Prasanta Kumar Mitra. *et al*. / *International Journal of Pharmacy & Therapeutics*, 7(2), 2016, 57-63.

e- ISSN 0976-0342
Print ISSN 2229-7456

International Journal of Pharmacy & Therapeutics

Journal homepage: www.ijptjournal.com

IJPT

ISOLATION AND CHARACTERIZATION OF A COMPOUND FROM THE LEAVES OF *ARTEMISIA VULGARIS* L.

Prasanta Kumar Mitra[1*], Tanaya Ghosh[1], Prasenjit Mitra[2*], Chandan Sarkar[3], Sumanta Gupta[4], Basudeb Basu[4]

[1]Department of Medical Biotechnology, [2]Department of Biochemistry, Sikkim Manipal University, Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim, India.
[3]Department of Biochemistry, Calcutta Medical College, Kolkata, West Bengal, India.
[4]Department of Chemistry, University of North Bengal, Raja Rammohanpur 734430, Siliguri, West Bengal, India.
[*]Department of Biochemistry, All India Institute of Medical Sciences, Jodhpur, Rajasthan, India.

ABSTRACT

By solvent extraction, acid hydrolysis, chromatography followed by crystallization, a compound was isolated from the leaves of *Artemisia vulgaris* L. Infra red spectroscopy, mass spectroscopy and nuclear magnetic resonance studies revealed that the isolated compound was chemically 3,4 dihydroxy cinnamic acid.

Key Words:- *Artemisia vulgaris* L., Chromatographic techniques, 3,4 dihydroxy cinnamic acid.

INTRODUCTION

Titeypati (*Artemisia vulgaris* L.) is a perennial shrubby aromatic plant throughout the hills of India. It belongs to the family Asteraceae and is abundant in Sikkim and Darjeeling Himalayas in the middle and upper hill forest up to the height of 2000- 5000 ft. The plant has different names : Titeypati in Nepali, Tuk – gnyel in Lepcha, Dhama naga in Tibetan, Dona in Hindi, Nagdamini in Bengali, Barha in Sanskrit and Indian worm wood in English. The whole plant has medicinal values. Medical uses of the plant as recorded in Ayurvedic literature are: used as appetizer, cures "kapha", asthma and itching, prevents convulsion. Water extract of the plant is good larvicide like kerosene. It has also feeble insecticidal property. It is antibacterial and antifungal too (Gurung Bejoy, 2002; Chopra Col RN and Chopra IC, 1958).

Corresponding Author

Prasanta Kumar Mitra
Email:- dr_pkmitra@rediffmail.com

We have noted anti microbial property, hepatoprotective activity of Titeypati (Ghosh Tanaya *et al*., 2013; Mitra Prasanta Kumar, 2014a; Mitra Prasanta Kumar, 2014b; Mitra Prasenjit *et al*., 2016; Mitra Prasanta Kumar, 2014c). We also fould that the plant could induce body weight reduction in rats (Ghose Tanaya *et al*., 2013).

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

MATERIALS AND METHODS

Plant Material

Artemisia vulgaris L. leaves 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 of Medical Biotechnology, Sikkim Manipal Institute of Medical Sciences of Sikkim Manipal University, Gangtok, Sikkim, India for future references. Leaves were shade dried and powdered. The powder was used for extraction and isolation studies.

Extraction and Isolation

First step: 100g of the powder were extracted with 500 ml methanol- water mixture (1:1 v/v) for 30 min at 37 ± 2^0C 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 silicic acid as adsorbent. Six bands were separated. Elution was done by methanol-chloroform mixture (60:40, v/v).

Fourth step: Eluted fourth band was evaporated to dryness. Dry brown mass was obtained. It was extracted with 10 ml n-hexane for 5 min on a rotary shaker.

Fifth step: The n-hexane extract was further subjected to column chromatography using silica gel mesh (200-400 size) as adsorbent. Four fractions were separated. Elusion was done by ethyl format: formic acid mixture (1:1, 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 (80:20, v/v) mixture from the brown mass. A compound was crystallized. Yield was 5.8 mg.

Homogeneity of the active compound

This was ascertained by silica gel- G thin layer chromatography by using the following solvent systems; Methanol : chloroform : water - 80 : 10 :10; Ethanol : chloroform : water - 60 : 20 : 20; Chloroform : methanol - 80 : 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 were obtained.

