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ISOLATION OF EMODIN FROM THE LEAVES OF *AMARANTHUS SPINOSUS* L. (AMARANTHACEAE)

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ABSTRACT

A compound was isolated from the leaves of *Amaranthusspinosus* L. by solvent extraction, acid hydrolysis, chromatography followed by crystallization. Infra red spectroscopy, mass spectroscopy and nuclear magnetic resonance studies revealed that the isolated compound was chemically 1,3,5-trihydroxy-7-methylanthracene-9,10-dione, also known as emodin.

KEY WORDS:*Amaranthusspinosus* L.chromatographic techniques, emodin.

INTRODUCTION

Amaranthusspinosus L., a medicinal plant under the family of amaranthaceae, is widely distributedthroughout the tropics and warm temperate regions of Asia. The plant grows in cultivated areas as well as in waste places andis distributed in lower to middle hills (3000–5000 ft) of entire north eastern Himalayas. The plant is known as “prickly amaranthus” in English and “ban lure”or “dhutighans” in Nepali [1].Leaves of *Amaranthusspinosus*L. are stacked and alternate. [2]. Medicinal uses of *Amaranthusspinosus*L. as mentioned in Ayurvedictext[3]are: Leaf infusion is diuretic and used in anemia. Root paste is used in gonorrhea, eczema, menorrhea etc. The plant is further used as diuretic, stomachic, digestive, laxative, antipyretic etc. and in the treatment of improving appetite, biliousness, burning sensation, leprosy, bronchitis, blooddiseases, piles and leucorrhoea [4]. In modern research the plant is claimed to have anti-inflammatory and

anthelmintic property, immunomodulatory activity, effect on hematology, antihyperlipidemic, spermatogenic and anti diabetic property [5-10]. Ethnic use of this plant is seen among the village people of Sikkim who use leaf infusionof *Amaranthusspinosus* L. in stomach disorder specially in case of indigestion and peptic ulcer [1]. We also observed anti ulceractivity of the leaves of *Amaranthusspinosus* L. against ethanol and cysteamine induced peptic ulcer in albino rats [11].Considering the medicinal importance of *Amaranthusspinosus* L. phytochemical studies of the plant were extensively undertaken. Phenol, sitosterol, stigmasterol, essential oil, friedolin, and unidentified esters were found as active componentsof *Amaranthusspinosus* L. [12-13]. Recently we have isolated and characterize a compound from the leaves of *Amaranthusspinosus* L. Results are being reported in this communication.

MATERIALS AND METHODS

Plant Material

Fig. 1:*Amaranthusspinosus* L.

Leaves of *Amaranthusspinosus* L.were collected from the medicinal plants garden of the University of North Bengal and authenticated by the experts of the department of Botany of the said University. A voucher specimen was kept in the department for future reference. Leaves were shade dried and powdered. The powder was used for extraction and isolation studies.

Extraction and Isolation

First step

50g of the powder were extracted with 500 ml methanol for 15 min at 40^0Cusing a soxhlet apparatus.

Second step

The extract was concentrated to 10 ml under reduced pressure using a rotary evaporator.

Third step

This was then subjected to column chromatographyusing alumina as adsorbent. Six bands were separated. Elution was done by 50% methanol-chloroform mixture.

Fourth step

Eluted second band was evaporated to dryness.Dry brown mass was obtained. It was extracted with 10 ml ethyl acetate for 10 min on a rotary shaker.

Fifth step

The ethyl acetate extract was further subjected to column chromatography using silica gel mesh (200-400 size) as adsorbent. Seven fractions were separated. Elusion was done by ethyl format: formic acid mixture (100:5, v/v)

Sixth step

Eluted thirdfractionwas evaporated to drynessunder reduced pressure using a rotary evaporator. Dry brown mass was obtained.

Seventh step

Repeated crystallization was done using ethyl acetate–formic acid (50:50, v/v) mixture from the brown mass. A compound was crystallized. Yield was 4.8 mg.

