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Phytochemical screening, free radical scavenging, antioxidant activity and phenolic content of *Dodonaea viscose* Jacq.

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Abstract: The purpose of this study was to evaluate the antioxidant potential of *Dodonaea viscosa* Jacq. A methanolic extract of the plant was dissolved in distilled water and sequentially partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-butanol. Phytochemical screening showed the presence of phenolics, flavonoids and cardiac glycosides in large amounts in the chloroform, ethyl acetate and *n*-butanol fraction. The antioxidant potential of all these fractions and remaining aqueous fraction was evaluated by four methods: 1,1--diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, total antioxidant activity, the Ferric reducing antioxidant power (FRAP) assay and ferric thiocyanate assay along with the determination of their total phenolics. The results revealed that the ethyl acetate soluble fraction exhibited the highest percent inhibition of the DPPH radical as compared to the other fractions. It showed 81.14±1.38 % inhibition of the DPPH radical at a concentration of 60 μg ml^{-1}. The concentration of this fraction leading to 50 % inhibition of the DPPH radical (IC_{50}) was found to be 33.95±0.58 μg ml^{-1}, relative to butylated hydroxytoluene (BHT), having an IC_{50} of 12.54±0.89 μg mL^{-1}. It also showed the highest FRAP value (380.53±0.74 μM of trolox equivalents) as well as the highest total phenolic contents (208.58±1.83 gallic acid equivalent (GAE) μg g^{-1}) and highest value of inhibition of lipid peroxidation (58.11±1.49 % at a concentration of 500 μg ml^{-1}) as compared to the other studied fractions. The chloroform fraction showed the highest total antioxidant activity, *i.e.*, 1.078±0.59 (eq. to BHT).

Keywords: *Dodonaea viscosa* Jacq.; phytochemical screening; DPPH assay; total antioxidant activity; FRAP value; total phenolics; inhibition of lipid peroxidation.

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INTRODUCTION

Medicinal plants constitute the major constituents of most indigenous medicines and a large number of Western medical preparations contain one or more component(s) of plant origin. The medicines that are in use today are definitely not the same as those that were used in ancient times or even in the recent past. Several modifications, improvement, sophistication and newer discoveries have continuously contributed to the type, quality, presentation and concept of medicinal preparation. In the development of human knowledge for therapeutic use, scientists endeavoured to isolate different chemical constituents from plants, subjected them to biological and pharmacological tests and then used them to prepare modern medicines.[1] There is increasing interest in the measurement and use of plant antioxidants for scientific research as well as for industrial (dietary, pharmaceutical and cosmetics) purposes. This is mainly due to their strong biological activity, excluding those of many synthetic antioxidants, which have possible activity as promoters of carcinogenesis. Therefore, the need exists for safe, economic, powerful and natural antioxidants to replace these synthetic ones. Obviously, there has been an increasing demand to evaluate directly the antioxidant properties of plant extracts.[2]

Many antioxidant compounds, naturally occurring in plant sources, have been identified as free radical or active oxygen scavengers.[3] A number of plants have been investigated for their biological activities and antioxidant properties.[4,5] Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants.[6] 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. Recent studies have highlighted the role of polyphenolic compounds of higher plants,[7] such as flavonols[8] and anthraquinones.[9]

In the search of plants as a source of natural antioxidants, some medicinal plants and fruits have been extensively studied for their antioxidant activity and radical scavenging in the last few decades.[10] Some antioxidant compounds are extracted from easy sources, such as agricultural and horticultural crops, or medicinal plants. Among them, medicinal plants are taking the main role for providing a large number of pure antioxidants. *Dodonaea viscosa* Jacq. is a traditional medicinal plant belonging to the family Sapindaceae. Plants of this family are utilized in folklore medicine in Pakistan for the treatment of various fungal skin diseases, such as *Tinea capitis*, *T. pedis*, *T. manum*, *T. corporis*, *etc*. The powdered leaves of *D. viscosa* applied over burn and scald wounds were found to possess febrifuge properties and to be useful for different skin diseases.[11] It is commonly used for skin diseases in Ethiopia.[12] Investigation of the aerial parts of *D. viscosa* led to the isolation of a new *ent*-labdane (*ent*-15,16-epoxy-9αH-labda-

