Iraqi Journal of Biotechnology, 2017, Vol. 16, No. 4, 37-46

Phytochemical, Antibacterial and Antioxidant Activities of *dodonea viscosa* Jacq. extracts Cultivated in Iraq

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Received: June 4, 2017 / **Accepted:** August 13, 2017

Abstract: In this present study, methanolic and aqueous crude extracts of leaves of *Dodonaea viscosa* were investigated for their antibacterial activity against *Shigella dysenteriae, Salmonella typhi* and *Bacillus cereus* using the agar well diffusion method. The high concentrations of methanolic and aqueous extracts (30 and 40 mg/ml) have inhibitory effects against microorganisms used in this study. The phytochemical analysis of the leaf extracts of *D. viscosa* were investigated the results show that the methanolic and aqueous extracts contain flavonoids, alkaloids, tannins, saponins, coumarins and steroids. In addition, the antioxidant and free radical scavenging activities were evaluated. The EC50 values of methanolic extract (8 µg/ml) has shown possess DPPH radical scavenging activity compared with reference substances BHT and vitamin C (EC50= 4 and 4.2 µg/ml) respectively.

Keywords: *Dodonaea viscosa,* Antibacterial, Antioxidant activity, DPPH, FTC, HPLC, Flavonoids.

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Introduction

Antioxidants are important in maintaining good health and there is a growing interest in the investigation of antioxidant activity of secondary metabolites from medicinal plants for compounds with higher potency and lower toxicities than the synthetic ones currently available (1). Plants offer a wide range of natural antioxidants due to the structural diversities of their secondary metabolites. Many medicinal plants have now been recognized as sources of natural antioxidant compounds which are mainly phenolic compounds (2, 3).

Many antioxidant compounds, naturally occurring in plant sources, have been identified as free radical or active oxygen scavengers (4). A number of plants have been investigated for their biological activities and antioxidant properties (5).

Dodonaea viscosa Jacq. which belongs to the family Sapindaceae, the center of origin of *D. viscosa* is believed to be Australia. In Iraq, *D. viscosa* widely cultivated as a hedge plant on the alluvial plain in the desert region. *D. viscosa* Jacq is a popular medicinal plant, leaves are used as anti-inflammatory, anti-ulcer, and in the treatment of fractures (6), antibacterial and antifungal agents (7), also leaves are used to relieve itching, fevers swellings, aches and can be used as a antispasmodic agent, leaves and roots as a painkiller to soothe toothaches and headaches and a lotion made from

unspecified plant parts to treat sprains, bruises, burns and wounds (8). The aim of this study is evaluation the antibacterial and antioxidant activities of aqueous and methanolic extracts of leaves of *Dodonaea viscosa*.

Materials and Methods

Plant material

Fresh leaves of *D. viscosa* were harvest during June 2016 from gardens of Genetic Engineering and Biotechnology Institute located in Baghdad University - Iraq, identification of the plant was carried out by Dr. Ali Al- Mosawy, Department of Biology, College of Science, University of Baghdad. The collected leaves were shade dried, coarsely powdered and used for the phytochemical study.

Extraction of Plant Material

Preparation of crude aqueous extract

Air dried leaves sample (50 gm) was soaked in 250 ml of water for 24 hr. at room temperature. The suspension was filtered through out filter of gauze to get rid of the large particles then filtered through a filter paper (Whatman no.1). The extracts were concentrated to near dryness under reduced pressure below 40 °C using rotary evaporator(9).

Preparation of methanol extract

A quantity of 50 g of plant powder was extracted with 250 ml of 95% methanol by soxhlet apparatus for 6 hrs at 40-60 °C, and then evaporated by using a rotary evaporator at 40 °C. The extracts were diluted to 20 mg/ml with 10 % dimethyl sulfoxide (DMS) solution and stored in air tight glass bottles in a refrigerator till further use(10).

Phytochemical Screening of Plant Extracts

Methanolic and aqueous extracts were tested for the presence of the phytoconstituents according to the following standard tests to detected Flavonoids, Alkaloids, Tannins, Saponins, glycosides, Coumarins, steroides (11, 12, and 13).