Homogeneity of the active compound

This was ascertained by silica gel- G thin layer chromatography by using the following solvent systems; Methanol : water - 80 : 20 ; Ethanol : chloroform : water - 50 : 25 : 25; Chloroform : methanol : water - 60 : 20 : 20

Structure determination

FT-IR spectrum of the sample was taken in KBr pellets using Shimadzu FT-IR 8300 Spectrophotometer. NMR spectrum was taken using Bruker AVH 300 Spectrometer

operating at 300 MHz (for ^{1}H) and 75 MHz (for ^{13}C) and in solvent, as indicated. ^{13}C NMR spectrum was run in ^{1}H-decoupled mode. The High Resolution Mass Spectral data for the compound was obtained in Mass Spectrometer (Model: Micromass Q-Tof Micro), run under Electron Spray Ionization (ESI) Positive Mode. Melting point was observed in an open sulfuric acid bath and is uncorrected.

RESULTS AND DISCUSSION

Homogeneity of the isolated compound

The isolated compound was pure as in all cases of thin layer chromatographic experiments using three different solvent systems single spot was obtained.

Structure elucidation

The isolated compound was a deep yellow crystalline solid, m. p. 249-255^{0}C.

FT-IR (KBr): ν_{max} were 3383, 3193, 1660, 1624, 1620 cm^{-1}.

The IR absorption data suggested the presence of hydroxyl group, conjugated carbonyl function and aromatic ring. The OH groups might have H-bonding also.

The ^{1}H-NMR (D_6-DMSO): δ 2.32 (s, 3H), 3.31 (br. s, 1H), 6.47 (d, 1H, J = 2.1 Hz), 6.96 (d, 1H, J = 2.1 Hz), 6.98 (d, 1H, J = 1.2 Hz), 7.28 (d, 1H, J = 1.2 Hz), 11.26 (br. s, 1H), 11.85 (br. s, 1H), 11.94 (br. s, 1H) ppm.

From ^{1}H-NMR spectral data, it appeared that there were four *meta*-coupled Ar-Hs, and one methyl group might be present as a substituent to the aromatic ring. On the other hand, the ^{13}C-NMR spectrum indicated twelve Ar-Cs and two carbonyl carbons appearing at δ 181.3, 189.8 ppm. By comparing with literature information and molecular structures for related compounds, the structure of the isolated compound was assigned as 1,3,5-trihydroxy-7-methylanthracene-9,10-dione, also known as Emodin.

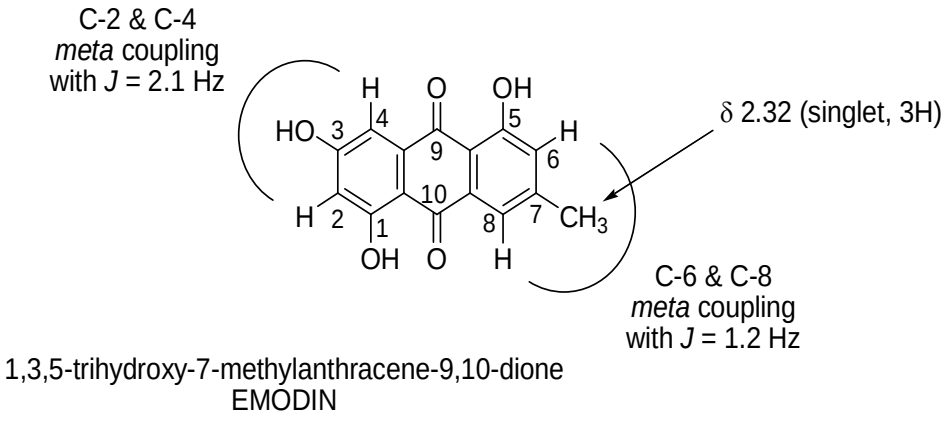

Fig. 2: Structure of the isolated compound

By comparison, the C2-H appears at 6.47 as a *meta*-coupled doublet with J = 2.1 Hz, which was coupled by C4-H appearing at 6.96 ppm (J = 2.1 Hz). Similarly, the other two Hs at C6 and C8 were appearing respectively at 6.98 and 7.28 ppm, again mutually meta-coupled doublets with J = 1.2 Hz. The aliphatic CH_3 appears as a singlet at 2.32 ppm, the shift position conformed to arylmethyl protons.