-13(16),14-diene-3β,8α-diol) and a novel *p*-coumaric acid ester of 1-L-*myo*-inositol (1-L-1-*O*-methyl-2-acetyl-3-*p*-coumaryl-*myo*-inositol).[13] Many flavonoids,[14–16] saponins,[17] and diterpenes[18] have also been isolated from *D. viscosa*. Notable among these compounds are pinocembrin, santin, penduletin, alizarin, 5-hydroxy-3,6,7,4'-tetramethoxyflavone, 5,7,4'-trihydroxy-3,6-dimethoxyflavone, isorhamnetin-3-rhamnosylgalactoside, 5,7-dihydroxy-3'-(4-hydroxy-3-methylbutyl)-3,6,4'-trimethoxyflavones,[14] 5,6,4'-trihydroxy-3,7-dimethoxyflavone,[16] viscosol,[15] hautriwaic acid,[16,18,19] dehydrohautriwaic acid, methyl dodonates,[18] dodonoside A, dodonoside B,[17] 5,7,4'-trihydroxy-3',5'-bis(3-methylbut-2-enyl)-3,6-dimethoxyflavone, and 5,7,4'-trihydroxy-3'-(4-hydroxy-3-methylbutyl)-50-(3-methylbut-2-enyl)-3,6-dimethoxyflavone, dodonic acid, hautriwaic lactone, (+)--hardwickiic acid, 5α-hydroxy-1,2-dehydro-5,10-dihydroprintzianic acid methyl ester, strictic acid, dodonolide, alizarin,[19] 3,5,7-trihydroxy-4'-methoxyflavone and 5-hydroxy-3,7,4'-trimethoxyflavone, 3,4',5,7-tetrahydroxyflavone (kaempferol),[20] and sakuranetin.[21,22]

D. viscosa Jacq. is a popular medicinal plant. It is used in folk medicine as a remedy for fever, rheumatism and gout. The crude extract has inhibitory effects against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Corynebacterium diphtherieae*, but no activity against *Escherichia coli* and *Pseudomonas aeruginosa*, thereby suggesting potential against notable Gram positive organisms.[23] Its leaves are used as anti-inflammatory, anti-ulcer, antibacterial and antifungal agents and in the treatment of bone fractures.[24]

To the best of our knowledge, no work has been performed on the comparative antioxidant potential of various fractions of *D. viscosa* Jacq. Therefore, the present study was undertaken to investigate the *in vitro* antioxidant potential of various fractions of this plant.

EXPERIMENTAL

Plant material

The plant *D. viscosa* Jacq. was collected from Kotli, Azad Kashmir in February 2010, and identified by Mr. Muhammad Ajaib (taxonomist), Department of Botany, Government College University, Lahore. A Voucher specimen (G. C. Herb. Bot. 965) has been deposited in the Herbarium of the Botany Department of the same university.

Extraction and fractionation of the antioxidants

The shade-dried ground whole plant (0.8 kg) was exhaustively extracted with methanol (5 L) on a Soxhlet apparatus. The extract was evaporated in rotary evaporator Laborta 4000--efficent (Heidolph) at 40 °C under vacuum to yield the residue (126 g), which was dissolved in distilled water (1 L) and partitioned with *n*-hexane (4×1 L), chloroform (4×1 L), ethyl acetate (4×1 L) and *n*-butanol (4×1 L). These four organic fractions and the remaining water fraction were concentrated separately on rotary evaporator (*n*-hexane at 34 °C, chloroform at 38 °C, ethyl acetate at 45 °C, *n*-butanol at 50 °C and water at 60 °C under vacuum) and the thus obtained residues were used to evaluate their *in vitro* antioxidant potential.

Chemicals and standards

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical, 2,4,6-tripyridyl-*s*-triazine (TPTZ), trolox, gallic acid, Folin–Ciocalteu phenol reagent and butylated hydroxytoluene (BHT) were obtained from the Sigma Chemical Company Ltd. (USA) and the organic solvents (*n*-hexane, chloroform, ethyl acetate, *n*-butanol), and other employed chemicals were from Merck (Pvt.) Ltd. (Germany).

