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Research Article

Isolation and Characterization of a Compound from the Leaves of Cassia Alata Linn.

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Abstract

By solvent extraction, acid hydrolysis, chromatography followed by crystallization a compound was isolated from the leaves of *Cassia Alata Linn*. 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.

Keywords: *Cassia Alata L; Spectroscopy; Chromatographic techniques*

Introduction

Cassia Alata Linn. (family, Caesalpiniaceae) is an erect tropical annual herb with leather compounded leaves. The plant was native to Ghana and Brazil, but it is now widely distributed throughout the world [1]. Even in India in the state of West Bengal, the plant grows everywhere up to 6 ft tall. *C. Alata* L. is widely known in the name of wild senna. It has other names also. They are: ringworm weed in English, dadmari in Hindi and cakramard in Sanskrit. Its therapeutic values as mentioned in Ayurvedic text [2,3] are: leaves are anti parasitic, used in eczema, bronchitis, asthma, ringworm and in poisonous insect bites, bark is used to treat skin diseases, extract of aerial parts is CNS depressant , diuretic and anti inflammatory. Modern researchers advocated the use of *C. Alata* L. for treatments of blennorrhagia, syphilis, diabetes, haemorrhoids, constipation, inguinal hernia and intestinal parasitosis [4-6]. Traditionally the plant is used as anti helminthic, in infection and in uterus disorder [7,8].

Makinde reported that all parts of *C. Alata* L. have one or more medicinal actions especially antimicrobial activities [9]. In 1998 Sakharkar and Patil confirmed antimicrobial activity of *C. Alata* L. [10]. We also noted that leaves of *C. Alata* L. could inhibit growth of *Staphylococcus aureus* [11].

Phytochemical studies revealed that *Cassia Alata* L. leaves contain alkaloids, saponins, anthraquinones, tannins, terpenes, steroids, flavonoids and carbohydrates [12, 13].

Recently we have isolated and characterize a compound from the leaves of *Cassia Alata* L. Results are being reported in this communication.

Materials

Plant Material

Figure 1: *Cassia Alata L.*

Leaves of *Cassia Alata* 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.

Reagents

Methanol, chloroform, ethyl acetate, acetic acid formic acid, alumina and silica gel were procured from MP Biomedicals India Pvt. Ltd.

Methods

Extraction and Isolation

First step: 100g of the powder were extracted with 500 ml methanol-chloroform mixture (60:40 v/v) for 10 min at 400C 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. Five bands were separated. Elution was done by methanol-chloroform mixture (60:40 v/v).

Fourth step: Eluted third band was evaporated to dryness. Dry brown mass was obtained. It was extracted with 20 ml ethyl acetate for 15 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. Six bands were separated. Elusion was done by ethyl format: formic acid mixture (80:20, v/v).

Sixth step: Eluted second 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 (80:20, v/v) mixture from the brown mass. A compound was crystallized. Yield was 5.7 mg.

Homogeneity of the Active Compound

This was ascertained by silica gel-G thin layer chromatography by using the following solvent systems; Methanol : water - 60 : 40 ; Ethanol : chloroform : water - 60 : 20 : 20; Chloroform : methanol : water - 50 : 25 : 25

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 1H-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.

Result and Discussion

Homogeneity of the isolated compound

The isolated compound was pure as in all cases of thin layer chromatographic an experiment 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): V_{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 1H-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 δ181.3, 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.

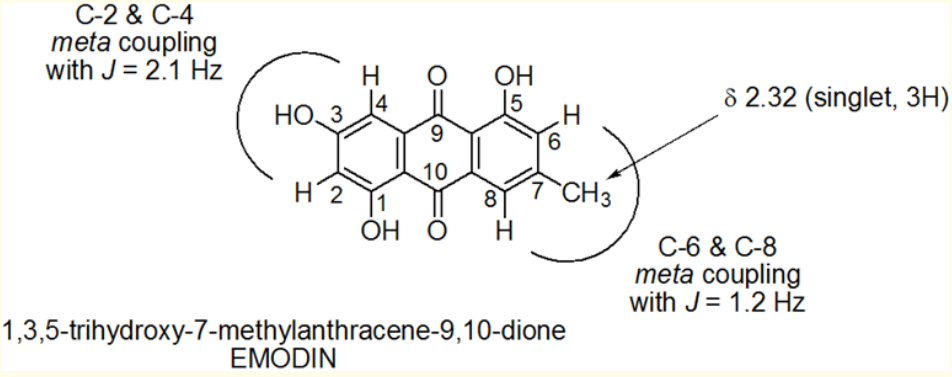

Figure 2: *Structure of the Isolated Compound.*

By comparison, the C2-H appears at δ 6.47 as a *meta*-coupled doublet with J = 2.1 Hz, which was coupled by C4-H appearing at δ 6.96 ppm (J = 2.1 Hz). Similarly, the other two Hs at C6 and C8 were appearing respectively at δ 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 δ 2.32 ppm, the shift position conformed to aryl methyl protons.

