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New prenylated flavonoids from the leaves of *Dodonea viscosa* native to the Sultanate of Oman

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ABSTRACT

Traditionally, in Oman, the whole plant is used to treat the body, including treatment of anaemia, arthritis and skin inflammation. Crude extracts were prepared from the leaves of *Dodonea viscosa* (*D. viscosa*) using different polarities of solvents to isolate flavonoid compounds from the highest activity crude extract of the selected plant species collected from AL-Jabal AL Akhdar, Nizwa. The plant samples were collected, processed and extracted with methanol using a hot extraction method. The prepared crude extract was dissolved in water and successively fractioned with different polarities of solvents to produce hexane, chloroform, ethyl acetate, butanol and water crude extracts. The chloroform crude extract was used for the isolation of flavonoid compounds by thin layer chromatography, column chromatography and preparative thin layer chromatography. The free radical scavenging activity of the isolated pure compound and the different polarities of crude extracts were determined using the 1,1-diphenyl-2-picrylhydrazyl method.

The highest antioxidant activity in crude extracts from the leaves of *D. viscosa* was in the hexane and chloroform crude extracts, and the lowest activity was in the water crude extract, followed by hexane > chloroform > ethyl acetate > methanol > butanol > water crude extracts. One new prenylated flavonoid along with one known compound were isolated from the chloroform crude extract of *D. viscosa* and were identified by their chemical structures using mass spectrometry, one and two dimension nuclear magnetic resonance. The isolated pure compound also showed significant antioxidant activity against DPPH. This is the first report of antioxidant compounds in the leaves of *D. viscosa*. The results obtained from this study might be a good natural antioxidant from the selected plant crude extracts.

1. Introduction

In the past, herbal medicines and traditional system have been used to provide a good quality of life for individuals. Herbal medicines are the oldest form of a primary health care system [1]. Human beings use raw plant materials or finished products for the treatment of different diseases. Plant-derived drugs remain an important resource, especially in developing countries, to combat serious diseases. Approximately 60–80% of the world's population still relies on traditional medicines for the treatment of common illnesses [2]. Plant materials and products are safer than pharmaceutical products in addition to being cost effective due to the availability of plants [1]. *Dodonea viscosa* (*D. viscosa*) is an important medicinal plant that belongs to the Sapindaceae family [1,2]. It is a flowering plant and is an evergreen shrub [3]. There are approximately 60 species of *Dodonea* found in the tropical parts of the world, including sandy, rocky or stony soil [3]. *D. viscosa* grows at 1700 m above sea level in Arabian countries [4], 7500 feet in Hawaii and approximately 800 feet in the continental United States [5]. The name is derived from the Flemish botanist Rembert Dodoens, who lived during the 16th century. Viscosa from the Latin name for viscosus, or sticky, refers to the leaves [4]. It has several common name variations from one place to another. In Oman, Dodonea are called Ashshahs [5]. In English, it is called the sticky hop-bush [5]. Japanese people call it hauchiwa-no-ki [4]. In Malaysia, there is also a special name, which includes seringan,

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seringan laut, berteh and letup letup [4]. *D. viscosa* is a medium-size tree that is approximately 2–4 m in height [6]. In some plants, there is only one trunk, and sometimes there are several trunks. Trunks have a reddish-brown to blackish grey colour. Additionally, the branches have a similar colour. The young branches have a green colour [7]. The leaves of *D. viscosa* are different in shape and size. Generally, leaves are long, approximately 1–4 inches [8]. Their flower also varies in colour. It ranges from greenish-white to red or yellow [4]. The plant contains di- and triterpenes, saponins, tannins, flavonoids, organic acids and sterols [9]. The plant showed different types of bioactivity, such as biopesticide, hypolipidemic, antioxidant, antimicrobial, anticandidal, antidiabetic, antifungal and antidiarrheal activities [10]. The leaves of *D. viscosa* are used as a medication for the treatment of itching, swelling, aches, trachoma, gout, bone fraction [3], rash [4,5], and fever [1,3], as well as an antispasmodic agent [1]. The leaves and roots combination is used for the treatment of toothaches, headaches, indigestion, ulcer, diarrhoea and constipation [1–3]. Traditionally, the mixture of leaves, steam and seeds with honey is used to treat malaria [1–3]. The literature search revealed that no work has been done on the Omani *D. viscosa* species. Due to the medicinal value of this plant, it is important to isolate the antioxidant principles of *D. viscosa*, which is abundantly available in Oman, mainly at AL-Jabal AL Akhdar. Therefore, the aim of this study was to prepare crude extracts from the leaves of *D. viscosa* collected from AL-Jabal AL Akhdar and to determine their antioxidant activity as well as to isolate the antioxidant compounds from the active chloroform crude extract.

2. Materials and methods

2.1. Chemicals and reagents

The chemicals used in this study, such as 2,2-diphenyl-1-pikryl-hydrazyl (DPPH), butanol, and ethyl acetate, were obtained from Sigma–Aldrich Company, Germany. Acetone was obtained from Analar Normapur, UK. Chloroform was obtained from Daejung, Korea. Filter papers were obtained from Whatman, France. Hexane 96% was obtained from Scharlau, Europe. Methanol was obtained from Analar Normapur, France. Dimethyl sulphoxide (DMSO, purity 99%) was obtained from Sigma, St. Louis, USA. All of the glassware used in this experiment came from Borosil, India.

