Isolation and identification of Ferulic acid from Syrian Urtica pilulifera

Shaza Sitrallah, Joumaa Merza

Abstract— A phytochemical study of Urtica pilulifera from Urticacea family collected in south west of Hama in Syria, led to isolate and identified the Ferulic acid from ethyl acetate extract. The purification, the isolation and the structural identification of this compound was achieved by means of the chromatographic (CC and TLC) analysis and the spectroscopic: nuclear magnetic resonance with different applications (^{1}H-NMR, ^{13}CNMR, COSY, HMQC, HMBC) and FT-IR spectroscopy.

Index Terms— Urtica pilulifera, Ferulic acid, ^{1}H-NMR, ^{13}CNMR, COSY, HMQC, HMBC.

1 Introduction

THE Urticaceae is a common family of plants which produces allergenic substances causing edema and inflammation in humans [1]. It is composed of over 48 genera and nearly 1300 species [2]. The main varieties identified under the Urtica species are Urtica dioica L., U. urens L., Urtica pilulifera L., U. cannabina L., U. membranacea Poiret, U. kiovensis Rogoff L. [2]. Urtica pilulifera L. is a member of family Urticaceae. Urtica pilulifera have been known for a long time as a medicinal plant for treatments of many diseases. Urtica sp. was reported as one of the most effective medicinal plant and widely used in folk remedy to treat hyperglycemia, hypertension and inflammation of some organs such as uvula and uterus, anemia, wound healing and it contains also various constituent's other ingredient as anhydrous lanolin and mint oil that used in preparation of wound–healing antimicrobial ointment [3-6]. Its extract reported as useful for bladder disorder and reduced postoperative blood loss, and prevented hemorrhagic and purulent inflammation following adenomectomy [7]. Therefore, this study will focus on describes the isolation and structural determination of the Ferulic acid from Urtica pilulifera cultivated in Syria.

2 Taxonomic descriptions of Urtica pilulifera

Urtica pilulifera (Roman Nettle) Stinging nettle (Urticaceae) are annual and perennial herbs, a plant with a square leg, its leaves are large serrated in the heart, its thickness and the leg are thick, thin hairs that carry in its bases a liquid composed of several chemicals, most important of which is histamine and formic acid. These capillaries are opened at their pointed tops once The small nettle flowers are colored in clusters that hang down, the seeds are dark yellow, and the two species that spread in the Arab environment are: the small nettle, up to 50 cm high, the large nettle, rising about one and a half meters and the leaves slightly larger than the nettle, but the two types are similar in their chemical components and therapeutic properties [8]. As for its whereabouts, it is found everywhere in the world, and the types of this plant spread in most of the Arab Mashreq countries. The whole herb, including roots and seeds, is harvested from the beginning of July until the beginning of September, and if only the roots are required to be harvested before flowering [9].

Fig. 1. photo of Urtica pilulifera

3 Experimental Procedure

3.1 Plant Material

Aerial parts of Urtica pilulifera, were collected and dried in August 2017, from South-Est of Hama, Syria. The plant was authenticated by the Atomic Agent in Syria. A voucher specimen of plant was deposited in the laboratory of chemistry of natural products, Department of chemistry, Faculty of sciences, AL Baath University, Homs, Syria.

3.2 Plant collection and Extraction method of the compound

The green parts of Urtica pilulifera were collected, and air-dried (500 g) were extracted with MeOH in soxhlet. The extracts were combined and concentrated under low pressure to give 32 g of extract I. The residue from extract I. was extracted once with C_6H_{14} Then extracted with CH_2Cl_2 and finally extracted with ethyl acetate concentrated under vacuum to give 4 g were loaded on chromatographic column (2 cm diameter, 120 cm. long) over silica gel (230 – 400 mesh, ASTM). The column has been eluted successively with: n-hexane (200 ml.), and chloroform (200 ml.), and Methanol: chloroform (20: 80, 200 ml.) Ferulic acid: was obtained from the latter fraction

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Methanol: chloroform (20: 80, 200 ml.), purified on preparative TLC by using mixture of MeOH/ $CHCl_3$ (30: 70), to give (35mg, the R_f = 0.65), it is a yellowish crystalline solid, its melting point 170-171°C.

4 RESULTS AND DISCUSSION

4.1 structure identification of Ferulic acid

The structural determination of the of Ferulic acid based on the usual spectral methods. The IR (KBr) cm-1: 3437, 1694, 1277, 1600, 1518. Figure 2

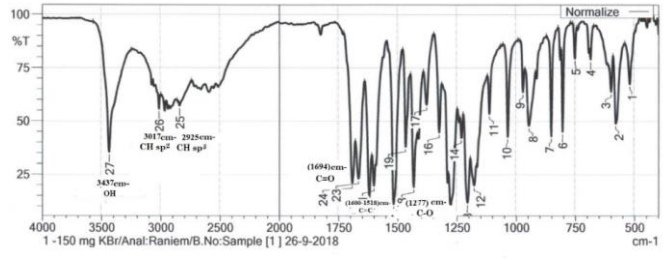


Fig. 2. IR of Ferulic acid in KBr

The ^{13}C-NMR exhibits 10 absorption signals indicating the presence of 10 carbon atoms in the molecule, these absorptions indicate the presence of five Aromatic carbons in the compound 1 (Table 1, Figure 3).

