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Bioactive properties of some selected Libyan plants

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The present work evaluates the antioxidant and antibacterial activities of four Libyan plants (*Arbutus pavarii*, *Pegnanum harmal*, *Pistachia atlantica*, and *Fagonia bruguieri*) using various assays including reducing power, Phosphomolybdenum, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and nitric oxide free-radical scavenging activity. *In vitro,* the antibacterial activity of crude and flavonoids extracts were determined against five strains, *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus faecalis*, *Escherichia coli*, *Salmonella typhi*. In the extracts of all plants under investigation, the total flavonoids and polyphenols were quantitatively estimated. Leaves of *A. pavarii* possess the highest antioxidant potential with significant free-radical-scavenging effects; the highest flavonoids content (1504.28±80.89 mg Rutin g^{-1}) and a relatively high content of phenolics (1217.14±50.52 mg GAE g^{-1}) compared to the other tested plants. Regression analysis of the antioxidant assays showed a strong correlation between phenolics or flavonoids content and nitric oxide scavenging activity as well as total antioxidant activity. Also the crude extracts especially *A. pavarii* showed greater growth inhibition capacity towards tested bacterial strains. Results of the antioxidant assays and antimicrobial screening showed that, all tested plants can act as radical scavengers and antimicrobial agents to a certain extent. Future study is recommended to further purify and examine individual bioactive compounds from these extracts and evaluate their *in vivo* antioxidant and bacterial activities.

Key words: *Arbutus pavarii*, *Pegnanum harmal*, *Pistachia atlantica*, *Fagonia bruguieri*, polyphenols, antioxidant activity, antibacterial activity.

INTRODUCTION

Increasing attention has been paid to the use of natural antioxidants, such as ascorbic acid, tocopherols, phenolic compounds including flavonoids, phenolic acids, and volatile compounds for preventing oxidation of biomolecules which can lead to cell injury and death (Patil et al., 2003). Also alkaloids has been showed of marked anti antioxidant ability (Maiza-Benabdesselam et al., 2007). Great number of substances of plant origin has been shown to exhibit antioxidant activity (Gulcin et al., 2003; Ali et al., 2008). The use of antioxidants that scavenge reactive oxygen species (ROS) has been studied by evaluating its potential and therapeutic applications. Antiradical antioxidants act by donating hydrogen atoms to lipid radicals. Radicals obtained from

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antioxidants with molecular structures such as phenols are stable species and will then stop the oxidation chain reaction (Morales-González, 2013).

The biostatic and biocidal activities of natural bioactive compounds including alkaloids, flavonoids, tannins, terpenoids, glycosides, saponins, anthraquinones, against a range of enteric pathogenic microbia were investigated (Enwa et al., 2013).

Many plant extracts have been shown to possess potent anti-oxidation activities (Tachakittirungrod et al., 2007), being able to scavenge a wide range of ROS (superoxide, hydroxyl and peroxyl radicals), reactive nitrogen species (RNS) (peroxynitrous acid) and chlorine species (hypochlorous acid) (Hernandez et al., 2009). Therefore, a number of plants have been utilized successfully for the treatment of free radical-mediated diseases in humans such as rheumatoid arthritis, atherosclerosis, cancer, Alzheimer's disease (AD), Parkinson's disease, aging and some inflammatory diseases (Das, 2002).

Plant extracts have also shown to display *in vitro* antimicrobial properties in which their bioactive constituents may exhibit different modes of action against enterotoxigenic bacterial strains. It range from interference with the phospholipoidal cell membranes which leading to an increase in the permeability profile and loss of cellular constituents, to the damage of the enzymes involved in the production of cellular energy and synthesis of structural components as well as destruction or inactivation of genetic material. In general, the mechanism of action is considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport mechanisms, and coagulation of cellular proteins (Kotzekidou et al., 2008).

Plants are a valuable source of new products. Despite the availability of different approaches for the synthesis of therapeuticals, natural products remain one of the best supplies of new antioxidants and antimicrobial agents. Therefore, the present investigation aims to evaluate the antioxidant and antibacterial abilities of some medicinal Libyan plants.

MATERIALS AND METHODS

Plant collection and crude extract preparation

Arbutus pavarii (Ericaceae) (flowers and leaves) were collected in January 2014 from Al Marj, Libya*, Pegnanum harmal (Zygophyllaceae)* (seeds), *Pistachia atlantica (Anacardiaceae)* (Fruits) were collected in May 2014 from Nafusa Mountain, Libya, and *Fagonia bruguieri* was collected in May 2014 from Ghadames*,* Libya. The three plants were authenticated by the Department of Botany, Faculty of Science, and University of Tripoli, Libya. After cleaning and drying plants, samples were powdered using electric blender.