Structure elucidation

The compound was a pale yellow solid, mp. 218-221 ^{o}C. 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.

From ^{1}H-NMR spectral data (Figure-2), it appeared that there were three aromatic protons, two olefinic protons and three broad singlets. The coupling patterns of the aromatic protons primarily 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}HNMR 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 dihydroxy cinnamic acid.

The FT-IR (KBr) absorption maxima, shown in Figure-3, (V_{max}) at 3423, 3179, 1675, 1657, 1603 cm^{-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, as shown in Figure 4, might be proposed :

The ^{13}C-NMR spectral data (Figure-5) 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-dihydroxycinnamic acid) might be ruled out. Out of other four structures, the literature value of the melting point of 3,4-dihydroxycinnamic acid (mp 223-25

^{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 clearly shown in Figure - 6 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 $H^{\square}$ $^{\square}$

Structure of the compound was further corroborated by the High Resolution Mass Spectral (HRMS) data (Figure-7), 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 Figure-8, may be assigned as 3,4-Dihydroxycinnamic acid .

Preliminary phytochemical screening of the methanolic extract of *Artemisia vulgaris* L. showed presence of phenols, flavonoids and saponins (Ashok Praveen Kumar and Upadhyaya Kumud, 2010; Kamarul Haniya A and Pr Padma, 2014). Twenty known flavonoids were isolated from this plant (Lee SJ *et al*., 1998).

Fig. 1. Leaves of Titeypati (*Artemisia vulgaris* L.)