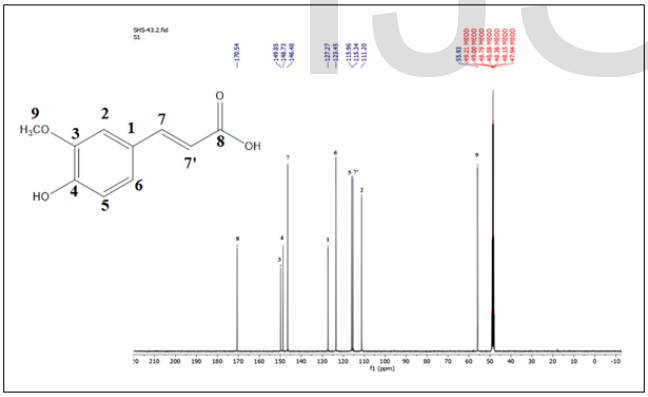


Fig. 3. 13C-NMR Spectrum Ferulic acid (400 MHz, CD_3OD, δ_{TMS} = 0 ppm)

The analysis of the DEPT-135 spectrum, indicate the presence of one primary carbon, 5 tertiary carbons and 4 quaternary carbons (Figure 4).

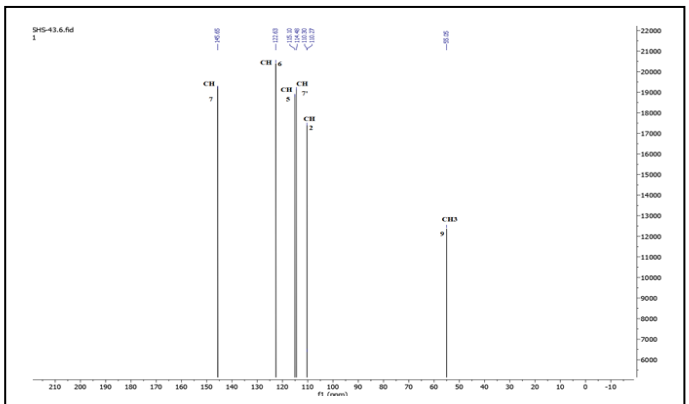


Fig. 4. DEPT- 135 Specter of Ferulic acid (400 MHz CD_3OD, δ_{TMS} = 0 ppm)

The1H-NMR spectrums shows 6 absorptions indicating the presence of 10 hydrogen atoms in the molecule 1: one isolated absorption at 3.81 (3H, s), two olefin protons at (δ_H =6.01ppm, $1H_{7'}$, d, J=15.8 Hz) and at (δ_H =7.30ppm, $1H_7$, d, J=15.8 Hz), and three aromatic protons: (δ_H=6.53 ppm, d, J=8.2 Hz), and doublet of doublet at (δ_H =6.75 ppm, dd, J^3=8.2, j^4=2.0 Hz), and singlet at (δ_H =6.85 ppm, s), see (Table 1, Figure 5).

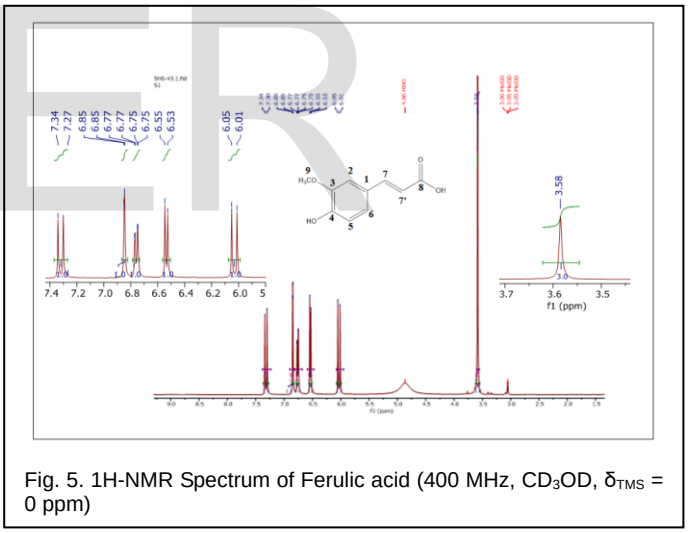


Fig. 5. 1H-NMR Spectrum of Ferulic acid (400 MHz, CD_3OD, δ_{TMS} = 0 ppm)

we can indicate from the COSY spectrum (Figure 6), the presence of one (spin – spin) scaling coupling system between the proton at (δ_H =6.01ppm, $1H_{7'}$, d, J=15.8 Hz) and the proton at (δ_H =7.30ppm, $1H_7$, d, J=15.8 Hz), we can confirm that the two protons are olefin protons in trans position, from the value of the coupling constant (15.8Hz) and the chemical shifts in the 1H-NMR Spectrum [15]. And one (spin – spin) scaling coupling system between the proton at (δ=6.53ppm, H_5) and the proton at (δ_H =6.75 ppm, H_6).