About 5 g of each sample (powdered) were soaked in 50 ml of methanol for 24 h with gentle shaking. It was then filtered using Whatman filter paper No.1 and the filtrate was evaporated to dryness under vacuum at 40°C using a rotary evaporator (Hidolph, Germany). The obtained crude extracts were preserved at 4°C until use.

Test organisms

The bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus faecalis*, *Escherichia coli*, *Salmonella typhi*) were provided and identified by the Microbiology Laboratory, Medical Research Center, Libya. Bacterial strains were routinely grown and preserved on Nutrient broth or Nutrient agar medium (2.0% agar was added whenever needed).

Phytochemical screening

Plants extracts were subjected to phytochemical screening as described by Harborne (1992).

Determination of polyphenols and flavonoids contents

The total phenolic content was determined using the Folin-Ciocalteu reagent (Sigma, USA) according to the method of Marinova (2005). 200 µl of the sample (0.1 g/ml) in triplicate were incubated with 1 ml of two fold diluted Folin-Ciocalteu reagent for 5 min. 1 ml of 7% Na_2CO_3 was then added to the reaction mixture which was incubated again for 90 min. Thereafter, the absorbance was read at 750 nm using Jenway UV-VIS 6305 spectrophotometer. The total phenolic content is expressed as gallic acid equivalent (GAE) in milligrams per gram of dry sample.

Total flavonoid content was estimated according to (Zhishen, 1999). 1 ml of plant extract (0.1 g/ml) was diluted with 4 ml of water and was mixed with 0.3 ml of $NaNO_2$ (5% w/v). After 5 min, 0.3 ml of $AlCl_3$ (10% w/v) was added followed by the addition of 2 ml of NaOH (1 M) six minutes later. The reaction volume was increased up to 10 ml by adding 2.4 ml distilled water and the sample was incubated at RT for 15 min. The absorbance was measured at 510 nm. The assay was performed in triplicate, and the flavonoids content was determined by interpolating the absorbance of the samples against a calibration curve constructed with rutin standard (1.0 to 5.0 mg/ml) and expressed as milligrams of rutin equivalent per gram of extract (mg RE/g).

Assessment of antioxidant activity

Reducing power

The reducing capacity of crude plant extracts were investigated according to the method of Oyaizu (1986). Various concentrations of plant extracts (1.25 to 10 mg/ml) were mixed with 2.5 ml of phosphate buffer (2 M, pH 6.6) and 2.5 ml of potassium ferric cyanide (1%), and the mixture was incubated at 50°C for 20 min. After which, 1.5 ml of 10% TCA was added to the reaction mixture and then centrifuged at 1000×g for 10 min. The supernatant (0.5 ml) was mixed finally with $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 665 nm. The higher the absorbance of the reaction mixture the greater is the reducing power. Concentrations of ascorbic acid were used to obtain a standard curve and the reducing power of extracts was expressed as ascorbic acid equivalents.

Evaluation of total antioxidant capacity (TAC) by phosphomolybdenum method

The total antioxidant capacity was evaluated by the

Table 1. Preliminary phytochemical screening of crude extract of some libyan plants.

Phytochemical compounds	*A. pavarii (Ericaceae)*		*P. harmala (Apiaceae)*	*P. atlantica (Euphorbiaceae)*	*F. bruguieri (Rhamnacea)*
	Used part				
	Flowers	Leave	Seeds	Fruits	Leave
Alkaloids	+++	+++	+	++	+
Flavonoids	+++	+++	+	+++	+
Tannins	+++	++	-	++	-
Terpenes	+++	++	-	++	-
Coumarins	+++	+	+++	++	-
Saponins	+	+++	-	-	+
Anthraquinones	+++	+++	-	+	-

(+++) high, (++) medium, (+) poor, (-) no found.

phosphomolybdenum method according to the procedure described by Prieto et al. (1999). 0.3 ml of extract was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). After that, the absorbance of the green phosphate/Mo complex was measured at 695 nm.