Microorganisms and media

The bacterial isolates *Shigella dysentry* and *Salmonella typhi* isolated from patients with food poisoning (gastrointestinal infections). While, *Bacillus cereus* isolated from spoiled rice, the bacteria were obtained, as clinical isolates, from Al–Yarmook Teaching Hospital, Baghdad, Iraq. Bacterial cultures were maintained on nutrient agar (NA) slops. Subcultures were made monthly and stored at 4 °C until required for use.

Culture preparation

A loopfull of 24 hr. surface growth on a NA slope of each bacterial isolate was transferred individually to 5ml of Brain heart infusion broth (pH 7.6) and incubated at 37°C for 24 hr. bacterial cells were collected by centrifugation at 3000 rpm for 15 min, washed twice and resuspended in 0.1% pepton water. Turbidity was adjusted to match that of

as McFarland standard (10^8 CFU/ml). Then 1:10 dilution of the cell suspension was performed to give an inoculums concentration of 10^7(CFU/ml) (14).

Antibacterial assay

0.2 ml volume of the standard inoculums (10^7 CFU/ml) of the test bacterial isolate was spread on Mueller Hinton Agar (MHA) with a sterile glass rod spreader and allowed to dry. Then 6 mm. diameter wells were bored using cork borer in the MHA. Plant extracts (10,20,30 and 40 mg/ml) were introduced into each well and allowed to stand for 1 hr. at room temperature to diffuse the plant extracts into medium before incubation at 37 °C for 24 hr.

The inhibition zone diameter (IZD) was measured by transparent ruler to nearest mm, diameter between 12 and 16 mm. was considered moderately active, and these with > 16 mm. were considered highly active (15).

Fourier transform infrared (FTIR) assay

The functional groups methanolic and aqueous extracts of *D. viscosa* were detected by using fourier transform infrared spectrophotometer (FTIR) and compared with standard value (16).

High-performance liquid chromatography (HPLC)

HPLC analysis was performed in a Shimadzu apparatus equipped with SPD-M10A Diode array detector using reverse phase column (Linchosorb RP-18, 25cm x 5mm) at room temperature. Elution was done using the mixture of Methanol, water and Phosphoric acid (100:100:1). The flow rate was about 1ml/min and peaks were detected at 270 nm (17).

Evaluation of Antioxidant activity DPPH assay

In order to obtain an indication of the antioxidant activity of lignan, 5 ml of a freshly prepared 0.004 % of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol was mixed with 50 μl of different concentration of methanolic and aqueous extracts of *D. viscosa* (5 , 10 , 25 , 50 , 75 and 100 mg/ml) and the absorbance of each dilution, after 30 minutes, was measured at 517 nm. Butylated hydroxytoluene (BHT) and vitamin C was the antioxidant used as positive control (18).

All tests were performed in triplicate and the methanol was used as blank solution. The percentage DPPH reduction (or DPPH radical scavenging capacity) was calculated as:

% Reduction = (Abs DPPH – Abs Dil.)/Abs DPPH x 100

Whereby:

Abs DPPH = average absorption of the DPPH solution

Abs Dil. = average absorption of the three absorption values of each dilution.

With the obtained values, a graphic was made using Microsoft Excel. The EC_{50} of each extract (concentration of extract or compound at which 50% of DPPH is reduced) was taken from the graphic.

Ferric thiocyanate (FTC) method

A mixture containing 4mg of the sample in 4 ml of 99% ethanol (final

concentration 0.02), 4.1ml of 2,52 % linoleic acid in 99% ethanol, 8ml of 0.05 M phosphate buffer (ph 7.0) and 3.9 ml of water was placed in a vial with screw cap and then placed in an incubator at 40 C in the dark. To 0.1 ml of this mixture 9.7 ml of 75% ethanol (v\v) and 0.1 ml of 30% ammonium thiocyanate were added. Precisely 3 minutes later the addition of 0.1 ml of 0.1 ml of 0.02M ferrous chloride in 3.5% hydrochloric acid was added to reaction mixture, the absorbance of red color indicated the antioxidant activity was measured at 500 nm for every 24 hours until the absorbance of the control reached maximum. The control and the standard were subjected to the same procedures as the sample except that for the control, only the solvent was used, and for the standard 4mg of the sample was replaced by 4 mg of vitamin C.(19).

