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ISOLATION AND CHARACTERIZATION OF A BIOACTIVE COMPOUND FROM *ABRUS PRECATORIUS* LINNAEUS LEAVES

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ABSTRACT

A compound was isolated from the leaves of *Abrus precatorius* Linnaeus (Family, Fabaceae). Isolation process involved solvent extraction, acid hydrolysis, chromatography followed by crystallization. Spectral studies like infra red spectroscopy, mass spectroscopy as well as nuclear magnetic resonance studies were undertaken to characterize the compound. Results showed that the isolated compound was chemically 3,4-Dihydroxycinnamic acid**.** 3,4-Dihydroxycinnamic acid, also known as caffeic acid, is a bioactive compound and has several pharmacological actions.

KEYWORDS: *Abrus precatorium* Linnaeus, chromatographic techniques, 3,4-Dihydroxycinnamic acid.

1. INTRODUCTION

Abrus precatorius Linnaeus (*A. precatorius* L.), commonly known as Indian liquorices, is a medicinal plant of the family 'fabaceae'. The plant has multidimensional medicinal properties. In traditional system of medicine as well as in Ayurvedic and Unani medicine, the

plant is reported to possess medicinal effects as an antidote, aphrodisiac, remove biliousness, cures leucoderma, itching, useful in eye diseases, skin diseases and wounds.[1-3]

A. precatorius L. leaf also called 'Chanoti' has been used in folk remedies by tribal.

population for over many years. Recent studies showed that leaves of *A. precatorius* L. have broad range of therapeutic effects like anti diabetic, antimicrobial, anti-migraine, anti-inflammatory, anti-serotonergic, antitumor, analgesic, antispasmodic etc. Leaves are also used for treatment of inflammation, wounds, ulcers, throat scratches and sores.[4] Report from our laboratory showed anti gastric ulcer activity of *A. precatorius* L. leaves in albino rats.[5]

Due to these medicinal values, people throughout the world consume *A. precatorius* L. leaves either as such or through traditional medicine to get rid of ailments.[6,7]

In the present communication we report isolation and characterization of a bioactive compound from the leaves of *A. precatorius* L.

2. MATERIALS AND METHODS

2.1 *Plant Material*

Leaves of *A. precatorius* L. were collected from the medicinal plants garden of the North Bengal University and authenticated by the experts of the department of Botany of the said University. A voucher specimen was kept in the department of Medical Biotechnology, Sikkim Manipal Institute of Medical Sciences of the Sikkim Manipal University, Gangtok, Sikkim, India for future references. Leaves were shade dried and powdered. The powder was used for extraction and isolation studies.

Fig. 1: *Abrus precatorius* Linnaeus.

2.2 *Chemicals*

Chemicals needed for isolation work were procured from Ranbaxy and SD Fine Chemicals, New Delhi, India as well as Sigma Chemical Company, Mumbai, India.

2.3 *Isolation studies*

Isolation studies involved the following steps:

1. Solvent extraction.
2. Acid reflux.
3. Solvent treatment.
4. Polyamide column chromatography.
5. Silica Gel G column chromatography.
6. Crystallization.

Principles of standard isolation procedures were followed.[8-12]

2.4 *Homogeneity of the active compound*

This was ascertained by silica gel- G thin layer chromatography by using the following solvent systems; Acetone: methanol: water-60: 20: 20; n-butanol: acetic acid: water - 80: 10: 10; Chloroform: methanol: water-40: 30: 330

2.5 *Characterization of the isolated compound*

Characterization of the isolated compound was done through standard methodologies.[12, 13-14] by following steps.

1. Infrared spectroscopy.
2. Nuclear magnetic resonance spectroscopy.
3. Mass spectroscopy.
4. Melting point determination.
5. Data analysis.

3. RESULTS AND DISCUSSION

3.1 *Diagrammatic scheme for isolation of active compound from leaves of Abrus precatorius Linnaeus*

Powdered leaves of *A. precatorius* L. (100 g)

↓

SOLVENT EXTRACTION

Extracted with 1000 ml of 10: 1 (v/v) acetone – chloroform mixture for ½hour at room temperature (12 ± 1^0C) on a rotary shaker. It was then centrifuged at 3000xg for 5min. Supernatant was collected and evaporated to dryness.

Brown mass

↓

ACID REFLUX

Refluxed with 100ml of 1(N) HCl for½ hour on a water bath at 100^0C. It was cooled and centrifuged at 3000xg for 5 min. Supernatant was evaporated to dryness.

