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3, 4 diHydroxyBenzoic acid Isolated from the Leaves of *Ageratum conyzoides* L.

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ABSTRACT

By different solvent extractions and chromatographic techniques an active compound was isolated from the leaves of *Ageratum conzoides* L. Infra-red spectroscopy, mass spectroscopy and nuclear magnetic resonance studies showed that the isolated compound was chemically 3,4 dihydroxy benzoic acid.

Keywords: *Ageratum conzoides* L., Chromatographic Techniques, 3,4 dihydroxy Benzoic Acid

1. Introduction

Ageratum conyzoides L. (family, asteraceae) is a medicinal plant, distributed throughout India, lower and middle hill in Sikkim and Darjeeling up to 6000 ft [1]. The plant is known as 'Goat weed' in English and 'Elame' and 'Namyew' in Nepali and in Lepcha respectively. Leaves, root, stem and flower of *Ageratum conyzoides* L. have medicinal use. Leaves are styptic effective in healing of wounds, used in boils and prevent tetanus. Leaf juice is also used as eye lotion. The root juice has antibiotic property. The plant is boiled with oil and applied externally in rheumatism. Leaves of *Ageratum conyzoides* L. are widely used in folk medicine in Sikkim and adjoining areas [2].

Considering the medicinal importance of *Ageratum conyzoides* L. phytochemical studies of the plant were extensively done. Phenol, essential oil, friedolin, sitosterol, stigmasterol and unidentified esters were found as active components of *Ageratum conyzoides* L.[3,4]. Recently we have isolated and characterize an active compound from the leaves of *Ageratum conyzoides* L. Results are being reported in this communication.

2. Materials and Methods

2.1 Plant Material

Leaves of *Ageratum conyzoides* 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.

2.2 Extraction and Isolation

First Step: 100g of this powder were extracted with 1000 ml of 10 : 1 (v/v) chloroform – ethyl alcohol mixture for 1h on a rotary shaker . It was then centrifuged. Supernatant was collected and evaporated to dryness. Dry brown mass was obtained.

Second step: Dry brown mass was refluxed with 200 ml of 1(N) HCL for 1h on a water bath at 100 degree centigrade. It was cooled and centrifuged. Supernatant was evaporated to dryness.

Third step: Dry brown mass thus obtained from the supernatant was extracted with 50 ml of ethyl acetate on a rotary shaker for 1h. It was evaporated to dryness.

Fourth step: Brown mass obtained was dissolved in 10 ml methanol and subjected to column chromatography using silica gel Gas adsorbent. 8 bands were separated. Bands were collected in separate beakers. Elution was done by 50% methanol – chloroform mixture.

Fifth step: First band was separately evaporated to dryness. The dry mass was extracted with 10 ml ethyl acetate for 15 minutes. It was then filtered. With filtrate polyamide column chromatography was done. Elution was made by ethyl formate: formic acid mixture (80: 20 v/v). Three bands were separated.

Sixth step: The first separated band was evaporated to dryness. Repeated crystallization was done from ethyl acetate–formic acid (50:50, v/v) mixture. Crystals obtained. Yield was 8.8 mg.

2.3 Homogeneity of the active compound

This was ascertained by silica gel- G thin layer chromatography by using the following solvent systems; Acetone : methanol : water - 80 : 10 : 10; n-butanol : acetic acid : water - 60 : 20 : 20; Chloroform : methanol : water - 50 : 25 : 25

2.4 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. Results and Discussion

3.1 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.

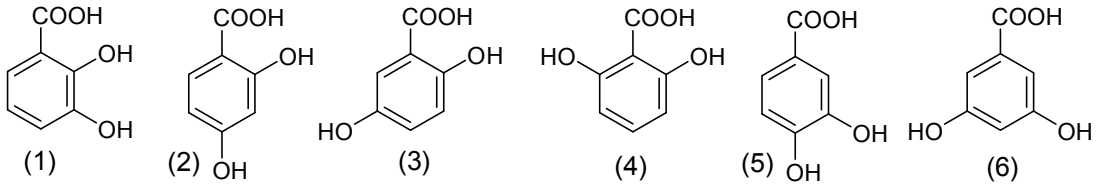
3.2 Structure Elucidation

The isolated compound was colorless solid, mp. 200-202 ^{o}C

The ^{1}H-NMR in D_6-DMSO displayed as: δ 6.80 (d, 1H, J = 8.1 Hz), 7.31 (dd, 1H, J = 8.1 & 2.1 Hz), 7.36 (d, 1H, J = 2.1 Hz), 9.30 (br. s, 1H), 9.67 (br. s, 1H), 12.36 (br. s, 1H) ppm.

