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Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam. trees

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Abstract

Barks extracts of four different trees (*Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam.) in three different solvents 80% methanol, 80% ethanol, and 80% acetone (solvent:water, 80:20 v/v) were evaluated for their antioxidant activity, total phenolic (TP), and total flavonoids (TF) contents. Antioxidant activity (AA) was determined by measuring reducing power, inhibition of peroxidation using linoleic acid system and 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activity. Significant ($P < 0.05$) differences were observed in the TP, TF, inhibition of linoleic acid oxidation and DPPH$^{\cdot}$ scavenging activity of different bark extracts. Nevertheless, minute variation was observed in reducing power. All the bark extracts exhibited wide range of total phenolic, 7.8–16.5 gallic acid equivalents and total flavonoid contents, 1.59–4.93 catechin equivalents. Reducing power at 10 mg/mL extract concentration ranged from 1.34 to 1.87. Different bark extracts inhibited oxidation of linoleic acid by 44–90% while DPPH radical scavenging activity ranged from 49% to 87%. Extraction efficacy of components with antioxidative properties was lowering in the following order: ethanol > methanol > acetone. Good correlation was observed between TP and DPPH scavenging activity among the extracts. *A. nilotica* bark had the highest amounts of TP, ranging from 9.2 to 16.5 g/100 g, while the highest AA as measurement by inhibition of linoleic acid oxidation is offered by bark from *E. jambolana* Lam. The same tree showed the highest DPPH scavenging activity and reducing power. The correlation among the results of different antioxidant assays although revealed a strong relationship between some of the assays, however, a number of different methods may be necessary to adequately assess the in vitro antioxidant activity of a specific plant material.

Keywords: Barks; Antioxidant activity; Total phenolic contents; Total flavonoids contents; Reducing power

1. Introduction

Reactive oxygen species (ROS) cover a wide range of chemical components, including superoxide anion, hydrogen peroxide, hydroxyl radicals, nitric oxide, peroxynitrite, these radicals have potential to initiate degenerative processes in human body (Wang et al., 2002). We are protected from oxidative stress induced by ROS by efficient defense systems, however, capacity of the defensive system is affected by age, diet, health status of individual (Chun, Kim, & Lee, 2003). To help keeping proper equilibrium between ROS and defense system components, there is a need to provide antioxidants as part of diet (Yu et al., 2002). Antioxidants also play important role preventing oxidative deterioration of food and indirectly eliminating radicals from it (Vagi et al., 2005). Synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate and tertiary butylhydroquinone are

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often used in foods to prevent oxidative degradation. Application of synthethic antioxidants in foods is negatively perceived by consumers due to safety and health effect (Iqbal, Bhanger, & Anwer, 2007; Jeong et al., 2004).

Potential sources of natural antioxidant have been searched in different types of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, tree barks, roots, spices and herbs (Rababah, Hettiarachy, & Horax, 2004). A number of plants such as rosemary and sage belonging to *Labiateae* family have provided effective antioxidative extracts, used for the protection of oils, fats and salad dressings (Bandoniene, Pukalskas, Venskutomis, & Gruzdiene, 2000).

Jayaprakasha, Singh, and Sakariah (2001) reported that grape seed extract exhibited good antioxidant activity in preservation of food products. Effectiveness of different natural antioxidant in stabilizing vegetable oils has been previously studied (Anwar, Jamil, Iqbal, & Sheikh, 2006; Jung, Lee, Hun, Kyung, & Chung, 2001; Shaker, 2006; Siddiq, Anwar, Manzoor, & Fatima, 2005). Spices and herbs are added to food not only for flavor but also for preservation and suppression of rancidity and oxidation of lipids. (Wang & Lin, 2000; Wyen, Takacsova, Jakubik, & Dang, 2000). Moreover, need to understand how different antioxidative compounds work, what components may form an antioxidative system, stimulate search for antioxidants, particularly from plant sources (Liu & Ng, 2000; Pietta, 2000).