Inhibition of lipid peroxidation (%) $= \{1 - (A \text{ sample}) / (A \text{ control})\} \times 100$

Determination of total phenolic contents

The amount of total phenolics in methanolic and aqueous extracts of *D. viscosa* was determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard (Figure 1) and the total phenolics were expressed as mg/g gallic acid equivalents (20). Concentration of 0.005, 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in methanol.Concentration of 50, 75, 100 and 150 mg/ml of methanolic and aqueous extracts of *D. viscosa* were also prepared and 0.5 ml of each sample were introduced into test tubes and mixed with 2.5 ml of a 10 fold dilute Folin- Ciocalteu reagent and 2 ml of 7.5 % sodium carbonate. The absorbance was at read at 760 nm spectrometrically. All determination was performed in triplicate. Thus total phenolic content can be determined (21, 22).

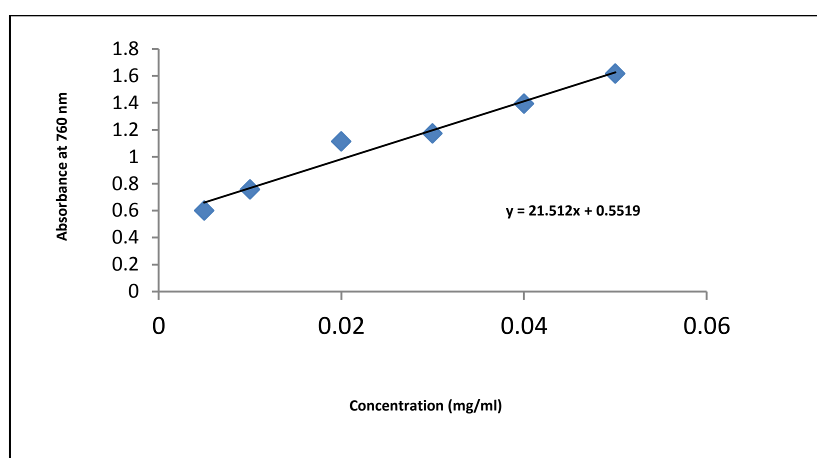


Figure (1): Standard curve of Gallic acid

Results and Discussion

Table 1 showed the phytochemical composition of the leaf extracts. Only glycosides was absent in methanolic and aqueous extracts.

Table (1): Phytochemical analysis of the leaf extracts of *D. viscosa*

Constituents	methanolic extract	aqueous extract
Flavonoids	+	+
Alkaloids	+	+
Tannins	+	+
Saponins	+	+
glycosides	-	-
Coumarins	+	+
steroides	+	+

(+) presence of constituents, (-) absence of constituents

FTIR Technique

FTIR spectrum of methanolic and aqueous extracts is shown in Figures 2 and 3. *D. viscosa* leaves extract contain many Functional Groups as shown in Table 2 with Standard groups (16).

Table (2): Frequencies of IR absorption of *D. viscosa* leaves extracts

The Functional Group	I.R Frequencies Standard groups (cm-1)	I.R. Frequencies of methanolic extract	I.R. Frequencies of aqueous extract
Phenolic–OH group stretching	3200-3600	3379.29	3387.00
C-H stretch	2850-3000	2927.94	2931.80
Aromatic C=C	1400-1600	1627.92	1604.77
N-O stretch	1345-1385	1346.31	1365.60
Aliphatic C–O	1000-1300	1145.72	1072.42