^{13}C-NMR (D_6-DMSO): δ 115.5, 116.9, 122, 122.3, 145.2, 150.3, 167.7 ππμ.

The ^{1}H-NMR spectral data displayed three aromatic hydrogens suggesting that other three positions of a benzene ring might be substituted. On the other hand, three broad singlets could be assigned for hydrogens attached with heteroatoms. By comparison, it may be suggested that these three broad singlets correspond to one carboxylic acid proton and two hydroxyl protons. Therefore the compound could be a dihydroxy benzoic acid. Based on three substituents in one aromatic ring, following isomers of dihydroxy benzoic acid are possible:



Two aromatic protons appearing at δ 7.36 and 7.31 ppm suggest that there might be some electron-withdrawing "deshielding" effect of the carboxylic acid group on the *ortho*-hydrogens. It is therefore presumed that the *ortho* positions of the carboxylic acid are un-substituted. As such, isomers (1) – (4) may be ruled out and we are left with the structures (5) or (6). In compound (6) i.e. 3,5-dihydroxybenzoic acid, two *ortho* Hs are chemically and magnetically equivalent and they should have same chemical shift and be displayed as *meta* coupled two Hs having coupled by the non-equivalent H at C-4. On the other hand, in compound (5), all three Ar-Hs are magnetically non-equivalent and would be displayed at different chemical shift (δ).