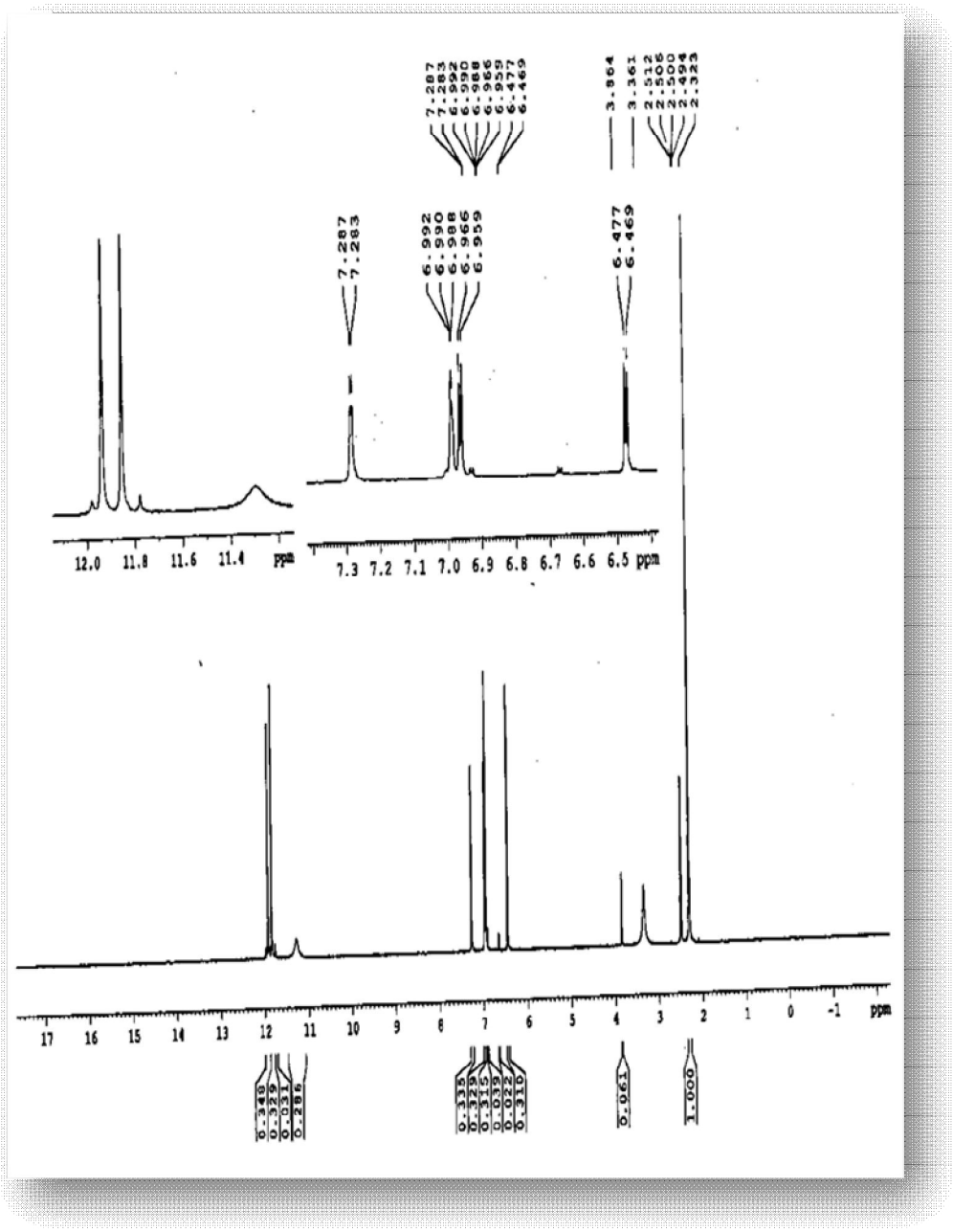

Fig. 3: The ^{1}H-NMR spectrum of the isolated compound

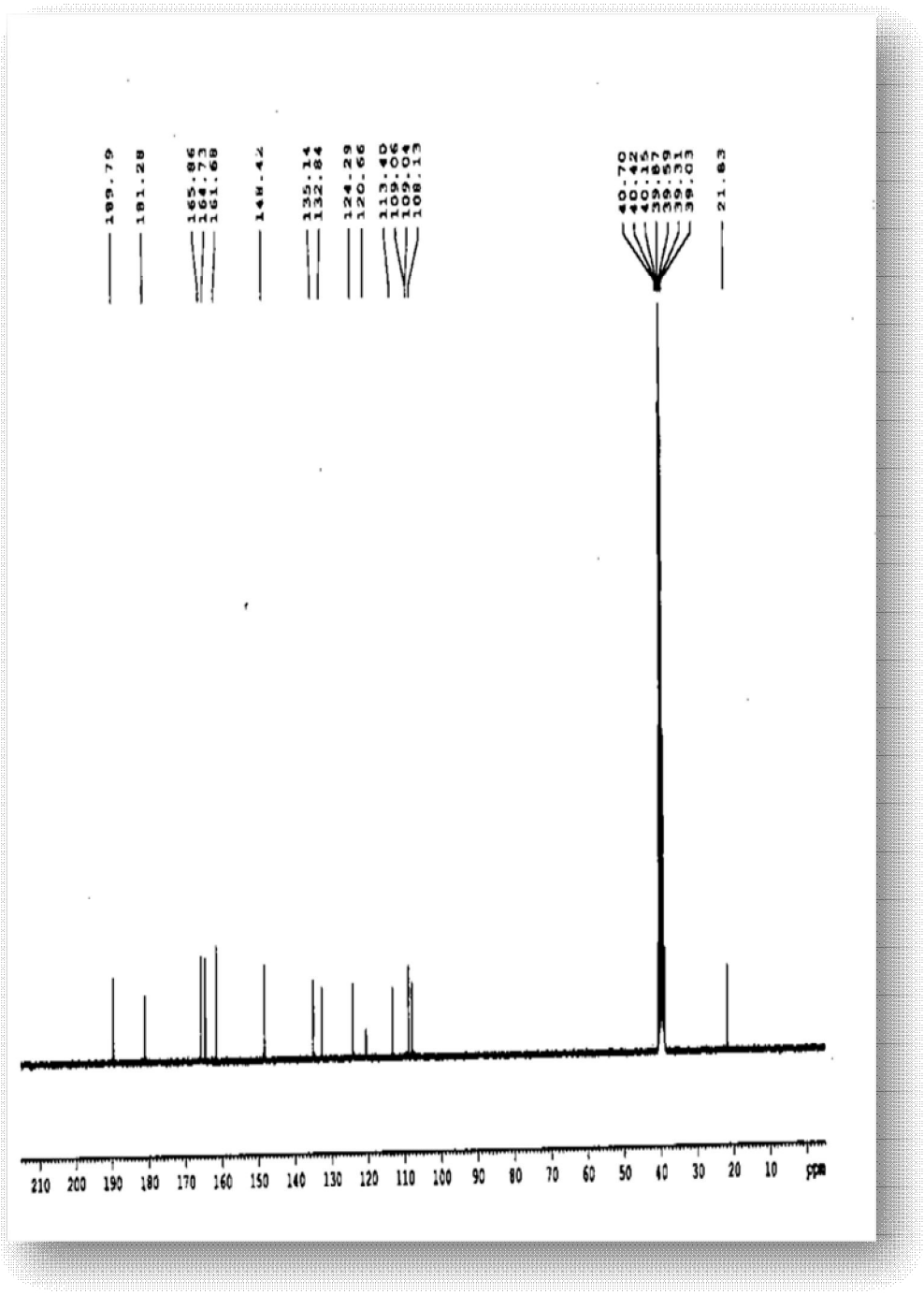

Fig. 4: The ^{13}C-NMR spectrum of the isolated compound

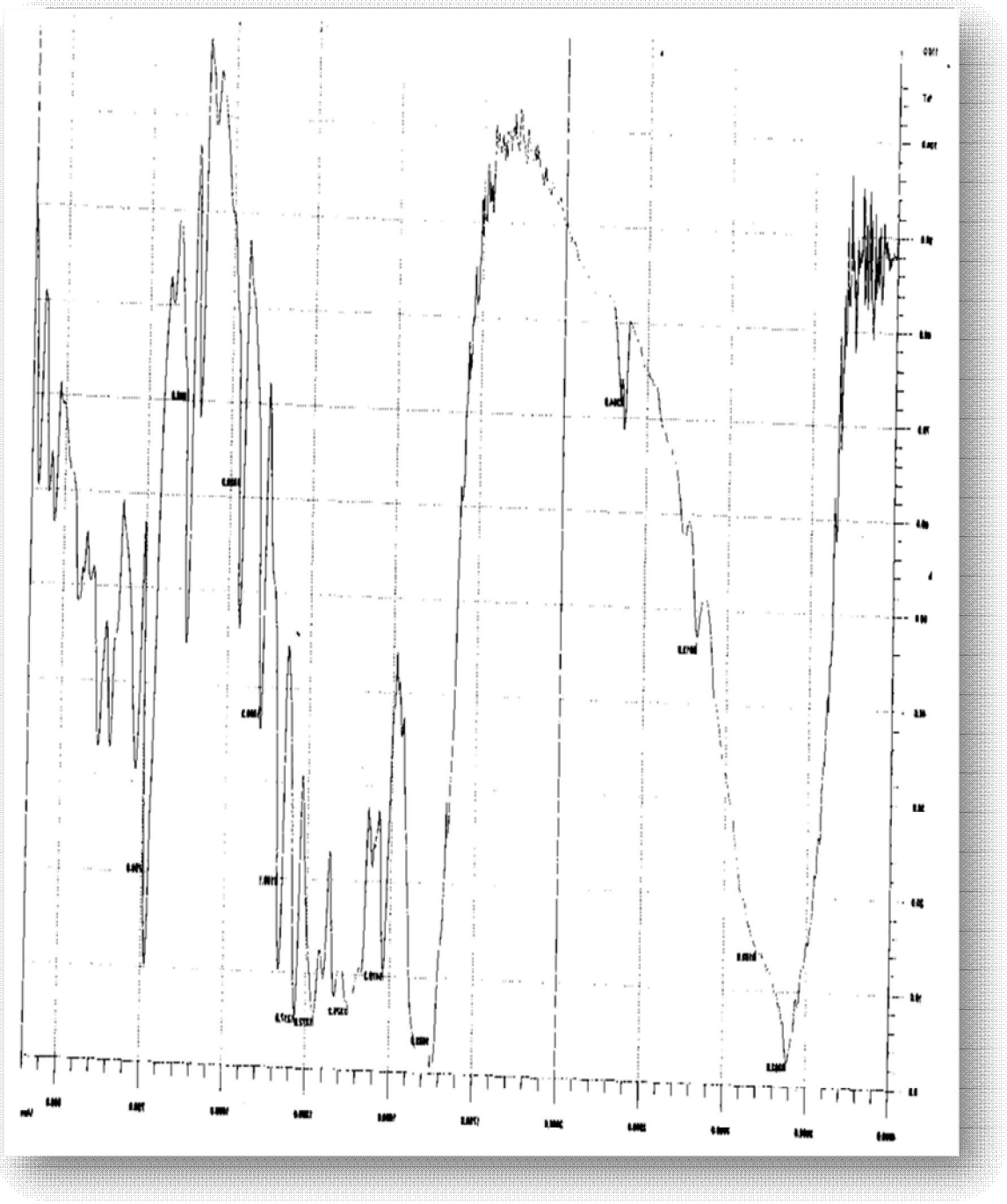

Fig. 5: The FT-IR spectrum of the isolated compound