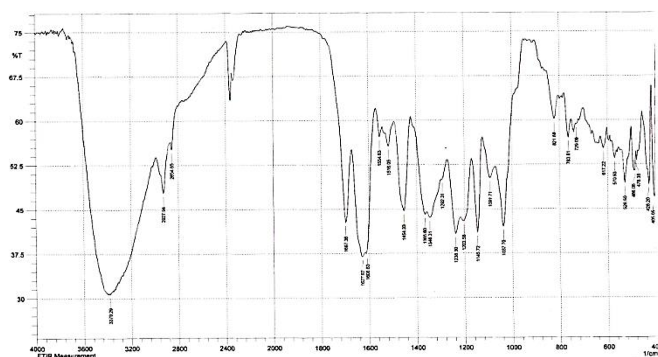


Figure (2): The infrared spectrum of methanolic extract

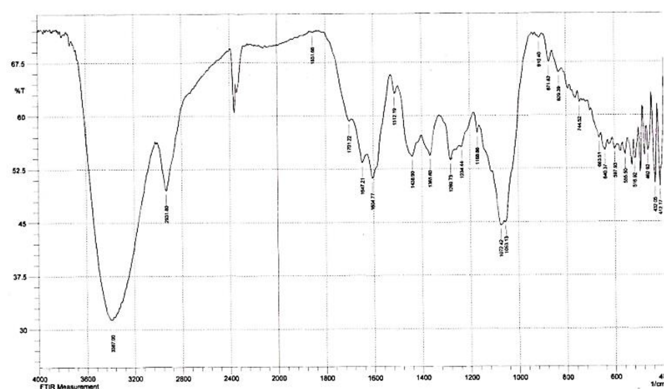
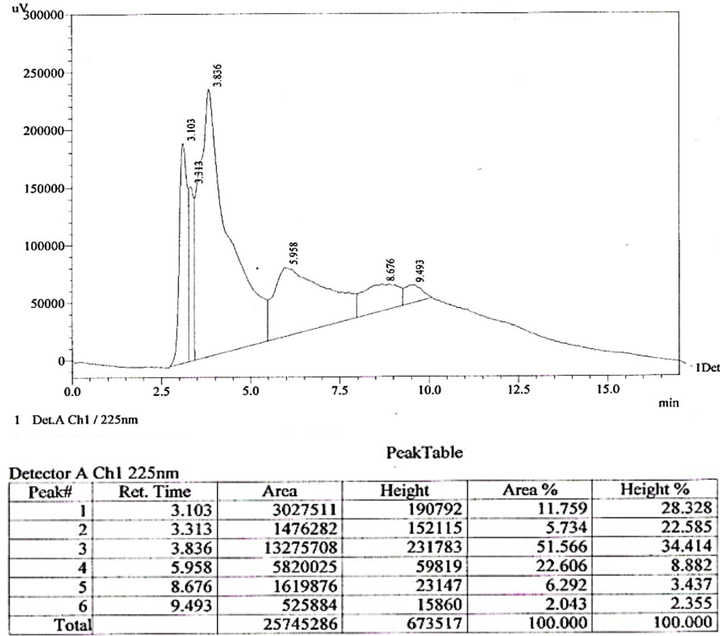


Figure (3): The infrared spectrum of aqueous extract

High-performance liquid chromatography (HPLC)

Methanolic and aqueous extracts of *Dodonaea viscosa* were analyzed by HPLC (Figure 4 and 5).

It is evident from the HPLC that there are several compounds in methanolic extract more than aqueous extract.

