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Quantification of Lupeol and Betulin in Ougenia Dalbergioides Bark by Column Chromatography and TLC

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ABSTRACT:

Ougenia dalbergioides Benth. (fabaceae) commonly known as sandan. It is reported to have good medicinal values in traditional system of medicine. According to Ayurveda the bark is used in dysentery, leucoderma, urinary discharge, ulcers, skin disease and blood disease, biliousness, and anaemia. A systematic HPTLC fingerprinting on the bark of *Ougeinia dalbergioides* has not been reported in literature to our knowledge. So, simultaneous HPTLC quantification of the bark of *Ougeinia dalbergioides* was carried out. Two compounds were isolated from the methanolic extract of *Ougeinia dalbergioides* by preparative TLC using toluene: chloroform: methanol (8.8:8.8:2.2) as mobile phase. These compounds were found to be triterpene by spraying with anisalehyde-H2SO4 as spraying reagent. Melting point, Co-TLC with standards and Spectroscopic data of UV and FTIR confirmed triterpene as betulin and lupeol. The simultaneous HPTLC quantification of lupeol and betulin in acetone and methanol extract were shows that amount of lupeol in acetone extract and methanol extract were 9.9 µg /mg of extract and 8.3 µg /mg of extract respectively. The method is validated for the parameters like linearity, accuracy, precision, specificity, limit of detection and limit of quantification. From the data it was found that the method was linear, accurate, précised and specific.

KEYWORDS: *Ougenia dalbergioides*, column chromatography, UV, IR, HPTLC, lupeol, betulin

1. INTRODUCTION:

The increasing interest in powerful biological activity of secondary metabolites outlined the necessity of determining their contents in medicinal plants. The present study intended to find out the active constituents. *Ougeinia dalbergioides* plant is rarely mentioned in published books and there is no data of showing which constituents are present in this plant Qualitative chemical examination of various successive extracts of bark of *Ougeinia dalbergioides* showed presence of triterpenes, carbohydrates, flavanoids and tannins were present. Isolation of terpenoids was done by column chromatographic technique, using different ratio of toluene: chloroform: methanol as an eluent and two compounds were isolated. These compounds were lupeol and betulin which are confirmed by TLC with Anisaldehyde in H_2SO_4 reagent. Spectroscopic data of UV, IR and HPTLC quantification of both done.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The plant material of *Ougeinia dalbergioides* were identified, confirmed and authenticated by the Taxonomist, Bioscience Dept., Sardar Patel University, Vallabh Vidyanagar, Gujarat, India. A voucher specimen (NAR/od-1/27/ARGH-11) has been deposited in the Department of Pharmacognosy of A.R. College of Pharmacy, Vallabh Vidyanagar.

2.2 Isolation of phytoconstituents by Column Chromatography: [1-3]

2.2.1 Isolation of compounds:

The column of about 60 cm long with an internal diameter of 3 cm having capacity for holding 50-100 gm absorbent was used. Silica gel was used for the packing as an adsorbent .The column was packed by wet packing. At the bottom of the column cotton plug was kept and wet slurry was made for packing of the column.

A] Wet packing:

Based on the report of Physicochemical Screening of the methanol extract of leaves contains flavonoids. Isolation of lupeol and betulin was done by column chromatographic technique, using different ratio of toluene: chloroform: methanol as an eluent and two compounds were isolated. These compounds were lupeol and betulin which are confirmed by TLC with Anisaldehyde in H_2SO_4 reagent.

Results are recorded in table 1

2.3 Identification of isolated compound:

(i) Co-TLC with standard: Co-TLC of both isolated compounds with standard was done. For Co-TLC precoated silica gel 60 F_{254} Aluminium plates were used as stationary phase. By using Toluene: chloroform: methanol (8.8: 8.8: 2.2) as mobile phase. Detection was done by spraying with Anisaldehyde sulphuric acid.

(ii) FTIR Spectroscopy[4]**:** The IR spectrum of the isolated compound and overlain IR spectrum of isolated compounds and standards were determined.

Scan Range : 15600 cm^{-1} -30 cm^{-1}

Scan time: 20 scan/second

Scan Range: 15600 cm-1 -30 cm-1

Scan time: 20 scan/second

Single Beam/Ratio: Single

Detector: MIRTGS & FIRTGC

Source NIR: 15000-1200 cm-1

Beam splitter opt KBr: 7800370cm1

Detector MIRTGS: 10000220cm-1

Optimum range: 7800-1200cm-1

Detector FIRTGS: 30-780 cm-1

OPD velocity: 0.20 cm/s Interferogram direction: Bi-direction

(iii) U.V.Spectroscopic analysis[4,5]: UV-VIS-NIR Spectrophotometer specification: Make:

Perkin Elmer, U.S.A.

