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COLUMN CHROMATOGRAPHIC ANALYSIS OF CRUDE EXTRACTS OF *M.SPICATA*

D. G. Karpe **K. R. Kamble** **M. D. Suryawanshi**

Post Graduate Department of Chemistry,

Shri Chhatrapati Shivaji College, Shrigonda, Dist-Ahmednagar, (MS)

ABSTRACT:

M. spicata plant material was collected and authenticated. Dry powdered plant material was extracted in organic solvents. Solvents were evaporated under reduced pressure. Crude extracts were screened for isolation of pure compounds. Column chromatographic analysis of crude extracts of aerial parts of *M.spicata*yielded pure compounds. Fractions obtained in column chromatography of ethyl acetate extract were further purified by HPTLC and pure compounds were characterized by spectroscopic methods.

Keywords: M. spicata, Extraction, Authentication

INTRODUCTION:

M. spicata is candy corn plant belong to family Caesalpinaceae. It is robust climber found in western ghat of India.It occurs abundantly in Maharashtra state, especially in Konkan region and on the Ghats near Mahabaleshwar[4].It is also found in Dajipur forest area of Tehsil Radhanagari, Dist-Kolhapur (LatitudeDMS: 16° 21’ 15.19” and Longitude DMS: 73° 54’ 44.88”). It occurs in Karnataka and Kerala hills up to altitude of 900 M and at Mount Abu in Rajasthan[5].The flowering season isusually in November and December.*M. spicata* is the medicinally important plant. The parts of the plants are useful in the treatment of various diseases. Roots of the plant are used to cure pneumonia and in treatment of pulmonary tuberculosis[5-8]. Crude ethanol extract of this plant shows hypotensive activity and its effects on respiration and

found to be active against respiratory and cardiovascular diseases[9].*M. spicata*possesses antiseptic properties[10].It also heals diabetic wounds[11].The bark is having applications in skin diseases. This plant also used to cure diorrhaea[11]. Literature survey reveals that the chemical constituents of aerial part of *M. spicata* are not yet reported. Only few compounds are reported but isolation methods of any compound was not reported yet. It was, therefore, decided to purify the crude extracts and identify its chemical constituents and their bioactivities.

MATERIAL & METHOD:

Collection of plant material: M. spicata plant material was collected from Radhanagari, Kolhapur. Plant material was shed dried, authenticate at Agharakar Research Institute, Pune. Dried plant material was pulverized.

Preparation of extract: Dry powdered plant material (400g) of *M. spicata* was extracted in ethyl acetate by soxhlet under optimal conditions.

Purification of crude *M. spicata* aerial part ethyl acetate extracts (MSEA): Crude ethyl acetate extract of *M. spicata* was purified by column chromatography.

Column packing: Crude ethyl acetate extract (4 g) was dissolved in ethyl acetate (20 ml) and activated silica gel (60 x 120 mesh, 4 g) was added to it. Solvent was carefully evaporated on rotary evaporator. Thus, the crude ethyl acetate extract was adsorbed on silica gel. The adsorbed dry powder was loaded on column (2.8 x 60 cm) of dry silica gel (60 x 120 mesh, 120 g). The column was eluted as shown in following table.

Table1: Details of the MSEA column elution

Sr. No	Elution	Volume fraction collected (ml)	Weight of the fraction(g)	Inference by TLC
1	Hexane (100%)	20 x100 ml	0.40	No spot
2	Hexane : ethyl acetate (9:1)	10 x 100 ml	0.10	Colored impurity
3	Hexane : ethyl acetate (8:2)	12 x100 ml	0.22	Colored impurity
4	Hexane : ethyl acetate (7:3)	16 x 100 ml	0.12	Mixture
5	Hexane : ethyl acetate (6:4)	12 x 100 ml	0.10	Mixture
6	Hexane : ethyl acetate (5:5)	14 x 100 ml	0.30	Complex Mixture
7	Hexane : ethyl acetate (4:6)	16 x 100 ml	0.20	Mixture
8	Hexane : ethyl acetate (3:7)	14 x 100 ml	0.42	Pure + small impurity
9	Hexane : ethyl acetate (2:8)	12 x 100 ml	0.15	Impurity
10	Hexane : ethyl acetate (1:9)	10 x 100 ml	0.15	Mixture
11	Ethyl acetate (100%)	5 x 100 ml	0.15	Mixture
12	Methanol : Ethyl acetate (1:9)	2 x 100 ml	0.20	Complex mixture
13	Methanol : Ethyl acetate (4:6)	2 x 100 ml	0.20	Complex mixture
14	Methanol : Ethyl acetate (6:4)	2 x 100 ml	0.30	No spot
15	Methanol : Ethyl acetate (8:2)	2 x 100 ml	0.25	No spot
16	Methanol (100%)	2 x 100 ml	0.40	Impurity
Total recovery: 3.66 g (91.5%)				

