

Original article

Estimating Antioxidant and Free Radical Scavenging Activity of *Arbutus Pavarii* Extracts

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ABSTRACT

The primary aim of the current study was to evaluate and compare antioxidant activity in leaves and flowers extracts of *arbutus pavarii*. For the purpose, the antioxidant potential of crude methanol extracts of *arbutus pavarii* was screened in vitro using reducing power, phosphor molybdenum assay, and radical scavenging activity by employing DPPH, NO, ·OH methods as well as ferric thiocyanate (FTC) and thiobarbituric acid (TBA) tests to confirm the antioxidant potential of these extracts. Resultantly, it was confirmed that the leaves extract (LE) has displayed higher reducing ability compared with flowers extract (FE). The maximum antioxidant activity was found in leaves methanol extract (199.38 ± 12.73 mg of ascorbic acid/g of dry weight). The Methanolic LE was able to reduce the DPPH concentration with an IC_{50} of $1.09 \pm$ mg/mL, which was noticeable stronger ($P < 0.01$) than that of the positive control (ascorbic acid), ($IC_{50} = 0.01 \pm 1.6$ mg/mL) and FE ($IC_{50} = 1.25 \pm$ mg/mL) as well. The LE showed slightly inhibited ·OH radical (IC_{50} , 0.78 mg/mL) compared with FE (IC_{50} , 0.91 mg/ml). The obtained results of this investigation indicated the usefulness of utilization of *arbutus pavarii* leaves as a reliable source of antioxidants for nutritive and industrial purposes.

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INTRODUCTION

Medicinal plants are an important source of antioxidants which appear to have such desired comparative advantages, hence the growing interest in natural antioxidants from plants. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases like cancer, stroke, neurodegenerative and heart diseases [1-3]. Antioxidants (AOX) are considered a promising therapeutic approach as they may play neuroprotective and neurodegenerative roles. The main characteristic of an antioxidant is its ability to trap free radicals [4]. Indeed, free radicals toward endogenous molecules (DNA, proteins, and lipids) play an important role (antimicrobial activity).

However, they are implied specifically in the patho-physiology of numerous affections such as atherosclerosis, heart failure, liver injury, ageing, ischemic and a plethora of other diseases. Within normal conditions, the body is equipped with defense mechanisms that scavenge reactive oxygen species (ROS) and protect the cell from oxidative damage. Undoubtedly, damages are made in proteins, lipids and nucleic acids signaling cascades which result in disruption of ion homeostasis and modification of the genetic apparatus parallel to consequence of apoptotic cell death. The relationship between free radicals and diseases can be explained by the concept of 'Oxidative Stresses'. In a normal healthy human body, the generation of pro-oxidants in the form of ROS and RNS (reactive nitrogen species) are effectively kept in check by the various levels of antioxidant defense [5].

It is widely believed that mammalian cells possess elaborate defense mechanisms for radical detoxification. Antioxidants are agents, which scavenge the free radicals and prevent the damage caused by them. Despite these in-built defense mechanisms, it seems more meaningful to utilize extra antioxidants available in diets, especially from fruits, vegetables and whole grains. Due to their minimal side effects, there are growing interests in using natural products for preventive and therapeutic medicine [6]. The mechanism of these antioxidant compounds includes suppression of reactive oxygen species formation either by inhibition of enzymes or by chelation of trace elements involved in free radical production, scavenging of reactive species and up-regulating or protecting antioxidant defense [7].

Over the past few decades most chemical research have shown that free radicals cause oxidation which can be controlled or prevented by a range of antioxidant substances [8]. In addition to such advantages of natural antioxidants in medicine, these compounds have many industrial uses, such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline.

Arbutus pavarii. Pampan (Ericaceae) known as "strawberry trees" are species which start flowering in late fall and fruits become mature in early winter. The fruit is globose and many-seeded berry, yellow to orange red when fully ripe. The fresh mature fruits are edible, but sometimes processed before consumption. In traditional folk medicine, the fruits of *Arbutus pavarii* are used as antiseptic, diuretic and laxative, beside treatment of arterial hypertension. Importantly, Originally, *Arbutus pavarii* grow in the coastal region of Cyrenaica, and considered endemic species in El-Jabal El-Akhdar (northeastern area of Libya) [9-11].

Similarly, *Arbutus* bark and leaves are commonly used as medicines for colds, stomach problems, tuberculosis, and are used as the basis for contraceptives. The fruit is edible but has minimal flavor. Besides, *Arbutus* fruits are broadly used to make jams and beverages.

Phytochemicals (from the Greek word phyto, meaning plant) are chemical compounds formed during the plants' normal metabolic processes. These chemicals are often referred to as "secondary metabolites" of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, polysaccharides, phenols, tannins, terpenes and terpenoids [12].

The preliminary phytochemical analysis of *Arbutus pavarii* leaves and flowers extracts showed the presence of alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins, and reducing sugars [13]. It is known to contain important antioxidant components such as flavonoids, tannins, glycosides, simple phenolics [14,15].

