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Evaluation of antimicrobial and antioxidant activity of areal part methanolic extract of *Paeonia mascula* (L.) Mill

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Abstract **Original Research Article**

Paeonia mascula is a plant used in folk medicine for treatment of antispasmodic, antitussive, tonic, inflammation, disturbances, the healing of wounds and gynecology. In this study, total phenolic, flavonoid and tannins contents, antioxidant and antimicrobial activities were evaluated. Antioxidant activity of areal part methanol extract was carried out by four different methods (DPPH, β-carotene, reduction power, and Iron chelation). However, the antimicrobial activity was realized by disc diffusion methods in using: Escherichia coli ATCC 25922 Salmonella typhimurium ATCC 13311, Staphylococcus aureus ATCC25923, klebsela pneumoniae ATCC70060, Shigella flexheri NCCB1406, Proteus mirabilis ATCC35659, Aspergillus niger 2CA936, Aspergillus flavius NRRL3357 and Candida albicans ATCC1024. Our results exhibit high antioxidant and antimicrobial activities.
Keywords: Antioxidant; Antimicrobial; *Paeonia mascula ;* methanol extract.

INTRODUCTION

Plants such as herbs have been used in folk medicine for centuries in most of the cultures throughout the world [1]. Many antioxidant phenolic compounds in plants, however, are most frequently present as a covalently bound form with insoluble polymers [2]. These compounds also proved to have anti-inflammatory, antibacterial, antifungal properties [3]. The initial response of plants to pathogen attacks is an oxidative burst with rapid and transient production of reactive oxygen species (ROS) [4]. This plant response indeed is a defense mechanism as ROS can kill the pathogenic bacteria or inhibit their growth [5]. ROS production is usually followed by the hypersensitive response (HR) to pathogens leading to rapid cell death [6]. This oxidative damage is a major factor implicated in many chronic diseases such as cancer, diabetes, hypertension and other cardiovascular disorders [7].

The fact that microorganisms nowadays tend to develop resistance towards drugs, coupled to the undesirable side effects of certain antibiotics offer considerable potentials for the development of new effective antimicrobial agents; medicinal plants being a prolific source. Various plant extracts possess bacteriostatic and bactericidal effects due to secondary metabolites they contain, namely alkaloids, tannins, flavonoids, and phenolic compound.

Paeonia is a genus of family Paeoneacae comprises of 35 species, which are mainly distributed in the North Temperate Zone especially in the Mediterranean region and Asia Only one species, namely *Paeonia mascula*, occurs in Algeria [4]. Pony herbaceous perennial plant 0.5–1.5 meters tall, the Wild Peony has leaves which are divided into three segments and large red flowers.

MATERIALS AND METHODS

Methanolic Extraction

The plant material (10 g) was extracted with methanol 1L (80%) at room temperature in a steel tank. The combined extract was concentrated in the rotary evaporator at 40°C under vacuum to get The crude methanol extract from the leaves has been conserved at 4°C(2)

Total Phenolic Content (TPC)

The total phenolic compounds in the stem extract and leaf extracts were determined by method deFolin-Ciocalteu assay [8] a volume of 0.1 mL of the extract was introduced into test tubes followed by 0.5 mL Folin- Ciocalteu. reagent (diluted 10 times with water). The solution was then kept in dark for 5 min and then 0.4 mL sodium carbonate (7.5% w/v) and incubated for 30mins; absorbance was measured at

750nm against blank. The same procedure was followed for the standard solution of Gallic acid. The total phenolic content in the extract was expressed as mg of Gallic acid equivalent (GAEs) per g of extract (GA mg/g).

Total Flavonoid (TF)

Total flavonoid content was measured by Aluminium chloride colorimetric assay [9] as described by. The extract (0.2mg/1ml) in methanol was mixed with 1ml of methanol, 1ml of 2% aluminum chloride. After 30min of incubation, the absorbance was measured at 415nm with UV/Visible spectrophotometer. Quercetin was used as standard and the flavonoid content is expressed in terms of mg of Quercitin equivalents (QE) per g of extract.