It has also been proposed that antioxidant activity of plant origin components can be mainly ascribed to the presence of phenolic compounds (Heim, Taigliaferro, & Bobilya, 2002). Phenolic compounds are not evenly distributed in plant parts; they are present at elevated amounts in the outer parts of the fruits, leaves and barks (Kahkonen et al., 1999). Tree bark is usually rich in compounds with medicinal properties, and many cultures use it for centuries. Asians, Polynesian and American indigenous people used bark components to treat heart failure among other medicinal applications (Duncan, 1998). Following the great success of Taxol (a chemotherapic anticancer drug), there has been a massive search by ethno botanists and biochemists for bark components, leading to the discovery of a number of useful products (Wansi et al., 2006).

Pakistan is an agricultural country, rich in endogenous medicinal and aromatic plants that are used as natural health care products in traditional medicine (Anonymous, 2003). A number of plants have been investigated for their biological activities and antioxidant principles (Baris et al., 2006; Saleem, Ahotupa, & Pihlaja, 2001).

Azadirachta indica, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam. trees are known as versatile source of components with bioactive properties. Recent discovery of antioxidants in *T. arjuna* which may be helpful in treatment of some cardiovascular problems, including ischemic heart disease (Anonymous, 2005). Components present in leave stem bark, flower buds and fruits of *E. jambolana* Lam. showed antibiotic activitie (Morton & Miami, 1987). *A. nilotica* offers variety of bioactive components which showed spasmogenic, vasoconstrictor, anti-hypertensive, antispasmodic, anti-inflammatory and anti-platelet aggregatory properties (Biswas, Chattopadhyay, Banerjee, & Bandyopadhyay, 2002).

In the present work, barks of four trees: *A. indica*, *T. arjuna*, *A. nilotica*, and *E. jambolana* Lam. grown in Pakistan were investigated for the presence of components with antioxidant activity to be applied in food system.

2. Materials and methods

2.1. Samples

Barks of four trees: *A. indica*, *T. arjuna*, *A. nilotica*, and *E. jambolana* Lam. commonly known as neem, arjun, desi kiker, and jaman, respectively, were collected in the vicinity of University of Agriculture, Faisalabad, Pakistan. Three samples for each bark were collected ($3 \times 3 \times 1$) to fulfill the requirements of statistical analysis.

2.2. Chemicals and reagents

Linoleic acid, (±) Catechin, Gallic acid and Folin–Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents, all of analytical grade, were from E. Merck (Darmstadt, Germany), unless stated otherwise.

2.3. Extraction

Air-dried samples of barks were ground into a fine powder in a grinding mill (Tector–Cemotec 1090 sample mill, Hognas, Sweden). Pulverized bark sample (20 g) was extracted with 200 mL of 80% methanol, 80% ethanol, and 80% acetone in water (80:20 v/v) using an orbital shaker (Gallenkamp, UK) for 8 h at room temperature. The extract was separated from the solids by filtration with Whatman No. 1 filter paper. The remaining solids were extracted twice with the same solvent and extracts combined. The extracts were concentrated under reduced pressure at 45 °C, in a rotary evaporator (EYELA, Tokyo, Japan). Concentrated extracts were stored in a refrigerator (−4 °C) until analysed.

2.4. Determination of total phenolics (TP)

Amount of TP were assessed using Folin–Ciocalteu reagent procedure as described by Chaovanalikit and Wrolstad (2004). Briefly, 50 mg of dry mass of barks extract was mixed with 0.5 mL of Folin–Ciocalteu reagent and 7.5 mL deionized water. The mixture was kept at room temperature for 10 min, and then 1.5 mL of 20% sodium carbonate (w/v) were added. The mixture was heated in a water bath at 40 °C for 20 min and then cooled in an ice bath, finally absorbance at 755 nm was measured (Hitachi U-2001 spectrophotometer, model 121-0032). Amounts of

TP were calculated using a calibration curve for gallic acid (10–100 ppm) ($R^2 = 0.9986$). The results were expressed as gallic acid equivalents (GAE) per dry matter. All samples were analyzed thrice and results averaged.

2.5. Determination of total flavonoids (TF)

TF were determined following the procedure by Dewanto, Wu, Adom, and Liu, (2002). One milliliter of aqueous extract containing 0.1 g/mL of dry matter was placed in a 10 mL volumetric flask, then 5 mL of distilled water added followed by 0.3 mL of 5% $NaNO_2$. After 5 min, 0.6 mL of 10% $AlCl_3$ was added. Afer another 5 min 2 mL of 1 M NaOH was added and volume made up with distilled water. The solution was mixed and absorbance measured at 510 nm. TF amounts were expressed as catechin equivalents per dry matter. All samples were analyzed thrice and results averaged.