Over the past few years, Hepatoprotective effects of the extracts were examined using mice pretreated orally with 200 and 400 mg/kg bw of flavonoids extracted from *Arbutus pavarii* leaves and flowers as well as their combination [16]. Furthermore, it has been reported that the most evident characteristics of *Arbutus pavarii* involve nutritive value and antioxidant content [17]. With regard to the aforementioned arguments, the present study aimed to screen the crude extracts of leaves and flowers of *Arbutus pavarii* to investigate the possible antioxidant activity in vitro.

MATERIAL AND METHODS

Chemicals

The chemicals used during the investigation process include dDiphenyl, Bicrylhydrazyl (DPPH), Catechin, Butylatedhydroxyl anisole (BHA), Phosphate buffered saline (PBS), Hydrogen peroxide, and Linoleic acid, all these chemicals were obtained from Sigma Chemical Company Ltd. (USA). Sodium nitroprusside (SNP), potassium ferric cyanide [K₃Fe(CN)₆], ferrous chloride, ferric chloride, ammonium molybdate, which were obtained from BDH chemicals (England). Finally, methanol, ethanol, N-(1-Naphthyl)- Ethylene Diamine Dihydrochloride (NED), 2,4-Dinitrophenylhydrazine (DNPH), Ethylene Diamine Tetra Acetic Acid (EDTA), Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), hydrochloric acid, sulfanilic acid, glacial acetic acid, gallic acid, ascorbic acid, vitamin-e were obtained from Merck (Pvt.) Ltd. (Germany). Solvents and other reagents were of analytical grade.

Plant materials collection

Arbutus pavarii (Ericaceae) (leaves and flowers) were collected from Al Marj, Libya. Collected leaves and flowers of *Arbutus pavarii* were dried at room temperature, ground into a powder, passed through a suitable mesh sieve and the dried powder was then stored at 4°C until used. Two grams of each sample (leaves and flowers of *Arbutus pavarii*) were soaked in 100 mL of methanol for 24 hr with gentle shaking. It was then filtered using Whatman filter paper No.1. The obtained crude extracts were preserved at 4°C until used [18].

Antioxidant study

The reducing property of the extracts was determined by assessing the ability of the extract to reduce FeCl₃ solution as described by Oyaizu [19]. An aliquot of extracts (2.5 mL) was mixed with 2.5 mL of phosphate buffer (2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (10 g/L) and then the mixture was incubated at 50° C for 20 min. After which, 1.5 mL of trichloroacetic acid (100 g/L) was added to the reaction mixture and then centrifuged at 3000 rpm for 10 min at room temp. Finally, 0.5 mL of the supernatant solution was mixed with 1.0 mL of distilled water, 0.5 mL of FeCl₃ (0.1%) and the absorbance was measured at 700 nm. Higher absorbance means higher reducing power.

Total antioxidant capacity

The total antioxidant capacity of the extracts was evaluated by the phospho-molybdenum method according to the procedure described by Prieto et al. [20]. A portion of 0.3 mL of extracts was mixed with 3 mL of reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate and 4 mM phosphor-ammonium molybdate). In case of blank, 0.5 mL of 45% ethanol was used in place of sample. The tubes were incubated at 95 °C for 90 min. After that, the absorbance of the green phosphate/Mo complex was measured at 695 nm. The higher absorbance value indicated higher antioxidant activity [21]. The results were expressed as ascorbic acid equivalent using the following linear equation: [Y= 3.9981X + 0.4436; R²= 0.9945] where Y is the absorbance at 695 nm and X is the concentration of ascorbic acid equivalent (mg/mL). The values are presented as the means of triplicates.

Free radical scavenging activity

DPPH (1, 1'-diphenyl-2-picrylhydrazyl) radical scavenging activity

The free radical scavenging activity of the extracts against 'DPPH was evaluated as explained by Wong et al., [21]. A 40 µL of extracts from leaves and flowers of tested plant at different concentrations (2.5, 5, 10 and 20 mg/mL) was added to 3.0 mL of 'DPPH (0.1 mM) in methanol solution, the mixture was left in the dark for 30 min and the absorbance was measured at 517 nm. The percent of DPPH scavenging effect was calculated as follows:

$$\% \text{ DPPH radical scavenging activity} = ([A_C - A_S] / A_C) \times 100$$

Where A_C was the absorbance of the control reaction and A_S was the absorbance in the presence of the extracts. The results were compared with ascorbic acids and BHA as positive control. The IC₅₀ was calculated as the number of antioxidants present in the sample necessary to reduce the initial 'DPPH concentration by 50%.

