International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Purification of Crude Ethyl Acetate Extract of *M. Spicata* Leads to Isolation of Pure Compounds by Different Chrmatographic Methods

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Abstract: ***Moullava spicata is a candy corn plant belongs to family Caesalpinacae; it is also called as Wagatea spicata. Crude extracts of this plant shows various prominent biological activities. Reports on presence of various phytoconstituents from M. spicata are available. In present investigation, fresh plant material was collected, shade dried, pulverized and extracted in different solvents with their increasing polarity. Extraction is done in Soxhlet apparatus, solvents are removed under reduced pressure. Dried extracts were screened for TLC and HPLC analysis. Repeated Column chromatography, Preparative Thin Layer Chromatography and Recrystallization techniques are used to isolate pure compound from crude extracts. Characterization of isolated compound was done by spectroscopic methods and compared with authentic sample, it was identified as Quercetin.***

Keywords: Moullava spicata, Isolation, Chromatography, Quercetin, Preparative TLC

1. Introduction

Plants are the main sources of food, shelter and clothing, also considered to be the first living entity on the earth. Plants are a great source of medicines even for life - threatening diseases. Environment and climate are completely interlinked with plants. Temperature, humidity and rainfall are influenced by the presence of plants. Plants belonging to the family Caesalpinacae contain various classes of compounds such as tannins, alkaloids, terpenoids, flavonoids, saponins. Biological importance of plants is due to the presence of various classes of compounds[1, 2]. *Moullava spicata* is also called as *Wagatea spicata,* it belongs to family Caesalpinacae and it shows various biological properties. In most of the cases the biological activities of plant extracts are the result of the presence of chemical constituents in it, therefore, thought adequate to understand the chemical composition of aerial part extract of *M. spicata* responsible for biological activities. Lakshmi *et al*[1] have isolated 'Vakerin' **(I)** from roots and characterized it by physical, spectral and chemical methods. Other compounds identified as epifriedelin, friedelin, lupeol, taraxerol, β - sitosterol, lignoceryl alcohol, mellisic acid, β - sitosterol - β - D - glucoside and quercetin[1, 5, 6]. Daulatabad *et al*[2] reported 9 - keto - octadec - cis - 12 - enoic acid in *Wagatea spicata* seed oil. Crude extracts showing promising antimicrobial activity[4]. Joshi *et al*[3]isolated Vakerin and its pentacetyl derivative. Identification of some biologically active constituents are reported by HPLC and GC analysis[5, 6]. Lupeol was isolated from crude extracts of M. spicata by chromatographic methods[7]. Gallic acid was reported from crude extracts of M. spicata[8]. Literature survey reveals that the chemical constituents of aerial part of *M. spicata* are not yet reported. Only few compounds are reported and isolation methods of few compounds were reported. It was, therefore, decided to purify the crude extracts and identify its chemical constituents.

2. Materials and Methods

Collection of plant

Plant material was collected from Radhanagari, District – Kolhapur, Maharashtra and authenticated at Plant Science Divison, Agharkar Research Institute, Pune (MS).

Preparation of extract

Dry powdered plant material (200 g) of *M. spicata* was defatted with hexane and then extracted in ethyl alcohol by using soxhlet extractor for 10 hours. Crude ethanol extract (23.5 g) was obtained by evaporation of solvent under reduced pressure. Dried ethanol extract was fractionated using hexane and ethyl acetate. For this, dried ethanol extract was taken in a R. B. flask and hexane (200 ml) was added. The mixture was stirred at 60^0C for 2 hours and after cooling, it was filtered through whatmann filter paper, solid mass was dried and again taken into R. B. flask and ethyl acetate (200 ml) was added into it. Then, it was stirred for 2 hours at 70^0C on hot plate with magnetic stirrer (Remi). The mixture was filtered through whatmann filter paper and solvent was evaporated. The same procedure was repeated thrice to yield the ethyl acetate extract (3 g). The extract obtained was subjected to column chromatography.