2.2. Instruments for sample analysis

The 1D and 2D NMR spectra were recorded on a Bruker (600 MHz) instrument in $CDCl_3$ with TMS as an internal standard (chemical shifts in (ppm)). Mass spectra were recorded on a Waters

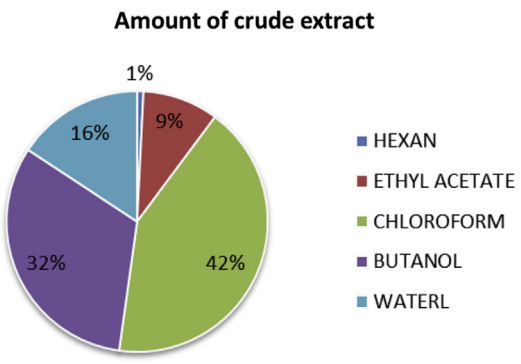

Fig. 1. Amount of crude extract isolated from *D. viscosa*.

Table 1
Antioxidant activity of leaves crude extracts of *D. viscosa*.

Crude extracts	Conc (μg/ml)	Inhibition (%)
Hexane	12.5	70.7
	25	75.0
	50	76.6
	100	93.1
	200	95.4
Ethyl acetate	12.5	69.6
	25	71.4
	50	78.0
	100	91.5
	200	94.3
Chloroform	12.5	63.3
	25	70.2
	100	82.9
	200	94.3
Butanol	12.5	73.2
	25	76.5
	50	77.9
	100	85.7
	200	93.3
Methanol	12.5	67.0
	25	69.0
	50	77.0
	100	93.3
	200	93.2
Water	12.5	68.0
	25	71.4
	50	75.1
	100	93.1
	200	92.2

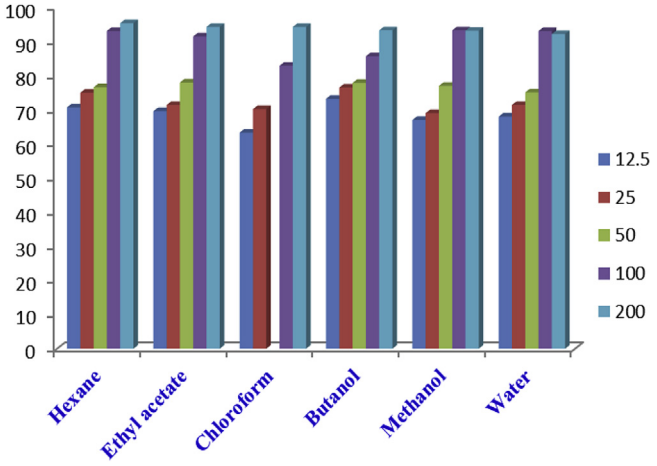

Fig. 2. Antioxidant activity of leaves crude extracts of *D. viscosa*.

Quattro Premier XE Tandem Quadrupole system (Waters, Inc. USA). The electron multiplier (ESI^+) voltage was obtained from autotune. All data were obtained by collecting full-scan mass spectra within a scan range of 50–850 amu. Silica gel GF_{254} was used for the preparation TLC (E. Merck, Germany). Shimadzu UV spectrophotometer, Model: UV-1800, Japan was used for measuring the absorbance of the plant crude samples.

2.3. Sample collection

The leaves of *D. viscosa* samples for this study were collected from Al Jabal Al Akhdar, Oman, during the month of December 2015 at 8 am. The plant was identified using photo images, histological appearance and herbalists among the local people, as well as the voucher specimen (NP. 023). The photos were deposited in the Natural Product Laboratory, University of Nizwa, Oman.

Table 2
ID and 2D NMR ($CDCl_3$) data of compound **1**.

	δ_C	DEPT/HSQC	δ_H	COSY	HMBC
1	179.20	>C<			
2	159.57	>C<			
3	129.94	>C<			
4	152.26	>C<			
5	151.79	>C<			
6	138.33	>C<			
7	154.88	>C<			
9	130.39	>C<			
10	129.94	>C<			
11	129.48	>CH–	6.93 (d, 1H, J = 2.28 Hz, H-5′)	12	15, 2, 21
12	127.89	>CH–	7.92 (dd, 1H, J = 2.28 & 8.63 Hz, H-6′)	11,15	10, 2
13	122.37				
14	121.66	>CH–	5.31 (m, 1H, $-CH_2-CH=$)	21,23	21, 23
15	109.18	>CH–	7.87 (d, 1H, J = 8.7 Hz, H-2′)	12	14, 10, 22
16	106.18				
17	93.07	>CH–	6.52 (s, 1H, H-8)		16, 10, 4
18	60.91	$-OCH_3$	4.01 (s, 3H, $-OCH_3$)		10, 20
19	60.07	$-OCH_3$	3.81 (s, 3H, $-OCH_3$)		7
20	55.52	$-OCH_3$	3.89 (s, 3H, $-OCH_3$)		2, 18/19
21	28.34	$-CH_2-$	3.34 (d, 2H, J = 7.38 Hz, $-CH_2-CH=$)	14,23	14, 8, 2
22	25.84	$-CH_3$	1.75 (s, 3H, $-CH_3$)		14, 8, 23
23	17.79	$-CH_3$	1.71 (s, 3H, $-CH_3$)	14, 22	14, 8, 22
		–OH	12.93 (s, 1H, –OH)		
24	156.59	–COOH			
	133.45	>C<			

Table 3
Antioxidant activity of pure compound isolated from *D. viscosa*.