Hydroxyl radical (·OH) scavenging activity

In order to calculate hydroxyl radical scavenging activity, the Fe³⁺-ascorbate-EDTA-H₂O₂ system (Fenton reaction) was used to investigate the effects of the various fractions of extract to scavenge the hydroxyl radicals [22]. The reaction constituents involved mixing 500 µL of deoxyribose (2.8 mM) in phosphate buffer solution (50 mM, pH 7.4), 200 µL of premixed ferric chloride (100 mM) and EDTA (100 mM) solution (1:1; v/v), 100 µL of H₂O₂ (200 mM) with or without the extract solution (100 µL). The chemical reaction was activated by mixing 100 µL of 300 mM ascorbate and incubated for an hour at 37°C. Then, 0.5 ml of the mixture was added to 1.0 mL of trichloroacetic acid (2.8%; w/v), followed by adding 1.0 mL of TBA (1%; w/v) to the reaction mixture. Later, the mixture was incubated for 15 mins on a boiling water bath.

Overwhelmingly, the absorbance was noted after cooling at 532 nm against a blank. Interestingly, the calculation of scavenging activity on hydroxyl radical was made using this chemical equation (% Hydroxyl radical scavenging activity = ([A_C-A_S]/A_C) × 100).

Nitric oxide radical (NO·) scavenging activity

Garrat Method [23] was employed to assess nitric oxide radical scavenging activity spectrophotometrically. Accordingly, 1.0 mL of sodium nitroprusside (10 mM) in phosphate buffer was mixed with 0.5 mL of each extract of *Arbutus pavarii* kept at 25°C for 150 mins. After that, 0.5 mL of the mixture containing nitrite ions was removed and

1.0 mL of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) was added, shaken and allowed to stand for 5 mins. Next to that step, 1.0 mL of N-(1-Naphthyl)-ethylene diamine dihydrochloride (0.1%) was mixed and allowed to stand for 30 mins. The absorbance of the mixture was calculated at 540 nm against the corresponding blank. Ascorbic acid and butylated hydroxyl anisole (BHA) were used as positive control. The scavenging activity on nitric oxide radical was calculated as follows:

$$(\% \text{ Nitric oxide radical scavenging activity}) = ([A_C - A_S] / A_C) \times 100$$

Antioxidant activity determination in linoleic acid system

The antioxidant activity of the extracts against lipid peroxidation was determined using Ferric thiocyanate (FTC) and TBA methods. The FTC method was used to evaluate the peroxides at the initiation of lipid peroxidation, and TBA method was used to evaluate the secondary products of peroxide oxidation such as aldehyde and ketone.

Ferric thiocyanate (FTC)

The antioxidant activity of the extracts was determined using the ferric thiocyanate method in linoleic acid emulsion [24]. A mixture containing 4.0 mg of each extract of *Arbutus pavarii* [or methanol (as control) or BHA/vitamin E (as standard)] was mixed with 4 mL of pure ethanol (99.5%), 4.1 mL of linoleic acid (2.52%) in pure ethanol, 8 mL of phosphate buffer (0.05 M, pH 7.0) and 3.9 mL of distilled water was placed in a vial with screw cap and then placed in a rotary incubator (150 r/mins, 40° C) in a dark place. 0.1 mL of this mixture, 9.7 mL of ethanol (75%), and 0.1 mL of ammonium thiocyanate (30%) were combined altogether. Precisely 3 mins later the addition of 0.1 mL of ferrous chloride solution (20 mM in 3.5% HCl) acid was added to the reaction mixture. Hence, the absorbance of red color that indicates the antioxidant activity was measured at 500 nm for every 24 hr until the absorbance of the control reached maximum. The percent inhibition of linoleic acid peroxidation in an emulsion was calculated following the equation: (% inhibition of peroxidation (% IP)) = $([A_C - A_S] / A_C) \times 100$.

Thiobarbituric Acid (TBA)

TBA method was first introduced and explained by Halliwell in 1999. This method implied mixing 2.0 mL of trichloroacetic acid (20%) and 2.0 mL of TBA (0.67%) with 2.0 mL of the mixtures containing a FTC pre-prepared sample. This mixture was kept in water bath (100 °C) for 10 mins and after cooling to room temperature, it was centrifuged at 3000 rpm for 20 mins. The absorbance of the supernatant, containing TBA-MDA complex was read at 532 nm. The anti-lipid peroxidation activity percentage was calculated using the formula:

$$(\% \text{ Anti-lipid peroxidation activity}) = ([A_C - A_S] / A_C) \times 100.$$

RESULTS AND DISCUSSION

In vitro* antioxidant activity of crude extracts of leaves, flowers of *Arbutus pavarii

Antioxidant activity should not be concluded on a single antioxidant test model. Several *in vitro* assays are carried out for evaluating antioxidant activities with the samples of interest. Anti-oxidative abilities of the extracts were analyzed by phosphomolybdenum and reducing power methods. The free radical scavenging activities such as nitric oxide, hydroxyl, DPPH, and anti-peroxidation like thiobarbituric acid reactive substance (TBARS), FTC were carried out.