Column Packing

Crude ethyl acetate extract (3 g) was dissolved in ethyl acetate (3 ml) and activated silica gel (60 x 120 mesh, 3 g) was added to it. Solvent was carefully evaporated on rotary evaporator. The crude ethyl acetate extract was adsorbed on silica gel. The adsorbed dry powder was loaded on column (2.1 x 46 cm) of dry silica gel (60 x 120 mesh, 90 g). The column was eluted as shown in Table 1.1

Table 1.1: Details of the column elution of MSEA

Sr. No	Elution	Volume of fraction collected (ml)	Weight of the fraction (g)	Inference by TLC
1	Pet. ether (100%)	20 x 100 ml	0.20	Complex Mixture
2	Pet. ether: ethyl acetate (9: 1)	12 x 100 ml	0.30	Complex Mixture
3	Pet. ether: ethyl acetate (8: 2)	14 x 100 ml	0.20	Colored Impurity
4	Pet. ether: ethyl acetate (7: 3)	10 x 100 ml	0.10	Mixture
5	Pet. ether: ethyl acetate (6: 4)	20 x 100 ml	0.12	Mixture
6	Pet. ether: ethyl acetate (5: 5)	20 x 100 ml	0.16	No any spot
7	Pet. ether: ethyl acetate (4: 6)	20 x 100 ml	0.12	No any spot
8	Pet. ether: ethyl acetate (3: 7)	20 x 100 ml	0.18	Mixture
9	Pet. ether: ethyl acetate (2: 8)	25 x 100 ml	0.60	Pure + Small Impurity
10	Pet. ether: ethyl acetate (1: 9)	20 x 100 ml	0.40	One major spot + small impurity
11	Ethyl acetate (100%)	5 x 100 ml	0.30	Mixture
12	Methanol (100%)	4 x 100 ml	0.20	Mixture
Total recovery: 2.88 g (96%)				

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. Initial fractions Sr. No 1 to 8 of table no.1.1 are showing complex mixture but the fractions eluted in 80% ethyl acetate in petroleum ether showed a major spot along with small impurities. The combined fractions of three columns (1.5g) were recrystallized in hot alcohol to get the pure compound (0.45 g) labeled as MSEA - I.

3. Results

To study the chemical constituents of fresh aerial part of *M. spicata* was extracted by using various solvents. Examination of crude extract by TLC indicated to be a complex mixture. It was decided to purify the crude extract by using different chromatographic methods. The quantity of the total crude extract was more, initially the part of the crude extract was purified by simple column chromatography on large scale using column grade silica gel as stationary phase (60 x 120 mesh), Crude extract was adsorbed on silica gel (60 x 120 mesh) and it was loaded on a dry column. The columns were eluted with solvents of their increasing polarities. The quantity of the fractions was not sufficient for subjecting them to another column. Therefore, it was obtained by repeated column chromatography. The identical fractions were pulled together. The enriched fractions were examined and attempts were made to understand the nature of major compounds in them. Based on this information and the quantity of fractions available, further methods of purification were employed. Methods like conventional column chromatography, Preparative TLC were used for the purifications of extract/fractions. The chromatographic procedures were repeated till pure compounds were obtained. The details of chromatographic purification of ethyl acetate extract yielded pure compound which is characterized by spectral methods.

4. Discussion

It was obtained as yellow solid with melting point 316°C. Elemental analysis indicated molecular formula is $C_{15}H_{10}O_7$, it suggests that it is to be an aromatic compound. It is abbreviated as MSEA - I. Its IR spectrum showed presence of broad band between 3371 cm^{-1} due to the phenolic groups. A band at 1664cm^{-1} was due to the carbonyl group conjugated with a phenyl ring. Presence of medium band at 1640 cm^{-1} confirmed the presence of double bond. Bands at 1560 and 1460cm^{-1} are due to aromatic ring. The ^{1}H NMR spectrum showed presence of aromatic protons. Doublets at δ 6.18, 6.40, 6.88, 7.67 and doublet of doublet at δ 7.53 were due to the aromatic protons. On the basis of physical data, IR, ^{1}H NMR spectral data and comparison with literature, the tentative structure of MSEA - I was assigned as Quercetin.