Figure 2. 1 H NMR spectrum of the isolated compound

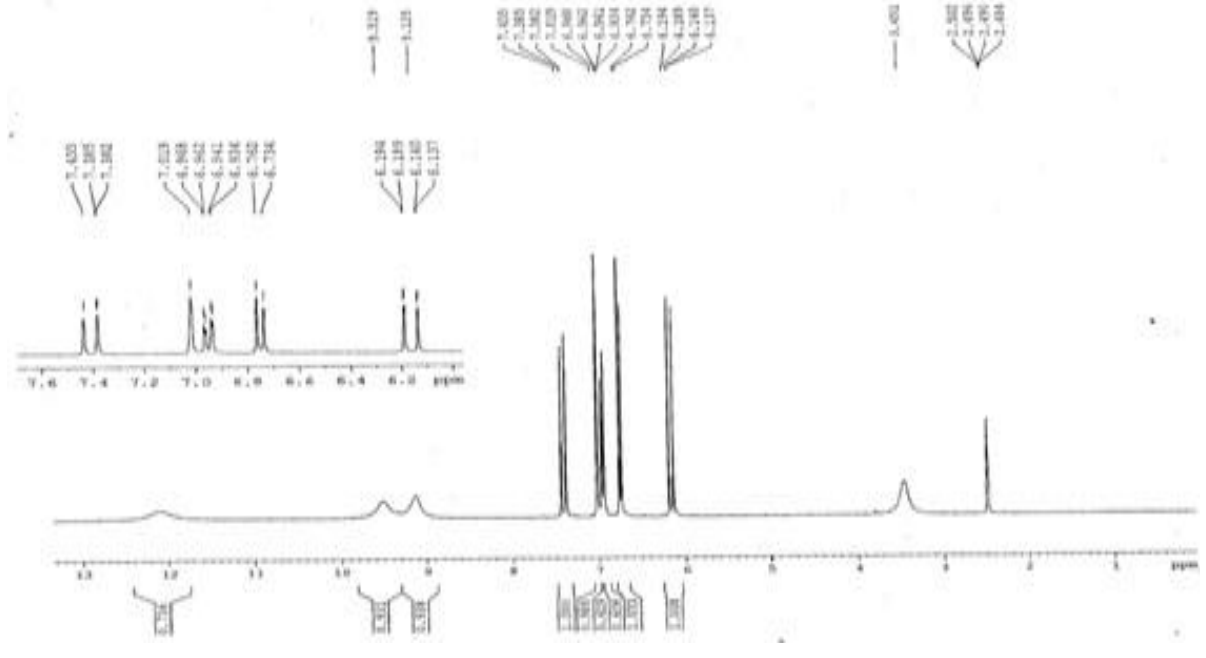


Figure 3. IR spectrum of the isolated compound

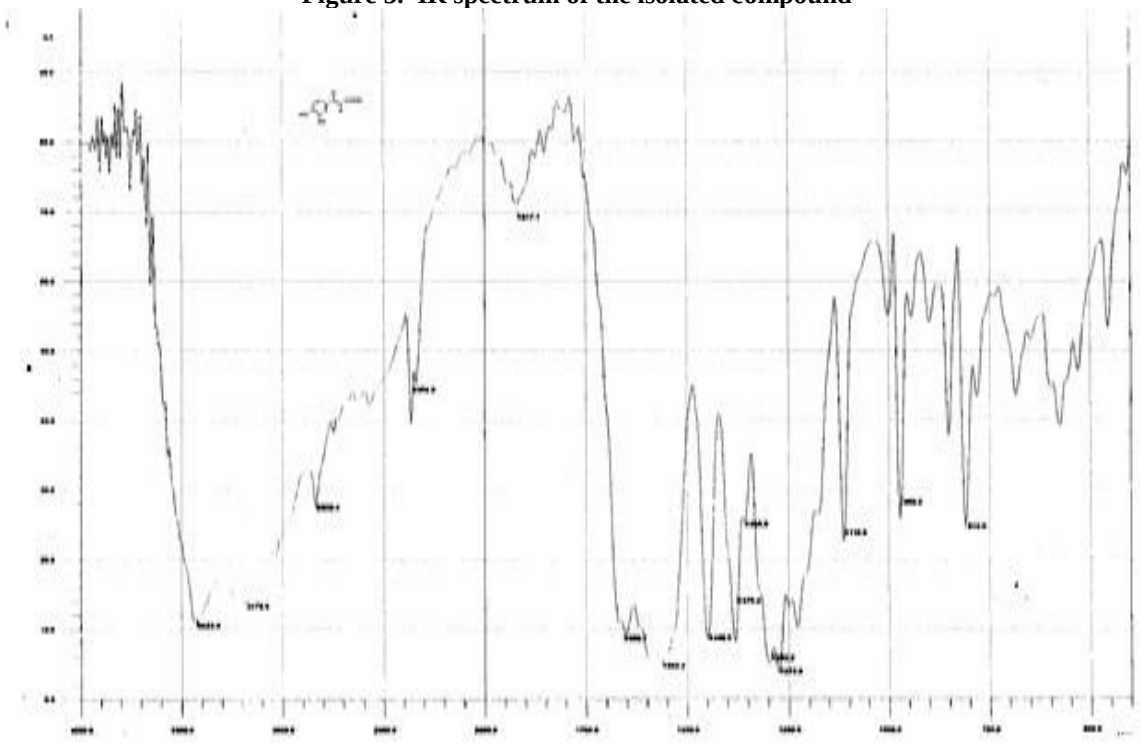


Figure 4. Possible structures of the isolated compound