Determination of total tannins contents

Tannin content was evaluated using the hemoglobin precipitation assay. An aliquot of 0.5 ml of each extract is mixed with 0,5ml of hemolysis bovine blood to reach a final concentration of 1 mg/ml then the mixture was centrifuged at 480g for 20 min and the absorbance was measured at 578nm [10].

In the same time, tannic acid at various concentrations (100-600 µg / ml) was used in the same manner as the samples. The result is expressed as mg equivalent of a tannic acid gram of extract E TA/GE.

DPPH Radical Scavenging Activity

The assay was conducted on the basis of scavenging activity of the stable DPPH free radical following the method described by Brits and Bucar [11] with some minor modificatio50 µl of different dilutions of the extracts were added to 1250 µl of 4% solution DPPH dissolved in methanol. The absorbance of all the samples was determined at 517nm (UVD 2960, Labomed, Inc.) after an incubation period of 30minutes. The percentage inhibitory activity was determined according to the following formula.

DPPH scavenging effect (%) = [(A0 – A1)/ A0] × 100

Where:
$A_{0:}$ is the absorbance of the control at 30 min
$A_{1:}$ is the absorbance of the sample at 30 min.
BHT was used as a positive control. Samples were analyzed in triplicate

Beta carotene bleaching method

B-Carotene (0.5 mg) was dissolved in 1 ml of chloroform. A 4 ml aliquot of the solution was added to a conical flask with 25mg linoleic acid and 200 mg Tween-40. Chloroform was removed with a rotary evaporator at 50 °C. Oxygenated distilled water (100 ml) was added to the β-carotene emulsion mixed well and aliquots (2.5 ml) of the oxygenated β-carotene emulsion and 0.2 ml of methanolic extracts were placed in capped culture tubes and mixed well, taking absorbance at 470nm BHT was used as a positive standard. The antioxidant activity was calculated as the following equation:

AA%= Abs sample/Abs BHT * 100

Reducing Power

The reducing power was determined according to the method of Oyaizu [12]. Each extract (0.5–10 mg/ml) in methanol (2.5 ml) was mixed with 2.5 ml of 200 mg/mg sodium phosphate buffer (pH 6.6) and 2.5 ml of 10 mg/ml potassium ferricyanide, and the mixture was incubated at 50 C° for 20 min. After 2.5 ml of 100 mg/ml trichloroacetic acid were added, the mixture was centrifuged at 200g for 10 min. The upper layer (5 ml) was mixed with 5ml of deionized water and 1ml of 1 mg/ml ferric chloride, and the absorbance was measured at 700nm against a blank.

A higher absorbance indicates a higher reducing power. IC_{50} value (mg extract/ml) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis. Ascorbic acid, BHT was used for comparison [13].

Antimicrobial activity

Agar well diffusion method

The determination of the inhibitory effect of the methanolic extract on test bacteria was carried out by the agar diffusion method [14]. Bacterial cultures were grown at 37°C for 24 h in Nutrient Broth. Petri dishes with 9ml of Nutrient Agar were prepared. After incubation, of the suspension of the tested microorganism containing 108 CFU/ml. Filter paper discs (6 mm in diameter) were impregnated with 10 µl of different concentration of the extracts and placed on the inoculated plates. These plates were incubated at 37°C for 24 h. Gentamicin (10µg/disc) was used as a standard and dimethylsulfoxide DMSO as a control.

The antibacterial activity was determined by measuring of inhibition zone diameters (mm) and was evaluated according to the parameters suggested by Alves *et al.* [15]: inhibition zones <9 mm, inactive; 9–12 mm, less active; 13–18 mm, active; >18 mm, very active. The measurements were done basically from the edge of the zone to the edge of the well.