2.6. Antioxidant activity determination in linoleic acid system

The antioxidant activity of bark extracts was also determined in terms of measurement of % inhibition of peroxidation in linoleic acid system following a reported method of Iqbal and Bhanger (2005). Extracts (5 mg) of each treatment were added to a solution mixture of linoleic acid (0.13 ml), 99.8% ethanol (10 ml) and 10 ml of 0.2 M sodium phosphate buffer (pH 7). Total mixture was diluted to 25 ml with distilled water. The solution was incubated at 40 °C and the degree of oxidation was measured following thiocyanate method (Yen, Duh, & Chuang, 2000) with 10 ml of ethanol (75%), 0.2 ml of an aqueous solution of ammonium thiocyanate (30%), 0.2 ml of sample solution and 0.2 ml of ferrous chloride ($FeCl_2$) solution (20 mM in 3.5% HCl) being added sequentially. After 3 min of stirring, the absorption values of mixtures measured at 500 nm were taken as peroxide contents. A control was performed with linoleic acid but without extracts. Synthetic antioxidants; butylated hydroxytoluene (BHT) and ascorbic acid (200 ppm) were used as positive control. The maximum peroxidation level observed as 360 h (15 days) in the sample that contained no antioxidant component was used as a test point. Percent inhibition of linoleic acid peroxidation, 100 – [(Abs. increase of sample at 360 h/Abs. increase of control at 360 h) × 100], was calculated to express antioxidant activity.

2.7. Determination of reducing power

The reducing power of the extracts was determined according to the procedure described by Yen et al. (2000), with modification. Equivalent volume of bark extract containing 2.5–10.0 mg of dry matter was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL, 1.0%); the mixture was incubated at 50 °C for 20 min. Then 5 mL of 10% trichloroacetic acid added and centrifuged at 980*g* for 10 min at 5 °C in an refrigerated centrifuge (CHM-17; Kokusan Denki, Tokyo, Japan). The upper layer of the solution (5.0 mL) was diluted with 5.0 mL of distilled water and ferric chloride (1.0 mL, 0.1%), and absorbance read at 700 nm (Hitachi U-2001). The measurement was run in triplicate and results averaged.

2.8. DPPH radical scavenging assay

Free radical scavenging activities of bark extract was measured by using procedure described by Iqbal and Bhanger, 2005. Briefly, to 1.0 mL of bark extract containing 25 μg/mL of dry matter in methanol, 5.0 mL of freshly prepared solution of 2,2′-diphenyl-1-picrylhydrazyl (DPPH) at concentration 0.025 g/Lwas added. Absorbance at 0, 0.5, 1, 2, 5 and 10 min was measured at 515 nm (Hitachi U-2001 spectrophotometer, model 121-0032). The remaining amounts of $DPPH^{\cdot}$ radical was calculated from calibration curve. Absorbance measured at 5th minute was used in comparison of radical scavenging activity of bark extracts.

2.9. Statistical analysis

Three samples of each bark were assayed. Each sample was analyzed individually in triplicate and data is reported as mean ($n = 3 \times 3 \times 1$) ± SD ($n = 3 \times 3 \times 1$). Data were analyzed using two-way analysis of variance ANOVA using Minitab 2000 Version 13.2 statistical software (Minitab Inc. Pennysalvania, USA) at 5% significance level.

3. Results and discussion

3.1. Total phenolic and total flavonoid contents

The yield of crude bark extracts, content of total phenolics and total flavonoids in Table 1 are shown. The amount of components extracted from different barks using different solvents varied widely. The highest amounts were extracted with 80% ethanol from *A. indica*, *T. arjuna* barks. Whereas, 80% methanol extracted more components from *A. nilotica* and *E. jambolana* Lam. Differences in the yield of extracts from barks might be attributed to the availability of different extractable components, define by the chemical composition of barks, nature of soil and agro-climatic conditions (Hsu, Coupar, & Ng, 2006). Among other parameters effectiveness of the extracting solvent to dissolve endogenous compounds. Methanol and ethanol has been proven as effective solvent to extract phenolic compounds (Siddhuraju & Becker, 2003). Ethanol is preferred for the extraction of antioxidant compounds mainly because it lowers toxicity (Karadeniz, Burdurlu, Koca, & Soyer, 2005; Tung, Wu, Kuo, & Chang, 2007).