Reductive activity

Reducing power activity

It is thought that chemical components of some plants exhibit direct correlation between antioxidant activities and reducing power [25-27]. Therefore, reducing power may serve as a significant reflection of the antioxidant activity [28]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants [29]. In the current study, the reducing power of all the extracts increased in concentration. Maximum antioxidant activity was observed in the extracts of the highest concentration (20mg/mL) for crude leaves and flowers extracts as compared with the ascorbic acid (Figure 1). This difference in reducing powers could be due to their hydrogen- or electron-donating ability [30].

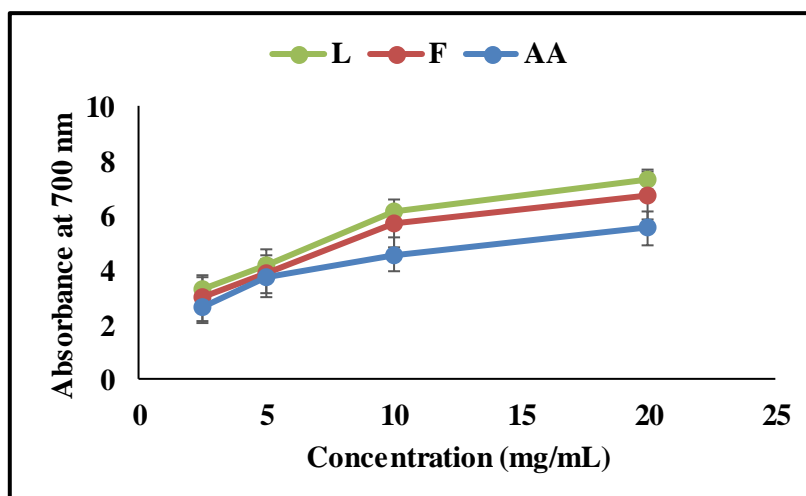


Figure 1. Reducing potential of crude extracts of *A. pavarii* (leaves and flowers), each value represented as mean \pm SD ($n=3$); L: leaves, F: flowers, Asca: ascorbic acid.

Total antioxidant capacity by phosphomolybdenum

Total antioxidant capacity by phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the crude extracts and the subsequent formation of green phosphate/Mo(V) complex at acidic pH. The phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid [20]. Overall, the results showed that leaves extracts showed higher antioxidant potential than flower extracts of *Arbutus pavarii* (figure 2). The maximum antioxidant activity was exhibited significantly by the methanol extract of leaves (0.4005 ± 0.041 mg of ascorbic acid/g dw).

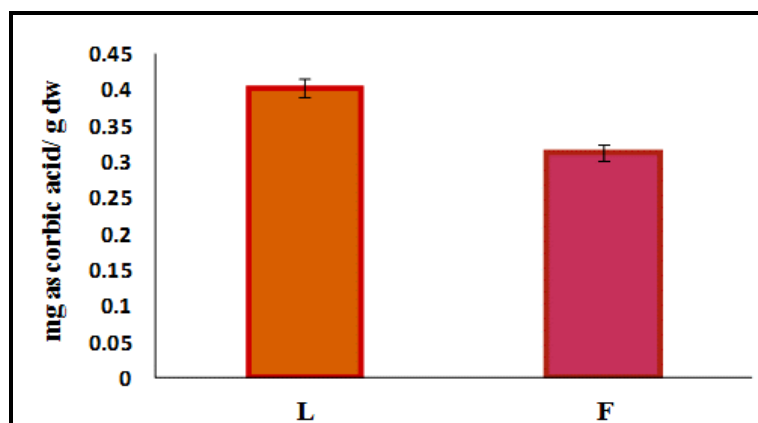


Figure 2. Total antioxidant capacity of crude extracts of *A. pavarii* (leaves and flowers). Each value represented as mean \pm SD ($n=3$); L: leaves, F: flowers, (ascorIc aId), dw; dry weight.

Free radical scavenging activity

DPPH radical scavenging activity

2, 2-Diphenyl-1-picrylhydrazyl ('DPPH') is a stable organic free radical which is capable to accept an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capacity of 'DPPH' was determined by the decrease in its absorbance at 517 nm induced by antioxidant molecules and it is visually noticeable as a discoloration from purple to yellow [31]. The ability to scavenge 'DPPH' has been extensively used as an easy, rapid, and sensitive way to evaluate free radical-scavenging capacities of natural antioxidants. The percentage inhibition of 'DPPH' radical was highlighted in Figure 3. The IC_{50} was expressed as the amount of antioxidant exists in the sample necessary to decrease the initial 'DPPH' concentration by 50%. The lower the IC_{50} value, the higher is the antioxidant activity. The methanol extract of leaves was able to reduce the 'DPPH' concentration and higher inhibition of 'DPPH' with an $IC_{50} = 1.09$ mg/mL as compared with flowers extract ($IC_{50} = 1.25 \pm 1.3$ mg/mL) but it was less than ascorbic acid, ($IC_{50} = 10 \pm 1.6$ μ g/mL).

