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ISOLATION, CHARACTERIZATION OF PHYTOCONSTITUENTS FROM CRUDE EXTRACT OF M. SPICATA

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ABSTRACT:

M. spicata is candy corn plant belong to family Caesalpinaceae. Extraction of shed dried plant material in various solvents with their increasing polarity was carried out. Extraction of plant material in ethyl alcohol gave better yield. Ethyl alcohol extract was further fractionated in hexane, ethyl acetate and methanol. Thin layer chromatographic analysis showed complex mixture. Purification of ethyl acetate extract on column chromatography yielded green coloured mixture. PTLC of this mixture was done and white solid was obtained in pure form, it was characterized by physical and spectral methods as gallic acid.

Keywords: M. spicata, Gallic acid, PTLC, crude extracts

INTRODUCTION:

M. spicata shows wide range of biological activities[1]. Crude extracts showing promising antimicrobial activity. 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* have isolated 'Vakerin' from roots and characterized it by physical, spectral and chemical methods[2]. Other compounds identified as epifriedelin, friedelin, lupeol, taraxerol, ß-sitosterol, lignoceryl alcohol, mellisic acid, ß-sitosterol-ß-D-glucoside and quercetin[2]. However the detailed isolation procedures and characterization of these compounds was not yet reported from this plant. Daulatabad *et al* reported 9-keto-octadec-cis-12-enoic acid in *Wagatea spicata* seed oil[3]. Identification was done on the basis of spectrometry and chemical

degradation[3].GC MS analysis of crude extracts was reported various phytochemicals[8].

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.

MATERIAL AND METHOD:

Collection, Authentication and extraction of plant material:

M. spicata plant material was collected from Radhanagari, Kolhapur. Collected plant material was shed dried for a week. Authenticated at Agharkar Research Institute, Pune. To study the chemical constituents of fresh aerial part of *M. spicata* was extracted by using various solvents. Examination of crude extracts by TLC indicated to be a complex mixture. It was decided to purify the crude extract by using different chromatographic methods.

Purification of crude *M. spicata* aerial part ethyl acetate extracts:

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

Table-1: Details of the column elution of Ethyl acetate extract

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	Complex mixture
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 are showing complex mixture but the fractions eluted in 80% ethyl acetate in petroleum ether showed a major spot along with small impurities.

Purification of fractions eluted with 90% ethyl acetate in petroleum ether:

Fractions eluted with 90% ethyl acetate in petroleum ether Sr. No. 10 of Table-1 showed major green colored spot along with impurities on TLC. Fractions from three columns were combined (1.2 g) and purified by using PTLC to get pure compound (0.4 g). It was identified as Gallic acid by studying its spectral data and comparing it with authentic sample. The authentic sample was obtained from Botany Group, Agharkar Research Institute, Pune.

RESULTS AND DISCUSSION:

Characterization:

It is white yellow crystalline solid having M.P. 260^{0}C (decomposed).

Literature M.P: 258-265^{0}C (dec.)[18], Mixed M. P. 260^{0}C

Physical data:

Molecular formula: $C_7H_6O_5$

Molecular weight: 170

Elemental composition

Found: C49.32%, H 3.58%, O 47.00%

Required: C 49.40%, H 3.50%, O 47.00%

Spectral data:

IR spectrum:

3500 (strong, acidic OH), 1649 (C=C), 1545, 1432 cm^{-1} (aromatic)

^{1}H NMR Spectrum (DMSO D6; δ, ppm):

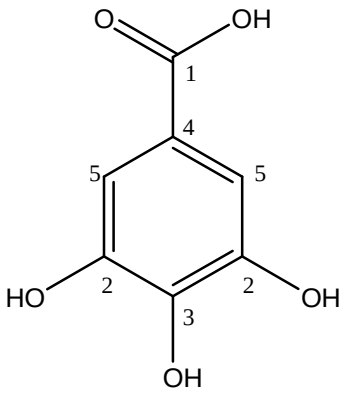
6.9 (2H, s, C_5H)

^{13}C NMR (DMSO):

Table-2: ^{13}C NMR and DEPT assignments

Sr. No	δ (ppm)	DEPT	Assignment
1	CH and CH	109.17	C_5
2	Quaternary	120.89	C_4
3	Quaternary	138.44	C_3
4	Quaternary	145.86	C_2
5	Quaternary	167.91	C_1

Comparison with literature indicated pure compound to be Gallic Acid. It was whitish solid with melting point 260˚C (decomposed). Its elemental analysis indicated molecular formula $C_7H_6O_5$ indicated the compound is aromatic. Its IR spectrum showed the broad band at 3500cm^{-1} indicating presence of free as well as hydrogen bonded hydroxyl groups. The band at 1649 cm^{-1} was due to the presence of carbonyl group. The bands at 1545 and 1432 cm^{-1} are due to aromatic region. The ^1H NMR spectrum showed peak at δ 6.9 which appeared as a singlet indicating that both aromatic protons are equivalent. Thus IR and ^1HNMR spectrum assigned tentative structure of the isolated compound as Gallic Acid. The ^{13}C NMR spectrum showed the presence of seven carbons. The DEPT spectrum showed the presence of two methine and five quaternary carbons. The^{13}C NMR spectrum and DEPT spectrum supports tentative structure assignment. The physical data and spectral data of the compound was compared with authentic Gallic acid and found to be identical. The physical and spectral data was compared with literature, it was found to be identical[9-11]. Thus It was identified as **Gallic acid.**



Gallic Acid

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