	Conc (μm/ml)	Absorbance (nm)	Inhibition (%)
Compound **1**	200	0.227	86.21
	100	0.214	86.93
	50	0.209	87.31
	25	0.175	89.37
	Gallic acid	0.155	90.58
	DPPH	1.647	–

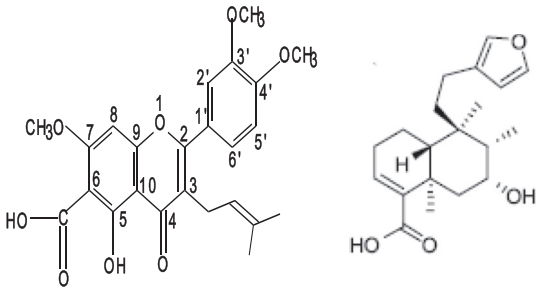

Fig. 3. Structure of compound **1** & **2**.

2.4. Sample preparation

The leaves of the selected plant were washed carefully with water, and the good quality leaves were separated for processing. The washed leaves were dried at 25 °C in the shade. The dried leaves were ground into powder by using a kitchen grinder. Finally, the powder of the leaves was kept in an amber colour bottle for extraction.

2.5. Extraction

The powdered sample of selected plant leaves (252.80 gm) was extracted with methanol (800 ml) by using a hot extraction method for 2 days. The solvent was evaporated from the mixture by using a rotary evaporator at 22 °C under reduced pressure for 6 h. After evaporating the solvent, the crude extract (116.72 gm) was suspended in water (250 ml). Then, the whole extract, including water, was transferred into a separatory funnel and extracted successively with increasing polarities to produce hexane (6.18 gm), chloroform (63.1 gm), ethyl acetate (14.12 gm), butanol (48.13 gm) and water (23.61 gm) crude extracts. The obtained crude extracts were used for determining the *in vitro* antioxidant activity and for the isolation of active plant constituents [11].

2.6. Antioxidant activity

Each crude extract (2 mg) was dissolved by methanol in a 10-ml volumetric flask. The concentration of the prepared solution was 200 μg/ml. Four dilutions, 100, 50, 25 and 12.5 μg/ml, were prepared with methanol by using a serial dilution technique. 1,1-Diphenyl-2-picrylhydrazyl (DPPH, 4 mg) was dissolved in a 100-ml volumetric flask, and the concentration was 40 μg/ml. The volumetric flask was covered with aluminium foil to protect it from the light. The free radical scavenging activity of all of the prepared crude extracts was examined through the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method described by Hossain et al. [12]. Each crude extract (30 μl) was taken separately in a 5-ml test tube, and 1.2 ml of DMSO solvent was added.

The mixture was shaken vigorously, and 2.7 ml of a DPPH solution was added. All of the test tubes were kept in a dark place for 1.5 h to complete the reaction. Finally, the absorbance of each crude extract was measured at 517 nm against methanol as a blank by a UV-visible spectrophotometer. The percent of free radical scavenging activity was calculated by using the formula:

$$\text{Free radical scavenging activity}(\%) = (Abs\ \text{control} - Abs\ \text{sample})/Abs\ \text{control} \times 100$$

2.7. Extraction of antioxidant compounds

The highest activity chloroform crude extract (4.8 gm) was subjected to column chromatography and was eluted initially with

