SUPPLEMENTARY MATERIAL

Phenyl propanoid rich extract of edible plant *Halosarcia indica* exert diuretic, analgesic, and anti-inflammatory activity on Wistar albino rats

Bhanuvalli R. Shamprasad, Robert Lotha, and Aravind Sivasubramanian*

Natural products and organic synthesis laboratory, Department of Chemistry, School of Chemical and Biotechnology,
SASTRA Deemed To Be University, Thanjavur, India

***Corresponding Author:**

Aravind Sivasubramanian,

Senior Assistant Professor,

Natural products and organic synthesis laboratory,

Department of Chemistry,

School of Chemical and Biotechnology,

SASTRA Deemed To Be University,

Thanjavur, India.

Telephone: +91-9789092505

E-mail: arvi@biotech.sastra.edu

Abstract

Halosarcia indica (*Amaranthaceae*), an Edible food used in Indian traditional folk medicine. The present study aims to evaluate the diuretic, analgesic, and anti-inflammatory properties of *Halosarcia indica* aqueous extract (HAE) on Wistar albino rats. HAE was found to contain chlorogenic acid, sinapic acid, caffeic acid, ferulic acid, scopoletin, quercetin 3-O-β-D-glucoside and β-sitosterol-D-glucopyranoside. HAE increased the urine excreted (Diuretic Index: 1.62-1.96 over 2-10 h) and curbed writhing responses significantly (40%) compared to aspirin (54.56%). It showed significant reduction in carrageenan plantar edema (42%) similar to indomethacin (48%). Anti-inflammatory activity by Cotton Granuloma method proved that HAE significantly reduced weights of pellets (dry weight 44.93 mg, wet weight 127.45 mg) similar to diclofenac sodium (dry weight 33.2 mg, wet weight 123.58 mg). Acute toxicity studies showed HAE to be safe until 2000 mg/kg. The above findings evidently propose that HAE has diuretic, analgesic and anti-inflammatory activity as observed with rodent models.

2. Experimental

2.1 Plant material, isolation and characterization of compounds

Halosarcia indica (*Salicornia brachiata*) were collected from the coast of Bay of Bengal, near Pichavaram, Tamil Nadu, India. *H. indica* is as Halophytic, erect herb 25-45 cm height, branchlets are succulent and much branched, jointed and constricted at nodes, matured stems are yellow in colour. Leaves decussate, obscure, connate in to fleshy cup. Spikes are compact, bracts decussate, fleshy and connate; braceoles are minute which is similar to the reported (Patel 2016). The collected aerial part of the plant was authenticated by Dr. Jayendran, Department of Botany, Government Arts College, Ootacamund, India. A voucher specimen (JSB4113) [the authenticated herbarium sample (5555) was matched with, Regional plant resource center, Puri, India] and then deposited at herbarium collection center, Government Arts College, Ootacamund, India. The aerial parts (~1 kg) were shade dried and were ground to a fine powder. The powder (100 g) was soaked in double distilled water, and the extract after 24 h was filtered through a Whatman No 41 filter paper (repeated three times) and followed by concentration *in vacuo* and the obtained extracts then lyophilized to get a HAE 35g. Then 10 g of HAE was partitioned between water (distilled) and ethyl acetate, the ethyl acetate soluble fraction was carefully separated using a separating funnel. The resulting ethyl acetate fraction was concentrated *in-vacuo* which afforded a residue of 2.5g (yield from HAE=25%). This ethyl acetate residue of HAE was engaged for silica gel column chromatography (100-200 mesh) packed in a glass column (diameter-1 cm x length-10 cm) and eluted successively with chloroform, chloroform-methanol [2, 4,8,16 and 20% (v/v)] and methanol. 2% and 4% methanol-chloroform fractions afforded quercetin 3-O-β-D-glucoside and β-sitosterol-D-glucopyranoside, respectively.

Further HAE (5g) was further subjected to column chromatography with Sephadex LH- 20, as matrix (diameter-1 cm x length-10 cm) and methanol-chloroform as mobile phase [30%-70% (v/v)] which afforded chlorogenic acid, sinapic acid, caffeic acid, ferulic acid and scopoletin respectively.

2.2 Animals

Wistar albino rats (220-250 g) of both sexes were obtained and protocol for the animal experiments was approved by the Institutional Animal Ethics Committee. All the animals were housed in clean polypropylene cages with suitable temperature (20 ± 2 °C), and

12 h light/dark cycle. Animals were fed with commercial rat and mouse food (Amrut Brand, Pranav Agro Industries Ltd., Baroda, India) and water *ad libitum*.

2.3 Acute toxicity

The acute oral toxicity study was performed also in accordance as per OECD-423 guidelines (OECD, 2001). Six groups consisting control, vehicle control and treated groups (5, 50, 300 and 2000 mg/kg body weight) with 3 females in each. Single dose was administered orally to each animal and signs of toxicity, behavior, body weight and feed and consumption patterns of each animal were observed every 5 h on initial day, later every day for 14 days.

2.4 Diuretic test

For the diuretic assessment, the rats were divided into five groups of equal numbers (n=6) and were kept fasted and in water deprived conditions for 18h. Each animal was administered 2.5 ml of 0.9 % NaCl/100 g of body weight (Jadhav et al., 2010) to establish uniformity in diet and salt. HAE was dissolved in 1 % gum acacia (vehicle) and given to different groups at dosage pattern of 100, 200 and 400 mg/kg, respectively. The volume of administration was kept uniform. Control group received vehicle alone as alternative dosage in addition to saline described above. Furosemide (10 mg/k) served as standard administered to one of the groups. The amount of urine excreted by animals was measured after 2,4,6,8 and 10 h of treatment.