Among solvents used in this study, 80% ethanol showed the best effectiveness extracting phenolic components. The amount of TP and TF extracted from barks in different solvent ranged from 7.8 to 16.5 GAE and from 1.6 to 4.9 CE,

Table 1
Yield, contents of total phenolics and flavonoids expressed as percentage, gallic acid and catechin equivalents, respectively

Component	*Azadirachta indica*			*Terminalia arjuna*			*Acacia nilotica*			*Eugenia jambolana*		
	A	B	C	A	B	C	A	B	C	A	B	C
% Age yield of crude extract	25.0 ± 0.50	13.8 ± 0.28	13.2 ± 0.40	37.2 ± 0.74	23.3 ± 0.93	7.70 ± 0.23	15.7 ± 0.63	31.6 ± 0.95	23.3 ± 0.47	13.5 ± 0.27	14.1 ± 0.56	3.60 ± 0.11
TPC	12.0 ± 0.36	9.30 ± 0.37	9.60 ± 0.28	12.8 ± 0.26	7.80 ± 0.39	8.20 ± 0.49	16.5 ± 0.66	11.2 ± 0.33	9.20 ± 0.37	9.00 ± 0.45	8.30 ± 0.49	8.20 ± 0.25
TFC	3.14 ± 0.09	3.31 ± 0.16	2.52 ± 0.10	3.49 ± 0.11	2.13 ± 0.13	1.59 ± 0.08	4.93 ± 0.15	3.21 ± 0.12	2.14 ± 0.04	2.10 ± 0.06	1.72 ± 0.07	1.68 ± 0.05

TPC, total phenolic contents; TFC, total flavonoid contents; A, 80% ethanol; B, 80% methanol; and C, 80% acetone.
Data are mean ($n = 3$) ± SD ($n = 3$, $P < 0.05$).
All calculations made on dry mass basis.

respectively, however, ethanolic extract of all the barks showed significantly ($P < 0.05$) higher TPC and TFC ranging from 9.0 to 16.5 GAE and 2.10 to 4.93 ± CE g/100 gm of dry matter, respectively. Barks from *A. nilotica* offered significantly ($P < 0.05$) higher amounts of TP and TF (9.2–16.5; 2.13–4.93), lower levels were extracted from *E. jambolana* Lam. (8.2–9.0; 1.68–2.10).

The interest in the phenolics has increased outstandingly due to their prominent free radical scavenging activity. Phenolic compounds could be classified as simple phenols, a single aromatic ring bearing at least one hydroxyl group, and polyphenols with at least two phenol subunits like flavonoids or three and more phenol subunits called tannins (Robbins, 2003). The extracted amounts of TP and TF from investigated tree barks in the study were lower than found from *Acacia confusa* bark (Tung et al., 2007). However, were higher than amounts extracted from *Bauhinia macrostachya*, *Cecropia obtuse*, *Davilla kunthii*, and *Inga edulisbark* (Silva, Souza, Rogez, Rees, & Larondella, 2006). The content of TP in the presently investigated barks was higher than in *Acacia auriculiformis* bark (Singh, Singh, Kumar, & Arora, in press). The amounts of flavoinoids extracted in this study were higher than those found in the barks of *Cercropia palmate*, *Cedrela odorata*, *Davilla rugosa*, and *Stryphnodendron barbadetiman* (Silva et al., 2006).

Difference in the amounts of TP and TF in different barks could be explained by the fact that presence of phenolics is affected by plant species, maturity at harvest, growing conditions, soil conditions and post-harvest treatment (Jaffery et al., 2003). Many studies confirmed that amounts and composition of phenolic compounds is diversified at sub-cellular level and within the tissues (Macheix, Fleuriet, & Billot, 1990, Randhir et al., 2004). Concentration of simple phenolic compounds such as caffeic acid ferulic acid etc. are generally higher in younger tissues, later then different phenolic acids condense to form complex phenolic compounds such as flavonoids, tannins and lignin etc. Hence, barks due to accumulation of phenolic compounds with the maturity of the plant possess relatively higher amounts of TFC than other plant organs (Siddhuraju, Mohan, & Becker, 2002; Wang & Lin, 2000).