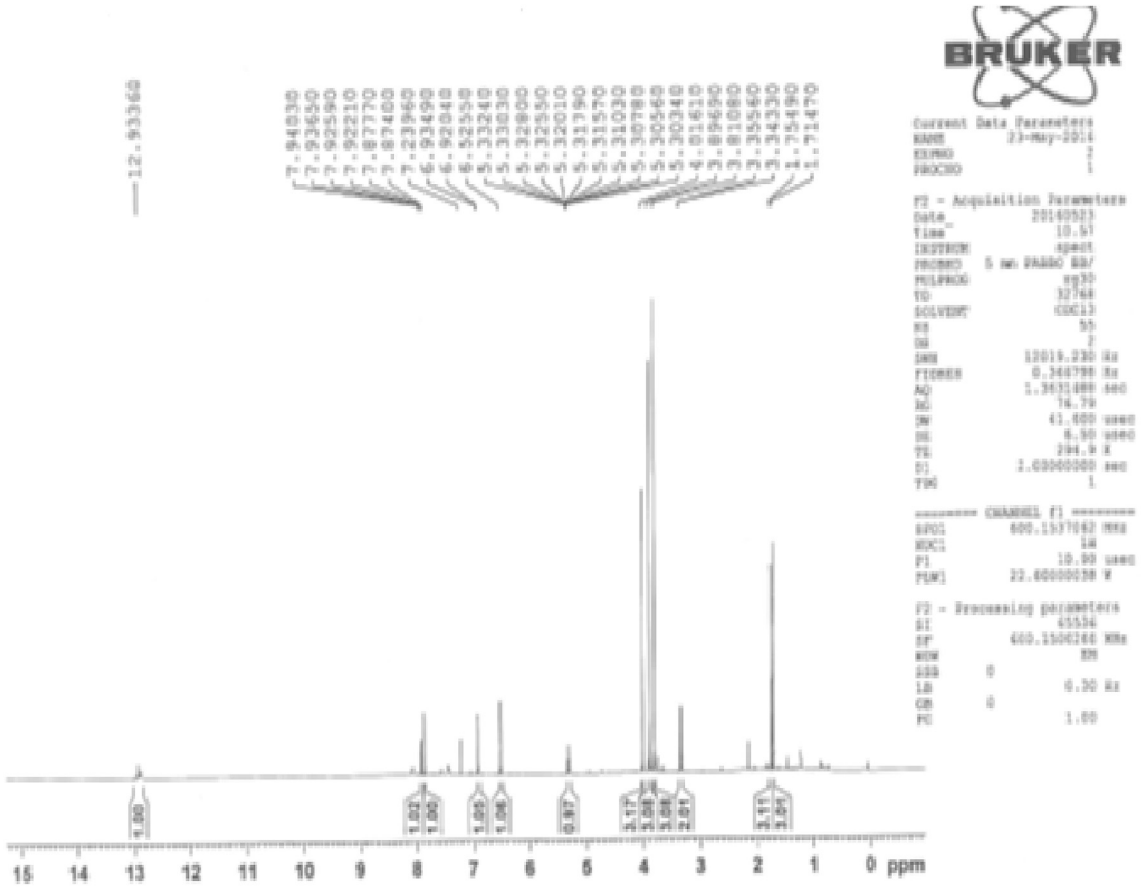

Fig. 4. ^{1}H NMR spectra of compound **1**.

hexane. Then, the mixture solvent hexane: ethyl acetate (2:1) was used as the mobile phase, with an increasing proportion of ethyl acetate. These elutes from the column were collected in a series of test tubes, with 4 ml in each test tube. All of the collected test tubes were examined by TLC. Based on the similar TLC behaviour, these elutes were combined to give Fraction 1, Fraction 2, Fraction 3, Fraction 4, Fraction 5, Fraction 6, Fraction 7 and Fraction 8. The solvent from all of the combined fractions was evaporated at room temperature inside the fume hood.

2.8. Fraction 3

Fraction **3** obtained from the column was further purified by column chromatography to give several major and minor fractions (A-D). Major fraction **C** was further purified by preparative thin layer chromatography (PTLC) to give two compounds (**1** and **2**). Compound (**1**) was collected from preparative TLC at a high amount by a spatula. Compound (**1**) was separated from the silica gel by acetone. The amount of purified compound (**1**) was 13 mg of weight. Finally, compound (**1**) was crystallized from ethyl acetate to give yellow crystals (12 mg), m.p.177 °C: R_f 0.54 (hexane-ethyl acetate; 7:2); M^+, 428; 1D and 2D NMR (600 MHz, $CDCl_3$) (See Table 2 and Fig. 3).

Similarly, compound (**2**) was collected from preparative TLC by a spatula, and compound (**2**) was separated from the silica gel by the same solvent. Compound (**2**) was not crystallized from any solvent to give a pale yellow oil (3.5 mg), R_f 0.61 (hexane-ethyl acetate; 7:2). On the basis of the spectral data, compound (**2**) was identified as dodonic acid [13–15].

3. Results

The leaves of *D. viscosa* are used for the preparation of crude extract and were collected from Al Jabel al Akhtar at Nizwa. Methanol was used for the preparation of crude extract. The crude extract was defatted with water and finally fractionated with different polarities of solvents. The amount of different crude extracts is presented in Fig. 1.

3.1. Antioxidant activity

The free radical scavenging activity of the fractionated crude extracts was examined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method [11]. The highest antioxidant activity was found in the hexane and chloroform crude extracts, and the lowest activity was found in the water crude extract (Table 1 and Fig. 2). The isolated pure compound showed significant antioxidant activity against DPPH (Table 3).

3.2. 5-Hydroxy-7,3′4′-trimethoxy-6-acetoxy-3-prenylflavone (*1*)

A pure compound was isolated from the leaves of the chloroform crude extract of *D. viscosa* with different chromatographic techniques. The separated compound was crystallized from ethyl acetate to give yellow crystals. The pure isolated compound was identified on the basis of MS and ^{1}H NMR, ^{13}C NMR and 2D NMR (Table 2, Figs. 3–11).