Fig. 6. COSY Spectrum of Ferulic acid (400 MHz, CD_3OD, δ_{TMS} = 0 ppm)

we determinate from the HMQC spectrum, the heteroatom correlations between hydrogen systems and the carbon atoms carrying these protons (Figure 7).

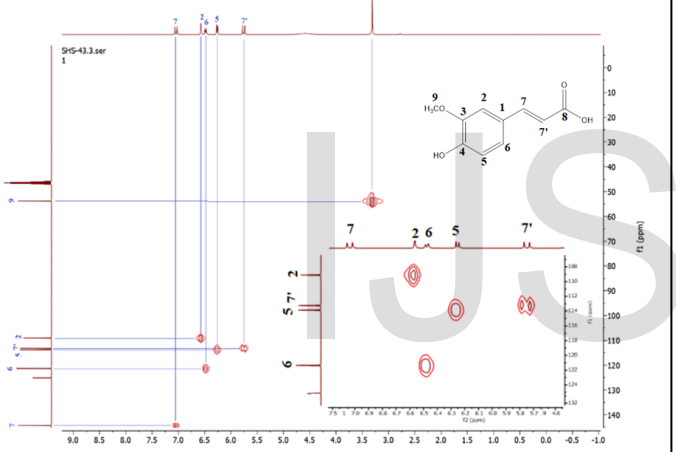


Fig. 7. HMQC Spectrum of Ferulic acid

To determaine the hetero-atomes correlation (J^2, J^3, J^4, J^5) for obtaining the detail of the skelet of compound 1, we analysed the HMBC spectrum (figure8)

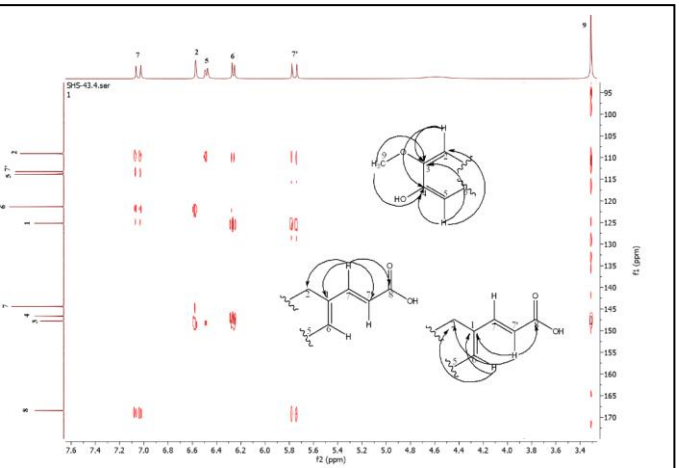
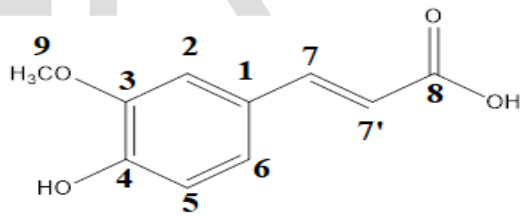


Fig. 8. HMBC Spectrum of Ferulic acid

TABLE 1
DEPT, COSY AND HMBC DATA OF COMPOUND FERULIC ACID

C	^{13}C δ_C (ppm)	DEPT (135-90)	HMQC δ_H (j =H_Z)	^1H-^1HCOSY δ_H (ppm)	HMBC
9	55.93	CH_3	3.81 (s)	--	**C_3, C_4**
2	111.20	CH	6.85 (s)	--	**C_3, C_4**
7'	115.34	CH	6.01 (d, *J* = 15.9 Hz)	7.30	**C_1, C_2, C_8**
5	115.96	CH	6.53 (d, *J* = 8.2 Hz)	6.75	**C_4, C_3, C_2**
6	123.45	CH	6.75 (dd, *J* = 8.2, 2.0 Hz)	6.53	**C_1, C_2**
1	127.27	C	--	--	**--**
7	146.48	CH	7.30 (d, *J* = 15.8 Hz)	6.01	**$C_{7'}$, C_1, C_5, C_6, C_8**
4	148.73	C	--	--	**--**
3	149.85	C	--	--	**--**
8	170.54	C	--	--	**--**

So we can sugest the structure of compound 1 ($C_{10}H_{10}O_3$) as: compound Ferulic acid



3-(4-hydroxy-3-methoxyphenyl) acrylic acid

4.2 Conclusion

In summary, we demonstrated in this article compound identity Ferulic acid which is a new compound. The compound seems a yellow crystalline solid, fully dissolved in ethyl acetate, and R_f account for this compound in a sentence (MeOH: $CHCl_3$) (20:80) was R_f=0.65.

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