2.5 Analgesic test

The analgesic activity of HAE was done by dividing Wistar albino rats into 6 groups (n=6 each). Group A served as control group which received 1% gum acacia (vehicle). Group B, C and D received HAE at 100, 200 and 400 mg/kg of body weight respectively mixed with 1 % gum acacia and administered orally. Group E and F received Aspirin (35 mg/kg) and Morphine (4 mg/kg, s.c) as standard drugs respectively. After 1h of administration, 1 % acetic acid was injected (i.p) and then the animals were observed for their number of writhes (contraction of the abdominal musculature and extension of the hind limbs) over 15 min (Wang et al., 2013).

Inhibition percentage of the writhing was calculated using the following equation

$$\% \text{ Inhibition} = (100\text{-}C_t/C_c) \times 100$$

Where C_t is the number of writhes in the group treated and C_c is the number of writhes in the control group.

2.6 Anti-inflammatory test

2.6.1 Carrageenan induced edema

The assessment of anti-inflammatory activity exhibited by HAE was determined by carrageenan plantar edema test (Guerrero et al., 2013). Six animal groups (n=6) were used and divided. Group A which served as control group received only the vehicle (1 % gum acacia). Group B, C and D received HAE (100, 200 and 400 mg/kg body weight respectively) orally. Group E and F received standard drugs indomethacin 10 mg/kg and diclofenac sodium 6 mg/kg body weight respectively. After 1 h of oral administration, 0.1ml of 1% w/v carrageenan (Sigma) suspension (in normal saline) was injected subcutaneously into dorsal region of sub planar surface of hind paw of rat using 26 G needle. The paw volumes were measured initially before drug administration and after 1, 3 and 6 h using plethysmometer (Ugobasile®, Italy). Change in paw volumes at different time intervals were observed. Percentage inhibition of edema was calculated for each group with respect to its control group based on formula

$$\% \text{ Inhibition} = 100 - [(\text{Experimental paw thickness}/\text{Control paw thickness}) \times 100]$$

2.6.2 Cotton pellet induced inflammation test

Cotton pellets (50±1 mg) were sterilized at 121°C, 15 psi pressure using an autoclave. An incision was made at the dorsal side of the rats, after applying local anesthesia. Then, the cotton pellets were implanted subcutaneously on the dorsal side. HAE at 100, 200 and 400 mg/kg respectively) or standards (indomethacin10 mg/kg or diclofenac sodium 6 mg/kg body weight) were administered continuously for 7 days, followed by which the animals were sacrificed and granuloma was dissected out and weighed (wet), dried in the oven at 60°C for 24 h and again weighed (dry).

2.7 Statistical analysis

All the data were analyzed with the Graph Pad Prism Software. When required, comparison between two groups was made using one way ANOVA test followed by a Turkey test for multiple comparisons. The data are represented as Mean ± Standard Mean Error. A p-value less than 0.05 and 0.001 were considered statistically significant.