Phytochemical screening

Qualitative phytochemical screening of all five crude extracts, *i.e.*, the *n*-hexane soluble fraction, the chloroform soluble fraction, the ethyl acetate soluble fraction, the *n*-butanol soluble fraction and the remaining aqueous fractions, was performed to identify the phytochemical constituents, *i.e.*, alkaloids, terpenoids, saponins, tannins, sugars, phenolics, flavonoids and cardiac glycosides, using standard procedures.[25–27]

Antioxidant assays

The following antioxidant assays were performed on all the studied fractions.

DPPH radical scavenging activity

The DPPH radical scavenging activities of each crude extract of plant were examined by comparison with that of a known antioxidant (BHT), using a reported method.[28] Briefly, various amounts of the samples (1000, 500, 250, 125, 60, 30, 15 and 8 μg mL^{-1}) were mixed with 3 ml of methanolic solution of DPPH (0.1 mM). The mixture was shaken vigorously and allowed to stand at room temperature for 1 h. Then, the absorbance was measured at 517 nm against methanol as a blank in a spectrophotometer (CECIL Instruments CE 7200, Cambridge, UK). The lower the absorbance of the reaction mixture, the higher was the free radical scavenging activity.

The percent of DPPH decolouration of the samples was calculated according to the formula:

$$\text{Antiradical activity (\%)} = (A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \times 100$$

Each sample was assayed in triplicate and the mean values were calculated.

Total antioxidant activity by the phosphomolybdenum method

The total antioxidant activities of various fractions of the plant were evaluated by the phosphomolybdenum complex formation method.[29] Briefly, 500 μg mL^{-1} of each crude extract was mixed with 4 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in sample vials. The blank solution contained 4 mL of reagent solution. The vials were capped and incubated in water bath at 95 °C for 90 minutes. After the samples had been cooled to room temperature, the absorbance of mixture was measured at 695 nm against the blank. The antioxidant activity is expressed relative to that of BHT. All determinations were assayed in triplicate and mean values were calculated.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed according to Benzie and Strain[30] with some modifications. The stock solutions included 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM hydrochloric acid, and 20 mM ferric chloride hexahydrate solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL ferric chloride hexahydrate solution, which was then warmed to 37 °C before use. The solutions of plant samples and that of trolox were prepared in methanol (250 μg mL^{-1}). 10 μL of each of crude extracts was taken in separate test tubes and 2990 μL of FRAP solution was

added to each to make a total volume of 3 mL. The plant crude extracts were allowed to react with the FRAP solution in the dark for 30 min. The absorbance of the coloured product (ferrous tripyridyltriazine complex) was checked at 593 nm. The FRAP values are expressed as micromoles of trolox equivalents (TE) per mg of the sample using the standard curve constructed for different concentrations of trolox. The results are expressed in μmol TE mL^{-1}.

Total content of phenolics

The total phenolics of the various fractions of plant were determined by a reported method.[31] An aliquot of 0.1 mL of each crude extract (0.5 mg mL^{-1}) was combined with 2.8 mL of 10 % sodium carbonate and 0.1 mL of 2 M Folin–Ciocalteu reagent. After 40 min, the absorbance at 725 nm was measured using a UV–visible spectrophotometer. The total phenolics are expressed as micrograms of gallic acid equivalents (GAE) per gram of sample using a standard calibration curve constructed for different concentrations of gallic acid. The curve was linear between 50 and 400 μg mL^{-1} of gallic acid. The results are expressed in μg GAE g^{-1}.

Ferric thiocyanate (FTC) assay

The antioxidant activities of the various fractions of the plant on the inhibition of linoleic acid peroxidation were assayed by the thiocyanate method.[32] Each crude extract (0.1 ml, 0.5 mg mL^{-1}) was mixed with 2.5 mL of linoleic acid emulsion (0.02 M, pH 7.0) and 2.0 mL of phosphate buffer (0.02 M, pH 7.0). The linoleic emulsion was prepared by mixing 0.28 g of linoleic acid, 0.28 g of Tween-20 as emulsifier and 50.0 mL of phosphate buffer. The reaction mixture was incubated for 5 days at 40 °C. The mixture without extract was used as the control. A 0.1 mL aliquot of the mixture was taken and mixed with 5.0 mL of 75 % ethanol, 0.1 mL of 30 % ammonium thiocyanate and 0.1 mL of 20 mM ferrous chloride in 3.5 % hydrochloric acid and allowed to stand at room temperature. Precisely 3 min after the addition of ferrous chloride to the reaction mixture, the absorbance was recorded at 500 nm. The antioxidant activity is expressed as follows:

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

The antioxidant activity of BHT as reference standard was assayed for comparison.