DPPH free radical-scavenging activity

The free radical scavenging activity of plant extracts was measured by DPPH (2, 2-diphenyl-1-picrylhydrazyl) (Sigma, USA) according to Wong et al. (2006) method. Briefly, 40 µl of methanolic extract of plant at different concentrations (5, 12.5, 25 and 50 mg/ml) or 0.165, 0.25, 0.5 and 1 mg/ml for *A. pavarii (flowers and leaves)* were added to 3 ml of DPPH (0.1 mM) in methanol solution, shaken vigorously and allowed to stand for 30 min at room temperature. The absorbance was measured at 517 nm using a UV- visible spectrophotometer (U-6305 model, Jenway, Japan). The percent of DPPH scavenging effect was calculated as follows:

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

Where A_C was the absorbance of the control reaction and As was the absorbance in the presence of the sample plants.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was measured spectrophotometrically according to Garrat (1964) method. 1 ml of sodium nitroprusside (10 mM) in phosphate buffer was added to 0.5 ml of sample (1.25 mg/ml) and incubated at 25°C for 150 min. Thereafter, 0.5 ml of the reaction mixture containing nitrite ions was taken; 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) was added. After being shaken, the samples were allowed to stand for 5 min. then 1 ml of naphthylethylene diamine dihydrochloride (0.1%) was added, mixed and allowed to stand for 30 min. The absorbance of the mixture was measured at 540 nm against the corresponding blank. The percentage of scavenging activity was measured with reference to ascorbic acid as standard.

Antimicrobial assay

The antibacterial activity of the crude and flavonoids plant extracts was screened against some Gram-negative and Gram-positive bacteria using the Agar diffusion method (Nair et al., 2005) at a concentration of 100 mg/ml for crude extract and 5 mg/ml for flavonoids extracts. Nutrient agar (Difco™ Tryptic Soy Agar, Becton Dickinson and Company, USA) was inoculated with 250 µl the suspension of the respective 24 h cultured organism and poured into a sterile Petri dish. Ceproxazine (Merck, Germany) was used as a positive control, the solvent of each extract as a negative control. A pre-diffusion for 3 h at 4°C was guaranteed. Diameters of inhibition zones (DIZs) were measured in mm after 18 h incubation at 37°C. The inhibition zones were measured excepting the hole diameter (8 mm). The results were recorded as the mean of triplicate experiments. Inhibition zones >15 mm were declared as strong, from 8 to 15 mm as moderate and from 1 to 8 mm as weak activities. Minimal inhibitory concentration (MIC) values against selected strains were determined by standard serial broth microdilution assay (European Pharmacopoe, 1997).

Statistical analyses

The experiments were carried out in triplicate. The results are given as mean ±standard deviation (SD), Student's t-test was used for comparison between the means of samples and standards. A difference was considered statistically significant when $P<0.05$. Correlation analysis was carried out on total phenolics and flavonoids contents (TPC, TFC) and the three antioxidant models (total antioxidant activity, DPPH & Nitric oxide scavenging activity) using SPSS 12.0 for Windows (Statistical package).

RESULTS

Phytochemical screening

The phytochemical characters of the four investigated medicinal plants are summarized in Table 1. Flavonoids and alkaloids were the most common components present in investigated plants. Coumarins are found in *A. pavarii*, *P. harmal*, *P. Atlantica* while absent in *F. bruguieri*. Anthraquinones are present only in *A. pavarii*, and *P. Atlantica*, while Terpenoids are found in *A. pavarii* and *P. atlantica*. Tannins are present in *A. pavarii*, and

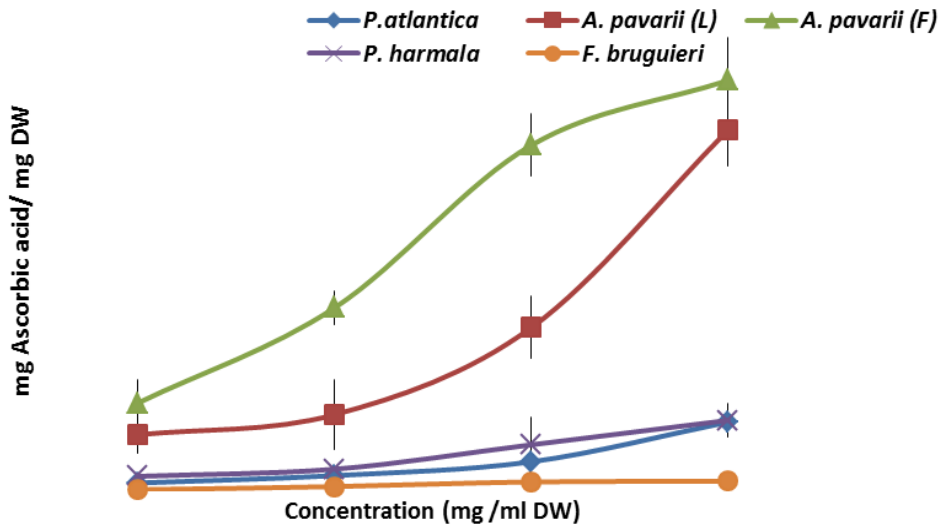

Figure 1. Total reducing power of crude extracts of the investigated plants estimated by ferrous cyanide. Each value is represented as mean ±SD (n=3).