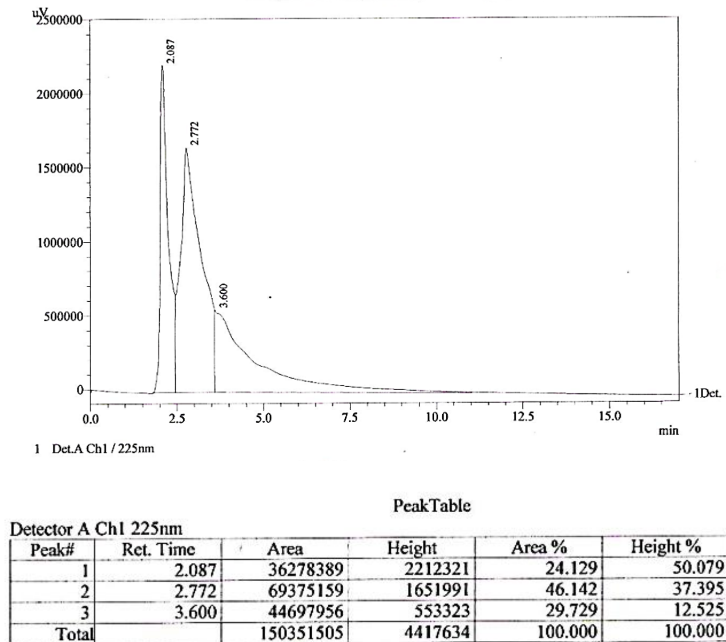


PeakTable

Detector A Ch1 225nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.103	3027511	190792	11.759	28.328
2	3.313	1476282	152115	5.734	22.585
3	3.836	13275708	231783	51.566	34.414
4	5.958	5820025	59819	22.606	8.882
5	8.676	1619876	23147	6.292	3.437
6	9.493	525884	15860	2.043	2.355
Total		25745286	673517	100.000	100.000

Figure (4): HPLC quantification of the methanolic extract



PeakTable

Detector A Ch1 225nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.087	36278389	2212321	24.129	50.079
2	2.772	69375159	1651991	46.142	37.395
3	3.600	44697956	553323	29.729	12.525
Total		150351505	4417634	100.000	100.000

Figure (5): HPLC quantification of the aqueous extract

Antibacterial activity

Dodonaea methanolic extract exhibited antibacterial activity against microorganisms at the concentrations (30 and 40 mg/ml). The diameter of the inhibition zones against *S. typhi* was (22 and 25 mm) at (30 and 40 mg/ml) respectively. Whereas, decreased to (15 and 21 mm) against *Sh. dysenteriae*. While *B. ceries* had the lowest inhibition zones which was (13 and 15 mm) at concentrations (30 and 40 mg/ml) respectively, as shown in Table 3.

Results displayed in Table 4 indicate that high concentrations of *Dodonaea* aqueous extract (30 and 40 mg/ml) had inhibitory effects against (*S. typhi)* with (15 and 18 mm) inhibition zones diameter respectively, when (13 and 20 mm) was recorded in the same concentrations against *Shigella dysenteriae*. While *B. ceries* gave (12 and 13 mm) in concentrations of (30 and 40 mg/ml) respectively.

Table (3): Antibacterial activity of methanolic extract on some pathogenic bacteria

Microorganism	Methanol extract			
	10 mg/ml	20 mg/ml	30 mg/ml	40 mg/ml
Salmonella typhi	19	21	22	25
Shigella dysentry	11	13	15	21
Bacillus cereus	-	9	13	15

(-) = no Inhibition

Table (4): Antibacterial activity of aqueous extract on some pathogenic bacteria

Microorganism	Aqueous extract			
	10 mg/ml	20 mg/ml	30 mg/ml	40 mg/ml
Salmonella typhi	12	14	15	18
Shigella dysentry	-	-	13	20
Bacillus cereus	-	10	12	13

(-) = no Inhibition

Determination of Antioxidant Activity

DPPH assay

Figure 6 illustrates the concentration of DPPH radical due to the scavenging ability of the extract and standards. BHA and ascorbic acid were used as references. The EC50 values of *Dodonaea* methanolic extract (8 µg/ml) had shown possess DPPH radical scavenging activity compared with reference substances BHT and vitamin C (EC50= 4 and 4.2 µg/ml) respectively, and this was higher than aqueous extract (EC50= 60 µg/ml). These findings showed that methanolic extract exhibited strong antioxidant and protective effects in quenching the DPPH.