Brown mass

↓

SOLVENT TREATMENT

Extracted with 100 ml of 1: 1 v/v mixture of water and isobutanol on a rotary shaker for 30 minutes. Isobutanol layer was separated from water layer and was evaporated to dryness.

Brown mass

↓

POLYAMIDE COLUMN CHROMATOGRAPHY

Dissolved in 10ml ethyl acetate and subjected to column chromatography using polyamide as adsorbent. Elution was done by ethyl formate: formic acid mixture (80: 20v/v). Five bands were separated.

First band was taken

↓

SILICA GEL G COLUMN CHROMATOGRAPHY

Eluent of first band was evaporated to dryness. The dry mass was extracted with 15ml acetone for 10 minutes. It was then filtered. With filtrate silica gel G column chromatography was done. Elution was made by methanol–chloroform mixture (1:1 v/v). Six bands were separated.

Fourth band was taken

↓

CRYSTALLIZATION

Eluent of fourth band obtained from the above step was evaporated to dryness. Repeated crystallization was done from ethyl acetate–cyclohexane (60:40, v/v) mixture.

Crystals obtained

(Yield, 9.8mg)

3.2 *Homogeneity of the active compound*

In all cases of thin layer chromatographic experiments using three different solvent systems single spot was obtained. Thus, it was a single compound.

3.3 *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.

3.3.1 *Infrared spectroscopy*

FT-IR spectrum of the sample was taken in KBr pellets using Shimadzu FT-IR 8300 Spectrophotometer. The FT-IR (KBr) absorption maxima (V_{max}) were recorded at 3423, 3179, 1675, 1657, 1603 cm^{-1}. IR spectrum was shown below in Fig - 2.