Statistical analysis

All the measurements were performed in triplicate and statistical analysis was realised by statistical software. All the data are expressed as ± *SEM*. Statistical analyses were determined using one way analysis of variance (ANOVA) followed by the *post-hoc* Tukey test.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening was performed on all the studied fractions and results are given in Table I. It can be observed from the results that the chloroform fraction, ethyl acetate fraction, *n*-butanol fraction and aqueous fraction contained phenolics and flavonoids, while the *n*-hexane fraction showed absence of these compounds. Cardiac glycosides were absent in all the fractions. Terpenes were detected in all the fractions except the aqueous fraction. Alkaloids were detected only in the chloroform fraction and the ethyl acetate fraction. Tannins and sugars were present in the ethyl acetate fraction, the *n*-butanol fraction and the aqueous

fraction but were not detected in the *n*-hexane fraction and chloroform fraction. Saponins were present in all the fractions except the *n*-hexane fraction.

TABLE I. Phytochemical constituents of the various fractions of *D. viscosa* Jacq. ("+" represents presence and "–" represents absence)

Test	*n*-Hexane soluble fraction	Chloroform soluble fraction	Ethyl acetate soluble fraction	*n*-Butanol soluble fraction	Remaining aqueous fraction
Alkaloids	–	++	+	–	–
Terpenoids	+	++	++	+	–
Saponins	–	++	++++	+++	++
Tannins	–	–	++	++	++
Sugars	–	–	++	++	++
Phenolics	–	+	++	+	+
Flavonoids	–	+	++	+	+
Cardiac glycosides	–	–	–	–	–

DPPH scavenging activity

This method is based on the reaction of DPPH that is characterized as a preformed stable free radical with a deep violet colour and any substance that can donate a hydrogen atom to DPPH reduces it to a stable diamagnetic molecule.[28] The effects of phenolic compounds on DPPH scavenging are thought to be due to their hydrogen donating ability.[33] It was reported that the decrease in the absorbance of the DPPH caused by phenolic compound is due to scavenging of the radical by hydrogen donation, which is visualized as a discoloration from purple to yellow.[34] The reduction of the DPPH was followed *via* the decrease in absorbance at 517 nm. The various fractions of *D. viscosa* significantly reduced the DPPH. The values of percent scavenging of DPPH are presented in Table II. It was observed that activity increased with increasing concentration of the fractions in the assay. For the various concentrations of ethyl acetate, soluble fraction exhibited the highest percent inhibition of the DPPH as compared to the other fractions. This fraction showed 81.14±1.38 % inhibition of DPPH at a concentration of 60 μg ml^{-1}. The various concentrations of the fractions which showed percent inhibition greater than 50 % the activities were found to be significant ($p < 0.05$).

The IC_{50} value is defined as the concentration of a substrate that causes 50 % loss of the DPPH activity and was calculated by linear regression of plots of the percentage antiradical activity against the concentration of the tested compounds.[1] The IC_{50} values of all the fractions were calculated and the results are given in Table III. The lower the IC_{50} value, the higher is the scavenging potential. The ethyl acetate soluble fraction exhibited the lowest IC_{50} value, *i.e.*, 33.95±0.58 μg ml^{-1} as compared to the other studied fractions. The chloroform soluble fraction and the *n*-butanol soluble fraction showed very similar IC_{50} va-

lues, *i.e.*, 79.42±0.97 and 78.48±0.47 μg ml^{-1}, respectively. The IC_{50} values of the *n*-hexane soluble fraction and the aqueous fraction were found to be 238.30±1.89 and 189.28±1.59 μg ml^{-1}, respectively.

TABLE II. 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activity of the various fractions of *D. viscosa* Jacq.