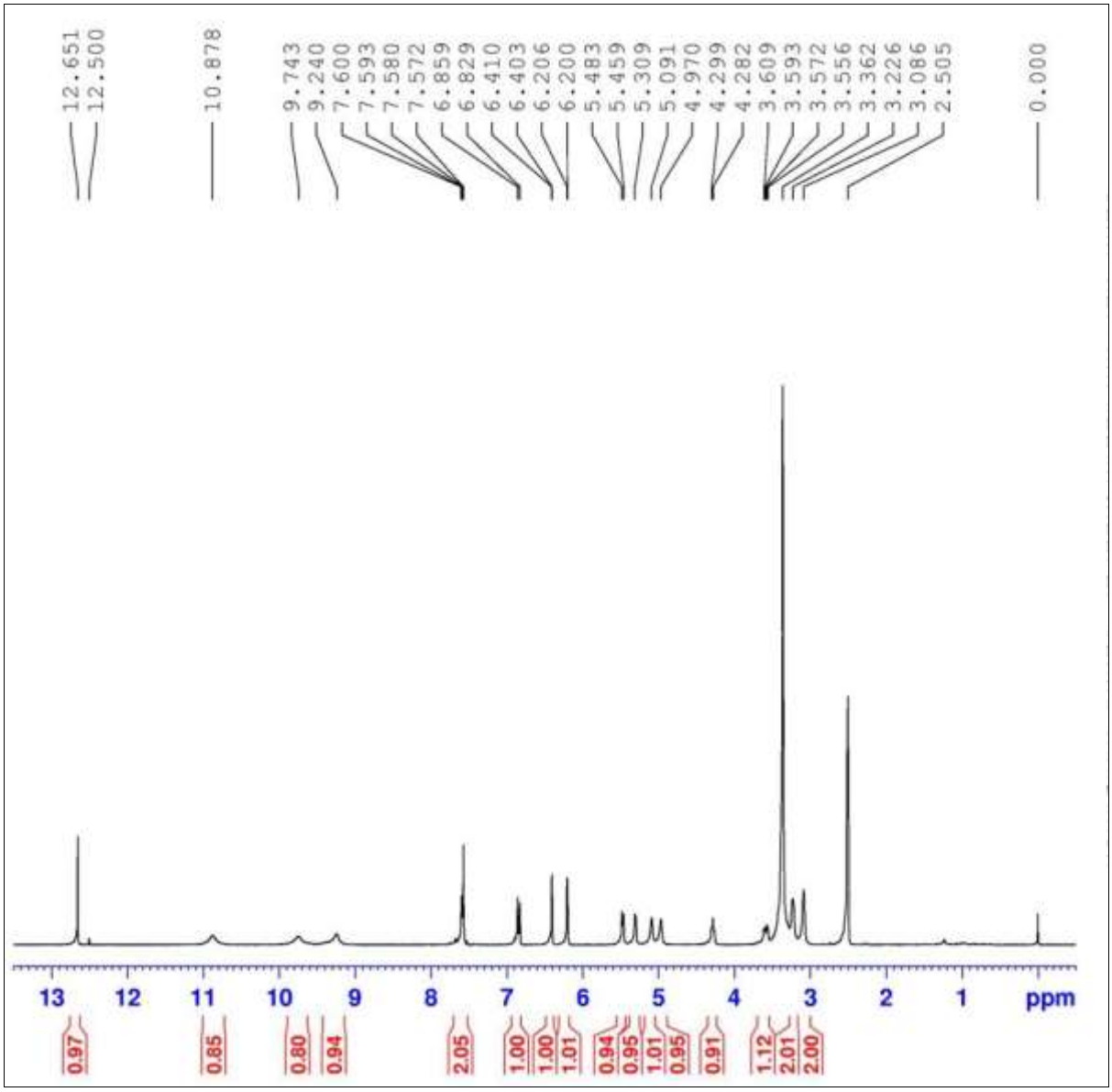

Fig S1: ^{1}H NMR Spectrum of Quercetin-3-O-glucoside

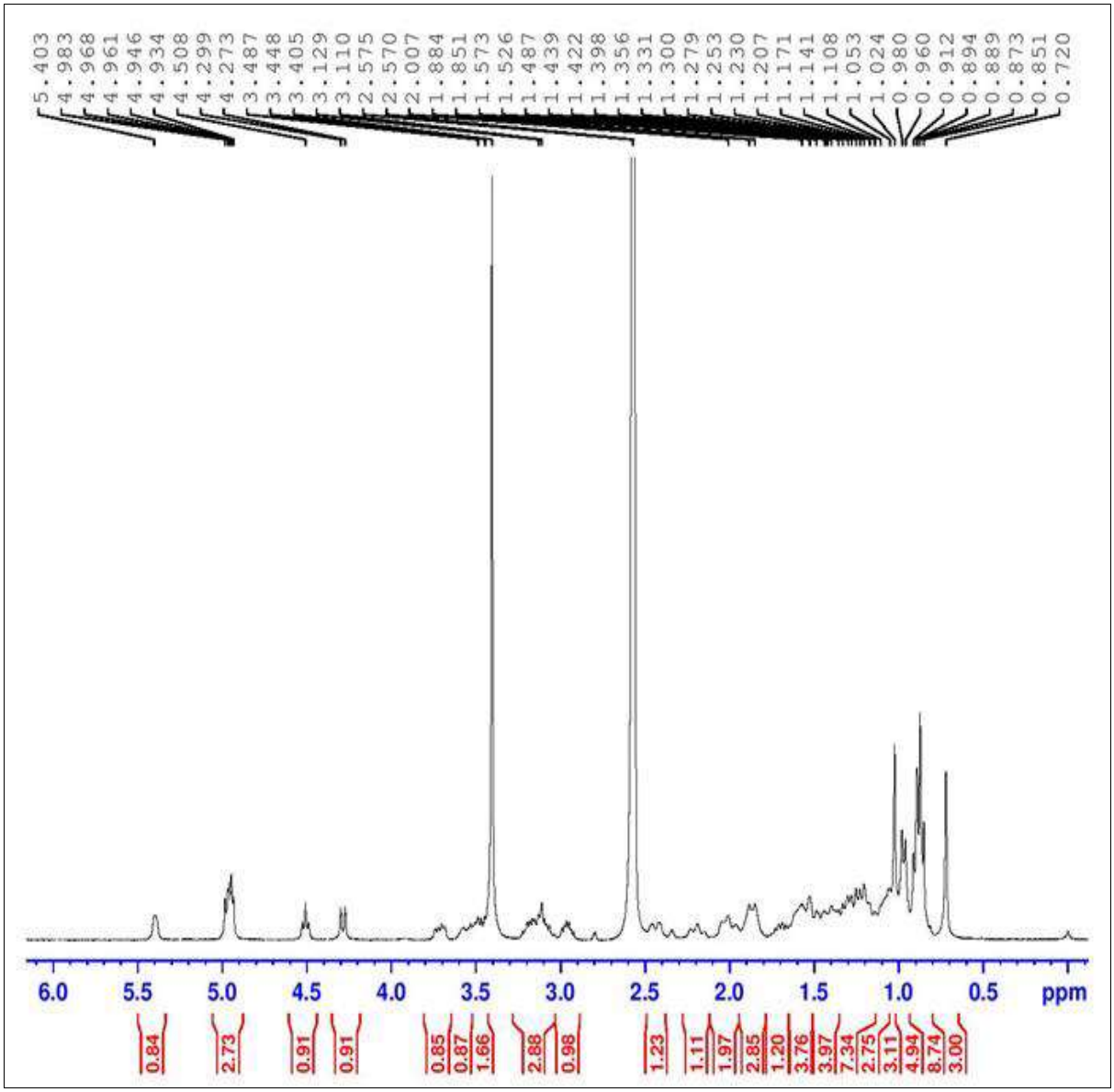

Fig S2- ^{1}H NMR Spectrum of β-sitosterol- β-D-glucoside

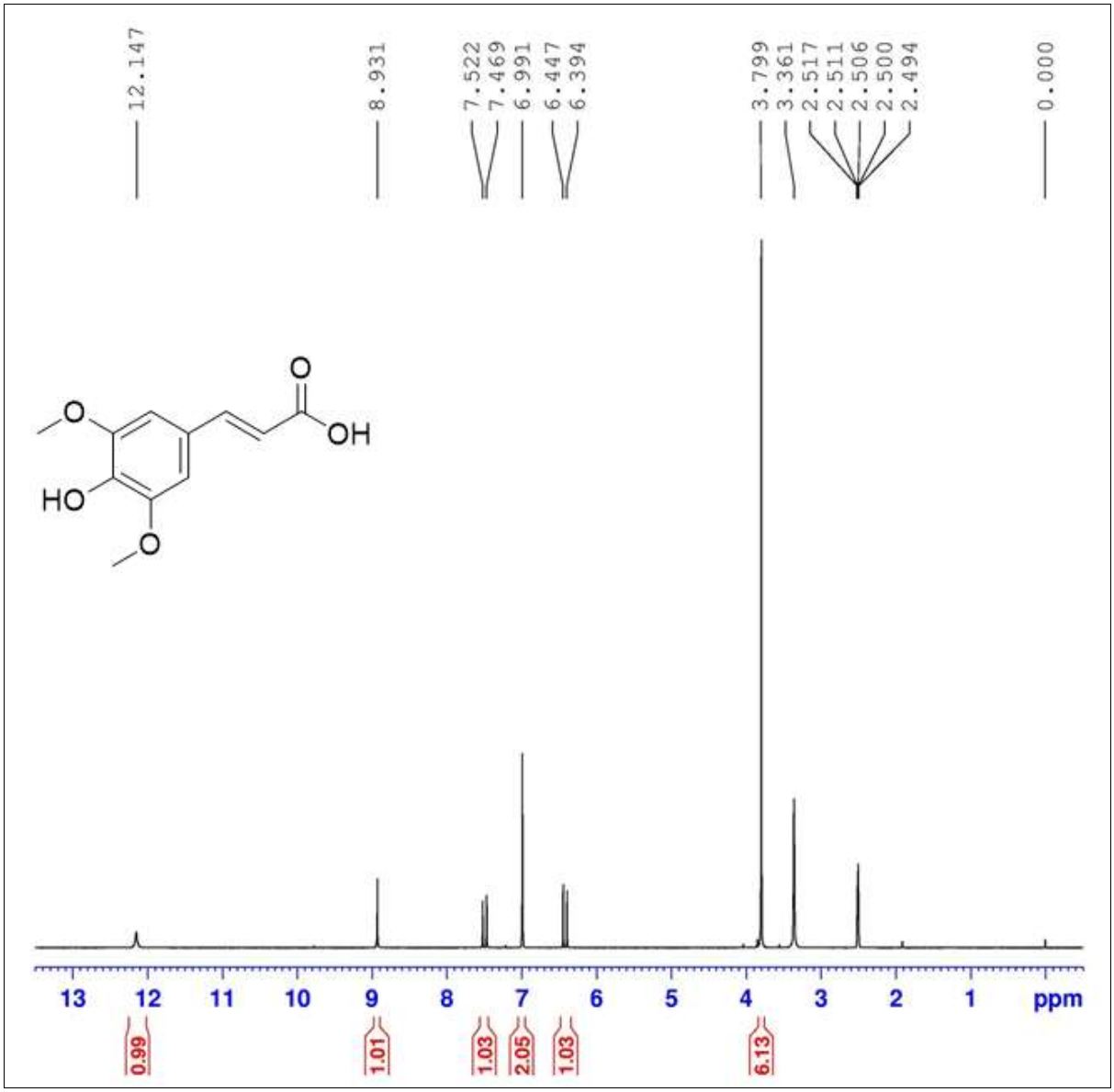

Fig S3- ^{1}H NMR Spectrum of Sinapic acid