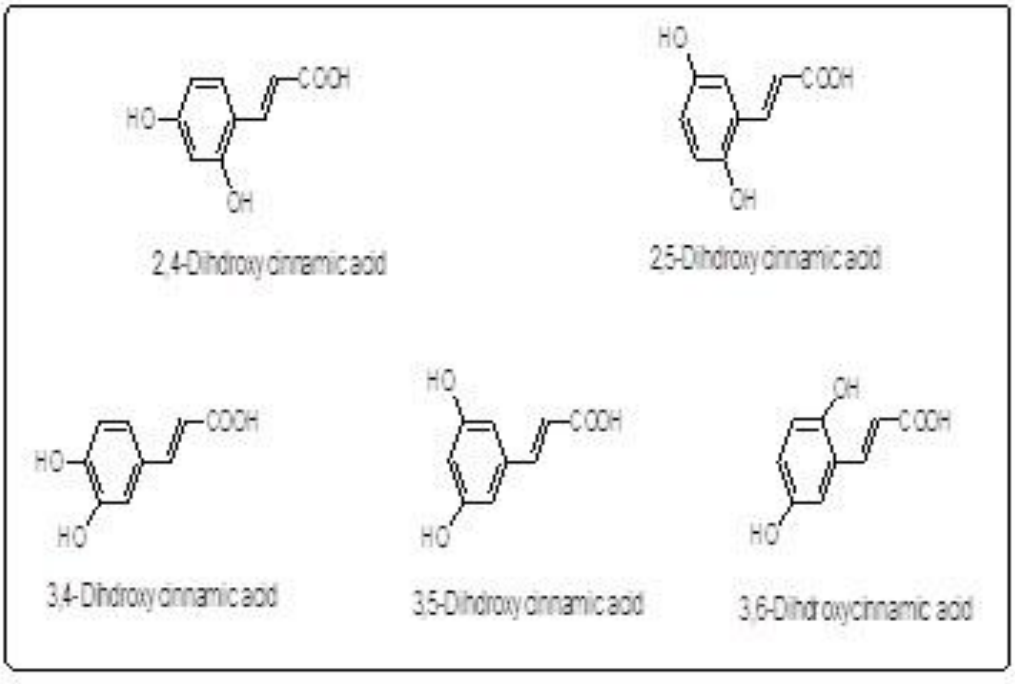


Figure 5. 13C NMR spectrum of the isolated compound

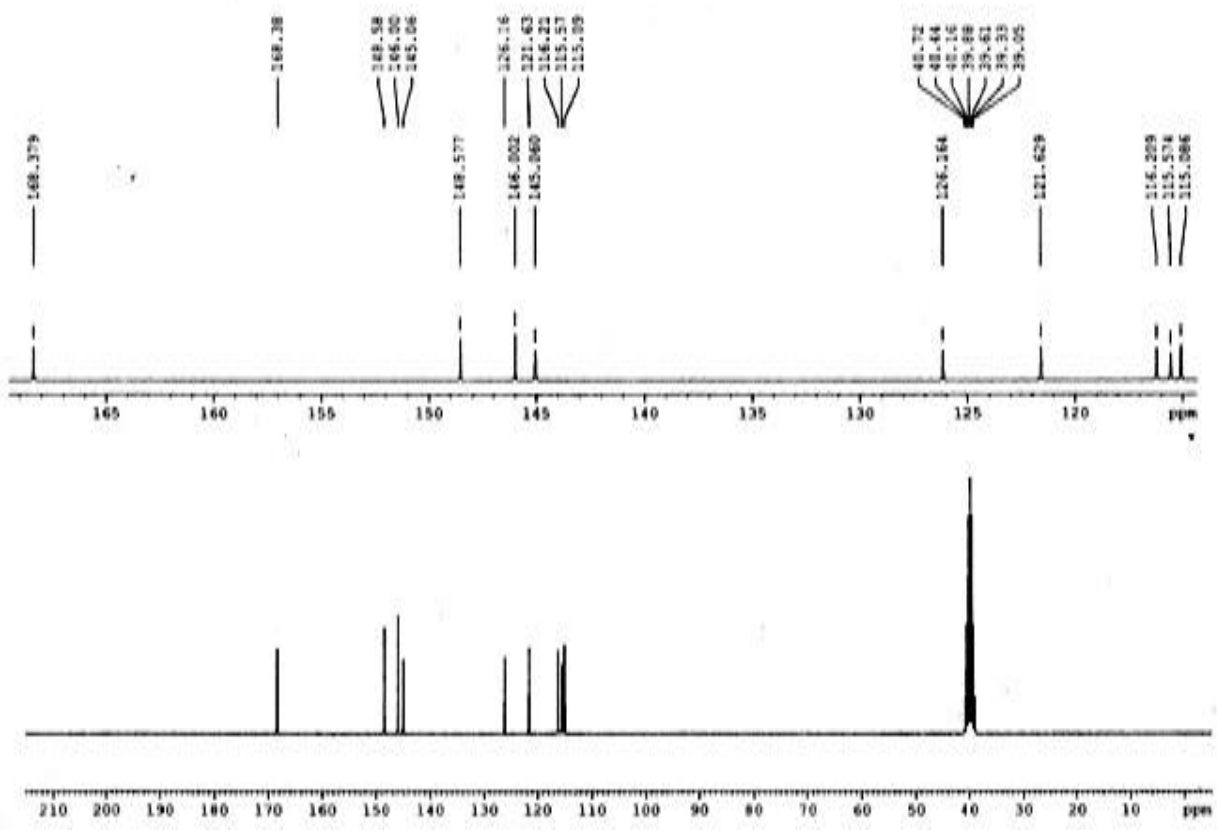


Figure 6. Spin-spin couplings between hydrogen in the structure of the isolated compound.