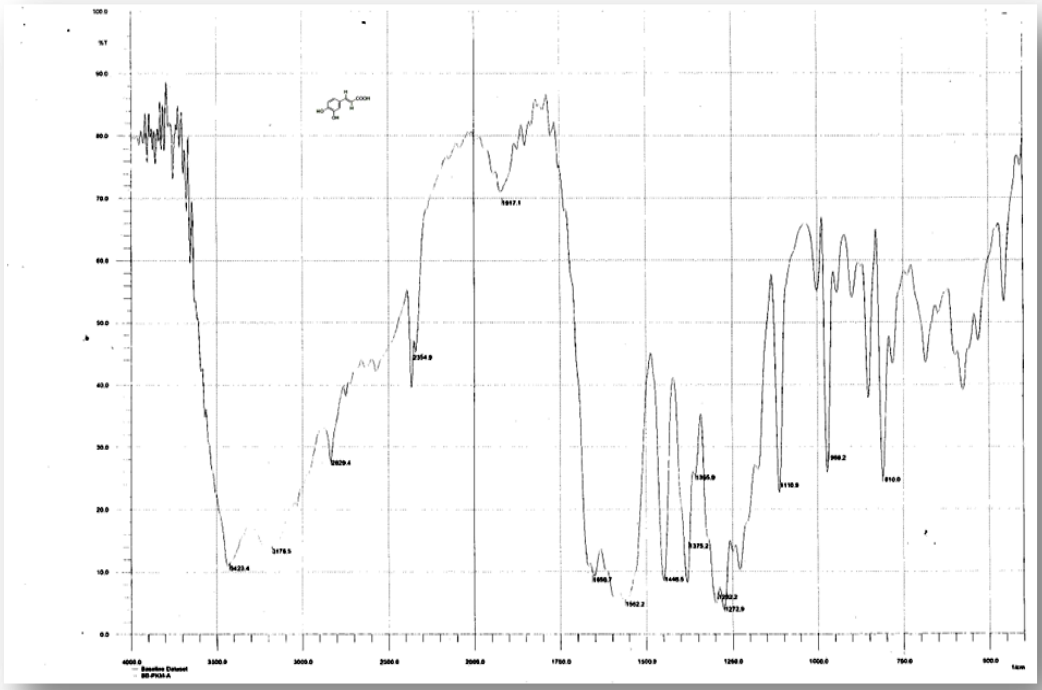

Fig: 2 IR spectrum of the isolated compound

3.3.2 Nuclear magnetic resonance spectroscopy

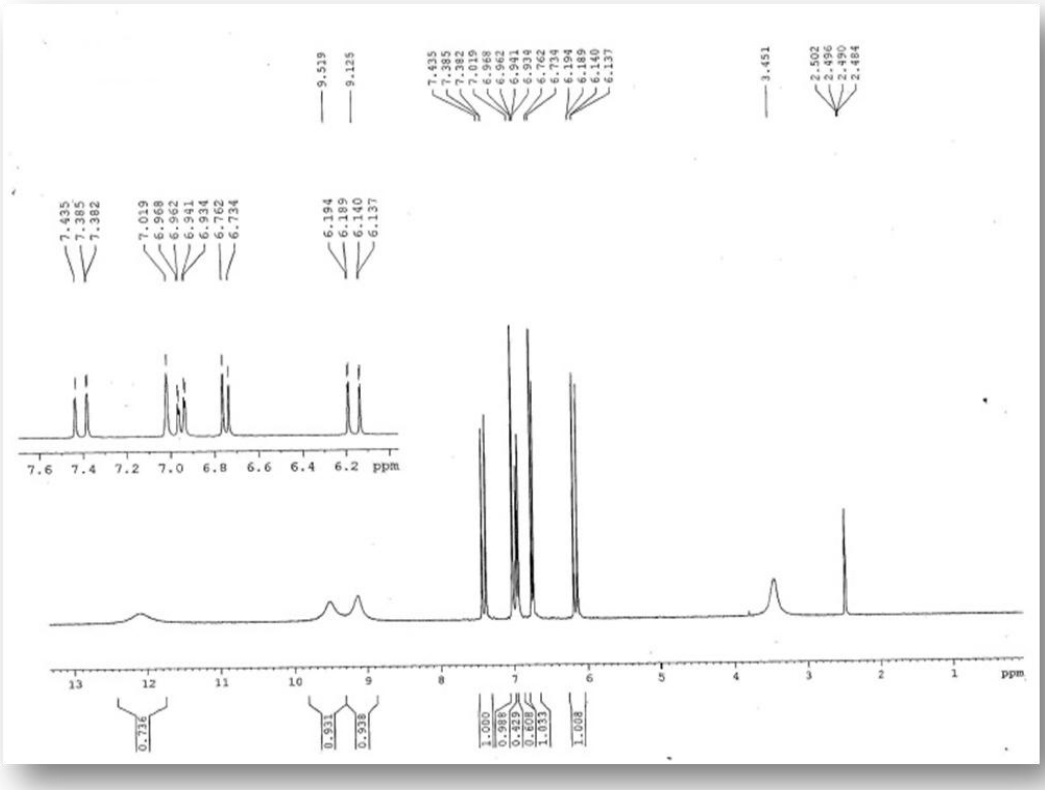

Fig. 3: 1 H NMR spectrum of the isolated compound

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. ^{1}H NMR spectrum and ^{13}C NMR spectrum were shown in Fig – 6 and Fig – 7 respectively. . NMR data were as follow :The ^{1}H-NMR (D_6-DMSO): δ 6.16 (d, 1H, J = 15.9 Hz), 6.75 (1H, d, J = 7.4 Hz), 6.95 (dd, 1H, J = 8.1 & 2.1 Hz), 7.02 (s, 1H), 7.41 (d, 1H, J = 15.9 Hz), 9.12(br. s, 1H), 9.52 (br. s, 1H), 12.11 (br. s, 1H) ppm. Its ^{13}C-NMR (D_6-DMSO): δ □115.1, 115.6, 116.2, 121.6, 126.2, 145.1, 146.0, 148.6, 168.4.