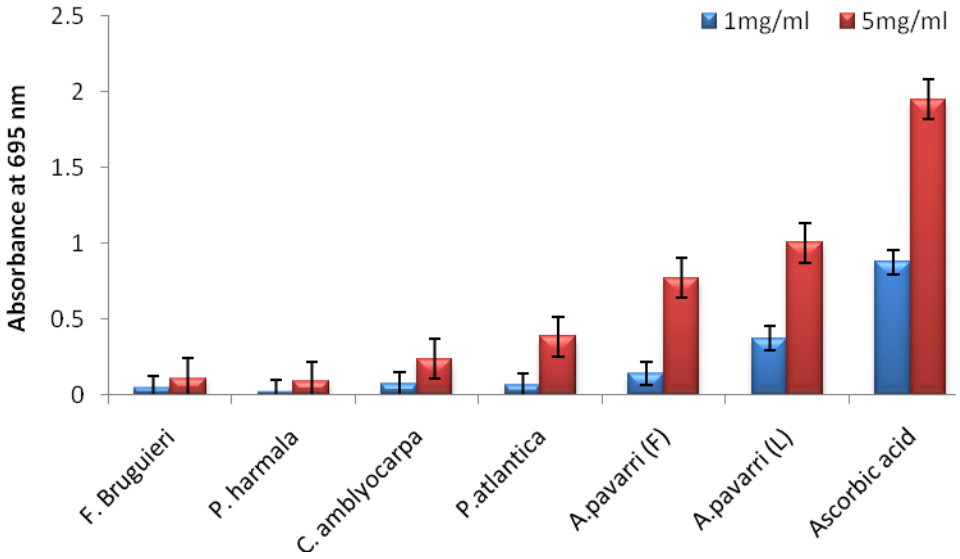

Figure 2. Total antioxidant capacities of extracts at two different concentrations (1 and 5 mg/ml) as determined by *Phosphomolybdate* method. Each value is expressed as mean ±SD (n=3).

P. harmal.

In vitro antioxidant activity

Antioxidant capacity of plant extracts were assessed and compared using four different antioxidant assays.

Reducing power activity

As shown in Figure 1, reducing power increased with the increase of extracts concentration. Flowers of *A. pavarii* had the highest ability to reduce Fe (III) while *F. bruguieri* extract had the lowest capacity. Also the reducing power of methanolic leaves extracts of *A. pavarii* was more than that of the *P. harmala*, and *P. atlantica* extracts.

Phosphomolybdate assay

Results showed in Figure 2 revealed that the antioxidant activities of the tested plant extracts were significantly

Table 2. IC_{50} of plants extracts in comparison with ascorbic acid as standard.

Plant	IC_{50} (mg /ml)
A. pavarii (L)	0.62±0.003**
A. pavarii (F)	1.30±0.004**
P. atlantica	50±3.75
P. harmala	100.30± 10.23
F. bruguieri	12.50± 1.15*
Ascorbic acid	3.05±0.005

Each value in the table is represented as mean ±SD (n=3).* indicates significance at $P<0.05$, ** indicates significance at $P<0.01$.