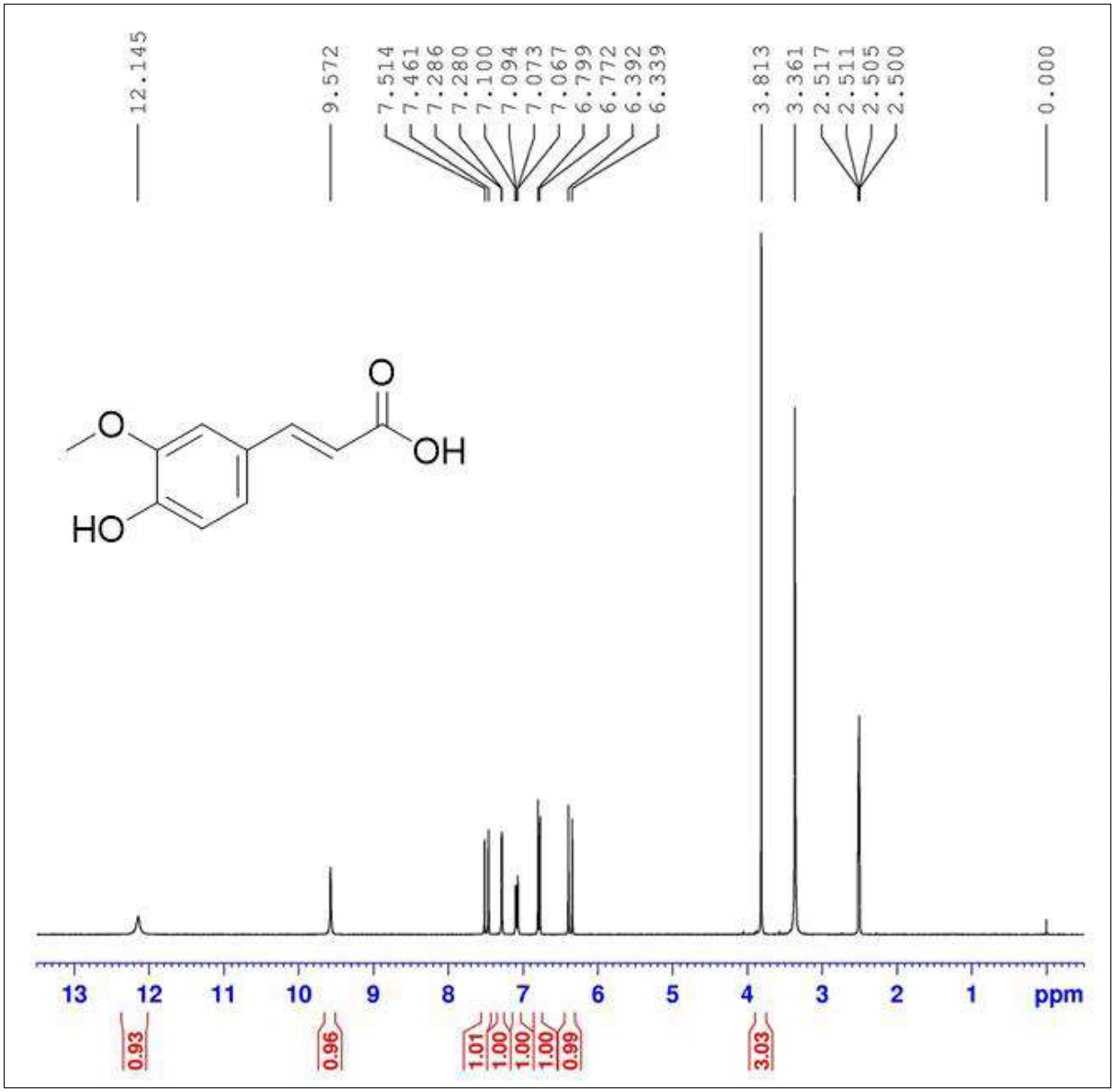

Fig S4- ^{1}H NMR Spectrum of Ferulic acid

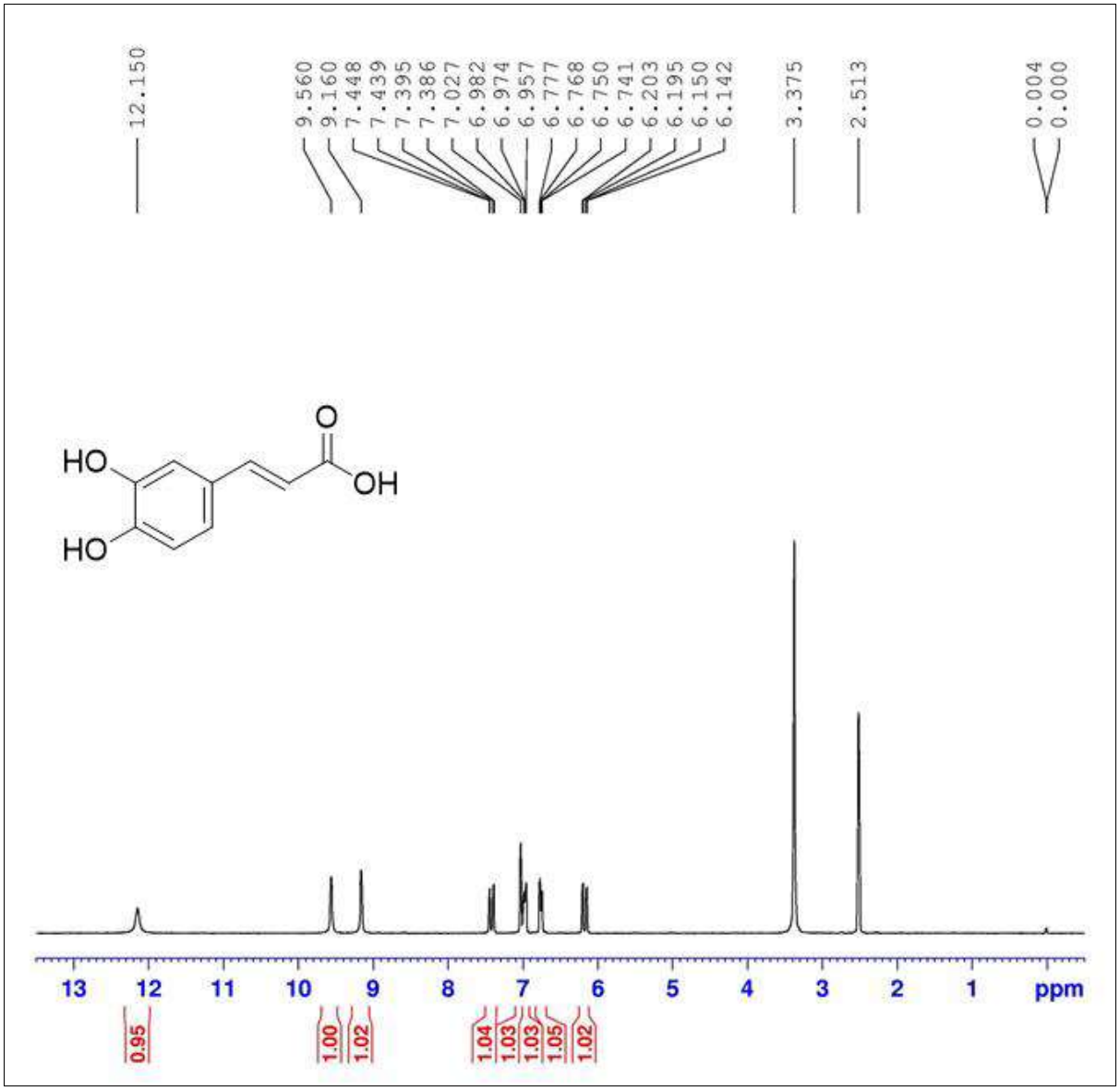

Fig S5- ^{1}H NMR Spectrum of Caffeic acid

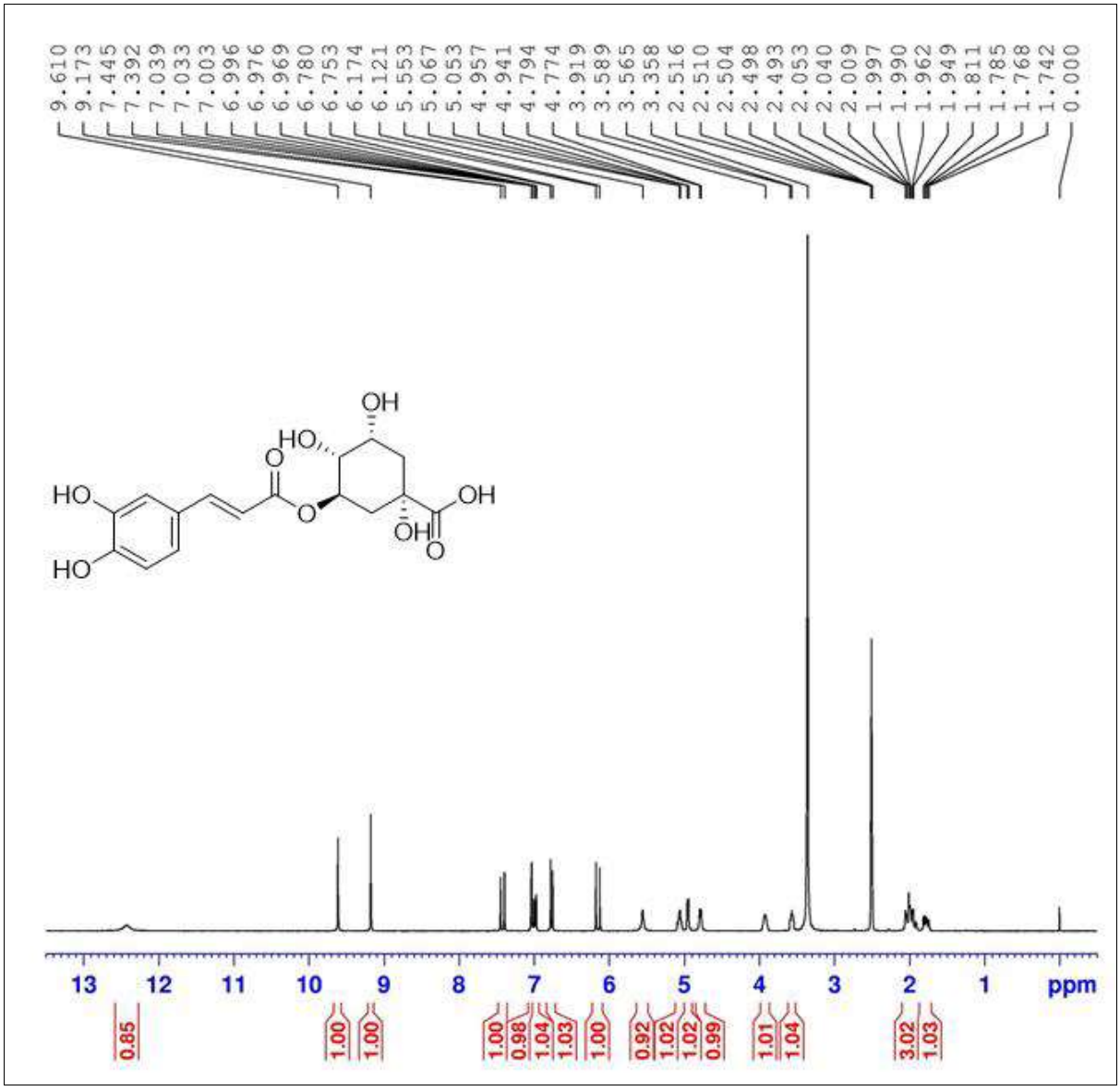

Fig S6- ^{1}H NMR Spectrum of Chlorogenic acid