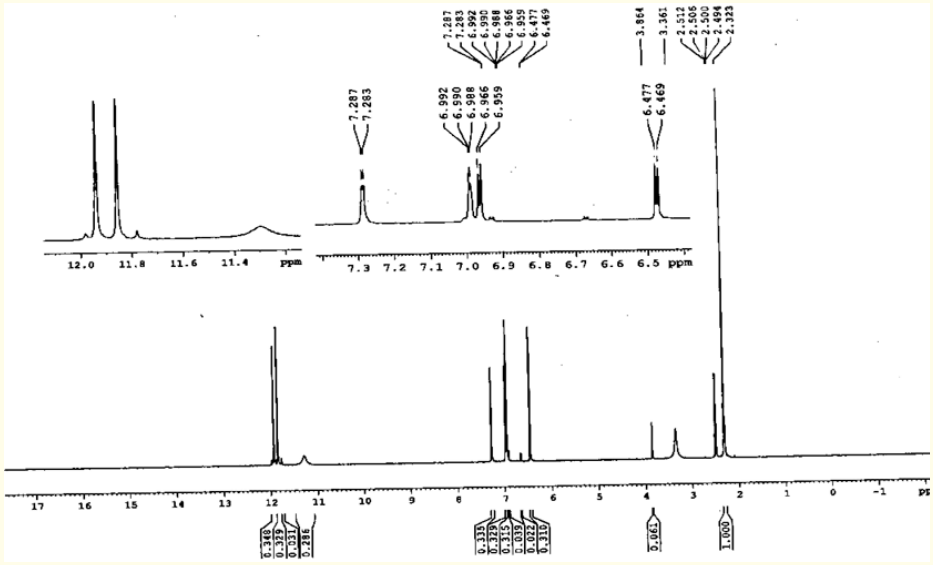

Figure 3: *The [1]H-NMR Spectrum of the Isolated Compound.*

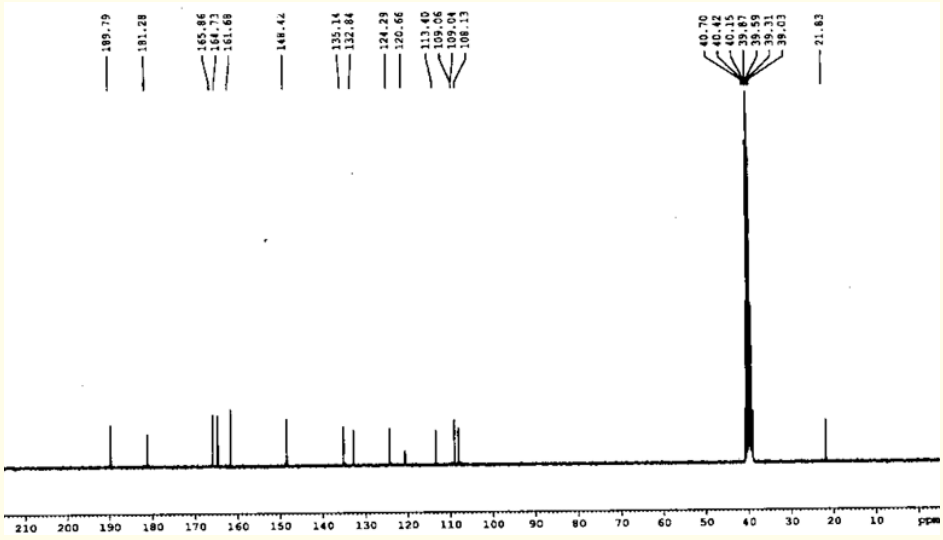

Figure 4: *The [13]C-NMR Spectrum of the Isolated Compound.*

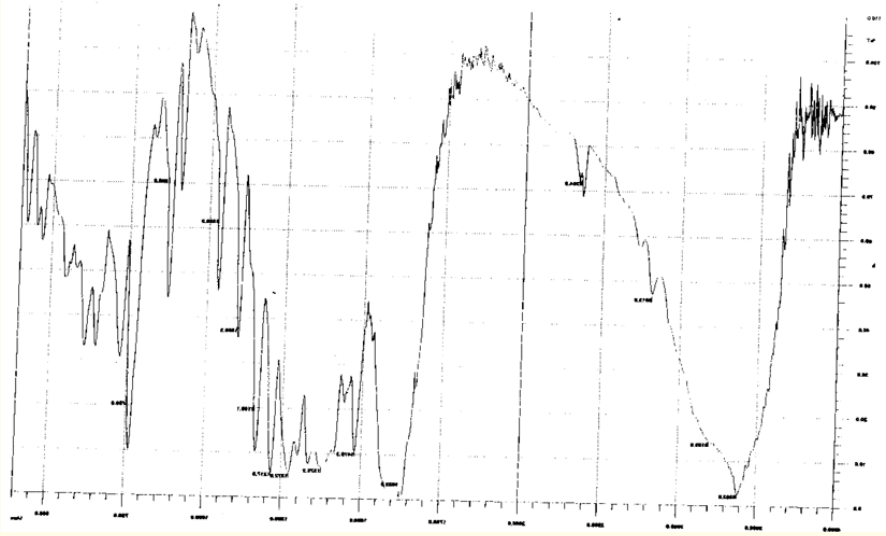

Figure 5: *The FT-IR Spectrum of the Isolated Compound.*

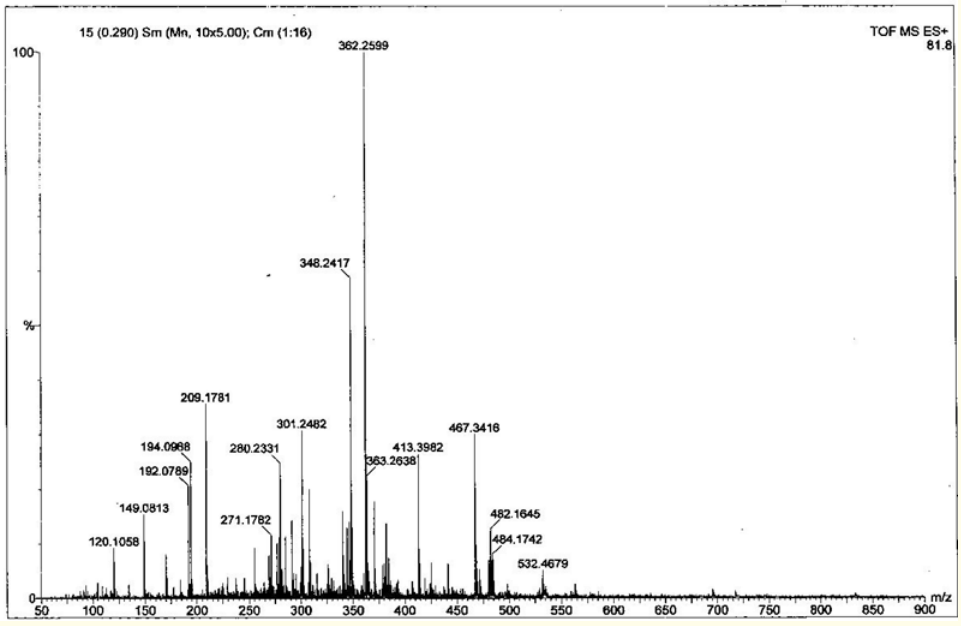

Figure 6: *The Mass Spectrum of the Isolated Compound.*

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: δ 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 or 1,3,5-trihydroxy-7-methylanthracene-9,10-dione. Structure is as follow:

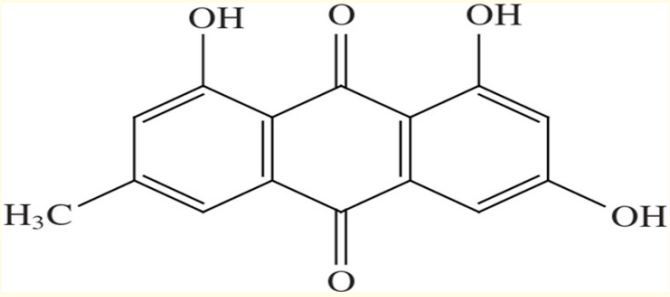

Figure 7: *1,3,5-Trihydroxy-7-Methylanthracene-9,10-Dione (Isolated Compound).*

Phytochemical studies of *Cassia Alata* L. leaves confirmed presence of alkaloids, anthraquinones, saponins, tannins, terpenes, steroids, flavonoids and carbohydrates [12, 13].

In the present study a compound was isolated from the leaves of *Cassia Alata* L. Characterization of the compound indicated that the compound was 1,3,5-trihydroxy-7-methylanthracene-9,10-dione.

Conclusion

A compound was isolated from the leaves of *Cassia Alata* L. From spectral data the compound was characterized as 1,3,5-trihydroxy-7-methylanthracene-9,10-dione. In the list of phytochemicals present in *Cassia Alata* L., 1,3,5-trihydroxy-7-methylanthracene-9,10-dione is included.

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