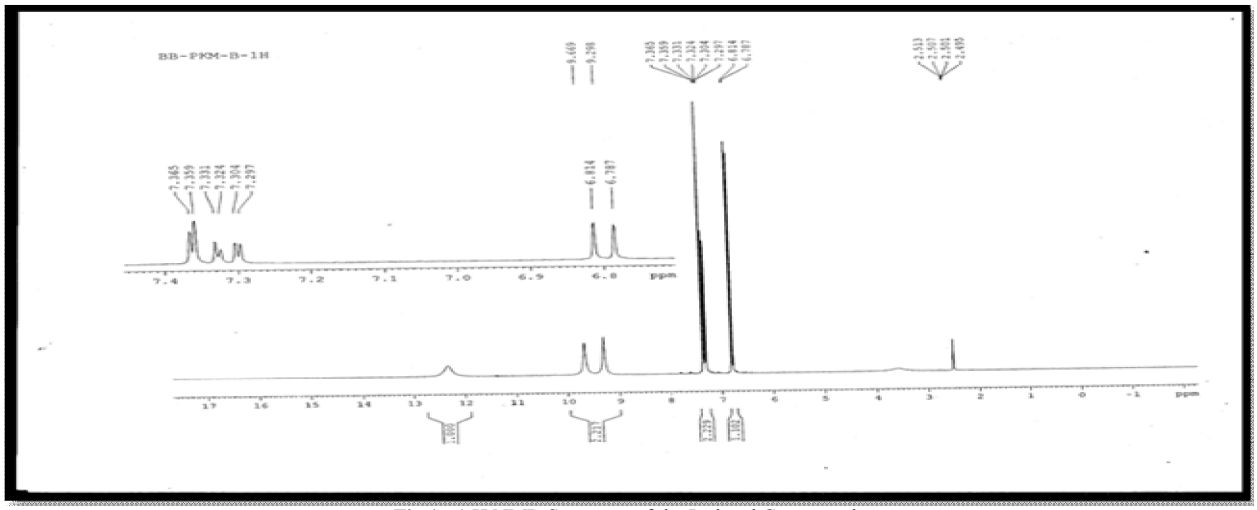


Fig 1: 1 H NMR Spectrum of the Isolated Compound

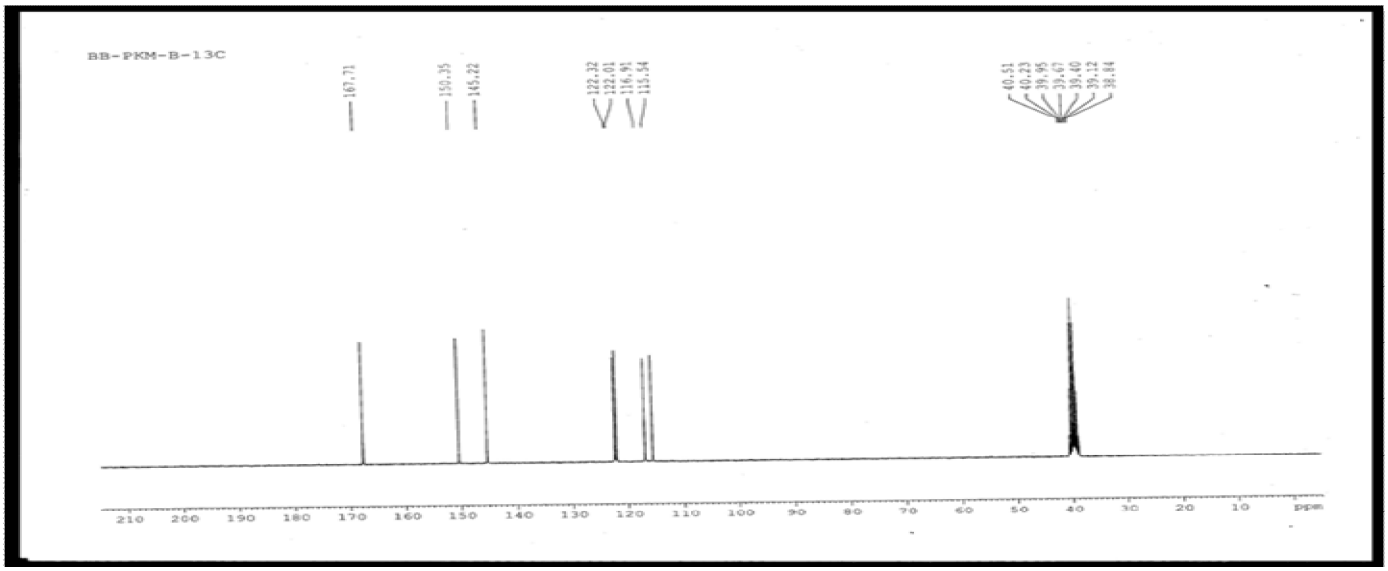


Figure 2. 13C NMR spectrum of the isolated compound

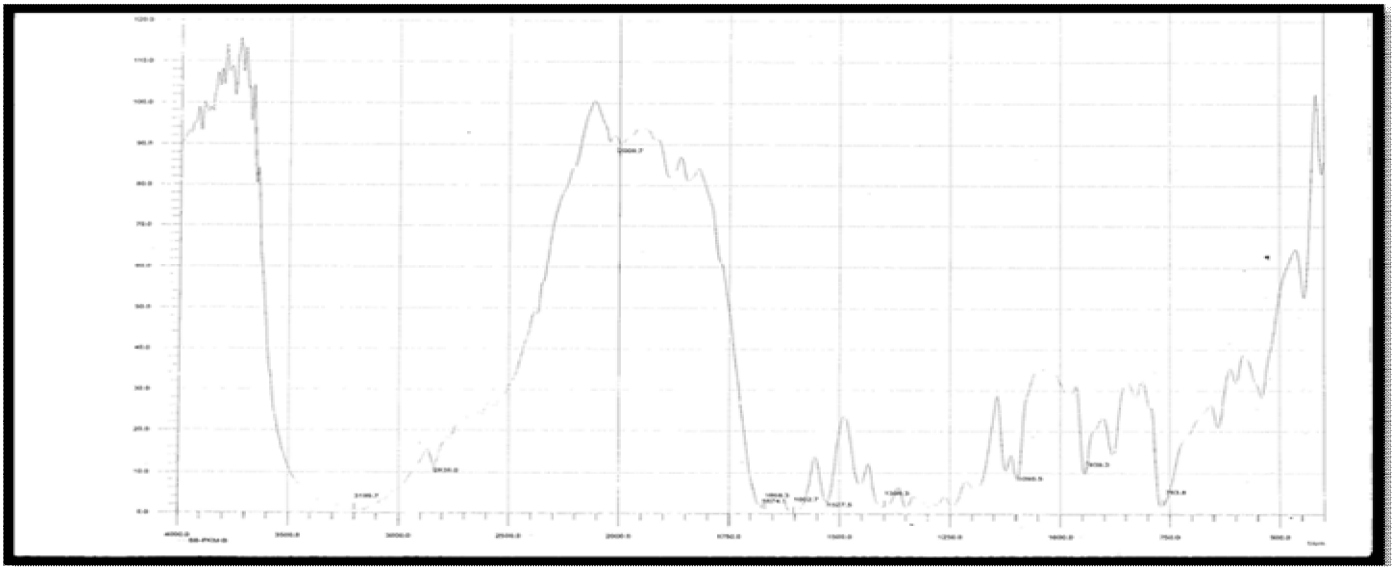


Fig 3: IR spectrum of the isolated compound

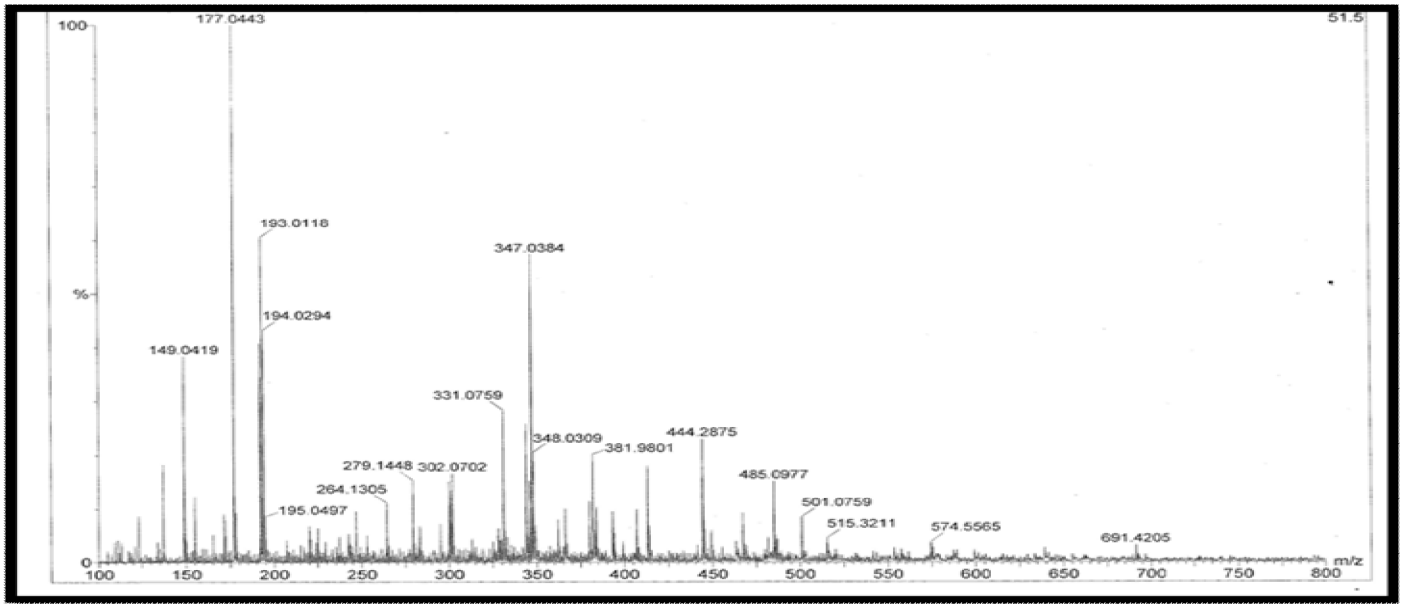
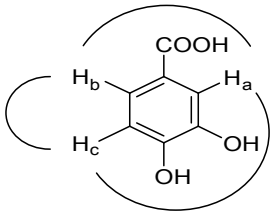


Fig 4: Mass spectrum of the isolated compound

Values and with different splitting patterns. We therefore assign our compound as the compound (5), i.e. 3,4-dihydroxy benzoic acid. The Ar-Hs are indicated as follows:



(5)

H_a is *meta* coupled by H_b with J = 2.1 Hz,
H_b is *ortho* and meta coupled by H_c and H_b respectively with J = 8.1 & 2.1 Hz,
H_c is *ortho* coupled by H_b with J = 8.1 Hz

Fig 5: Spin-spin couplings between hydrogen in the structure of the isolated compound.