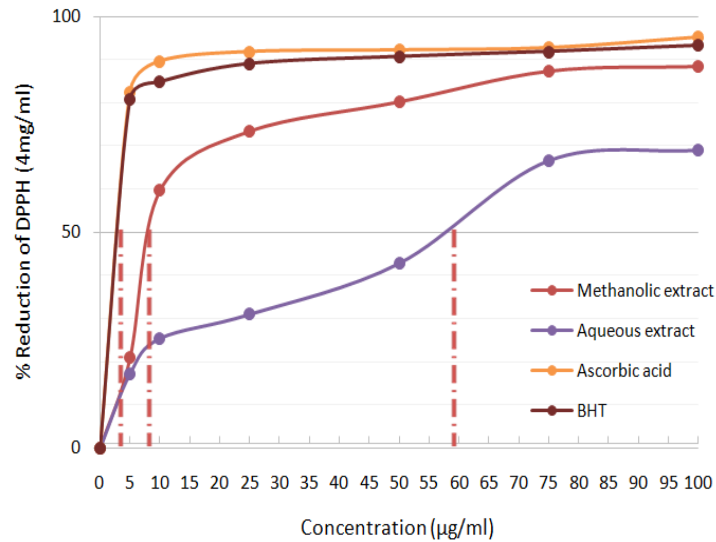


Figure (6): The reduction percentage of DPPH using methanolic and aqueous extracts of *D. viscosa*

Ferric thiocyanate (FTC) method

Ferric thiocyanate method was originally designed for measuring lipid peroxide content. The advantage of using ammonium thiocyanate over other coloring reagents is that binding of iron by thiocyanate ion is specific to Fe3+ only, and that the Fe3+ thiocyanate complex produces a single absorbance peak at 500 nm. Results obtained from FTC assay (Figure 7). revealed that methanolic extract carries the antioxidative potential for chain-breaking inhibition of lipid peroxidation as it has shown 94.16 % inhibition when compared with vitamin C (84.41 % inhibition), and approach with aqueous extract which was (83.12 % inhibition).

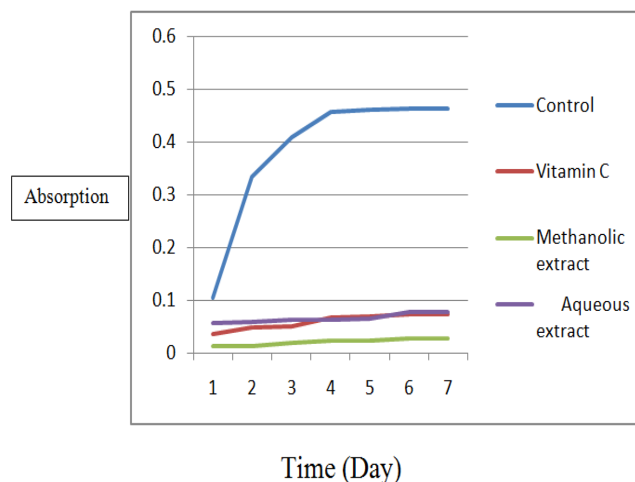


Figure (7): Antioxidant properties of methanolic and aqueous extracts of *D. viscosa* by FTC method

Determination of total phenolic content

The amount of total phenol was determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent using the standard curve equation: $y = 21.512 + 0.5519$, where y is absorbance at 760 nm.

The total phenolic content of the methanolic extract was higher than aqueous extract shown in Table 5.

Table (5): Total Phenolic content of *D. viscosa* extracts

***D. viscosa* extract**	**Concentration (mg/ml)**	**Total phenol (mg/g)**
methanolic extract	50	1.187
	75	1.750
	100	3.416
	150	5.291
aqueous extract	50	0.0937
	75	1.942
	100	2.729
	150	3.270

Water extracts of *D.viscosa* possess antioxidant activity in spite of containing low flavonoid levels (23). The methanolic extract showed a significant and much effective free radical scavenging activity in the DPPH assay and hence provides the prophylaxis against various diseases such as heart diseases, arteriosclerosis and cancers (24).

Flavonoids are well known for their antioxidant activities (25). Myricetin, quercetrin and rutin help to inhibit the production of superoxide radicals (26). Furthermore, (27) confirmed that flavonoids are the main components present in *D. viscosa* leaves extracts.

Conclusion

The results of the present study, showed that *D. viscosa* leaf extract possess bioactive constituents of pharmacological significance. The methanolic extracts showed prominent antibacterial and antioxidant activity more than aqueous extract

Therefore, further studies are recommended for the isolation and purification of the phytochemicals from methanolic extract which have great antibacterial and antioxidant activity.

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