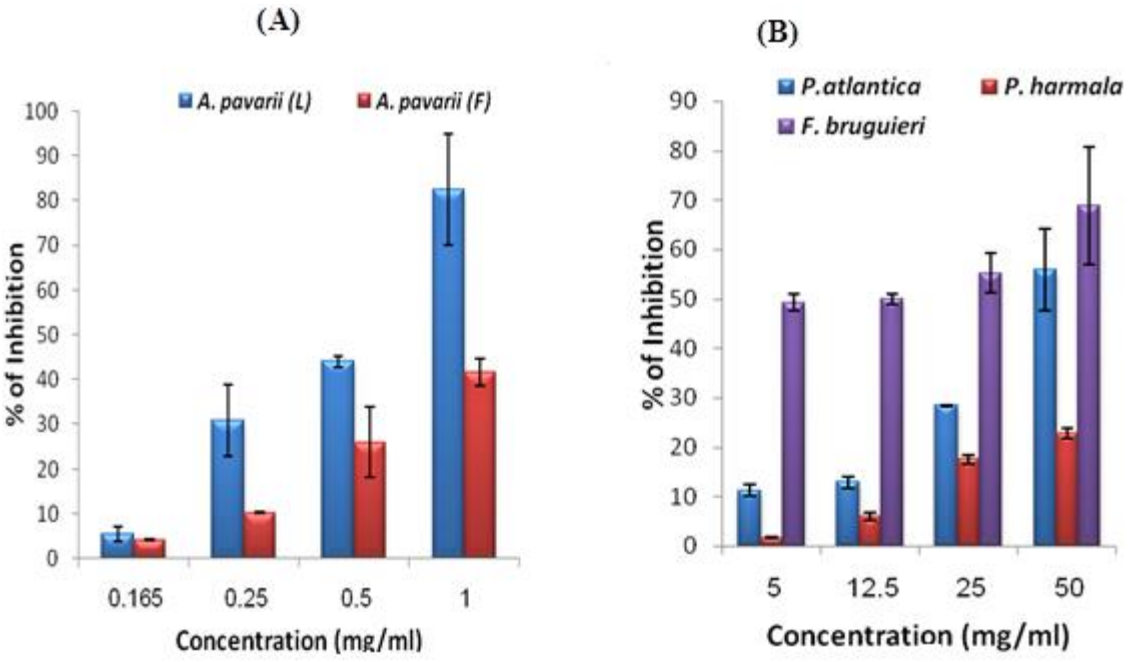

Figure 3. DPPH radical scavenging activity of plant extracts at various concentrations. Each value are represented as mean ± SD (n = 3).

different especially at high concentrations (2.5 to 5.0 mg/dL). Although *A. pavarri* (L) had the highest antioxidant capacity for phosphomolybdate reduction, its activity did not reach that obtained by ascorbic acid. *P. harmala* exhibited the lowest antioxidantion capacity for phosphomolybdate reduction.

DPPH radical scavenging activity

The results showed that *A. pavarii* possessed highest radical scavenging and reducing power activities (Table 2, Figures 1 and 3A). Leaves and flowers extracts of *A. pavarii* displayed strong radical scavenging effect compared with ascorbic acid at very low concentration (0.165, 0.25, 0.5 and 1 mg/mL) (Figure 3A). Compared to ascorbic acid a significant difference between the IC_{50} of *A. pavarii* (leaves and flowers) and ascorbic acid (0.62±0.003, 1.30±0.004 and 3.05±0.005 respectively) ($P< 0.001$), which means that the radical scavenging activities of these two extracts were higher than ascorbic acid (Table 2). In addition, the radical scavenging activity of *F. bruguieri, P. atlantica* and *P. harmala* was substantially low as compared with ascorbic acid ($P < 0.05$) (Figure 3B).

Nitric oxide scavenging activity

Figure 4 shows the comparative NO scavenging activity

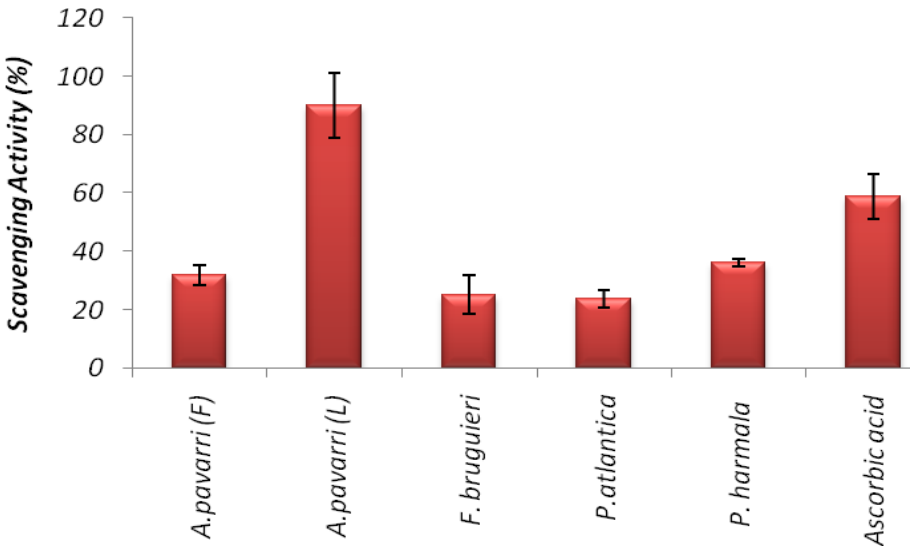

Figure 4. Nitric oxide radical scavenging activity of crude extracts under testing. Each value is represented as mean ±SD (n=3).

Table 3. Total polyphenols (TPC) and total flavonoids (TFC) concentrations of plants under investigations.