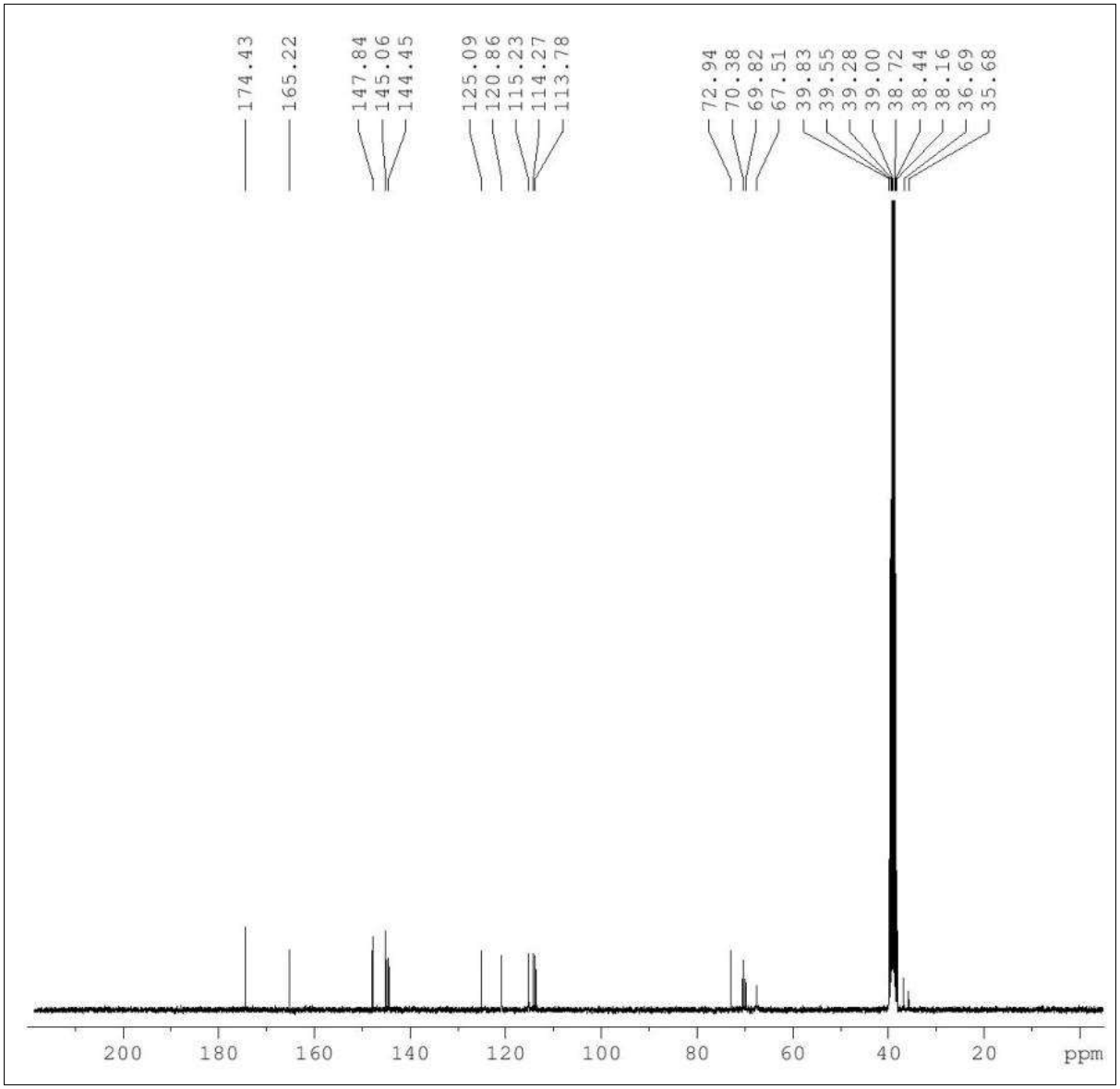

Fig S7- ^{13}C NMR Spectrum of Chlorogenic acid

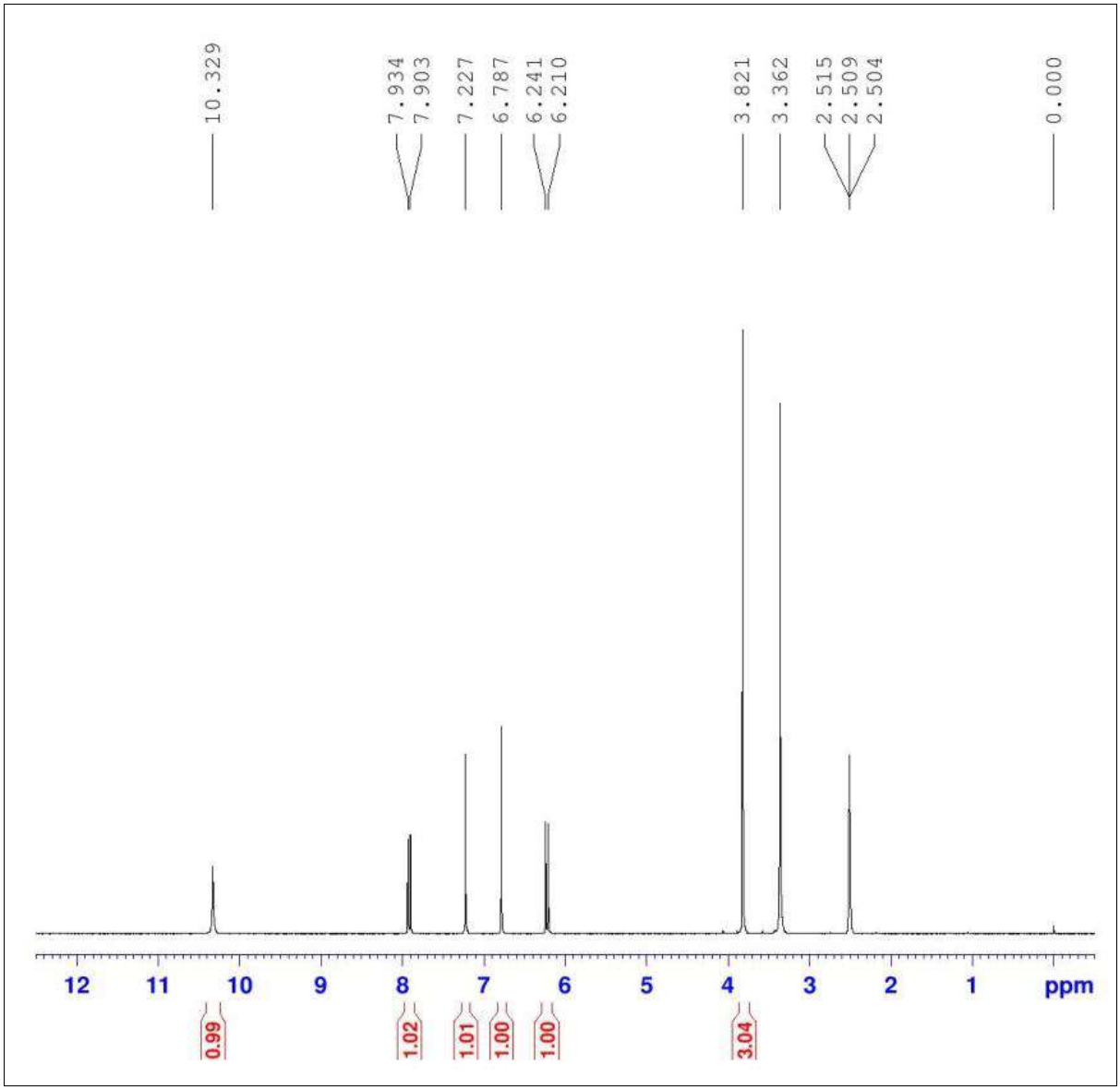

Fig S8 - ^{1}H NMR Spectrum of Scopoletein

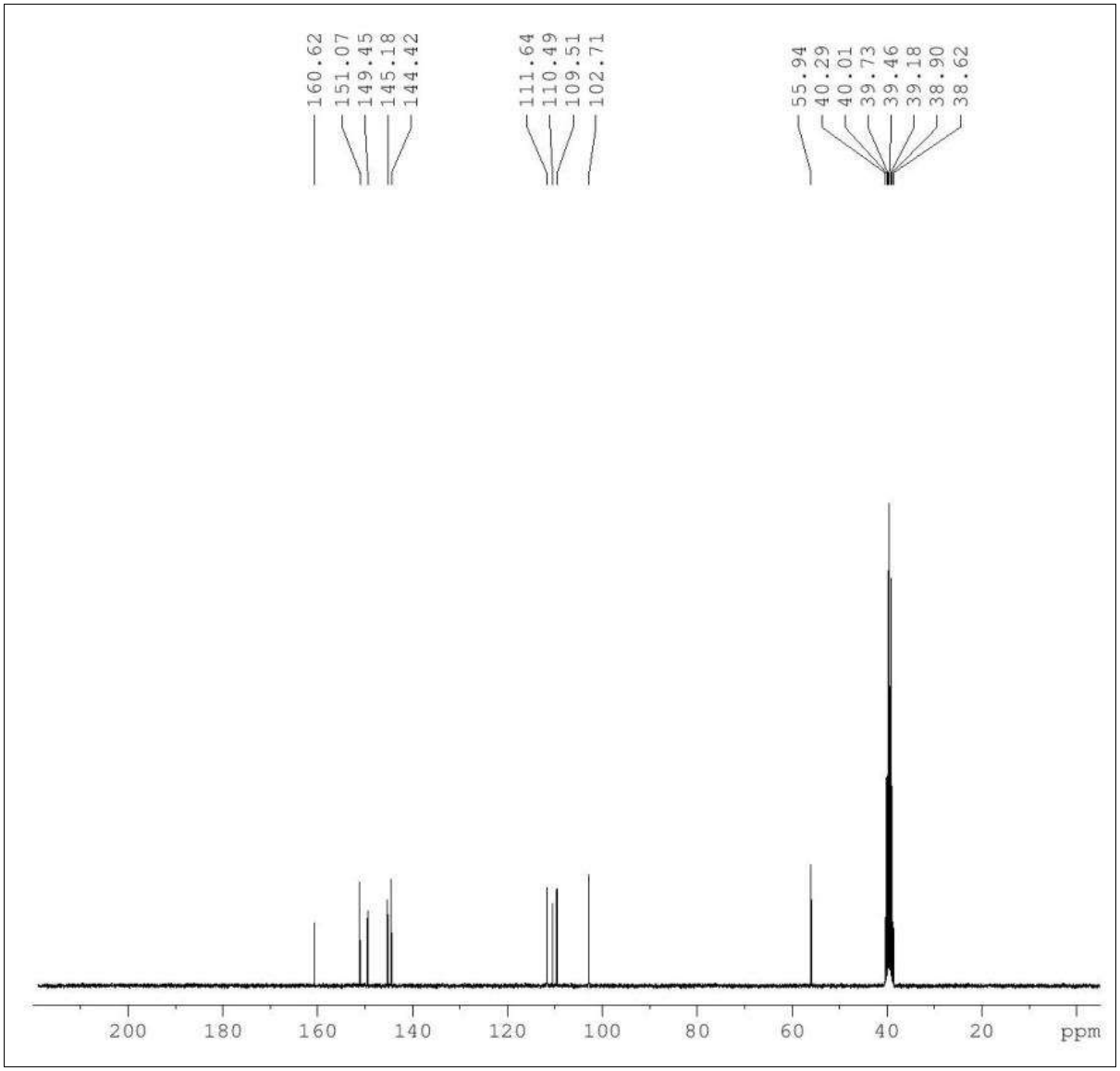

Fig S9- ^{13}C-NMR Spectrum of Scopoletein

References:

Guerrero E, Abad A, Montenegro G, Olmo E, Perez JLL, Feliciano AS. (2013). Analgesic and anti-inflammatory activity of podophyllotoxin derivatives. *Pharm. Biol* 51: 566-572.

Jadhav RB, Bhatnagar SP, Surana SJ. (2010). Diuretic activity of squamate mistletoe, *Viscum angulatum*. *Pharm. Biol* 48: 417-421.

OECD. (2001). Acute oral toxicity method. In: *OECD Guideline For Testing Of Chemicals, No. 423.* Organization for Economic Cooperation and Development, Paris, France

Patel S. (2016). *Salicornia*: evaluating the halophytic extremophile as a food and a pharmaceutical candidate. *3 Biotech* 6: 1-10.

Wang Y, Chen Y, Xu H, Luo H, Jiang R. (2013). Analgesic effects of glycoproteins from *Panax ginseng* roots in mice. *J Ethnopharmacol* 148: 946-950.