TLC's of all the fractions were recorded on precoated plates in 20% ethyl acetate- hexane. The plates were developed to visualize the spots using anisaldehyde- sulphuric acid reagent and fractions having same R_f were combined.In similar manner the column of ethyl acetate extract was repeated five times to get more quantity of fractions, so as to get the sufficient amount of pure compounds.All column fractions are monitored by TLC analysis in ethyl

acetate in hexane (8:2). TLC plates are developed under iodine/UV/ anisaldehydesulphuric acid reagent. The fractions eluted (Sr.no.8 of table 1) with 70% ethyl acetate: hexane system showed a major spot with some impurities. The fractions eluted in the same eluent from five columns were pulled together (2.0g)was dissolved in ethyl acetate (2 ml) and activated silica gel (60 x 120 mesh, 2.0 g) was added to it. Solvent was carefully evaporated on rotary evaporator. Thus, the fraction was adsorbed on silica gel and dry powder obtained was loaded on column (3.5 x 50 cm) of silica gel (60 x 120 mesh, 60 g). The column was eluted as shown in Table 2.

Table 2: Details of the column of combined fractions eluted in 70% ethyl acetate in hexane

Sr. No	Elution	Volume of fractions collected (ml)	Weight of the fraction (g)	Inference by TLC
1	Hexane (100%)	4 x 100ml	0.05	Colored Impurity
2	Ethyl acetate : hexane(0.5:9.5)	4 x 100 ml	0.05	Colored Impurity
3	Ethyl acetate : hexane(1:9)	4 x 100 ml	0.05	Colored Impurity
4	Ethyl acetate : hexane(1.5:8.5)	4 x 100 ml	0.12	Complex mixture
5	Ethyl acetate : hexane(2:8)	4 x 100 ml	0.10	Mixture
6	Ethyl acetate : hexane(2.5:7.5)	4 x100 ml	0.05	Mixture
7	Ethyl acetate : hexane(3:7)	2 x 100 ml	0.12	Complex mixture
8	Ethyl acetate : hexane(4:6)	2 x100 ml	0.12	Complex mixture
9	Ethyl acetate : hexane(5:5)	2 x100 ml	0.12	Mixture
10	Ethyl acetate : hexane(6:4)	2 x100 ml	0.10	Pure + small Impurity
11	Ethyl acetate : hexane(7:3)	6 x100 ml	0.45	Pure + small Impurity
12	Ethyl acetate (100%)	2 x100 ml	0.52	Complex mixture
13	Methanol (100%)	2 x100 ml	0.05	Mixture
Total recovery : 1.78 g (89 %)				

The initial fractions eluted (Sr. No. 1-9 of table no. 2) were combined which were showing coloring matter, no any separation on TLC was achieved. The fractions eluted in 60% and 70% ethyl acetate in hexane fractions(Sr. NO. 10 and 11 of table no. 2)were showed single spot along with minor impurity. The combined fraction (0.55g) was further purified by PTLC. The purity was checked by TLC and HPLC showed good purity. Pure compound was labeled as MSEA-1 (0.115g).MSEA-1 was identified as Lupeol by studying its spectral data and comparing it with authentic compound. The authentic compound was procured from Sigma Aldrich.