Sr. No.	Sample	Concentration in the assay μg ml^{-1}	Scavenging of DPPH ± *SEM*[a] %
1	*n*-Hexane soluble fraction	500	67.14±1.72[b]
		250	54.29±1.25[b]
		125	40.01±0.81
		60	30.71±1.68
2	Chloroform soluble fraction	125	78.14±1.67[b]
		60	33.57±1.22
		30	23.47±1.81
3	Ethyl acetate soluble fraction	60	81.14±1.38[b]
		30	52.85±1.55[b]
		15	22.14±1.79
4	*n*-Butanol soluble fraction	125	75.71±1.87[b]
		60	40.62±0.99
		30	22.12±1.21
5	Remaining aqueous fraction	250	57.85±1.69[b]
		125	45.01±0.81
		60	27.14±0.46
6	BHT[c]	60	91.35±0.14[b]
		30	75.46 ±0.08[b]
		15	42.57±0.05
		8	23.47±0.34

[a]All results are presented as mean ± standard mean error of three assays; [b]$p < 0.05$ when compared with the negative control, *i.e.*, blank/solvent ($p < 0.05$ is taken as significant); [c]standard antioxidant

The results are expressed relative to butylated hydroxytoluene (BHT), a reference standard having IC_{50} of 12.54±0.89 μg ml^{-1}. The IC_{50} values of the chloroform soluble fraction, the ethyl acetate soluble fraction, the *n*-butanol soluble fraction and the aqueous fraction were found to be significant ($p < 0.05$) while that of the *n*-hexane soluble fraction was found to be non-significant ($p > 0.05$) when compared with BHT.

Total antioxidant activity

The total antioxidant activity of the studied fractions was measured by the phosphomolybdenum complex formation method. This method is based on the reduction of molybdenum(VI) to molybdenum(V) by the antioxidants and the subsequent formation of a green phosphate Mo(V) complex at acidic pH values. Electron transfer occurs in this assay which depends on the structure of the antioxidant.[29] The phosphomolybdenum method usually detects antioxidants such as

ascorbic acid, some phenolics, tocopherols and carotenoids. The total antioxidant activities of these fractions were compared with the standard antioxidant BHT and the results are given in Table III. The results revealed that the chloroform fraction showed the highest total antioxidant activity, *i.e.*, 1.078±0.59, as compared to the other fractions. The total antioxidant activities of ethyl acetate, *n*-butanol and aqueous fraction were found to be 0.941±0.17, 0.636±0.32 and 0.375±0.29, respectively. The *n*-hexane fraction showed lowest total antioxidant activity (0.356±0.21). The results were compared with BHT, a reference standard having total antioxidant activity 1.219±0.37. The total antioxidant activity shown by the chloroform soluble fraction, the ethyl acetate soluble fraction and the *n*-butanol soluble fraction were found to be significant ($p < 0.05$), while those of the *n*-hexane and aqueous fraction were found to be non-significant ($p > 0.05$) when compared with BHT.

TABLE III. IC_{50}, total phenolics, total antioxidant activity, FRAP values and lipid peroxidation inhibition values of the different fractions of *D. viscosa* Jacq.

Sr. No.	Sample	DPPH-radical scavenging activity IC_{50} / μg L^{-1}	Total antioxidant activity (eq. to BHT)	FRAP value μmol TE mL^{-1}	Total phenolics μg GAE g^{-1}	Lipid peroxidation inhibition[a] %
1	*n*-Hexane soluble fraction	238.30±1.89	0.356±0.21	40.81±0.48	28.23±0.36	17.66±0.87
2	Chloroform soluble fraction	79.42±0.97[b]	1.078±0.59[b]	278.45±0.72[c]	140.55±1.21[c]	49.37±0.99[b]
3	Ethyl acetate soluble fraction	33.95±0.58[b]	0.941±0.17[b]	380.53±0.74[c]	208.58±1.83[c]	58.11±1.49[b]
4	*n*-Butanol soluble fraction	78.48±0.47[b]	0.636±0.32[b]	234.40±1.28[c]	132.76±1.53[c]	41.50±0.46[b]
5	Remaining aqueous fraction	189.28±1.59[b]	0.375±0.29	89.54±0.98[c]	95.17±1.95[c]	22.12±0.76
6	BHT[d]	12.54±0.89	1.219±0.37	–	–	62.73±0.96

[a]tested concentration at 500 μg mL^{-1}; [b]$p < 0.05$ when compared with the reference standard (BHT); [c]$p < 0.05$ when compared with negative controls, *i.e.* blank/solvent ($p < 0.05$ is taken as significant); [d]expressed relative to BHT

Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) assay measures the reducing ability of antioxidants against the oxidative effects of reactive oxygen species. Electron donating antioxidants can be described as reductants and inactivation of

oxidants by reductants can be described as redox reactions. This assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of tripyridyltriazine (TPTZ), whereby an intense blue Fe^{2+}–TPTZ complex with an absorbance maximum at 593 nm is formed.[30] Increasing absorbance indicates an increase in reductive ability. The FRAP values of the studied fractions were calculated and the results are presented in Table III. Among all the fractions, the ethyl acetate fraction showed the highest FRAP value (380.53±0.74 μmol TE mL^{-1}). The chloroform fraction, the *n*-butanol fraction and the aqueous fraction exhibited FRAP values of 278.45±0.72, 234.40±1.28 and 89.54±0.98 TE μmol TE mL^{-1}, respectively, while the *n*-hexane fraction showed a very low FRAP value, *i.e.*, 40.81±0.48 μmol TE mL^{-1}. The high FRAP values obtained for the polar solvent fractions may be partially ascribed to the presence of phenolics and flavonoids. The FRAP values of the chloroform soluble fraction, the ethyl acetate soluble fraction, the *n*-butanol soluble fraction and the aqueous fraction were found to be significant ($p < 0.05$), while that of the *n*-hexane soluble fraction was found to be non-significant ($p > 0.05$) when compared with the blank.

Chemical composition of the fractions

Phenolic compounds and flavonoids are very important plant secondary metabolites. These compounds have numerous defence functions in plants, and thus, several environmental factors, such as light, temperature and humidity, and internal factors, including genetic differences, nutrients, hormones, *etc*., contribute to their synthesis.[35] Similarly, other factors, such as germination, degree of ripening, variety, processing and storage also influence the content of plant phenolics.[36] It was reported that the phenolics are responsible for the variation in the antioxidant activity of plants.[37] They exhibit antioxidant activity by deactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals,[38,39] or chelate metal ions and protect against pathogens and predators.[40] The most frequently encountered flavonoids are flavonols, quercetin, flavanols and anthocyanins.

The concentrations of phenolics in the different fractions, expressed as micrograms of gallic acid equivalents (GAEs) per gram of fraction, are given in Table III. Among the studied fractions, the ethyl acetate soluble fraction showed the highest amount of total phenolic compounds, *i.e.*, 208.58±1.83 μg GAE g^{-1}. The chloroform fraction and the *n*-butanol fraction showed very similar values of total phenolic content (140.55±1.21 and 132.76±1.53 μg GAE g^{-1}, respectively). The total contents of phenolics in the *n*-hexane soluble fraction and the aqueous fraction were found to be 28.23±0.36, and 95.17±1.95 μg GAE g^{-1}, respectively. The results for the total phenolics contents in the chloroform soluble fraction, the ethyl acetate soluble fraction, the *n*-butanol soluble fraction and the aqueous frac-

tion were found to be significant ($p < 0.05$), while that of the *n*-hexane soluble fraction was found to be non-significant ($p > 0.05$) when compared with the blank.

Oxygen reacts with unsaturated double bonds on lipids, which results in the generation of free radicals and lipid hydroperoxides. Peroxidation of lipids occurs both *in vivo* and *in vitro* and gives rise to cytotoxic and reactive products. These products disturb the normal functioning of the cell and can give rise to damaged or modified DNA. Hydrogen-donating antioxidants can react with lipid peroxyl radicals and break the generation cycle of new radicals. The ferric thiocyanate assay, in which peroxide reacts with ferrous chloride to form ferric ions, is used to measure the amount of peroxide at the beginning of lipid peroxidation. The ferric ions then unite with ammonium thiocyanate and produce ferric thiocyanate, a reddish pigment.[41] The various fractions of the plant were tested by this assay and the results are given in Table III. Significantly lower absorbances, as compared to the control, were observed for the chloroform fraction, the ethyl acetate fraction and the *n*-butanol fraction, which indicated that these fractions had greater antioxidant activities than the others. The fractions which showed greater values of percent inhibition of lipid peroxidation might contain primary antioxidant compounds, which are able to react aggressively with free radicals, particularly hydroxyl radicals, thereby terminating the radical-chain reaction and retarding the formation of hydroperoxides.[42] The highest percentage inhibition of lipid peroxidation was exhibited by the ethyl acetate fraction (58.11±1.49 %) at a concentration of 500 μg ml^{-1}, while the *n*-hexane soluble fraction showed the lowest percentage of inhibition of lipid peroxidation (17.66±0.87 %). The chloroform, *n*-butanol and aqueous fractions exhibited percent inhibition of lipid peroxidation of 49.37±0.99 %, 41.50±0.46 % and 22.12±0.76 %, respectively. The inhibition of lipid peroxidation by BHT (standard) was found to be 62.73±0.96 %. The results for percent inhibition of lipid peroxidation of the chloroform soluble fraction, the ethyl acetate soluble fraction and the *n*-butanol soluble fraction were found to be significant ($p < 0.05$) while that of the *n*-hexane soluble fraction and the aqueous fraction were found to be non-significant ($p > 0.05$) when compared with BHT.