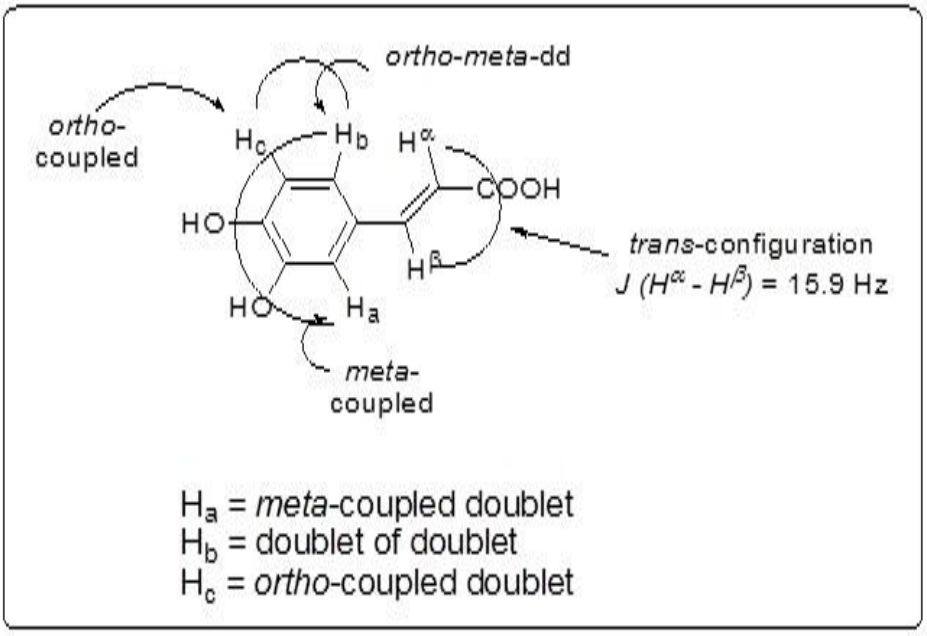


Figure 7. Mass spectrum of the isolated compound

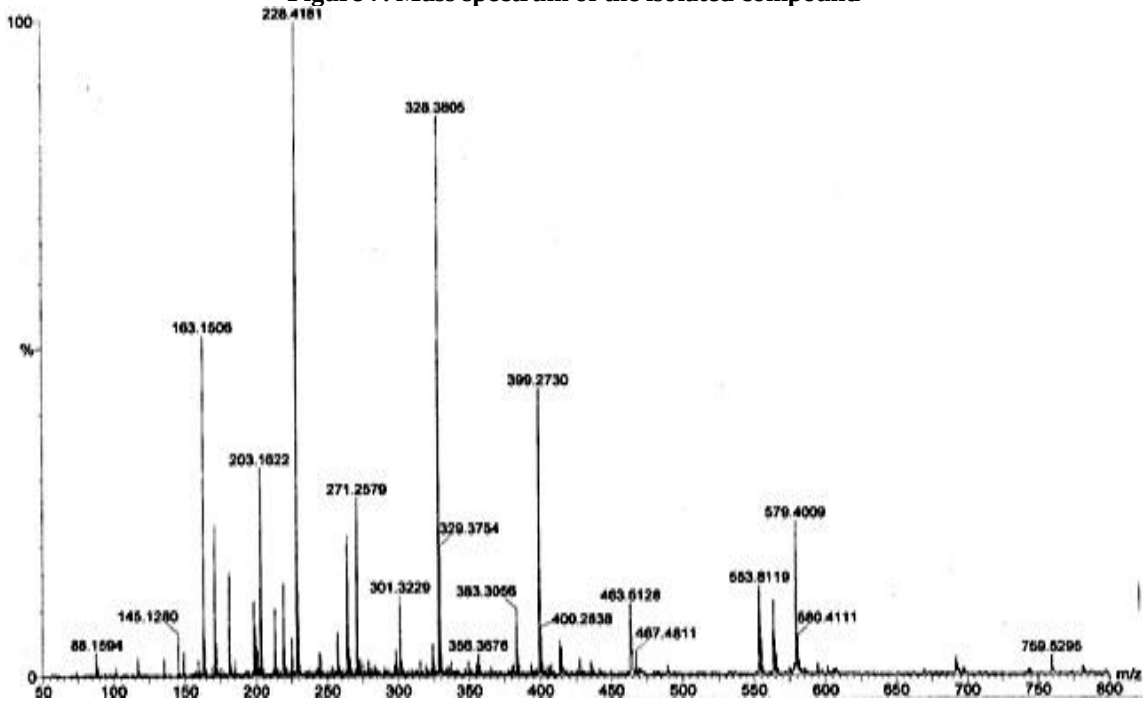
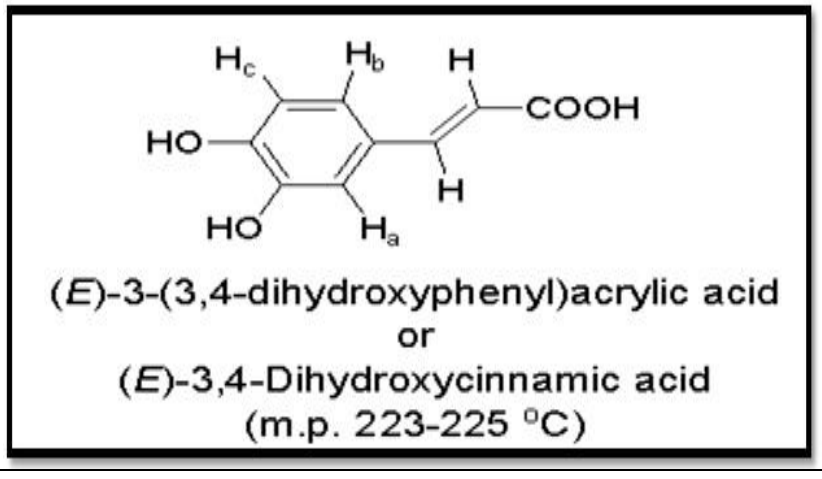


