Antibacterial and antioxidant activities of *Dodonaea viscosa* Jacq. extracts cultivated in Iraq

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**ABSTRACT**

In present study, methanolic and aqueous crude extracts of leaves of *Dodonaea viscosa* were investigated for their antibacterial activity against *Shigella dysentery*, *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. In addition, evaluate the antioxidant and free radical scavenging activities. The EC50 values of methanolic extract (8 μg/ml) was shown possess DPPH radical scavenging activity compared to reference substances BHT and vitamin C (EC50= 4 and 4.2 μg/ml) respectively.

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

**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. In addition, natural antioxidants have the capacity to improve food quality and stability and also act as nutraceuticals to terminate free radical chain reaction in biological systems, and thus may provide additional health benefits to consumers (5, 6).

*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 (7), antibacterial and antifungal agents (8), also leaves are used to relieve itching, fevers swellings, aches and can be used as an 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. Digestive system disorders, including indigestion, ulcers, diarrhea and constipation are commonly treated in traditional medicine with an orally-administered decoction of either the leaves or roots (9). 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 procured 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 a 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 (10).

**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 (11).

**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, steroids (12, 13, and 14).

**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) slopes. Subcultures were made monthly and stored at 4 °C until required for use.

**Culture preparation:** A loopful 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 cell 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 (108 CFU/ml). Then 1:10 dilution of the cell suspension was performed to give an inoculums concentration of 107(CFU/ml).

**Antibacterial assay:** 0.2 ml volume of the standard inoculums (107 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. Cephalothin (30 μg/ml) (Oxoid) was used as positive control inhibition zone with diameter less than 12 mm. were considered as having no antibacterial activity, diameter between 12 and 16 mm. were 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 were 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:

\[
\text{% Reduction} = \frac{(\text{Abs DPPH} – \text{Abs Dil.})}{\text{Abs DPPH}} \times 100
\]

Whereby: \(\text{Abs DPPH} = \text{average absorption of the DPPH solution}; \) \(\text{Abs Dil.} = \text{average absorption of the three absorption values of each dilution.}\)

With the obtained values, a graphic was made using Microsoft Excel. The EC50 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 (νvν) and 0.1 ml of 30% ammonium thiocyanate were add. 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 sample) / (A 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 Folien-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 Folien-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).

FT-IR Technique: FT-IR 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

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

RESULTS AND DISCUSSION

Table (1) shows 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

<table>
<thead>
<tr>
<th>Constituents</th>
<th>methanolic extract</th>
<th>aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>glycosides</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coumarins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>steroids</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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

**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. dysentery*. While *B. cereus* 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 *Sh. dysentery*. While *B. cereus* gave (12 and 13 mm) in concentrations of (30 and 40 mg/ml) respectively.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Methanol extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 mg/ml</td>
<td>20 mg/ml</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td><em>Shigella dysentery</em></td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>-</td>
<td>9</td>
</tr>
</tbody>
</table>

(-) = no Inhibition

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

**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) was shown possess DPPH radical scavenging activity compared to 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](image_url)
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).

![Absorption](image)

**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 show in (Table 5).

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

<table>
<thead>
<tr>
<th>D. viscosa extract</th>
<th>Concentration (mg/ml)</th>
<th>Total phenol (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>methanolic extract</td>
<td>50</td>
<td>1.187</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>1.750</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>3.416</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>5.291</td>
</tr>
<tr>
<td>aqueous extract</td>
<td>50</td>
<td>0.0937</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>1.942</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.729</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>3.270</td>
</tr>
</tbody>
</table>

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, shows 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 the phytochemicals from methanolic extract which have great antibacterial and antioxidant activity.

REFERENCES