CONCLUSIONS

The obtained results showed that the chloroform fraction, the ethyl acetate fraction, the *n*-butanol fraction and the aqueous fraction contained phenolics and flavonoids, while the *n*-hexane fraction showed an absence of these compounds. Due to the presence of such compounds, the chloroform fraction, the ethyl acetate fraction and the *n*-butanol fraction showed good antioxidant activity, the aqueous fraction showed moderate activity due to lower amounts of such compounds while the *n*-hexane fraction showed no activity because of the absence of all these compounds. The ethyl acetate soluble fraction exhibited the highest

percent inhibition of the DPPH radical as compared to the other fractions. It showed 81.14±1.38 % inhibition of the DPPH radical at a concentration of 60 μg ml^{-1}. The IC_{50} of this fraction was 33.95±0.58 μg ml^{-1}, relative to butylated hydroxytoluene (BHT), having an IC_{50} of 12.54±0.89 μg ml^{-1}. It also showed the highest FRAP value (380.53±0.74 μg of trolox equivalents) as well as the highest total phenolic content (208.58±1.83) and the highest value of inhibition of lipid peroxidation (58.11±1.49 % at a concentration of 500 μg ml^{-1}) as compared to the other studied fractions. The chloroform fraction showed the highest total antioxidant activity (1.078±0.59). Hence, it was concluded that chloroform fraction, ethyl acetate fraction and *n*-butanol fraction are rich in strong antioxidants. These fractions are potentially valuable sources of natural antioxidants and bioactive materials, which would be expected to increase shelf life of foods and fortify against peroxidative damage in living systems in relation to aging and carcinogenesis.

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ИЗВОД

ФИТОХЕМИЈСКА АНАЛИЗА, АНТИОКСИДАТИВНА АКТИВНОСТ И САДРЖАЈ ФЕНОЛА У БИЉЦИ *Dodonaea viscosa*

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Циљ студије је био утврђивање антиоксидативне активности биљке *Dodonaea viscosa* Jacq. Метанолском екстракту биљке је додата вода и извршена је екстракција *n*-хексаном, хлороформом, етил-ацетатом и *n*-бутанолом. Фитохемијском анализом је утврђено значајно присуство фенола, флавоноида и срчаних гликозида у хлороформском, етилацетатном и *n*-бутанолном екстракту. Антиоксидативни потенцијал ових фракција и заостале водене фракције је утврђен применом четири методе: DPPH, укупна антиоксидативна активност, FRAP тест и тест за одређивање укупних фенола. Етилацетатна фракција је била најпотентнији инхибитор DPPH радикала, у односу на друге фракције. Инхибиција је била 81,14±1,38 % при концентрацији 60 μg ml^{-1}. IC_{50} вредност ове фракције је била 33,95±0,58 μg ml^{-1}. Ова фракција је имала највећу FRAP вредност (380,53±0,74 μM тролокс еквивалента), највећи садржај фенола (208,58±1,83 GAE μg g^{-1}) и најизраженију инхибицију липидне пероксидације (58,11±1,49 % у концентрацији 500 μg ml^{-1}). Хлороформска фракција је имала највећу укупну антиоксидативну активност, 1,078±0,59 (апсорбанција на 695 nm).

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