3.2. Reducing power of the bark extracts

Measurement of reducing potential can reflect some aspects of antioxidant activity in the extracts. In this method ferric ions are reduced to ferrous ions and with it change in color from yellow to bluish green. The intensity of color depends on the reducing potential of the compounds present in the medium. Greater the intensity of the color, greater will be the absorption; consequently, greater will be the antioxidant activity (Zou, Lu, & Wei, 2004).

The data for the reducing potential of different bark extracts in Table 2 are presented. The reducing potential of the bark extracts measured for the concentration up to

Table 2
Reducing potential of bark extracts

Extracts	Conc. (mg/ml)	*Azadirachta indica*	*Terminalia arjuna*	*Acacia nilotica*	*Eugenia jambolana*
80% Ethanol	0.0	0.007 ± 0.002	0.002 ± 0.003	0.009 ± 0.001	0.003 ± 0.002
	2.5	0.321 ± 0.02	0.296 ± 0.02	0.354 ± 0.02	0.210 ± 0.03
	5.0	0.610 ± 0.02	0.435 ± 0.01	0.632 ± 0.04	0.542 ± 0.02
	7.5	0.933 ± 0.04	0.860 ± 0.04	1.896 ± 0.03	0.721 ± 0.04
	10.0	1.710 ± 0.03	1.340 ± 0.03	1.870 ± 0.05	1.600 ± 0.03
80% Methanol	0.0	0.004 ± 0.002	0.012 ± 0.003	0.005 ± 0.001	0.013 ± 0.002
	2.5	0.288 ± 0.03	0.326 ± 0.02	0.472 ± 0.02	0.391 ± 0.02
	5.0	0.508 ± 0.03	0.539 ± 0.03	0.931 ± 0.04	0.632 ± 0.02
	7.5	0.762 ± 0.02	0.920 ± 0.02	1.200 ± 0.05	0.829 ± 0.05
	10.0	1.460 ± 0.04	1.600 ± 0.04	1.520 ± 0.04	1.480 ± 0.04
80% Acetone	0.0	0.006 ± 0.004	0.011 ± 0.002	0.008 ± 0.005	0.002 ± 0.001
	2.5	0.421 ± 0.03	0.336 ± 0.02	0.394 ± 0.02	0.264 ± 0.02
	5.0	0.626 ± 0.02	0.537 ± 0.02	0.596 ± 0.03	0.492 ± 0.02
	7.5	0.932 ± 0.03	0.981 ± 0.04	0.882 ± 0.03	0.760 ± 0.04
	10.0	1.460 ± 0.06	1.740 ± 0.03	1.640 ± 0.05	1.480 ± 0.03

Data are mean ($n = 3$) ± SD ($n = 3$, $P < 0.05$).

10.0 mg/mL, showed general increase in activity when concentration increased. Reducing potential of different bark extracts at 10 mg/mL ranged from 1.34 to 1.87. The reducing power of the presently investigated barks was better compared to *Cassia fistula* leaves, flowers and pulp, however lower than the bark of the plant (Siddhuraju et al., 2002). Furthermore, the study results are comparable with the *Acacia auriculiformis* bark (Singh et al., in press). Indicating that these barks are a good source of antioxidants with high reducing power.

3.3. Antioxidant activity of different bark extracts in the linoleic acid peroxidation system

The antioxidants activity has also been assessed as ability to prevent from oxidation. Therefore, inhibition of linoleic acid oxidation was also used to asses the antioxidant activity of the bark extracts. Antioxidant activity (AA) of different barks extracts was determined by inhibition of peroxidation in linoleic acid system using thiocyanate method (Yen et al., 2000). Linoleic acid is a polyunsaturated fatty acid, upon oxidation peroxides are formed which oxidize Fe^{2+} to Fe^{3+}, the later forms complex with SCN^-, concentration of which is determined spectrophotometrically by measuring absorbance at 500 nm. Higher the absorbance higher will be the concentration of peroxides formed during reaction, consequently lower will the antioxidant activity.