Model: Lambda 19

Specification: Double beam double Monochromator, Ratio Recording Lamp: Deuterium (UV), Tungsten-Halogen (VIS/NIR)

Detector: Photomultiplier (UV/VIS), Lead sulphide cell (NIR)

Wavelength: 185-3200 nm

(iv) Simultaneous quantification of lupeol and betulin by HPTLC method: 3, 6, 9 12, 15 µl of each standard solution were applied on TLC plates. The plates were developed in mobile phase and scanned at 525 nm. Calibration curve for standard were prepared by plotting the graph of concentration vs peak area. For selection of detection wavelength: The sensitivity of HPTLC method that uses UV detection depends upon the selection of proper detection wavelength. Both standard and sample spots were scanned in visible region of 400-800 nm and the overlain spectrum were recorded.

3. RESULTS:

3.1 Column chromatography

Two compounds, OD-1 and OD-2 isolated by column chromatography were confirmed as lupeol and betulin from their melting point, UV spectra, IR Spectra and its Co-TLC with standard. shown in Table 1

From above all compounds the yield of OD-2, OD-3, OD-5 were less in amount (<2mg). While, the yield of OD-1

and OD-4 were 15 mg and 10 mg respectively. Detailed TLC studies and comparing the Rf value with data of literature found that that isolated compounds might be the triterpenoids. As the yield of OD-2, OD-3, OD-5 were less, they were not used for further studies. Melting point, spectral analysis, IR spectroscopic studies and Co-TLC with standard was determined for both compounds, OD-1 and OD-4.

3.2 TLC study

3.2.1 Identification of OD- 1

(I) Co-TLC of OD-1 with standard lupeol:

Co-TLC of isolated OD-1 was carried out by using mobile phase toluene: chloroform: methanol (8.8:8.8:2.2), silica gel GF_{254} as stationary phase and anisaldehyde-H_2SO_4 reagent. Their chromatogram (**Figure-1**) with single spot revealed the purity of OD-1 and its identity as lupeol.

3.2.2 Identification of OD- 4

(I) Co-TLC of OD-4 with standard betulin:

Co-TLC of isolated OD-4 was carried out by using mobile phase toluene: chloroform: methanol (8.8:8.8:2.2) , silica gel GF_{254} as stationary phase and anisaldehyde-H_2SO_4 reagent. Their chromatogram (**Figure 2**) with single spot revealed the purity of OD-4 and its identity as betulin.

3.3 HPTLC data[6-11]:

The HPTLC chromatograph of lupeol and betulin from bark *Ougeinia dalbergioides* is shown in **Figure 3**.

3.3.1 Method Validation

The following parameters have been used to validate the developed HPTLC method for simultaneous estimation of lupeol and betulin according to ICH guideline.

a) Linearity:

A calibration curve of lupeol or betulin were obtained by plotting the peak area of lupeol or betulin against the concentration of lupeol or betulin over the range of 3-15

µl/spot from stock solution [Conc.-0.15-075 µg/spot]. The regression equation was found to be **y=7619x+1351** for lupeol and **y=5411x+1757** for betulin.

b) **Precision:**

The precision of the method was studied by applying three different concentration of the standard. The proposed method was found to be precise as indicated by intermediate precision studies expressed as percent RSD (Relative Standard Deviation) for intra-day and inter-day variation that is less than 2% as shown in **Table- 2, 3,4 ,5.**

c) Accuracy

The accuracy of the method was determined from recovery studies. The proposed method when used for quanfication of marker after spiking with standard afforded recovery of lupeol or betulin in acetone extract and in methanol extract at three concentration levels as shown in **Table- 6,7, 8,9.**

d) Specificity

The identity of the bands in the sample extracts were confirmed by comparing the Rf and the absorption spectra with those of their respective standard (Figure 6, 7). The purity of the bands due to lupeol or betulin in the sample extract was confirmed by overlaying the absorption spectra recorded at start, middle and end position of the band in the sample tracks. There were no interfering spots by the plant constituents at the Rf values of the marker. The absorption spectra of standard marker lupeol (Rf 0.69) or betulin (Rf 0.46) and the corresponding spot present in extract matched exactly, indicating no interference by the other plant constituents.

e) Limit of Detection and Limit of Quantification

LOD and LOQ were determined based on the standard deviation or the intercept and slope estimated from the calibration curve of the lupeol and betulin.