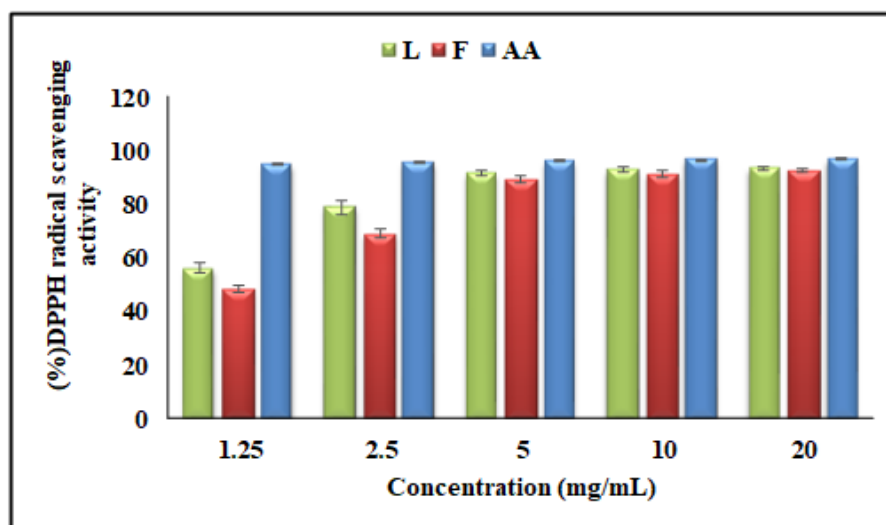


Figure 3. DPPH radical scavenging activity of crude extracts of leaves and flowers of *Arbutus pavarii*; each value represented as mean±SD (n=3) (p<0.01); L: leaves, F: flowers.

Hydroxyl radical scavenging activity

Among the reactive oxygen species (ROS), hydroxyl radicals ($\cdot\text{OH}$) are the most reactive and the dominant radicals generated endogenously during aerobic metabolism to initiate cell damage in vivo [32]. Thus, removing hydroxyl radical ($\cdot\text{OH}$) is important for the protection of living systems. $\cdot\text{OH}$ can be generated by Fenton reaction between ferrous iron and H_2O_2 . In the present study, the effect of methanol extracts of leaves and flowers on inhibition of the formation of hydroxyl radical ($\cdot\text{OH}$) was evaluated (Figure 4). Leaves extract showed slightly higher inhibition of produced hydroxyl radical (IC_{50} , 0.78 mg/mL) as compared with flowers extract (IC_{50} , 0.91 mg/mL) but it was still less than ascorbic acid (IC_{50} , 0.78 mg/mL).

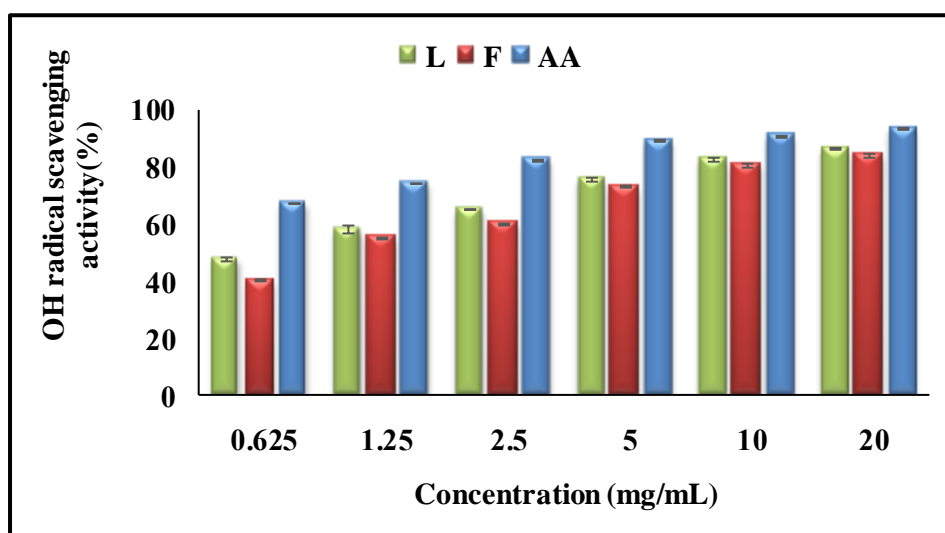


Figure 4. Hydroxyl radical scavenging activity of crude extracts of leaves and flowers of *A. pavarii*; each value represented as mean±SD (n=3); L: leaves, F: flowers, AA: ascorbic acid.

Nitric oxide radical scavenging activity

Nitric oxide ($\text{NO}\cdot$) is a potent inhibitor of physiological processes such as neuronal signaling, and inhibition of platelet aggregation, smooth muscle relaxation and regulation of cell-mediated toxicity [33]. The nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH reacts with oxygen to form nitrite. The extract inhibits nitrite formation by directly competing with oxygen in the reaction with nitric oxide. Figure 5 illustrates nitric oxide ($\text{NO}\cdot$) scavenging activity of leaves and flowers extracts at various concentrations. Although leaves extract exhibited

the higher NO[•] scavenging activity (47.43%) than flowers extract (34.46%) at 1.25 mg/mL but it was comparatively lower than ascorbic acid (85.73%).