All the bark extracts exhibited appreciable inhibition of peroxidation ranging from 44.4% to 90.2% and were compared with BHA, BHT and PG having inhibition of peroxidation 74.6%, 81.3%, and 63.7%, respectively (Fig. 1). 80%

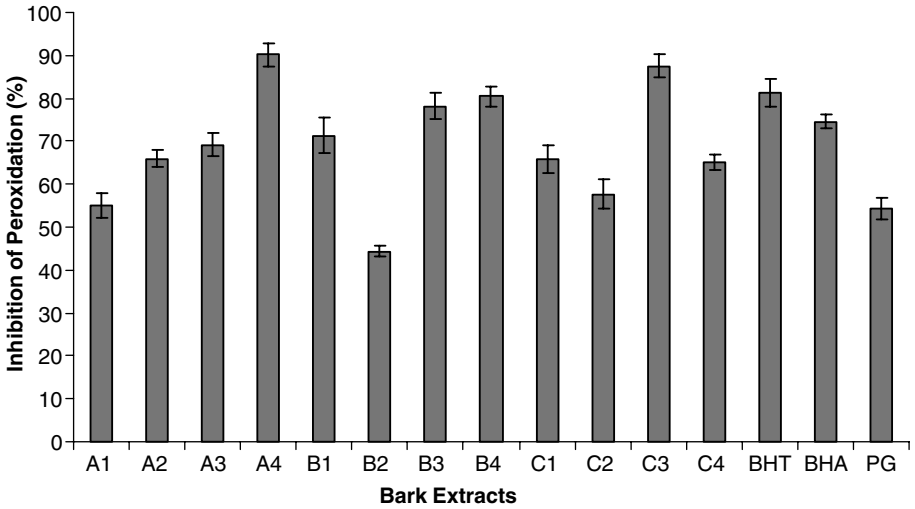

Fig. 1. Antioxidant activity of various solvent extracts (5 mg/25 ml (i.e. 200 ppm)) of different barks: A, 80% ethanol extract; B, 80% methanol extract; C, 80% acetone extract; 1, *Azadirachta indica*; 2, *Terminalia arjuna*; 3, *Acacia nilotica*; 4, *Eugenia jambolana*; BHT, butylated hydroxytoluene; BHA, butylated hydroxyanisole; PG, propyl gallate. Values are means of triplicate determination.

Ethanolic extract of *E. jambolana* bark and 80% acetonic extract of *A. nilotica* barks were found to be significantly ($P < 0.05$) more effective towards inhibition of peroxidation than BHT, BHA, and PG. With exception of 80% methanolic extract of *T. arjuna* all other bark extracts exhibited AA comparable to that of BHA, BHT, however, greater than PG. The effectiveness of these bark extracts towards inhibition of peroxidation was found to be greater than *C. fistula* L. (Siddhuraju et al., 2002). However, with the exception of methanolic extract of *T. arjuna* bark all other bark extracts exhibited nearly comparable inhibition of peroxidation to that of cinnamon bark (Mathew & Abraham, 2006). Considerable inhibition of peroxidation of bark could be attributed to presence of established antioxidants, such as xanthones, flavans, flavonols and di-antraquinones potentially responsible for the considerable activity of the bark extracts (Yen et al., 2000).

3.4. DPPH· radical scavenging activity

DPPH· is a very stable organic free radical with deep violet color which gives absorption maxima within the 515-528 nm. Upon receiving proton from any hydrogen donnor, mainly from phenolics, it loses it chromophore and became yellow. As the concentration of phenolic compounds or degree of hydroxylation of the phenolic compounds increases DPPH radical scavenging activity increases, and with it antioxidant activity (Sanchez-Moreno, Larrauri, & Saura-Calixto, 1999). Because this radical is very sensitive to active ingredients at low concentration and can accommodate a large number of samples in a very short time, this procedure is often used for measuring radical scavenging activity of different plant extracts. However, due to specificity of radical applied, which are not oxygen related radical species, antioxidant activity assessed can be related to them only. Absorbance in this assay was recorded at different time intervals from 0.5 to 10 min from the initiation of the reaction. Observed scavenging activity was similar at the beginning of the reaction and diverged with the increase of the reaction time until stabilized at 10th minute. Practical the highest differences between different extracts were observed at 5th minute of the reaction (Fig. 2).