CONCLUSION:

Two compounds were isolated from the methanolic extract of *Ougeinia dalbergioides* by preparative TLC using toluene: chloroform: methanol (8.8:8.8:2.2) as mobile phase. These compounds were found to be triterpene by spraying with anisalehyde-H_2SO_4 as spraying reagent. Melting point, Co-TLC with standards and Spectroscopic data of UV and FTIR confirmed triterpene as betulin and lupeol. The simultaneous HPTLC quantification of lupeol and betulin in acetone and methanol extract were shows that amount of lupeol in acetone extract and methanol

extract were 9.9 µg /mg of extract and 8.3 µg /mg of extract respectively. The amount of betulin in acetone extract and methanol extract were 6.29 µg /mg of extract and 12µg /mg of extract respectively. The method is validated for the parameters like linearity, accuracy, precision, specificity, limit of detection and limit of quantification. From the data it was found that the method was linear, accurate, précised and specific.

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REFERENCE:

1) Agrawal S. S., Paridhavi M., Herbal Drug Technology, Universities Press Private Limited, Hyderabad, India, 2007, pp271-286,297.

2) Wagner H, Bladt S. Plant drug analysis. Second Edition. Heidelberg, Springer, India 1996; 86-87, pp326-327.

3) Pach K., Tracy M.V., Modern methods of plant analysis Vol iii, 1980, Springer-verlerg, Berlin, Gottingen,Heidellerg, pp450-498.

4) orgchem.colorado.edu/hndbksupport/colchrom/colchrom.html - 8k

5) Silverstein R.M., Bassler G. Clayton, Morrill Terence C., Spectrometric Identification of Organic Compounds, 5th Edn, John Wiley & Sons, INC., New York, Chichester, Brisbane, Toronto, Singapore, 1991, pp 93-123.

6) Sethi P. D., HPTLC – High Perfomance Thin Layer Chromatography, 1st Edn, CBS Publishers, New Delhi, 1996, pp 1-68

7) Bailey L. C., Chromatography in the Science and Practice of Pharmacy (Remington), 14th Edn, Mark Publishing Company, Pennsylvania, Vol-I, 1995, pp 534-538.

8) Jaenchen D. E., Thin Layer (Planer) Chromatography in Handbook of Instrumental techniques for Analytical Chemistry , 1st Edn, 1997, pp 221-235.

9) Ranger B., "Quantitative Planer Chromatography as a tool in Pharmaceutical Analysis", Journal of AOAC, 76 (20), 1993, pp 7-13.

10) Jain P.S., Bari S.B., "Isolation of lupeol, stigmasterol, and campesterol from petroleum ether extract of woody stem of *Wrightia tintoria*", Asian Journal of plant science,9(3), 2010, pp 163-167.

11) Md. Enamal Haqe, Hussain Uddin Shekhar,AkimUddin Mohamad, Hafizur Rahman, AKM Mydul Islam, M. Sabir Hussain, "Triterpenoids fromstem bark of *Avicennia officinalis*". Journal Pharmaceutical Science, 5(1-2), 2006, pp 53- 57

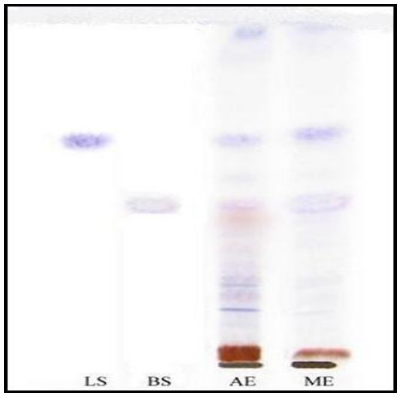
FIGURES:

LS-Lupeol standard

Figure.1: Co-TLC of OD-1 and lupeol standard after spraying with anisaldehyde- H_2SO_4