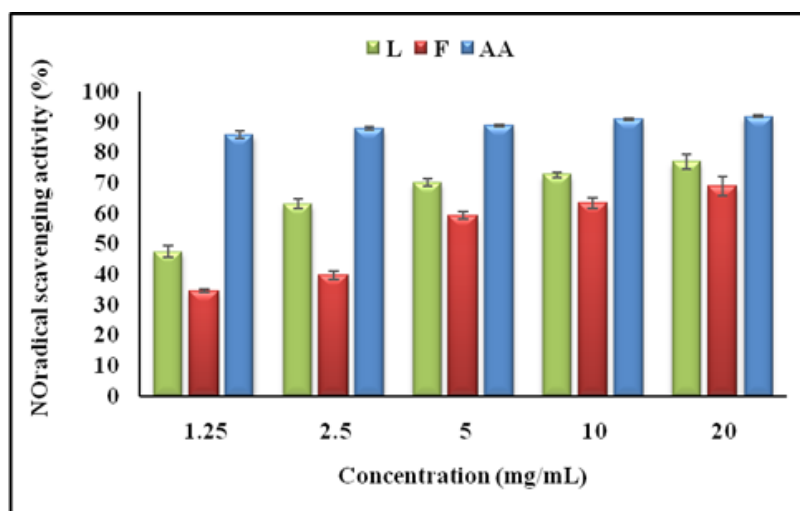


Figure 5. Nitric oxide radical scavenging activity of crude extracts under testing and ascorbic acid. Each value represented as means (n=3); L: leaves, F: flowers, AA: ascorbic acid.

Effect of extracts on the peroxidation of linoleic acid

FTC assay measured the number of primary products of lipid peroxidation (peroxides), while TBA assay was used to measure the secondary products of lipid peroxidation (MDA). Both assays obtained similar results whereas the maximum value of suppression of lipid peroxidation level was recorded in leaves extract (31.1%) which was significantly greater as compared with other extracts ($p < 0.05$) (Figure 6). Some of these active anti-lipid peroxidation compounds from plants were identified such as flavonoids, anthocyanidin and pro-anthocyanin [34-36].

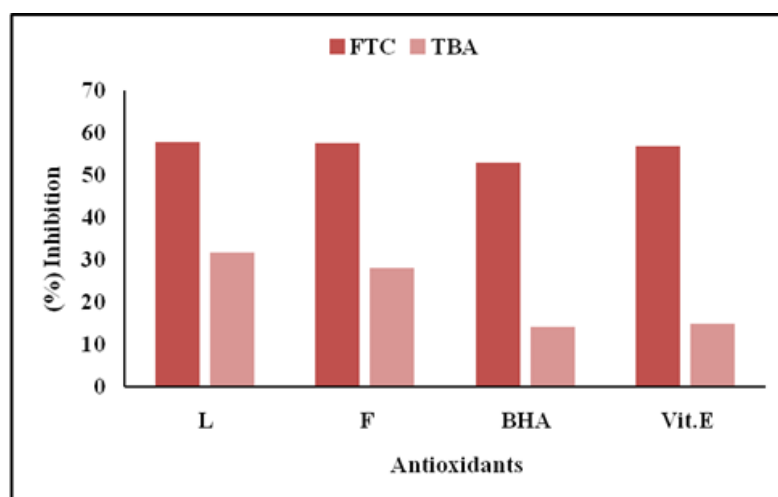


Figure 6: Inhibition of linoleic acid peroxidation (%) measured by the ferric thiocyanate (FTC) and thiobarbituric acid (TBA) antioxidant assays. Each value represented as mean \pm SD (n=3); L: leaves, F: flowers, BHA: Butylated hydroxyl anisole

CONCLUSION

The most obvious finding of this investigation demonstrated that the reducing ability was higher for the leaves extract compared with flowers extract. The total antioxidant activity of extracts was expressed as the number of equivalents of ascorbic acid. The maximum antioxidant activity was shown by the methanol extract of leaves (199.38 ± 12.73 mg of ascorbic acid/g of dry weight). Similar results were obtained from anti-lipid peroxidation activity outcomes; where the maximum values of suppression of primary and secondary products of lipid peroxidation were recorded in the sample incubated with leaves extract (31.1%). The methanol extract of the leaves was able to reduce the DPPH concentration with an IC₅₀ of $1.09 \pm$ mg/mL, which was noticeable stronger ($P < 0.01$) than that of positive control,

ascorbic acid, ($IC_{50} = 0.01 \pm 1.6$ mg/mL) as well as flowers extract ($IC_{50} = 1.25 \pm$ mg/mL). Furthermore, extracts of leaves showed slightly high inhibition OH radical (IC_{50} , 0.78 mg/mL) compared with flowers extract (IC_{50} , 0.91 mg/ml) but it was less than ascorbic acid (IC_{50} , 0.78 mg/mL). Leaves extracts exhibited higher $NO\cdot$ scavenging activity (76.86%) than flowers extract; it was significantly lower than ascorbic acid (91.77%) ($P < 0.05$).