Plant	Used part	TPC (mg GA/g DW)	TFC (mg Rutin/g DW)
P.atlantica	*Fruits*	585.47±8.98	110.07±3.83
A. pavarii	*Leaves*	1217.14±50.52**	1504.28±80.89**
	Flowers	883.33±169.64*	357.66±5.79
P. harmala	*Seeds*	654.28±90.94*	88.95±6.69
F. bruguieri	*Leaves*	249.28±54.48	349.28±54.48

Each value is represented as mean ±SD (n=3). *indicates significance at *P*<0.05.** indicates significance at *P*<0.01.

of the extracts at concentration 1.25 mg/ml. *A. pavarii* leave extract showed greater NO inhibition (90.27%) as comparative to other plant extracts and ascorbic acid (Figure 4). The maximum NO scavenging of *P. atlantica, P. harmala*, and *F. bruguieri* were 23.70, 36.075, 25.25 and 35.73% respectively, which was less than ascorbic acid (45%).

Total phenolics and flavonoids contents

Table 3 shows that, the highest amount of phenolic and flavonoids compounds were in *A. pavarii* (Leaves) (1217.14±50.52, 1504.28±80.89 mg/g Dw respectively), while *F. bruguieri* had the lowest content of phenolics and flavonoids (249.28±54.48 and 6.95±0.95 mg/g Dw respectively).

Correlation of values of antioxidant activities with TPC and TFC

Table 4, shows a high correlation (R^2 = 0.734) between phenolic compounds and flavonoids. The results show moderate correlation (R^2 = 0.585) between the DPPH radical scavenging activity and total phenolic compounds, but there was a low correlation (R^2= 0.420, Table 4) with flavonoids level. Also moderate correlation was found between TP content and NO scavenging activity (R^2 = 0.686) whereas highly significant correlation was observed between TF and NO scavenging activity (R^2 = 0.951) (*P<0.01*).

Antimicrobial activity of plants

In the present study, the antimicrobial activities of crude and flavonoids extracts against six test microorganisms were examined and their potency was qualitatively and quantitatively assessed by the presence or absence of inhibition zones. The results presented in Tables 5 and 6 showed moderate antimicrobial activities for crude extracts and high antimicrobial activity for flavonoids extracts against some microorganisms tested. There is no specifically previous report on evaluation of these plants against these set of microorganisms (Table 5).

Table 4. Correlation indexes between phytochemicals (phenolics and flavonoids) and the applied three methods for determination antioxidant potential in some Libyan plants.

Assays	Correlations R^2	
	Phenolics (TPC)	Flavonoids (TFC)
Flavonoids content	0.734*	-
DPPH radical scavenging ability	0.585	0.420
Nitric oxide scavenging activity	0.686*	0.951**
Phosphomolybdate antioxidant activity	0.798*	0.712*

*significant at *P*>0.05, **significant at *P*>0.01.

Table 5. Antibacterial activity of the crude and flavonoids extracts, and positive control against some bacteria strains. Results are expressed as diameter of inhibition zone (mm)[a].

Plant name	*B. sub*		*S. aur*		*S. faecalis*		*E. coli*		*P. aeruginosa*		*S. typhi*	
	C	F	C	F	C	F	C	F	C	F	C	F
A. pavarii (L)	27	30	13	23	26	22	20	25	15	25	20	25
A. pavarii (F)	25	45	20	32	25	35	30	25	20	33	28	35
P.atlantica	15	15	na	20	15	20	15	20	10	na	15	17
P. harmala	12	25	na	25	13	30	13	23	10	40	**30**	20
F. bruguieri	22	18	na	na	15	20	15	na	30	18	18	14
Ceproxazine (5 µg)	16		20		nt		18		nt		20	

a: Diameter of inhibition zone including hole diameter of 8 mm. **na**, not active; C: crude extract; F: Flavonoids extract, *B. sub: Bacillus subtilis, E. coli: Escherichia coli, S. aur: Staphylococcus aureus, P. aer: Pseudomonas aeruginosa, S. faecalis: Streptococcus faecalis, S. typhi: Salmonella typhi.*

According to the present results, the flavonoids extract of flowers of *A. pavarii* extract were found to be active against all tested bacteria. The strongest antibacterial activity was seen against *S. typhi* with a MIC value of 0.019, followed by *B. sub* and *E.coli with* MIC 0 .156 mg/ml, followed by *S. aureus* and *Ps. Aeruginosa,* MIC 0.3125 mg/ml. Whereas, *S. faecalis, S. typhi*, *Ps*. *Aeruginosa* and *B.Sub* showed best susceptibility towards the methanol extract of leaves of *A. pavarii* with a MIC value of 0.156 mg/ml followed by *E.coli* and *S. aureus* MIC 0.132 and 1.25 mg/ml respectively. The flavanoids extract of *P. harmal*, and *P. Atlantica demonstrated* moderate activities against tested bacteria with MIC ranged from 0.156 to 0.132 mg/ml.