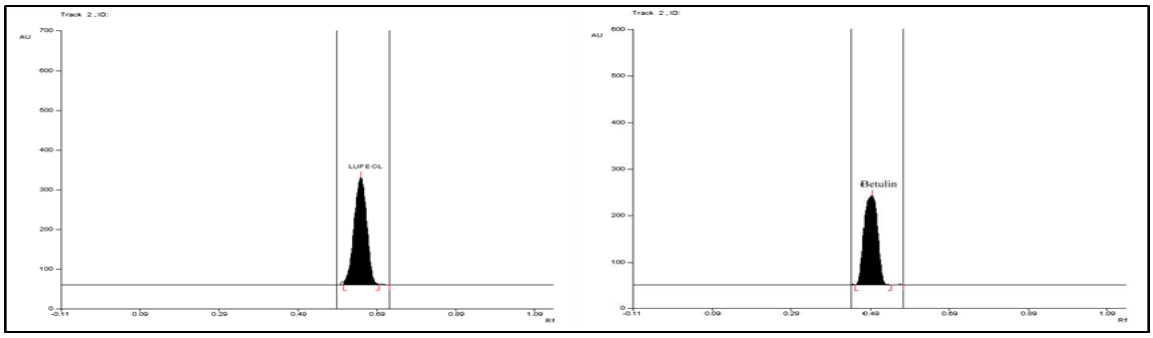
BS: Betulin standard

Figure 2 : Co-TLC of OD-1 and betulin standard after spraying with anisaldehyde- H_2SO_4



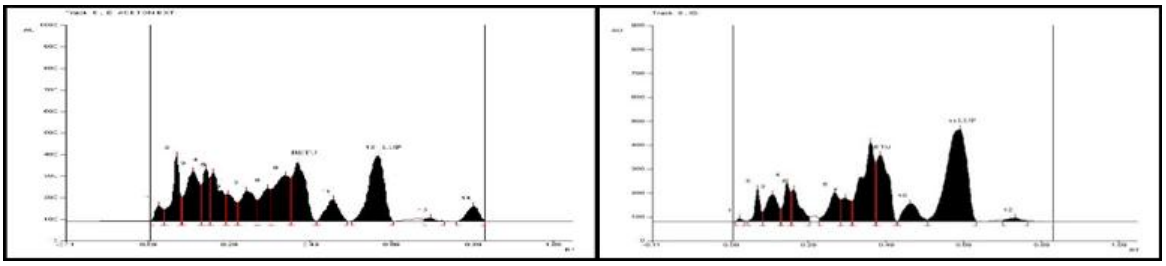
LS: Lupeol Standard, BS: Betulin Standard, AE: Acetone Extract, ME: Methanol Extract

Figure. 3: HPTLC chromatograph of standard lupeol, standard betulin, acetone extract and methanol extract after spraying spraying with anisalehyde-H_2SO_4.



(a) (b)

Figure. 4: HPTLC chromatogram of (a) standard lupeol (b) standard betulin



(a) **(b)**

Figure. 5: HPTLC Chromatogram of (a) Acetone extract (b) Methanol extract scanned at 525 nm.

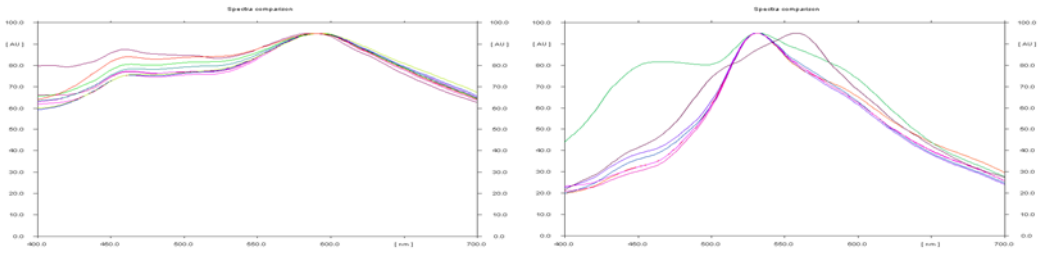


Figure6 Figure 7

Figure 6 : Overlain UV spectra of standard lupeol, acetone extract and methanol extract scanned at 525 nm.

Figure 7 : Overlain UV spectra of standard betulin, acetone extract and methanol extract scanned at 525 nm.