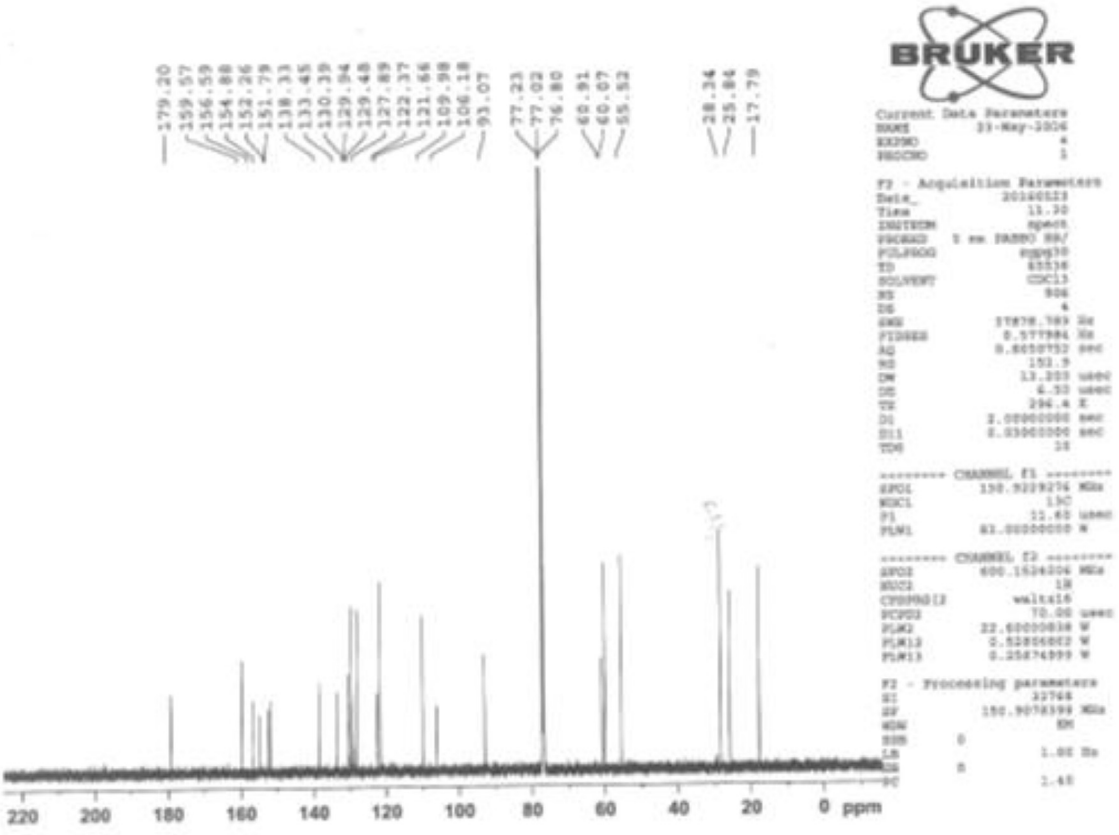

Fig. 5. ^{13}C NMR spectra of compound **1**.

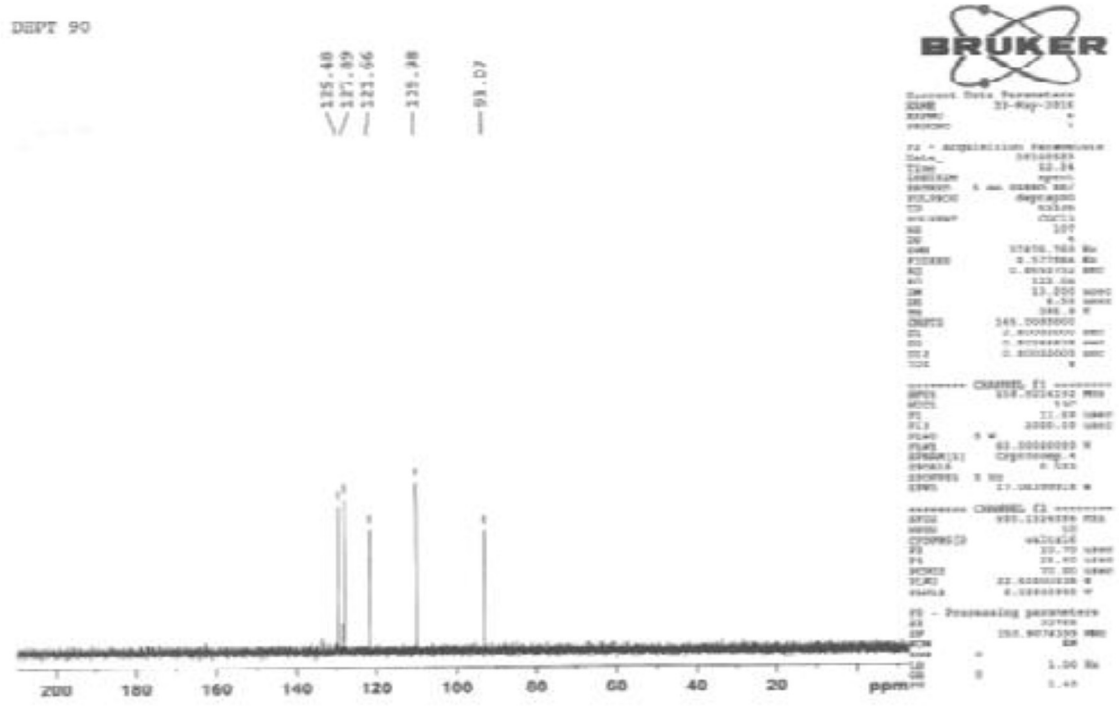

Fig. 6. Dept 90 spectra of compound **1**.