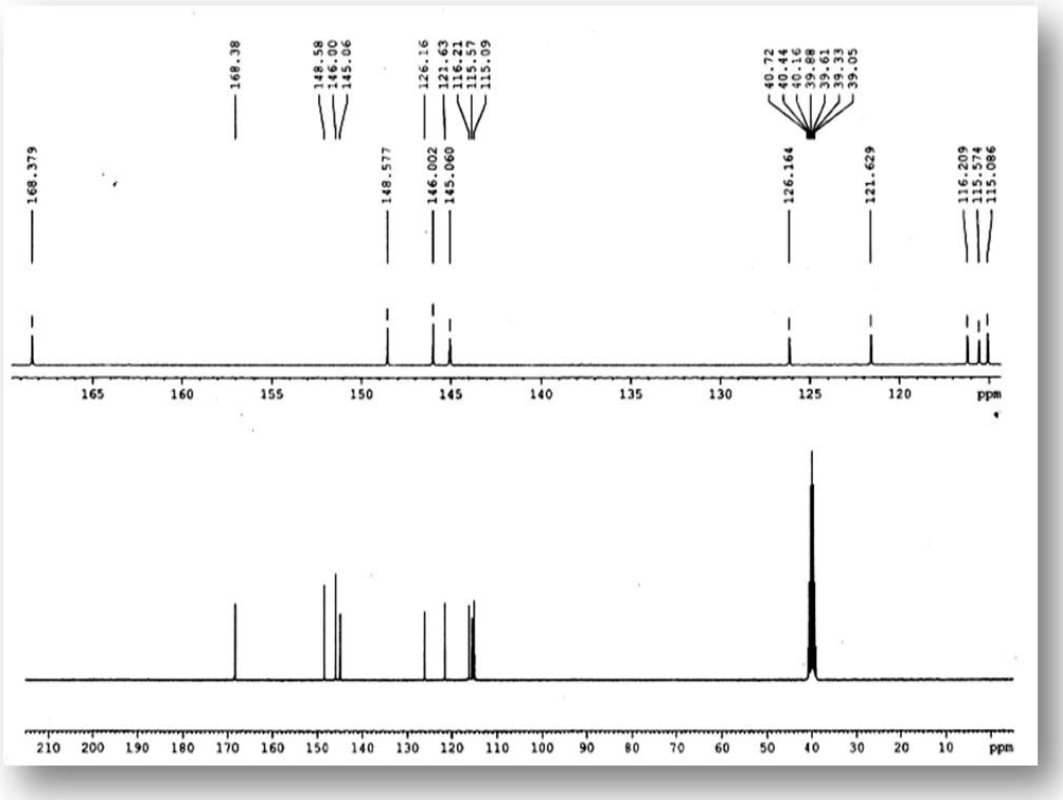

Fig: 4 13C NMR spectrum of the isolated compound

3.3.3 Mass spectroscopy

The High Resolution Mass Spectral data for the compound was obtained in Mass Spectrometer (Model: Micromass Q-T of Micro), run under Electron Spray Ionization (ESI) Positive Mode. Mass spectroscopy was shown below in Fig – 5.

3.3.4 Melting point determination

Melting point was observed in an open sulfuric acid bath and is uncorrected. Observed melting point of the compound was 218 - 221^{o}C.

3.4 Data analysis

From ^{1}H-NMR spectral data, it appeared that there were three aromatic protons, two olefinic protons and three broad singlets. The coupling patterns of the aromatic protons primarily.