DISCUSSION

The beneficial medicinal effects of plant materials typically result from the combinations of plant specialized products. These compounds are mostly secondary metabolites such as terpenoids, alkaloids, tannins, and flavonoids which are capable to play definite biological and pharmacological activities and may have potential to be used as chemotherapeutic agents or serve as starting material in the developing of new antibiotics (Sibanda, 2007). Therefore, this study was carried out to screen the presence of some of these phytochemical compounds in the selected four Libyan plants as well as their bioactivity. Flavonoids and alkaloids were the most common compounds present in the all plant extracts investigated. These finding correlated well with several earlier publications (Havsteen, 2002; Ncube et al., 2008; Abubakar et al., 2010).

Polyphenols which are commonly found in medicinal plants exist as a group of highly hydroxylated phenolic compounds including hydroxycinnamate derivatives, hydroxycoumarins, flavanols, flavanones, flavones, proanthocyanidins (tannins), anthocyanins, aurones, and hydroxystilbenes. Such bioactive compounds have showed many pharmacological activities (Sibanda, 2007). Tannins, saponins and terpenes are useful anti-inflammatory agents, as they have been shown to cure inflamed tissues (Li et al., 2003). Flavonoids are important constituents of plants, since they possess prominent antioxidant properties (Havsteen, 2002). Alkaloids have long history of use in pharmaceutical industry as antiallergy, analgesic, muscle relaxant and antimalarial (Yen and Chen 1995). Several studies have demonstrated a highly significant correlation between the phenolic content in a plant and the antioxidant activity (Madson et aI., 2000; Jagethia et aI., 2004; Abubakar et al., 2010). In this study, a high correlation (R^2 = 0.734) between phenolic compounds and flavonoids has been

Table 6. Antibacterial activity expressed as minimum inhibitory concentration (MIC)[a] of the crude and flavonoids extracts against some bacteria strains.

Plant name	*B. Sub*		*S. aur*		*S. faecalis*		*E. coli*		*P. aeruginosa*		*S. typhi*	
	C	F	C	F	C	F	C	F	C	F	C	F
A. pavarii (L)	1.0	0.156	8.0	1.25	0.5	0.156	0.5	0.312	2.0	0.156	0.25	0.156
A. pavarii (F)	0.25	0.156	1.0	0.156	0.5	0.156	0.25	0.156	2.0	0.156	0.25	0.019
P.atlantica	0.5	2.5	nt	0.312	4.0	0.312	1.0	0.312	4.0	nt	1.0	0.312
P. harmala	2.0	0.312	nt	0.625	8.0	0.156	0.5	0.625	1.0	0.625	4.0	0.321
F. bruguieri	1.0	2.5	8.0	5.0	8.0	nt	2.0	5.0	nt	nt	2.0	5.0

a: minimum inhibitory concentration values are given as mg/mL. nt, not tested. *B. sub: Bacillus subtilis, E. coli: Escherichia coli, S. aur: Staphylococcus aureus, P. aer: Pseudomonas aeruginosa, S. faecalis: Streptococcus faecalis, S. typhi: Salmonella typhi.*

statistically proved, which is in accordance with a previous recent study (Ibrahim et al., 2011).

Antioxidants (free radical scavengers) are substances that interact with, and neutralize free radicals, thus preventing them from causing cellular damage in the biological system (Rahman, 2007). Several methods have been used to determine the antioxidant activity *in vitro* in order to allow rapid screening of bioactive substances. Antioxidant activities have been attributed to various reactions and mechanisms such as prevention of chain initiation of lipid peroxidation, binding of transition metal ion catalysts, reductive capacity, and or radical scavenging effect (Frankel and Meyer, 2000; Huang et al., 2005).

In reducing power assay, the presence of the reductants in the solution causes the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by absorbance measurement at 700 nm. In this study, when the concentration of extracts was increased, the reducing ability was increased. This result is similar to that reported earlier (Noriham et al., 2004; Premanath and Lakshmidevi, 2010). Flowers of *A. pavarii* had the highest ability to reduce Fe (III) while *F. bruguieri* extract had the lowest one. Also the ability of reducing power for methanolic leaves extract of *A. pavarii* leaves were more than that of the *P. harmala*, and *P. Atlantica* extracts. Many studies suggested that the reducing power of plants might be related to the presence of reductans agents such as polyphenols (Duh, 1998; Sibanda, 2007; Knežević et al., 2011). A previous report suggested that the reducing properties have been shown to exert antioxidant action *via* donating a hydrogen atom to break the free radical chain (Gordon, 1990).