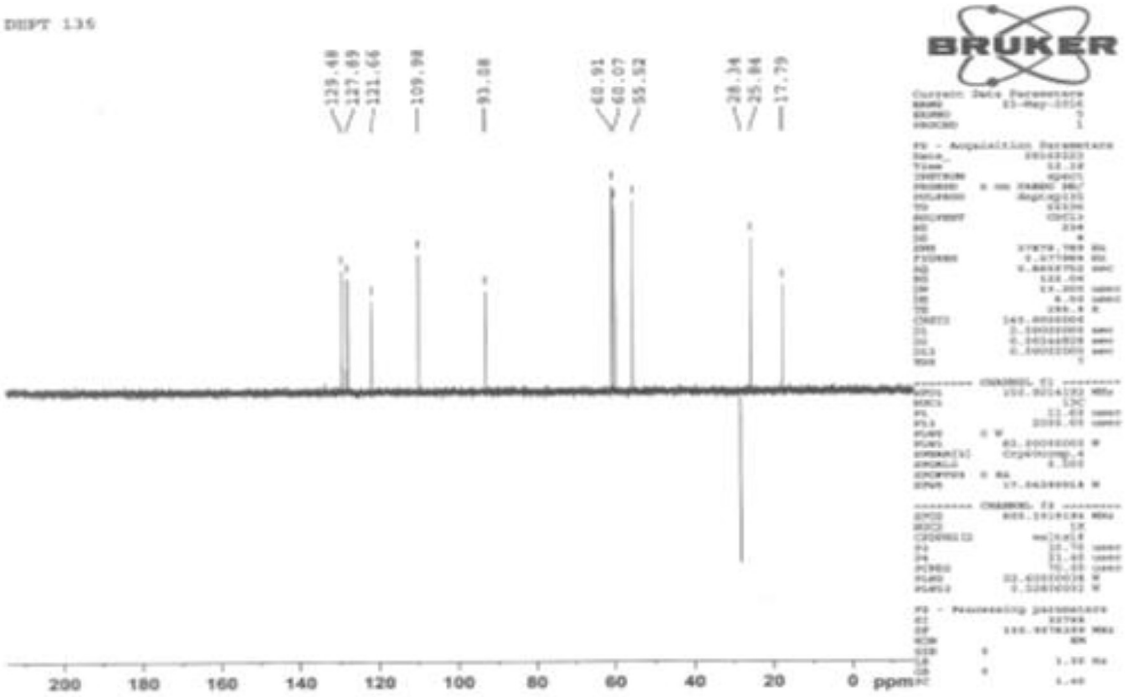

Fig. 7. Dept 135 spectra of compound **1**.

3.3. *Antioxidant activity of pure compound **1***

The antioxidant activity of isolated pure compound **1** from a chloroform extract was determined by the DPPH method described by Hossain et al. [11]. Compound **1** at all of the prepared concentrations showed strong antioxidant activity compared to standard gallic acid (Table 3).

4. Discussion

Usually, the plant crude extract is a combination of several types of chemical compounds. The biological activities of the plant depend on the compounds present in the plant. Therefore, the extraction, separation, isolation, identification and characterization of these plant compounds are essential to prove the biological activities. The selected plant of the present study showed potential biological activities, such as antioxidant, antimicrobial, anticandidal, antidiabetic, antifungal and antidiarrheal activities [10]. The leaves of the selected plant are used as medication for the treatment of itching, swelling, aches, trachoma,, gout, bone fraction [3], rash [4,5], and fever [1–3], as well as an antispasmodic agent [1]. The mixture of leaves and roots are used traditionally in Oman for the treatment of toothache, headache, indigestion, ulcer, diarrhoea and constipation [1–3]. In the Saudi Arabic traditional system, a mixture of leaves, steam and seeds with honey is used to treat malaria [1–3]. According to the antioxidant activity results of the selected plant, the crude extract with the highest activity should be used for the separation of bioactive compounds. In our experiment, the hexane crude extract showed the highest antioxidant activity. However, the amount of hexane extract was too small (Table 1). Therefore, it was very difficult to separate the compounds from the hexane crude extract using chromatographic methods. For that reason, we selected the extract with the second highest antioxidant activity, the chloroform crude extract, for the separation and characterization of compounds. The chloroform crude extract was used to separate the chemical compounds by different chromatographic techniques, such as thin layer chromatography (TLC), column chromatography (CC) and preparative TLC. Silica gel particles (60–120 mesh) were used as stationary phases in column chromatography for the separation of chloroform crude extracts for the determination of antioxidant compounds. The mobile phase used for the separation of compounds was a mixture of hexane: ethyl acetate with increasing polarities. All of the polarities of the solvent and the adsorbent play a significant role in the rate of separation of a mixture of components. Purification of separated compounds was performed by gravitational chromatography. By repeating chromatography and preparative TLC, one new prenylated compound (**1**) together with one known compound were isolated from fraction 3 and sub fraction C of the chloroform crude extract of *D. viscosa*. Compound (**1**) was obtained from the chloroform crude extract as yellow crystals. Compound **1** had a melting point of 177 °C. The molecular formula of $C_{23}H_{24}O_8$ was determined by MS and characterized by ^{1}H NMR, ^{13}C NMR and 2D NMR (See Table 2 and Fig. 3). The ^{1}H NMR spectrum of isolated compound (**1**) indicated the presence of a C– prenyl unit. Two singlets at δ 1.71 and δ 1.75 revealed the presence of a gem dimethyl group (Fig. 4). One double at δ 3.34 indicated the presence of $-C\mathbf{H}_2-CH=$; however, one multiplet at δ 5.31 indicated the presence of a $-CH_2-C\mathbf{H}=$ attached to an aromatic ring at position C-3. Three singlets at δ 3.81, 3.89 and δ 4.01 indicated the presence of three methoxy groups at position C-5, C-3'and C-4′ on the aromatic A and B rings. Two singlets at δ 6.52 and δ 12.93 indicated the presence of hydrogen at H-8 and 5-OH. Two doublets at δ 7.87 and δ 6.93 indicated the presence of H-2′ and H-5'. One doublet of doublets at δ 7.92 indicated the presence of a proton at H-6' (Fig. 4). The ^{13}C NMR, DEPT, COXY, HMBC and HSQC spectra showed 23 carbon atoms in the isolated molecules consisting of two $-CH_3$, three $-OCH_3$, one $-CH_2-$, six CH and twelve fully substituted carbons (Figs. 5–8). All of the carbon atoms in compound **1** were assigned according to the ^{1}H and ^{13}C NMR values and confirmed on the basis of HSQC and HMBC