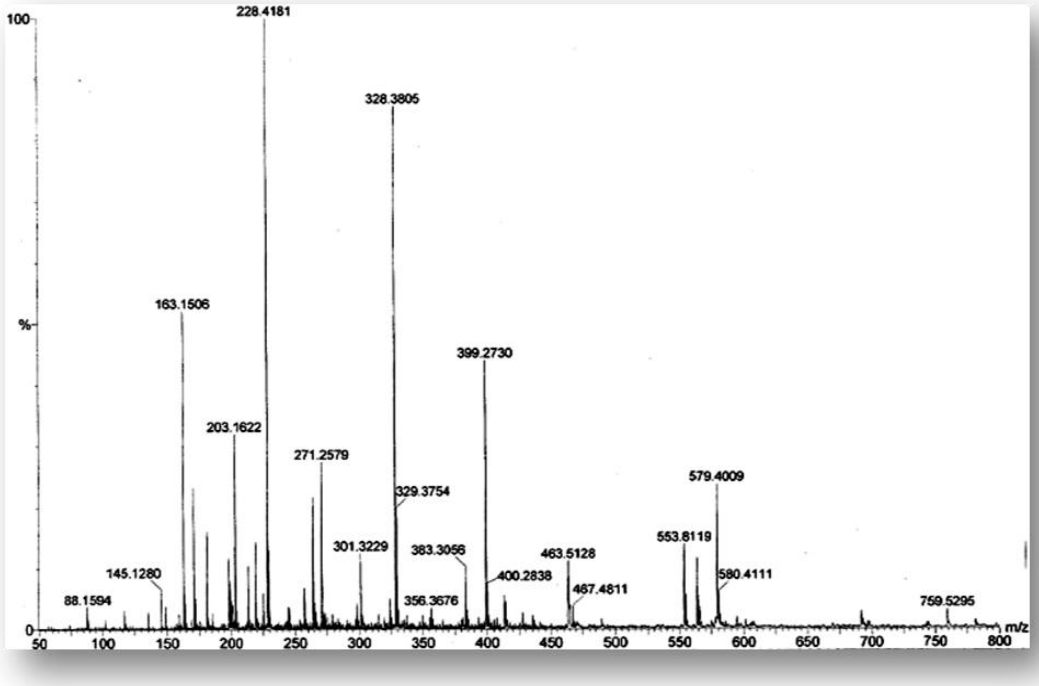

Fig. 5: Mass spectrum of the isolated compound

indicated one *ortho* coupled doublet and one *ortho-meta* coupled doublet of doublet (J = 8.1 & 2.1 Hz) and the other was possibly a *meta*-doublet, though appeared as a singlet (δ = 7.1 ppm). On the other hand, the olefinic protons with coupling constant, J = 15.9 Hz, indicated that the double bond was in *trans* configuration. Since there were three aromatic protons as seen by ^{1}H NMR spectral data, the other three positions of the aromatic ring might be substituted. Only one aromatic ring was considered because of low molecular mass of the compound. Also, there were only nine chemically non-equivalent carbons according to ^{13}C-NMR spectrum. As one substituent might be a C–C double bond, there were two other positions, might be substituted with two hydroxyl group (OH) that appeared as broad singlets. Out of three broad singlets, one broad singlets at δ=12.11 ppm could be assigned for the carboxylic (COOH) proton. The carboxylic acid group might be attached with the C–C double bond, leading to propose the assigned structure as di hydroxy cinnamic acid. The FT-IR (KBr) absorption maxima at 3423, 3179, 1675, 1657, 1603cm^{-1} also suggested the presence of hydroxyl, conjugated carboxyl and double bonds. Considering that the compound could be a dihydroxy cinnamic acid and based on the coupling pattern of three aromatic

protons (one *ortho*-doublets, one *ortho-meta* doublet of doublet and one *meta*-doublet), theoretically five possible structures are possible as below,

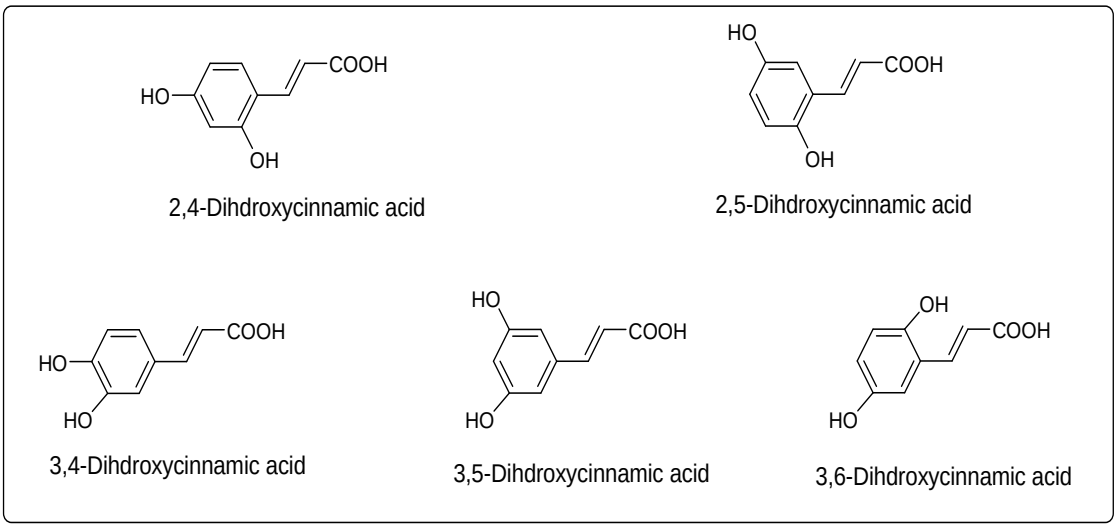

Fig: 6 Five possible structures of the isolated compound

The ^{13}C-NMR spectral data showed that there are nine chemically non-equivalent carbons, out of which three carbons were assigned for the acrylic acid side chain carbons (–C=C–COOH). Therefore, all aromatic ring hydrogens were non-equivalent. 3,5-Dihydroxycinnamic acid is having the axis of symmetry and thus it might have only four chemically and magnetically non-equivalent carbons. Accordingly, this structure (3,5- dihy droxycinnamic acid) might be ruled out. Out of other four structures, the literature value of the melting point of 3,4-dihydroxycinnamic acid (mp223-25 ^{o}C) fairly matched with the observed melting point of the compound (218-221^{o}C). Its coupling pattern was shown below with the possible coupling of the aromatic hydrogens as well as the *trans*-configuration of the carbon–carbon double bond, which was observed and calculated to be *J*=15.9 Hz). The *trans* configuration of the carbon–carbon double bond was assigned based on the fact that in the case of *cis*-configuration, the coupling constant (*J*) would have been within 6–12 Hz. The spin-spin couplings between hydrogens were for the aromatic hydrogens (marked as H^{a}, H^{b} and H^{c}) as well as for the carbon–carbon double bonds, marked as $H^{\square}$ and $H^{\square\square}$ and is as follow.