The antioxidant capacity of the extract was measured spectrophotometrically through phosphomolybdenum method, based on the reduction of Mo (VI) to Mo (V) by the test sample and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 765 nm. The present study revealed that leaves of *A. pavarri* (L) showed the highest antioxidant capacity for reduce phosphomolybdate. Previous studies have shown that many polyphenols including flavonoids contribute significantly to the phosphomolybdate scavenging character of medicinal plants (Sharififar et al., 2009; Khan et al., 2012).

In the radical scavenging assay, when the DPPH is exposed to antioxidant compounds the purple color of DPPH changes to yellow indicating the free radical scavenging ability of a sample (Ebrahimzadeh et al., 2008). The more yellowish color of DPPH observed the greater the antioxidant activity of the plants tested. In this work the results of radical scavenging activity showed that leaves and flowers of *A. pavarii* extracts possessed strong radical scavenging effect with low IC50 (0.62±0.003). Although many studies have suggested that polyphenolics and flavonoids are highly effective against free radicals (Havsteen, 2002; Amic et al., 2003), in this study a moderate correlation (R^2 = 0.585) was shown between the DPPH radical scavenging and total phenolic content of sample. Also a low correlation between plant flavonoid level and the DPPH radical scavenging (R^2= 0.420, Table 4). Therefore, it may be possible that the radical scavenging activity of a sample cannot be predicted on the basis of its total phenolic content (Kähkonen et al., 1999).

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, and is involved in the regulation of various physiological processes (Lata et al., 2003). NO scavenging capacity is determined by the decrease in the absorbance at 550 nm, induced by antioxidants. In this study, a moderate correlation was found between total phenolics content and NO scavenging activity (R^2 = 0.686) whereas highly significant correlation was observed between TF and NO scavenging activity (R^2 = 0.961) (*P<0.01*). These results were in line with previous studies which revealed that the scavenging activity of plants is related to their contents of polyphenolics and flavonoids (Madson et al., 2000; Jagetia et al., 2004). Results of this study suggest that the extracts especially *A. pavarri* contain phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge its possible potential cellular damage in living system.

Plant extracts have been used for many years,

especially in food preservation, pharmaceuticals, alternative medicine and natural therapies (Lis-Balchin and Deans, 1997; Aboud, 2015). It has long been acknowledged that some plants exhibit antimicrobial properties, this was a motive to investigate the metabolites of those plants precisely which have been used, in traditional medicine to improve the quality of healthcare. The mode of action of natural products toward microbial infections is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds (Tsuchiya et al., 1996).

There are many factors such as climate, soil composition, age and vegetation cycle stage that explain the differences of antimicrobial activities of medicinal plants belonging to different regions of the world on quantity, and quality of bioactive compound and composition of extracted natural products (Masotti et al., 2003; Angioni, 2006; Noumedem et al., 2013). In addition the effect of solvent polarity used in the extraction play an important role in the amount of biologically active materials (Al-Zubaydi et al., 2009; Bakht et al., 2011; Bedi, 2010; Anmar, 2015).

In the present study *A. pavarii* represents a good candidate as a reliable source for the extraction of some major bioactive compounds that inhibit the growth of microorganisms, thereby proving to be very effective as alternative source of antibiotics. The continued traditional medicinal use of these plants is therefore encouraged while it is suggested that further studies should be carried out to isolate, purify and possibly characterize the active constituents responsible for the activity of these plants. Most traditional medicinal plants in use today have no scientific data on their bioactivity and levels of safety or even how they are likely to affect each other when used as combinations in medicines. Furthermore, scanty research has been done on their mechanisms of action considering that most are orally consumed. Therefore, further studies are needed to find out the mode of action of the tested plant extracts against bacteria.

Conclusion

This research indicates that the tested parts of *A. pavarii*, especially leaves have prominent antioxidant and antibacterial activities. The presence of phenolic and flavonoids compounds which were found in high level could be attributable to the observed high antiradical properties of these extracts. However, further investigation is necessary to separate and characterize the component of each individual extracted sample and then evaluate the antioxidants activity of each component as well as to find out the possible mechanisms of antioxidant and antimicrobial activities *in vivo*. These plants could be added to the long list of promising medicinal plants that offer distinctive source of natural antioxidants that can be used on industrial or medical scales.

Conflict of Interests

The authors have not declared any conflict of interests.

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