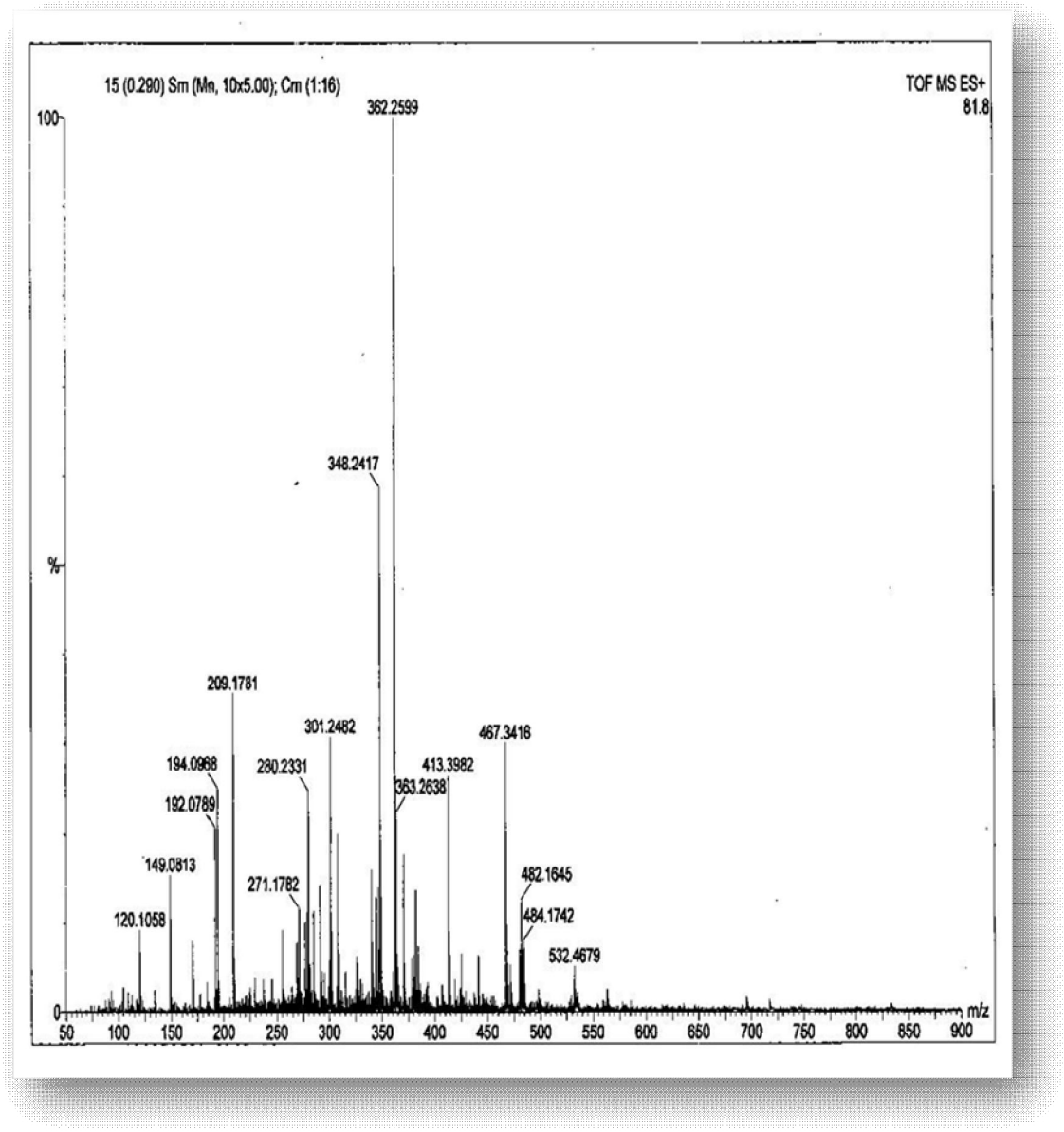

Fig. 6: The Mass spectrum of the isolated compound

The ^{13}C-NMR spectral data (H-decoupled) of emodinas reported in the literature matched quite satisfactorily with the sample and based on literature data, each Carbon was assigned as follows in D_6-DMSO: □□21.8 (CH_3), 108.1(C2), 109.0 (C4), 109.1 (C6), 113.4(C1-C-C10), 120.7(C8), 124.3 (C5-C-C9), 132.8 (C4-C-C9)), 135.1 (C8-C-C10), 148.4 (C7), 161.7 (C5), 164.7 (C1), 165.9 (C3), 181.3 (C9), 189.8 (C10) ppm. Most downfield two carbons were for the keto-carbonyl carbons at C-9 and C-10. Other carbon chemical shift values were consistent and compared with the literature values. The FT-IR spectral data also suggested presence of the functional groups like the hydroxyl, conjugated keto-carbonyl and the aromatic double bonds.