All the bark extracts exhibited appreciable scavenging activity ranging from 49.0% to 87.0%. For ethanolic extract from *A. nilotica* significantly ($P < 0.05$) highest and for methanolic extract of *T. arjuna* the lowest values were observed for scavenging activity. The results for *A. nilotica* were similar while for other bark extracts different from those reported for *Cassia fistula* bark (Siddhuraju et al., 2002). Substantial DPPH· radical scavenging capacity of the bark extracts could be explained by the presence of phenolic componens (Siddhuraju et al., 2002). Furthermore, flavan-3-4-diol (fustucacidin) is usually the major component identified in plant barks and it expected donation of hydrogen from it to quench DPPH radicals.

3.5. Comparison between different antioxidant assays

The comprehensive evaluation of antioxidant activity of natural products, using different tests had been shown importance in assessing antioxidant activity of endogenous compounds. It is attractive for researchers to have a convenient, fast and universal method for overall quantification of antioxidant efficacy of the natural material. However, such a test yet has to be developed. A total antioxidant activity assay using one chemical reaction seems to be rather unrealistic, yet there are numerous published methods claiming to measure total antioxidant activity in vitro. It has also been reported that the extracts of medicinal plants exhibit their protective effects through diverse mechanism, like free radical scavenger and by acting as suppressing agents (Shahidi, 1997).

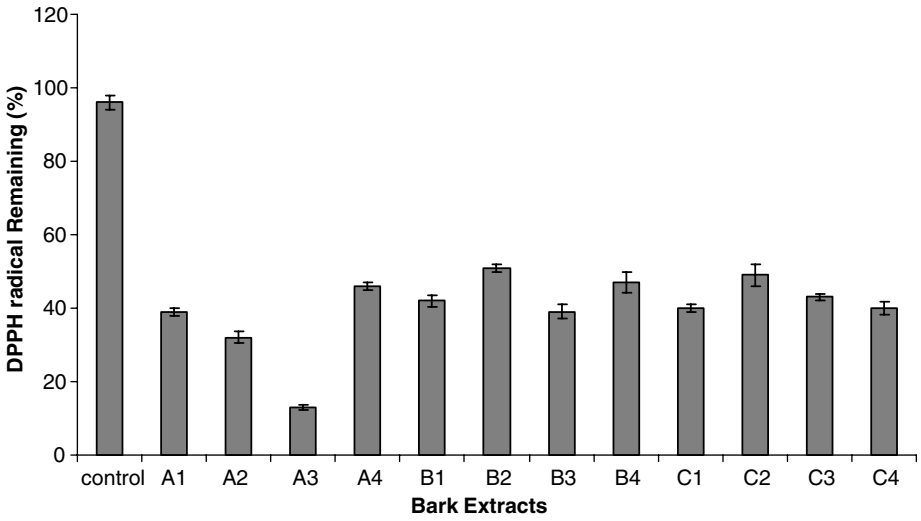

Fig. 2. DPPH radical scavenging activity of various solvent extracts of different barks A, 80% ethanol extract; B, 80% methanol extract; C, 80% acetone extract; 1, *Azadirachta indica*; 2, *Terminalia arjuna*; 3, *Acacia nilotica*; 4, *Eugenia jambolana*. Values are means of triplicate determination.

Table 3
Comparison between different antioxidant assays as represent by correlation coefficient (r) ($n = 12$)

Variable	Reducing power	Total phenolics	DPPH	Inhibition of oxidation	Flavonoids
Reducing power	–	0.846	0.753	0.138	0.671
Total phenolics	0.846	–	0.940	0.532	0.930
DPPH	0.753	0.940	–	0.637	0.875
Inhibition of oxidation	0.138	0.532	0.637	–	0.033
Flavonoids	0.671	0.930	0.875	0.033	–

Taking into account the free radical and oxidation of lipoprotein theory of diseases as the objective parameter, methodology assessing variety of parameters is required. In the present study different antioxidant assays such as inhibition of lipid oxidation, DPPH$^{\cdot}$ radical scavenging capacity, measurement of reducing potential combined with the estimation of total phenolic and total flavonoid contents were employed to assess the antioxidant effects of the bark extracts. The results of the different antioxidant assays used in the present investigation of bark extracts were compared and correlated with each other.