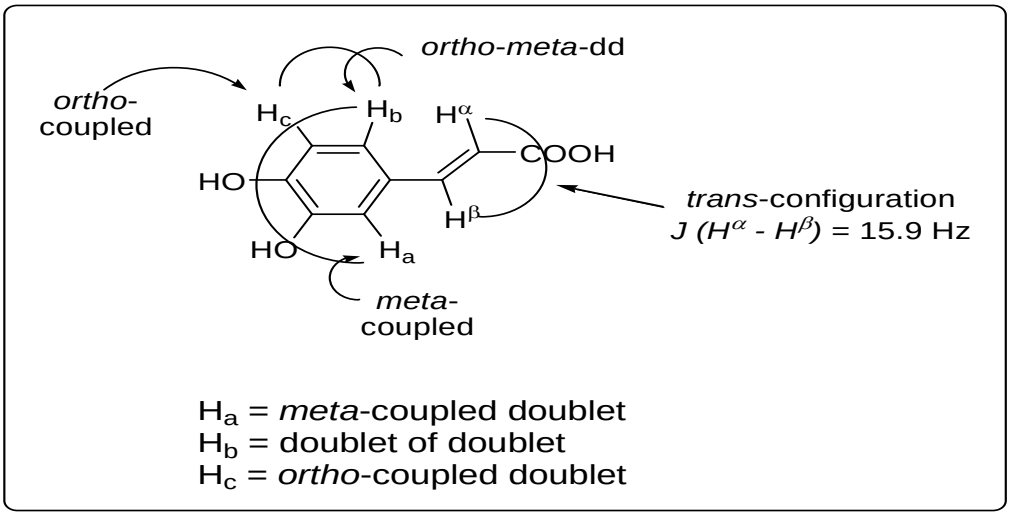

Fig: 7 Spin-spin couplings between hydrogen in the structure of the isolated compound

Structure of the compound was further corroborated by the High Resolution Mass Spectral (HRMS) data (Figure-8), run under Electron Spray Ionization (ESI) Positive Mode. In HRMS the exact mass for compound with mf $C_9H_8O_4Na$ [M^+Na] was calculated to be 203.1472 and observed as 203.1622. Therefore, the structure of the isolated compound, shown in below, may be assigned as 3,4-Dihydroxycinnamic acid.

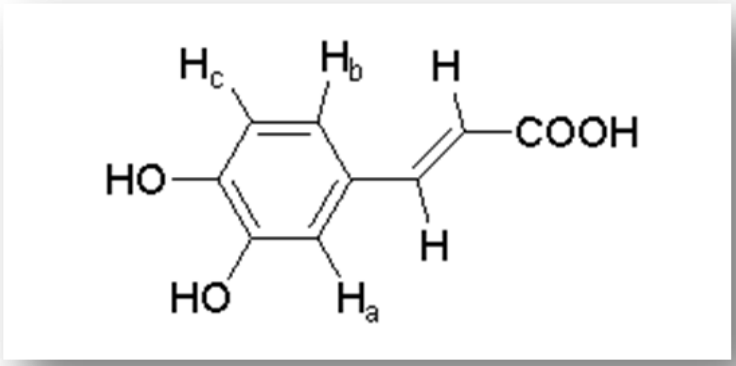

Fig. 8 Structure of the isolated compound (3,4-Dihydroxycinnamic acid)

Phytochemicals like abrine, abraline, abruslectone, abrussic acid, anthocyanins, abrasine, abrusgenic acid-methylester, abricin, abrin, abrisin etc. are present in *A. precatorius* L.[15,16]

The present study includes 3,4-Dihydroxycinnamic acid in the list of phytochemicals in *A. precatorius* L. leaves. 3,4-Dihydroxycinnamic acid, also known as caffeic acid, is a bioactive compound known to perform various biological activities.[17]

4. CONCLUSION

The present study showed presence of 3,4-Dihydroxycinnamic acid (caffeic acid) in *A. precatorius* L. leaves. Due to presence of this highly bioactive compound leaves of *A. precatorius* L. exerts various pharmacological actions.

5. CONFLICT OF INTEREST

Nil.

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