Characterization of MSEA-1

It is white crystalline solid having M.P 215^{0}C. Literature M.P: 215^{0}C[16], Mixed M.P.:215^{0}C

Experimental: $[\alpha]_D$ = +27.8 Literature: $[\alpha]_D$ = +27.2

Physical data

Molecular Formula: $C_{30}H_{50}O$ **Molecular Weight:** 426

Elemental analysis

Found: C84.20%, H 11.80%, O 3.80% **Required:** C84.50%, H 11.70%, O 3.75%

Spectral data

IR :3305(broad OH), 1658(C=C), 817cm^{-1}(C=C)

^{1}H NMR spectrum (DMSO D6, δ, ppm)

δ 0.66 (3H, s,C_{27} H), 0.78 (3H, s, C_{24}H), 0.88 (3H,s, C_{26}H), 0.92 (3H, s,C_{25}H) , 0.99 (3H,s, C_{28}H), 1.15(3H,s, C_{30}H), 1.18(3H, s,C_{23}H), 1.20-1.69 (21H, m, C_2H, C_6H, C_7H, C_9H, C_{11}H, C_{12}H, C_{13}H, C_{15}H, C_{16}H, C_{18}H, C_{21}H, C_{22}H), 2.33-2.57 (1H, m, C_{19}), 2.95-3.0 (1H, m, C3H) , 4.55 (1H, d, J=17.01Hz, C_{29}Ha), 4.69 (1H, d, J=17.01Hz, $C_{29}H_b$).

^{13}CNMR spectrum (DMSO D6, δ, ppm)

Table 3: ^{13}C NMR and DEPT assignments

Sr. No	δ (ppm)	DEPT	Assignment
1	14.32	CH_3	C_{27}
2	15.69	CH_3	C_{24}
3	15.78	CH_3	C_{26}
4	15.91	CH_3	C_{25}
5	17.76	CH_3	C_{28}
6	17.95	CH_2	C_6
7	18.95	CH_3	C_{30}
8	20.41	CH_2	C_{11}
9	24.68	CH_2	C_{12}
10	26.99	$CH_2$and CH_2	C_2 and C_{15}
11	27.15	CH_3	C_{29}
12	28.09	CH_2	C_{20}
13	29.19	CH_2	C_7
14	33.83	CH_2	C_{16}
15	35.06	Quaternary	C_{10}
16	36.67	CH	C_{13}
17	37.56	CH_2	C_1
18	38.26	Quaternary	C_4
19	38.49	CH_2	C_{21}
20	40.32	Quaternary	C_8
21	42.35	Quaternary	C_{14}
22	42.53	Quaternary	C_{17}
23	47.38	CH	C_{19}
24	47.77	CH	C_{18}
25	49.83	CH	C_9
26	54.85	CH	C_5
27	76.78	CH	C_3
28	109.65	CH_2	C_{23}
29	150.19	Quaternary	C_{22}

RESULTS & DISCUSSION:

It was obtained as white crystalline solid with M.P. 215°C. Its elemental analysis indicated the molecular formula $C_{30}H_{50}O$ suggested being a triterpenoidal nature. Its IR spectrum showed the presence of a broad band at 3367 cm^{-1} due to the hydroxyl group. The band at 1660 cm^{-1} confirms the

presence of olefinic groups in the molecule. Sharp band at 850cm^{-1} indicated the presence of a terminal double bond.^{1}H NMR spectrum showed triterpenoidal skeleton. It showed the presence of seven tertiary methyl groups at δ 0.66, 0.78, 0.88, 0.92, 0.99, 1.15 and 1.18. The proton on the carbon bearing hydroxyl group appeared as a multiplet at δ 2.95 – 3.0 while the olefinic protons on the terminal methylene appeared at δ4.55 and 4.69. Based on physical and spectral data generated the tentative structure of MSEA-1 assigned as Lupeol.The ^{13}C NMR spectrum showed the presence of thirty carbons. The DEPT spectrum showed the presence of seven methyl, ten methylene, six methine and seven quaternary carbons in the molecule. The ^{13}C NMR spectrum and DEPT spectrum supports the tentative structure assignment.The physical data and spectral data of MSEA-1 were compared with the physical and spectral data of authentic lupeol and were found to be in complete agreement. The mixed melting point with authentic lupeol was 215°C, which was identical with that of MSEA-1. The physical and spectral data was compared with literature, it was found to be identical[4-6]. Thus MSEA-1 was identified as Lupeol.

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