TABLES :

Table 1: Colour and R*F* value of isolated compounds by preparative TLC

Sr.	Fraction No.	Rf value	Compounds	Color of Spot after spraying with Anisaldehyde-H2SO4 reagent
1	01-Jul	-	-	-
2	Oct-21	0.71	OD-1	Violet
4	22-36	0.63 0.71	OD-2 OD-1	Light blue Violet
5	37-48	0.55 0.63	OD-3 OD-2	Dark blue Light blue
6	49-65	0.49 0.55 0.63	OD-4 OD-3 OD-2	Purple Dark blue Light blue
7	66-80	0.3 0.49	OD-5 OD-4	Blue Purple
8	80-100	-	-	-

Table-2 Intermediate precision studies for lupeol (Acetone extract):

Concentration (µg/spot)	Concentrat **Mean[a]**	Intrad **%RSD**	Concentrat **Mean[a]**	Interd %RSD
0.5	0.504	0.440	0.501	**0.17**
1.0	0.997	0.379	0.995	**0.24**
1.5	**1.499**	**0.570**	**1.496**	**0.68**

Mean[a] : Mean of three determination %RSD: Relative standard deviation

Table-3 Intermediate precision studies for lupeol (Methanol extract):

Concentration (µg/spot)	Concentration (µg/spot) Meana	Intraday **%RSD**	Concentration (µg/spot) **Meana**	Interday **%RSD**
0.5	0.502	0.36	0.498	0.67
1	1.007	0.568	1.005	0.357
1.5	1.502	0.69	1.499	0.185

Mean[a] : Mean of three determination %RSD: Relative standard deviation

Table-4 Intermediate precision studies for betulin (Acetone extract):

Concentration (µg/spot)	Concentr **Mean[a]**	Intraday **%RSD**	Concentr **Mean[a]**	Interda %RSD
0.5	0.507	0.500	0.502	**0.350**
1.0	1.006	0.802	1.003	**0.470**
1.5	**1.503**	**0.540**	**1.500**	**0.860**

Mean[a] : Mean of three determination %RSD: Relative standard deviation

Table-5 Intermediate precision studies for Betulin (Methanol extract):

Concentration (µg/spot)	Concentration (µg/spot) **Meana**	Intraday **%RSD**	Concentration (µg/spot) **Meana**	Inter **day** **%RSD**
0.5	0.503	0.36	0.49	0.76
1	1.008	0.8025	1.001	0.278
1.5	1.507	0.69	1.505	0.86

Mean[a] : Mean of three determination %RSD: Relative standard deviation

Table-6 Recovery studies of lupeol in acetone extract.

Amount of Sample (mg)	Amount of lupeol in sample (µg)	Amount of standard lupeol added (µg)	Total amount of lupeol taken (µg)	Total amount of lupeol found (µg)	% **Recovery**
5	25.6	2	27.6	27.3	98.91
10	33	4	37	36.8	99.46
15	41.5	6	47.5	47.52	100.04

Table -7 Recovery studies of lupeol in methanol extract.

Amount of sample (mg)	Amount of lupeol in sample (µg)	Amount of standard lupeol added (µg)	Total amount of lupeol taken (µg)	Total amount of lupeol found (µg)	% **Recovery**
20	34.6	2	36.6	36.7	100.27
25	49.3	4	53.3	53.1	99.62
30	58.1	6	64.1	63.7	99.38

Table-8 Recovery studies of 120 betulin in acetone extract.

Amount of sample (mg)	Amount of betulin in sample (µg)	Amount of standard betulin added (µg)	Total amount betulin taken (µg)	Total amount of betulin found (µg)	% Recovery
12	18.3	2	20.3	20.4	100.5
14	22.6	4	26.6	26.2	98.5
16	26.4	6	32.4	32.1	99

Table-9 Recovery studies of betulin in methanol extract.

Amount of sample (mg)	Amount of betulin in sample (µg)	Amount of standard betulin added (µg)	Total amount of betulin taken (µg)	Total amount of betulin found (µg)	% Recovery
22	27.4	2	29.4	29.1	98.98
24	32.8	4	36.8	36.6	99.46
26	36.5	6	42.5	42.4	99.76

Table 10: Summary of the method validation parameters for the simultaneous quantification of lupeol and betulin by the proposed method.

Sr.no	Parameters	Lupeol	Betulin
1	Wavelength	525	525
2	Linearity range	0.15-0.75	0.15-0.75
3	Regression	y=7619x+135	y=5411x+175
4	Correcorrelation Coefficient	R^2 = 0.998	R^2 = 0.996
5	Limit of	0.004	0.003
6	Limit of	0.012	0.009
7	Specificity	Specific	Specific