HRMS: The exact mass for the isolated compound with mf $C_{15}H_{10}O_5$ as $[M+H]^+$calculated to be 271.0606 and observed as 271.1782, thus confirming the structure for Emodin or1,3,5-trihydroxy-7-methylanthracene-9,10-dione. Structure is as follow:

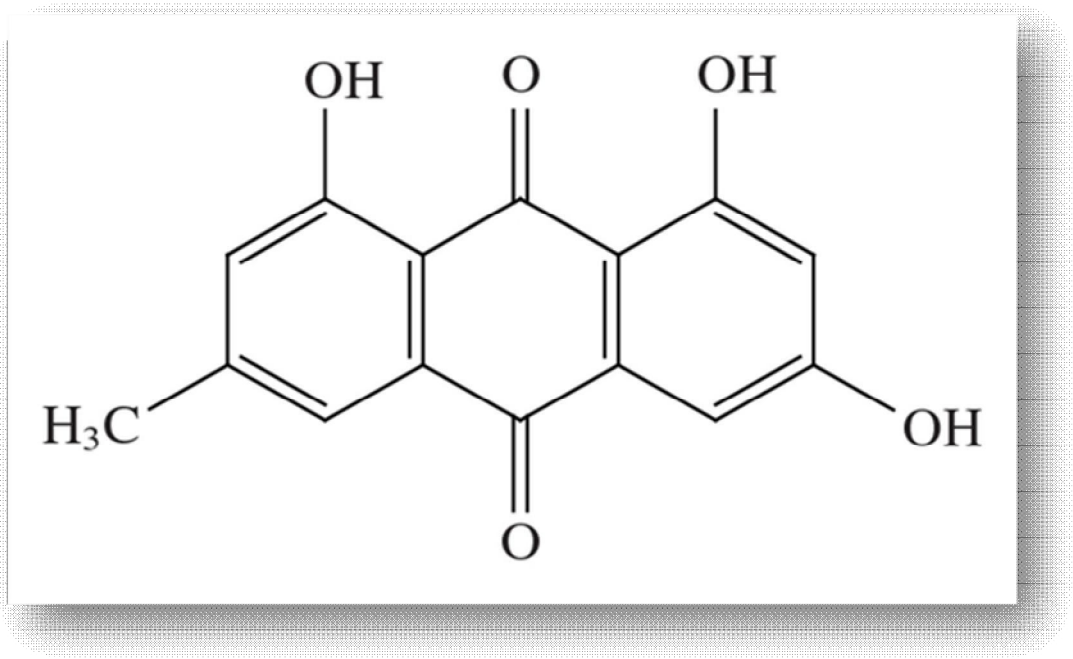

Fig. 7: Emodin - the isolated compound

Phytochemical studies of *Amaranthusspinosus* L. confirmed presence of various chemical compounds like betasitosterolglucoside, hydroxycinnamates, quercetin,7-p-coumaroyl apigenin 4-O-beta-D-glucopyranoside,betaxanthin, betacyanin,coumaroyl flavone glycoside called spinoside,beta-D-ribofuranosyl adenine,gomphrenin, betanin, xylofuranosyl uracil, and kaempferol glycosides, betalains; b-sitosterol, stigmasterol, linoleic acid,rutin, amaranthine and isoamaranthine, and beta-carotene [12-13].

In the present study a compound was isolated from the leaves of *Amaranthusspinosus* L. Characterization of the compound indicated that the compound was1,3,5-trihydroxy-7-methylanthracene-9,10-dione, commonly known as Emodin.

CONCLUSION

A compound was isolated from the leaves of *Amaranthusspinosus* L. From spectral data the compound was characterized as 1,3,5-trihydroxy-7-methylanthracene-9,10-dione, also known as Emodin. In the list of phytochemicals present in *Amaranthusspinosus* L. emodin was, thus, included.

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Authors Column

Prof. (Dr.) Prasanta Kumar Mitrais a very senior medical teacher and researcher. He has completed thirty seven years in medical teaching and about forty years in research. His research area is 'Medicinal plants of India'. He has four Ph.D.s to his credit and published one hundred five research papers in national and international journals. Fifteen students did their Ph.D. work under his guidance. He was co-supervisor of the research projects of five MD students.

Prof. Mitra was Editor-in-Chief of the European Journal of Biotechnology and Biosciences. He is now Editor, Associate Editor and Member of Editorial Board of many national andinternational research journals.

On behalf on Govt. of West Bengal Prof. Mitraworked as Coordinator of World Bank and GTZ projects for Health Sector Development in North Bengal.

Prof. Mitrais awell knownwriter and science popularizer. He has written fifteen hundred eighty seven popular science articles in different newspapers / magazines. He is the recipient of Rajiv Gandhi Excellence award for his academic excellence and outstanding contribution in the field of popularization of science in society.