Thus, H_a would be coupled by H_b (*meta*, J = 2.1 Hz) and appears as a doublet. H_b would be coupled by H_a (*meta*, J = 2.1 Hz) and by H_c (*ortho*, J = 8.1 Hz) and appears as a doublet of a doublet (dd). H_c would appear as a doublet, only coupled by H_b as *ortho*-couple (J = 8.1 Hz).

In the H-decoupled ^{13}C-NMR spectrum of compound, it is expected that all six aromatic carbons would be magnetically non-equivalent and adding the carboxylic carbon, there should be total seven different carbons. Indeed, there are seven peaks in the H-decoupled ^{13}C-NMR spectrum. The assignment of various carbons may be made based on possible substituent effect: 115.5 (C-5), 116.9 (C-2), 122 (C-6), 122.3 (C-1), 145.2 (C-3), 150.3 (C-4), 167.7 (COOH),

FT-IR: (KBr) ν_{max} 3200, 2839, 1674, 1603 cm^{-1}.

The OH groups and COOH having conjugated with the benzene ring in its IR spectrum are displayed at ν_{max} 3200 and 1674 cm^{-1}, respectively, while the benzene ring double bonds showed absorption at 1603 cm^{-1}. Such decrease in absorption frequency for the carboxyl carbonyl function is acceptable because it is conjugated to the benzene ring.

HRMS: The exact mass for compound with mf $C_7H_6O_4Na$ [M^+Na] calculated to be 177.0164 and observed as 177.0443 again confirm for the compound. Hence the structure of Compound is 3, 4-dihydroxybenzoic acid.

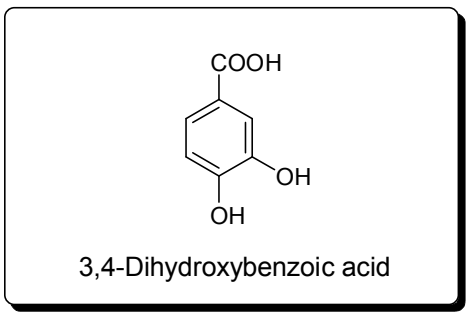


Fig 6: Name and structure of the isolated compound

Phytochemical studies of *Ageratum conyzoides* L. confirmed presence of various chemical compounds like 2,2-dimethylchromene-7-*O*-glucopyranoside, 6-(1-methoxyethyl). 7-methoxy-2,2-dimethylchromene, 7-methoxy2,2-dimethylchromene, encecalin, dihydrodemethoxyencecalin, demethoxyencecalin, 6-vinyl-7-methoxy-2,2-dimethylchromene, 6-(1-ethoxyethyl). 7-methoxy-2,2-dimethylchromene, 6-angeloyloxy-7-methoxy-2,2-dimethylchromene, dihydroencecalin, demethylencecalin as well as 2(1-oxo-2-methylpropyl.-2- methyl-6,7-dimethoxychromene, 6-(1-hydroxyethyl)-7-methoxy-2,2 dimethylchromene, mixture of encecanescins, benzofuranderivatives, 2(2'-methylethyl)-5,6dimethoxybenzofuran, 14-hydroxy-2H, β-3-dihydroeuparine, chromone derivatives, 3-(2-methylpropyl)-2-methyl-6,8-dimethoxychrom-4-one and 2-(2 methylprop-2-enyl.-2-methyl-6,7-dimethoxychroman-4-one[5-7].

Ageratum conyzoides L. also contains scutellarein-5,6,7,4-tetrahydroxyflavone, quercetin, quercetin-3-rhamnopiranoside, kaempferol, kaempferol -3-rhamnopiranoside and kaempferol 3,7-diglucopiranoside, phytol, sesamine, fumaric acid, caffeic acid etc[8-10].

In the present study a compound was isolated from the leaves of *Ageratum conyzoides* L. Characterization of the compound indicated that the compound was 3,4 dihydroxy benzoic acid

4. Conclusion

A compound was isolated from the leaves of *Ageratum conyzoides* L. From spectral data the compound was characterized as 3, 4 dihydroxy benzoic acid. In the list of phytochemicals present in Ageratum *conyzoides* L. 3, 4 dihydroxy benzoic acid was, thus, included.

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