REFERENCES

1. Rice-Evans C. Flavonoids and isoflavones: absorption, metabolism and bioactivity. *Free Rad Biol. Med.* 2004. 36: 827-828
2. Chanda S, Dave R. Invitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties. *An overview African J Micr Res.* 2009;13: 981-996.
3. Prior R L, Cao G. Antioxidant phytochemicals in fruits and vegetables. Diet and health implications. *Hortic Sci.* 2000;35: 588-592.
4. Mon M, Maw S, Oo Z. Quantitative Determination of Free Radical Scavenging Activity and Anti-tumor Activity of Some Myanmar Herbal Plants. *World Acad Sci Eng Tech.* 2011;51.
5. Devasagayam T, Tilak J, Bolor K, Ketaki S, Saroj S, Lele R. Free Radicals and Antioxidants in Human Health Current Status and Future Prospects. *J Gerontol.* 2004;11:298-300.
6. Baskar R, Shrisakthi S, Sathyapriya B, Shyampriya R, Nithya R, Palanisamy P. Antioxidant Potential of Peel Extracts of Banana Varieties (*Musa sapientum*) *Food and Nutrition Sciences*, 2011;2:1128-1133.
7. Darsini D, Maheshu V, Vishnupriya M, Sasikumar J. In vitro antioxidant activity of banana (*Musa spp.* ABB cv. PisangAwak). *Indian J Biochem Biophys.* 2012;49:124-129.
8. Bjelakovic G, Nikolova D, Gluud L, Simonetti R, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *JAMA.* 2007;297(8): 842-857.
9. Kabi H, Hegazy A, Lovett-Doust L. Demography of the threatened endemic shrub, *Arbutus pavarii*, in the Al-Akhdar mountainous landscape of Libya. *J For Res.* 2016;27:1295-1303.
10. Elshatshat S. Increasing anthropogenic impacts on restricted-range taxa of Libya from 2011 to 2015. *Archives of Applied Science Research*, 2015;7(5):91-96.
11. Siddiqi M. *Arbutus pavarii* in: *Flora of Libya. Ericaceae.* Ed. 1978;54: 6-8.
12. Liu R. Potential synergy of phytochemicals in cancer prevention: mechanism of action. *J Nutrition.* 2004;134:3479-3485.
13. Al-ghazeer R, Abourghiba T, Ibrahim A, Zreba E. Bioactive properties of some selected Libyan plants. *J Med Plants Res.* 2016;10(6): 67-76
14. Al-sabri S, El-Basir H, Rmeli N, Mohamed S, Allafi A, Zetrini A, El-Baseir M. Phytochemical screening, antioxidant, antimicrobial and anti-proliferative activities study of *Arbutus pavarii* plant. *J Chem Pharm Res.* 2013;5(1):32-36.
15. El-Darier S, El-Mogaspi F. Ethnobotany and relative importance of some endemic plant species at El-Jabal El-Akhdar region (Lybia). *World J Agri Sci.* 2009;5(3):353-360.
16. Alghazeer R, Elgahmasi S, Elnfati A, Elhensheri M, Al-Griw M, Awayn N, El-Nami M. Antioxidant Activity and Hepatoprotective Potential of Flavonoids from *Arbutus pavarii* against CCl₄ Induced Hepatic Damage. *Biotech J Inter.* 2018;21(1):2456-7051.
17. Elshatshat S, Elshibani F. Characteristics, Nutritive Value and Antioxidant Content of The Libyan Endemic (*Arbutus Pavarii*Pamp.) Strawberry Tree Fruits. *EPRA Inter J Res Develop.* 2020;2455-7838.
18. Al-ghazeer R, El-Saltani H, Saleh N, Al-Najjar A, Hebail F. Antioxidant and antimicrobial properties of five medicinal Libyan plants extracts. *Natural Science.* 2012;4:324-335.
19. Oyaizu M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese J Nutr.* 1986;44: 307-315.
20. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry.* 1999;269:337-341.
21. Wong C, Li H, Cheng F. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. *Food Chemistry.* 2006;97:705-711.
22. Ilavarasan R, Mallika M, Venkataraman S. Anti-inflammation and antioxidant activities of *Cassia fistula* Linn. bark extracts. *African J Trad Complement Altern Med.* 2005;2:70-85.
23. Garrat D. The quantitative analysis of drugs. Chapman and Hall Ltd, Japan. *Analytical and Bioanalytical Chemistry.* 1964;3:456-458.
24. Kikuzaki H, Nakatani M. Antioxidant effects of some ginger constituents. *Journal Food Science*, 1993. 58 (6): 1407-1410.
25. Duh, P.D. Antioxidant activity of burdock (*Arctiumlappa*Linne.): It is scavenging effect on free radical and active oxygen. *Journal of the American Oil Chemists' Society.*1998;75:455-461.
26. Duh P, Du P, Yen G. Action of methanolic extract of mung beans hulls as inhibitors of lipid peroxidation and non-lipid oxidative damage. *Food and Chemical Toxicology.* 1999;37:1055-1061.