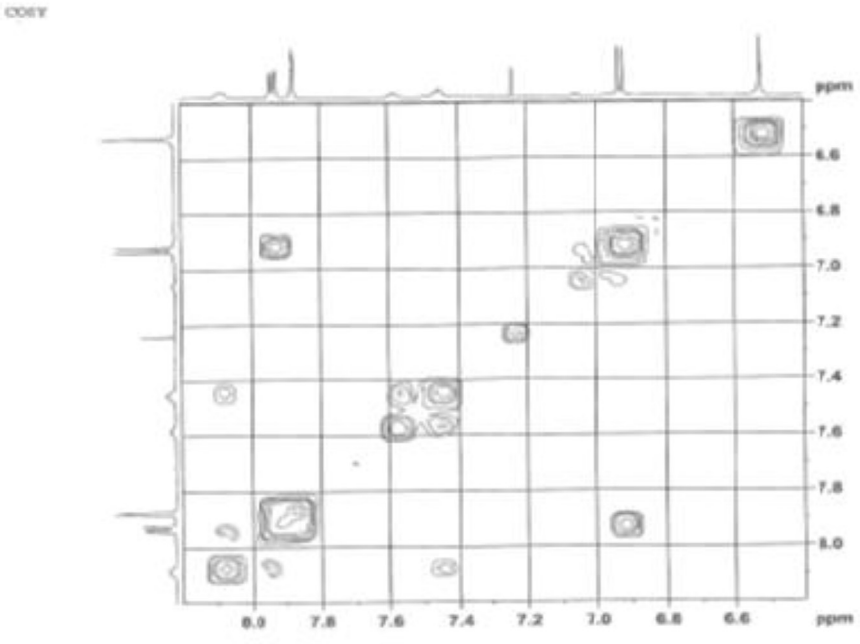

Fig. 8. COSY spectra of compound **1**.

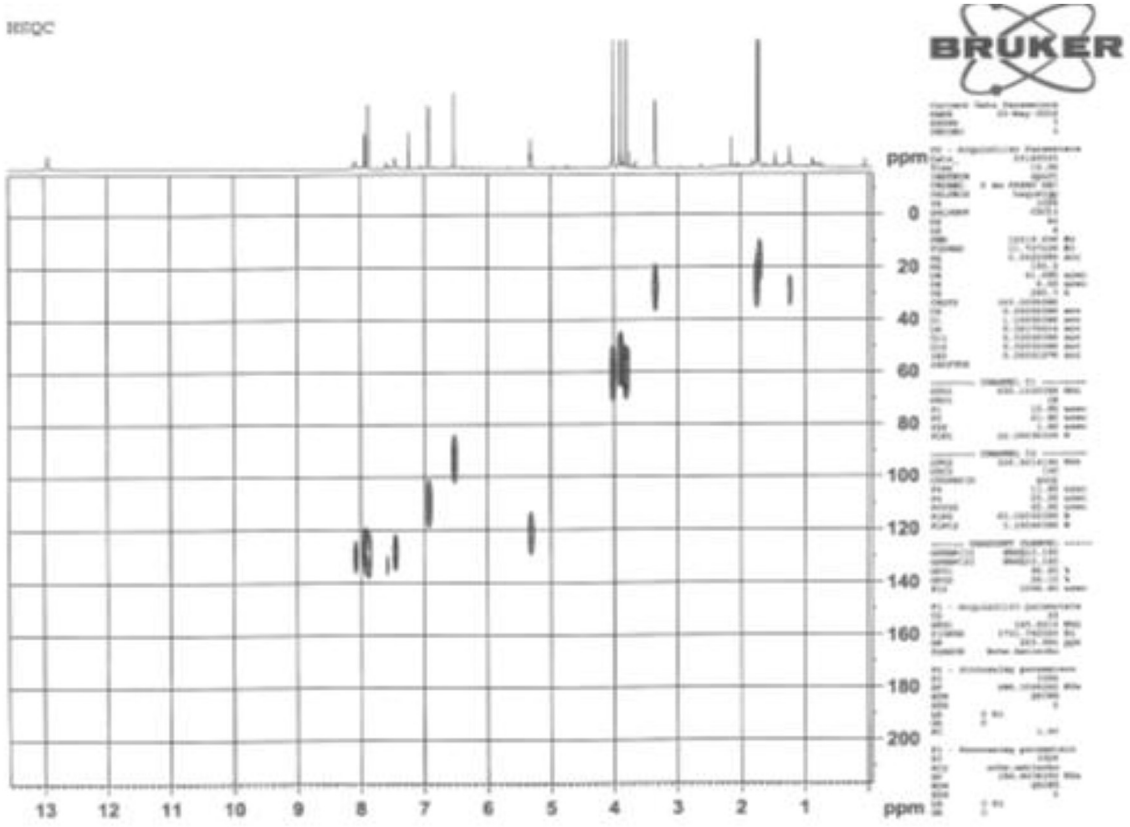

Fig. 9. HSQC spectra of compound **1**.

correlations (Figs. 9 and 10 and Table 2). The structure of compound **1** was further supported by the COSY and HMBC correlations, as shown in Fig. 11 and Table 2. On the basis of the above data structure, compound (**1**) was identified as 5-hydroxy-7,3′4′-trimethoxy-6-acetoxy-3-prenylflavone (**1**, Fig. 3). It was isolated from the selected plant species for the first time.

The free radical scavenging activity of crude extracts and compound **1** was determined by a well-established DPPH method [11].

