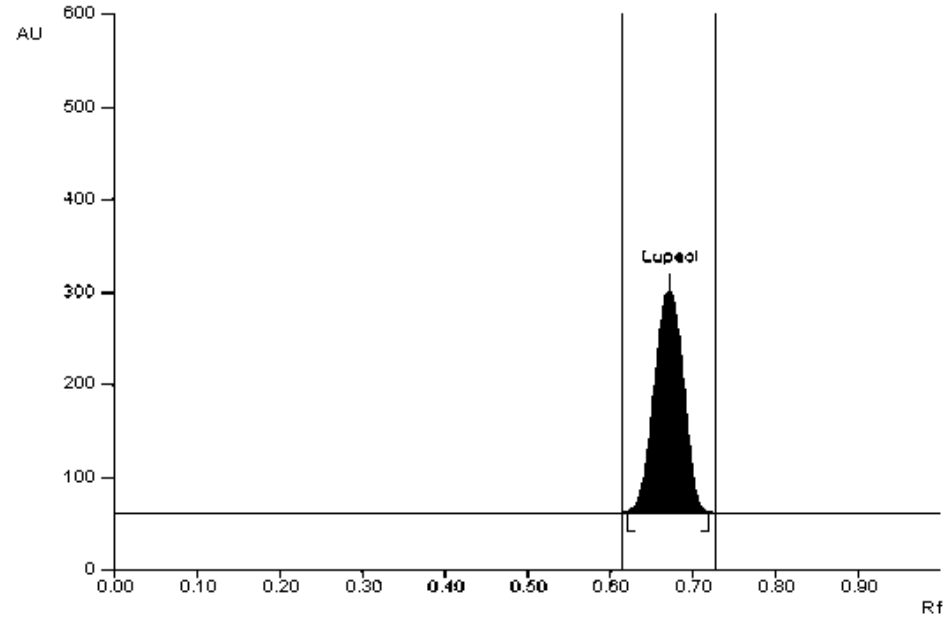


Fig. 1: Peak densitogram display of lupeol (Scanned at 366nm)

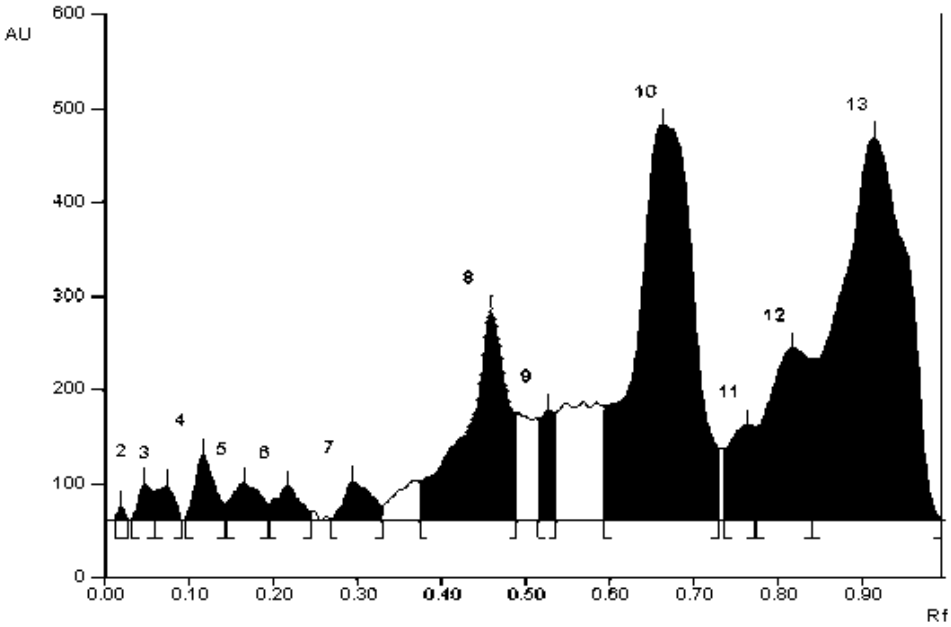


Fig. 2: Peak densitogram display petroleum ether extract of *S.ciliatus* (Scanned at 366nm)

CONCLUSION

Lupeol was isolated from the aerial part of *S.ciliatus* and it was characterized by spectral data. Lupeol was found to be major constituent of *S.ciliatus* and it was quantified by HPTLC method. This method is simple, rapid, and precise.

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