27. Yildirim A, Mavi A, Oktay M, Kara A, Algur O, Bilaloglu V. Comparison of antioxidant and antimicrobial activities of tilia (TiliaaarenteaDesf. Ex. D.C.) Sage (Salvia triloba L.) and black tea (Camellia sinensis L.) extracts. Journal of Agricultural and Food Chemistry. 2000;48(10):5030-5034.
28. Oktay M, Gulcin I, Kufrevioglu O. Determination of in vitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts. Lebensmittelwissenschaft and Technologie. 2003;36:263-271.
29. Yen G, Chen H. Antioxidant activity of various tea extracts in relation to their antimutagenicity. Journal of Agricultural and Food Chemistry. 1995;43:27-32.
30. Shon M, Choi, Kahng G, Nam S, Sung, N. Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onions. Food and Chemical Toxicology. 2004;42:659-66.
31. Hseu Y, Chang W, Chen C, Lia J, Huang C, Lu F, et al. Antioxidant activities of Toonasinensis leaves extracts using different antioxidant models. Food and Chemical Toxicology. 2008;46(1):105-114.
32. Park E, Pezzutto J. Botanicals in cancer chemoprevention. Cancer and Metastasis Reviews, 2002;21:231-255.
33. Hagerman A, Riedl K, Jones G, Sovik K, Ritchard N, Hartzfeld et al. High molecular weight plant poly-phenolics(tannins) as biological antioxidants. Journal of Agricultural and Food Chemistry. 1998;46:1887-1892.
34. Pedrielli P, Pedulli G, Skibsted L. Antioxidant mechanism of flavonoids. Solvent effect on rate constant for chain breaking reaction of quercetin and epicatechin in autoxidation of methyl linoleate. Journal of Agricultural and Food Chemistry, 2001;49:3034-3040.
35. Gabrielska J, Oszmianski J. Antioxidant Activity of Anthocyanin Glycoside Derivatives Evaluated by the Inhibition of Liposome Oxidation. ZeitschriftfürNaturforschung Journal, 2005;60:399-407.
36. Smeriglio A, Barreca D, Bellocco E, Trombetta D. Proantho-cyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. British Pharmacological Society, 2016.

تقدير النشاطية المضادة للأكسدة و كسح الجذور الحرة لمستخلصات *Arbutus pavarii*

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المستخلص

هدفت هذه الدراسة إلى تقدير و مقارنة النشاطية المضادة للأكسدة في المستخلصات الكحولية لأوراق و أزهار نبات الشماري. و قد تم فحص الخاصية المضادة للأكسدة للمستخلصات الميثانولية في *A. pavarii*، باستخدام خاصية القدرة الاختزالية، والتقدير بالفوسفومولبدنوم ، و نشاطية كسح الجذور الحرة باستخدام طرق OH،NO،DPPH و بالمثل استخدمت اختبار انثيوسيانات الحديدك (FTC) وحمض الثيوباربيتوريك (TBA) لتأكيد إمكانات هذه المستخلصات كمضادات للأكسدة. و اظهرت نتائج البحث بأن مستخلص الأوراق (LE) يتميز بقدرة اختزال أعلى مقارنة بمستخلص الازهار (FE). حيث تبين أعلى نشاط مضاد للأكسدة في المستخلص الميثانولي للأوراق (12.73 ± 199.38 ملجم من حامض الاسكوريك / جم من الوزن الجاف). و تم التوصل الى نتيجة ان هذا المستخلص له القدرة على تقليل تركيز DPPH بتقدير IC50 الى $1.09 \pm$ ملجم/مل، والذي كان أقوى بشكل ملحوظ ($P < 0.01$) من المرجع الإيجابي (حمض الأسكوريك)، (0.01 ± 1.6 IC50 ملجم/مل) و كذلك للمستخلص الميثانولي للازهار ($1.25 \pm$ IC50 ملجم/مل) و بالتوازي مع هذه النتائج فقد أظهر مستخلص الاوراق LE تثبيطاً طفيفاً لجذر ($0.78 = IC50$ OH ملجم/مل) مقارنةً بمستخلص الازهار (FE، $0.91 = IC50$ ملجم/مل). و بالمجمل فإن النتائج المتحصل عليها من هذه الدراسة أشارت إلى امكانية الاستفادة من أوراق نبات *A. pavarii* كمصدر موثوق لمضادات الأكسدة للأغراض الغذائية والصناعية.

الكلمات الدالة: النشاطية المضادة للأكسدة ، *Arbutus pavarii* ، مقايسة DPPH ، كسح الجذور الحرة.