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

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ABSTRACT
A compound was isolated from the leaves of Amaranthus spinosus 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: Amaranthus spinosus L. chromatographic techniques, emodin.

INTRODUCTION
Amaranthus spinosus L., a medicinal plant under the family of amaranthaceae, is widely distributed throughout the tropics and warm temperate regions of Asia. The plant grows in cultivated areas as well as in waste places and is distributed in lower to middle hills (3000–5000 ft) of entire north eastern Himalayas. The plant is known as “prickly amaranth” in English and “ban lure” or “dhutighans” in Nepali. Leaves of Amaranthus spinosus L. are stacked and alternate. Medicinal uses of Amaranthus spinosus L. as mentioned in Ayurvedic text are: Leaf infusion is diuretic and used in anemia. Root paste is used in gonorrhea, eczema, menorrhrea 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, blood diseases, piles and leucorrhoea. 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 infusion of *Amaranthus spinosus* L. in stomach disorder specially in case of indigestion and peptic ulcer \([1]\). We also observed anti ulcer activity of the leaves of *Amaranthus spinosus* L. against ethanol and cysteamine induced peptic ulcer in albino rats \([11]\). Considering the medicinal importance of *Amaranthus spinosus* L. phytochemical studies of the plant were extensively undertaken. Phenol, sitosterol, stigmasterol, essential oil, friedolin, and unidentified esters were found as active components of *Amaranthus spinosus* L. \([12-13]\). Recently we have isolated and characterize a compound from the leaves of *Amaranthus spinosus* L. Results are being reported in this communication.

**MATERIALS AND METHODS**

**Plant Material**

![Amaranthus spinosus L.](image)

Fig. 1: *Amaranthus spinosus* L.

Leaves of *Amaranthus spinosus* 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°C using 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 chromatography using 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. Elution was done by ethyl format: formic acid mixture (100:5, v/v)

Sixth step
Eluted third fraction was evaporated to dryness under 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°C.

FT-IR (KBr): $\nu_{\text{max}}$ 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): $\delta$ 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 $\delta$ 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-methylnanthracene-9,10-dione, also known as Emodin.

![Fig. 2: Structure of the isolated compound](image-url)
By comparison, the C2-H appears at 6.47 ppm 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
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.

Fig. 6: The Mass spectrum of the isolated compound
HRMS: The exact mass for the isolated compound with mf C\textsubscript{15}H\textsubscript{10}O\textsubscript{5} as [M+H]\textsuperscript{+} calculated to be 271.0606 and observed as 271.1782, thus confirming the structure for Emodin or 1,3,5-trihydroxy-7-methylantracene-9,10-dione. Structure is as follow:

![Fig. 7: Emodin - the isolated compound](image)

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 \cite{12-13}.

In the present study a compound was isolated from the leaves of *Amaranthusspinosus* L. Characterization of the compound indicated that the compound was 1,3,5-trihydroxy-7-methylantracene-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-methylantracene-9,10-dione, also known as Emodin. In the list of phytochemicals present in *Amaranthusspinosus* L. emodin was, thus, included.
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Prof. (Dr.) Prasanta Kumar Mitra is 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 and international research journals. On behalf of West Bengal Prof. Mitra worked as Coordinator of World Bank and GTZ projects for Health Sector Development in North Bengal. Prof. Mitra is a well-known writer 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.