Characterization of MSEA - I

It is yellow crystalline solid having M. P.316^{0}C. Literature M. P: 314°C[11, 12], Mixed M. P.315^{0}C

Ferric Chloride Test: 2 mg MSEA - I was mixed with a dilute aqueous solution of ferric chloride (0.1 ml). Intense violet coloration was observed.

Physical data

Molecular Formula: $C_{15}H_{10}O_7$**, Molecular Weight:** 302**,**

Elemental analysis:

Found: C 59.66%, H 3.36%, O 36.95%, **Required: C** 59.60%, H 3.31%, O 37.00%

Spectral data:

IR Spectrum: Bands at 3371 (br. strong OH) 1664 (C=O), 1640 (C=C) 1560, 1460, 1120, 998, 710 cm^{-1} (aromatic).

^{1}H NMR spectrum (DMSO D6; δ, ppm)

6.18 (1H, d, J=2 Hz,), 6.4 (1H, d, J=2 Hz,), 6.88 (1H, d, J= 8.48Hz,), 7.53 (1H, dd, J=8.48 and 2.2Hz,), 7.67 (1H, d, J=2.1 Hz,)

^{13}C NMR spectrum (DMSO - D_6):

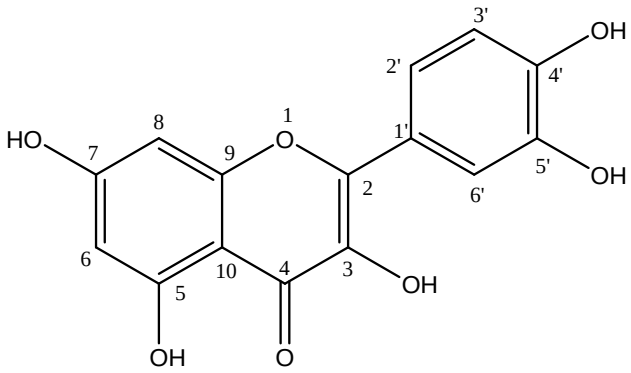
Table 1.2: ^{13}C NMR and DEPT assignments

Sr No	δ (ppm)	DEPT	Assignment
1	93.40	CH	C_8
2	98.24	CH	C_6
3	103.03	Quaternary	C_{10}
4	115.10	CH	$C_{5'}$
5	115.64	Quaternary	$C_{4'}$
6	120.02	CH	$C_{2'}$
7	122.00	Quaternary	$C_{1'}$
8	135.76	Quaternary	C_3
9	145.10	Quaternary	$C_{3'}$
10	146.83	CH	$C_{6'}$
11	147.74	Quaternary	C_2
12	156.18	Quaternary	C_9
13	160.75	Quaternary	C_5
14	163.99	Quaternary	C_7
15	175.87	Quaternary	C_4

The ^{13}C NMR spectrum showed the presence of fifteen carbons. The DEPT spectrum showed the presence of five methine and ten quaternary carbons.1^{3}C NMR spectrum and DEPT spectrum supports the tentative structure assignment.

5. Conclusion

It was identified as quercetin by studying its spectral data and comparing it with authentic sample. The authentic sample was obtained from Botany group, Agharkar Research Institute, Pune. The physical data and spectral data of MSEA - I was compared with authentic quercetin sample and found to be identical. Mixed M. P was 316°C, which is same as that of MSEA - I**.** The physical and spectral data was compared with literature, it was found to be identical. Thus **MSEA - I** was identified as **Quercetin.**



Quercetin

Acknowledgement:
I take this opportunity to express my deep sense of gratitude to my research guide and mentor Prof. (Dr.) S. P. Lawande, I sincerely thank Dr. D. G. Naik, Scientist - G (Retired) Agharkar Research Institute Pune, Project Coordinator, Maharashtra Education Society, Pune, for his guidance and fruitful suggestions in my work. I take this opportunity to thank Dr. R. J. Waghole, ARI, Pune for the suggestions and timely help in analytical aspects. I am thankful to Dr. M. N. Datar, Scientsist, ARI, for their help in plant identification and collection.

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