Figure 8. Structure of the isolated compound



CONCLUSION

A compound was isolated from the leaves of *Artemisia vulgaris* L.. From spectral data the compound was characterized as 3,4- dihydroxy cinnamic acid. In the list of phytochemicals present in *Artemisia vulgaris* L. 3,4-dihydroxy cinnamic acid was, therefore, included.

REFERENCES

Ashok Praveen Kumar and Upadhyaya Kumud. Preliminary phytochemical screening and physico-chemical parameters of aerial parts of *artemisia vulgaris. International Journal of Research in Ayurveda & Pharmacy,* 1(1), 2010, 206-211.

Chopra Col RN and Chopra IC. Indigenous drops of India, U. N. Dhar and sons Private Limited, Kolkata, 1958, P. 562.

Ghose Tanaya, Mitra Prasenjit, Mitra Prasanta Kumar**,** Effect of Leaves of *Artemisia vulgaris* L. on Growth of Rats. *Int. J. Herb Med.,* 1(2), 2013, 30-34.

Ghosh Tanaya, Mitra Prasenjit, Mitra Prasanta Kumar, Antibacterial activity of leaves of Titeypati (*Artemisia vulgaris* Linn). *Bioscience Guardian,* 3(1), 2013,107-112.

Gurung Bejoy. The medicinal plants of Sikkim Himalaya, J B Gurung (Pub), Gangtok, Sikkkim, 2002, p. 35.

Kamarul Haniya A and Pr Padma. Phytochemical investigation of methanolic extract of *artemisia vulgaris*. l. leaves. *Int J Pharm Bio Sci*., 5(2), 2014, 184 - 195

Lee SJ, Chung HY, Maier CGA, Wood AR, Dixon RA, Mabry TJ. Estrogenic flavonoids from *Artemisia vulgaris* L. *J of Agri & food chem*, 46(8), 1998, 3325- 29.

Mitra Prasanta Kumar. Antibacterial activity of an isolated compound (AV – 2) from the leaves of Titeypati (*Artemisia vulgaris* Linn.). *Mintage Journal of Pharmaceutical & Medical Sciences*, 3(3), 2014a, 12-14.

Mitra Prasanta Kumar. Antibacterial activity of an isolated compound (AV-1) from the leaves of Titeypati (*Artemisia vulgaris* Linn.). *World Journal of Pharmaceutical Research*, 3(3), 2014b, 4925 – 33.

Mitra Prasanta Kumar. Seasonal variation in antibacterial activity of leaves of titeypati (*Artemisia vulgaris* L.). *J Pharma Biol.*, 4(4), 2014c, 173-176.

Mitra Prasenjit, Tanaya Ghosh and Prasanta Kumar Mitra. Seasonal Variation in Hepatoprotective Activity of Titeypati (*Aartemisia vulgaris* L.) Leaves on Antitubercular Drugs Induced Hepatotoxicity in Rats. *SMU Medical Journal,* 3(1), 2016, 763-774.