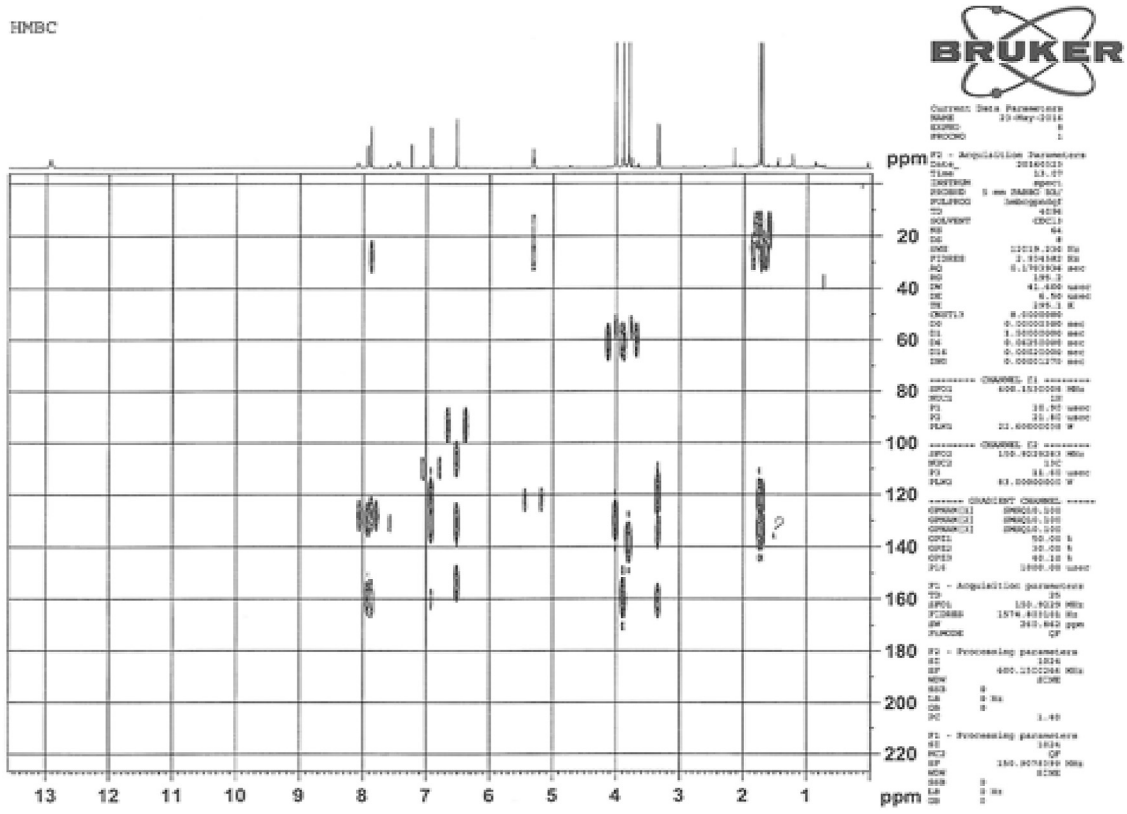

Fig. 10. HMBC spectra of compound **1**.

In our experiment, we found that hexane, ethyl acetate and chloroform crude extracts showed the highest antioxidant activity, followed by chloroform > ethyl acetate > methanol > butanol > water crude extracts (Table 1). Isolated pure compound **1** from a chloroform crude extract at all of the employed concentrations showed a strong antioxidant activity compared to standard gallic acid (Table 3). The strong antioxidant activity of pure compound **1** may be due to it being phenolic in nature. The −OH groups were free in compound **1**, and this significantly enhanced its antioxidant activity. This observation is in agreement with an earlier structural activity study [16–18]. Our experimental results indicate that compound 1 had strong activity; however, it was still less than that of the positive control gallic acid.

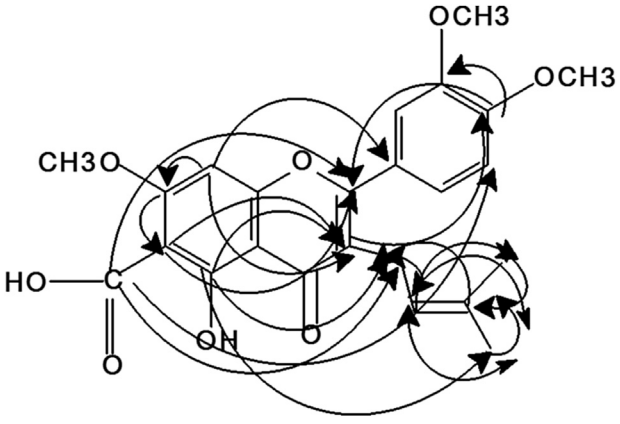

Fig. 11. COSY and HMBC correlation of compound **1**.

5. Conclusion

One new prenylated flavonoid along with one known compound was isolated from the leaves of the chloroform extract of *D. viscosa* by different chromatographic techniques. The new compound was identified as 5-hydroxy-7,3′4’-trimethoxy-6-acetoxy-3-prenylflavone (**1**) on the basis of extensive 1D (1H and 13C) and 2D (COSY, HMQC and HMBC) NMR as well as high resolution mass spectral (HRMS) data. The isolated pure compound showed significant antioxidant activity against DPPH. This is the first report of an antioxidant compound from the leaves of *D. viscosa*. The results obtained from this study might be a good natural antioxidant from the selected plant crude extracts. This plant possesses many medicinal, traditional, and pharmacological uses, which makes it a very useful plant, and the extracts could be useful in therapeutic treatment, but this treatment must ultimately be validated by in vivo experiments.

Conflict of interest

The authors declare no conflict of interest.

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