Correlation between the results of different antioxidant assays is shown in Table 3. A good correlation between TP and TF was verified ($r = 0.930$). Phenolic compounds are attracting considerable interest in the field of food chemistry and medicine due to their promising antioxidant potential (Singh et al., in press). Antioxidant activity of phenolic compounds is often associated with their redox properties, which allow them to act as reducing agents (Yıldırım et al., 2000; Siddhuraju et al., 2002), similar results were observed in the present study. Ethanolic extract from *A. nilotica* contained the highest amounts of TP and with it the highest reduction potential. While methanolic extract of *T. arjuna* confirmed opposite relation. The correlation between TPC and reducing power was high ($r = 0.846$), however correlation between TFC and reducing power was moderate ($r = 0.671$). This less correlation between TFC and reducing power might be attributed to the fact that most of the flavonoids are in their glycoside form, which are less effective as compared to their respective aglycone forms (Shahidi, 1997).

The results of the present study indicated that phenolic compounds are powerful scavenger of free radicals as demonstrated by a good correlation of DPPH$^{\cdot}$ scavenging activity with TP ($r = 0.940$) and TF ($r = 0.875$) when analyzing the barks of four different trees in three different solvents. Sequentially a good correlation ($r = 0.753$) was observed between reducing power and DPPH radical scavenging activity. For the correlation analysis the samples of *T. arjuna* were not taken into account, somehow this sample was at the other extreme compared to other samples. A comparable correlation coefficients were observed by Cai, Luo, Sun, and Corke (2004) Javanmardi, Stushnoff, Locke, and Vivanco (2003).

Efforts were made to establish correlation between % inhibition of oxidation with other antioxidant assays. The bark extracts showed relatively high inhibition of oxidation. Moderate correlation was observed between % inhibition and TP ($r = 0.532$) and DPPH scavenging capacity assay ($r = 0.637$) when 80% ethanolic extract of *A. nilotica*, 80% methanolic extract of *T. arjuna*, and 80% acetone extract of *T. arjuna* were not taken into account. TPC were determined by using Folin–Ciocalteu reagent that react nonspecifically to phenolic compounds as it can be reduced by many nonphenolic compounds e.g., vitamin C, Cu(I), etc. Hence, TPC determined by this method could not reflect the exact amount of phenolic antioxidants.

Poor correlation between reducing power and % inhibition ($r = 0.138$) was observed in the present investigation. This poor correlation could be explained by the facts that there are some antioxidative compounds (tocopherols, carotenoids, flavonoids, etc.) that not only exhibit their antioxidant activity by donating hydrogen but also by scavenging oxygen (Cuppett, Schnepf, & Hall, 1997). Consequently extracts containing such antioxidant compounds might exhibit higher levels of % inhibition as compared to their reducing power and thus can display a poor correlation.

Furthermore, little correlation was also observed between TFC and % inhibition of oxidation ($r = 0.033$) when all data were combined. This poor correlation between TFC and % inhibition of peroxidation could be explained on the facts that not only the flavonoids but many other compounds such as tocopherols, carotenoids etc. also contribute to antioxidant activity in terms of measurement of % inhibition of peroxidation (Karadeniz et al., 2005). Furthermore, Variation in correlation coefficient among different antioxidant assays indicates that a single assay is not sufficient to evaluate the total antioxidant activity (Frankel & Meyer, 2000; Silva et al., 2006). Overall, from the comparison and correlation of the results of different antioxidant assays in the present investigation of barks it could be stated that DPPH$^{\cdot}$ radical scavenging assay is preferred method over inhibition of oxidation test to asses the antioxidant efficacy of the phenolic compounds due to its fastness and direct relation with amount of phenolic compounds.

4. Conclusion

In this study, it was demonstrated for the first time that extracts from barks of investigated trees: *A. indica*, *T. arjuna*, *A. nilotica*, and *E. jambolana* Lam., exhibited outstanding antioxidant activity as measured by various antioxidant assays. Sizeable amounts of TPC and TFC were found in analyzed bark extracts. Among different

barks, *E. jambolana* Lam. was found to offer the most efficient antioxidant activity. The results of the present study would certainly help to ascertain the potency of the crude extracts of different barks as potential source of natural antioxidants. However, further research is needed to identify individual components forming antioxidative system and develop their application for food and pharmaceutical industries.

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