Antifungal Activity

The antifungal activity was tested by disc diffusion method [16]. The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper discs (5 mm in diameter) impregnated with 100 mg/ml concentrations of the extracts were placed on test organism-seeded plates. The activity was determined after 72 h of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

Statistical analysis

Results were expressed as the mean ± standard deviation. Data were statistically analyzed using one-way ANOVA as primary test followed by Newman Keuls with the criterion of P values < 0.05 to determine whether there were any significant differences between methanolic extract of and standards, using GraphPad Prism 5 Demo Software.

RESULTS AND DISCUSSION

Total polyphenols, flavonoids and tannins contents

In the present study, total phenolic, flavonoids and tannins contents. The total phenolic contents by methanolic extract for *Paeonia mascula* was measured using the Folin Ciocalteu method and also shown in Table 1.the phenolic content (37±1mg GAE/g). Flavonoids were quantified using AlCl3 method. The results with methanolic extract (11.5±0.046) (mg QE/g). Tannins are complex polyphenols found in plant extracts and could be good candidates, in a perspective of reducing the cost of prevention of lipid oxidation (52.66± 1.70) (mg TAE/g).

Table-1: Total polyphenols, flavonoids and tannins contents in extact methanolicof paeonia mascula(L.)Mill

Extract	Total phenolic content (mg GAE/g)	Total flavonoids (mg QE/g)	Total tannins (mg TAE/g)
Extract methanolic	37± 1	11.5 ± 0.046	52.66 ±1.70

Antioxidant activity

Scavenging of DPPH radicals

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517 nm induced by antioxidants. The methanolic extract of *p. mascula* and positive control BHT. The (EC5010 ± 0, 07) (µg/ml) of extract is less than two BHT (69, 96 ± 1, 23) (µg/ml).

B-Carotene linoleic acid bleaching assay

In this method, the linoleic acid free radical formed attacks the highly unsaturated β -carotene molecules and in the absence of an antioxidant rapidly bleached the orange color of β-carotene. The extent of discoloration is monitored spectrophotometrcially at 490 nm [17]. The lowest β -carotene discoloration rate exhibited the highest antioxidant activity. As shown in Fig.2, β-carotene in a very important level compared to BHT (92.4 ± 0.61% inhibition)extract metanolic is the best inhibitor of the oxidation of β-carotene in 24h (61.9 ± 1.147%).

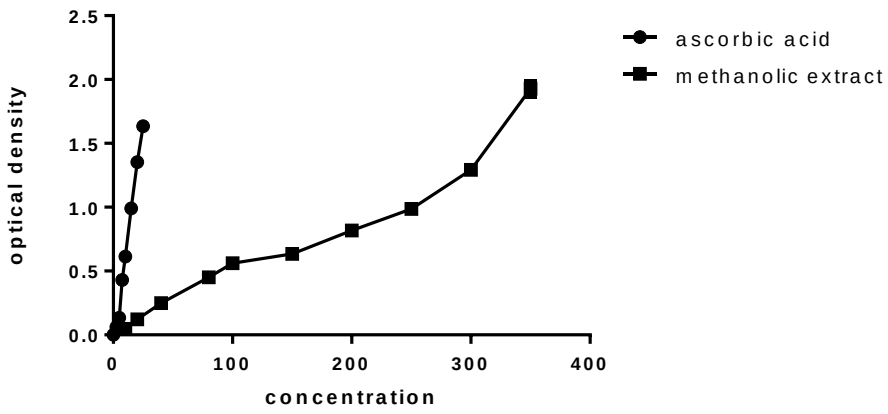

Fig-1: Antioxidant activities of flavonoids of *paeonia mascula* (L.)Mill. by reducing power test

Reducing power

The extract methanolic *of Paeonia mascula*(L.)Mill. show a low capacity to reduce the ferric cyanide *Salmonella typhimurium* complex with EC_{50} reach (105.43±0.9) mg/mL (Figure 2). In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of each compound. The presence of reducers in the test solution results in a reduction of the Fe3+/ferricyanide complex to the ferrous form. The reducing power of both species was excellent .an important capacities of extract methanolic in reducing ferric cyanide complex to the ferrous but lower capacity than ascorbic acid.

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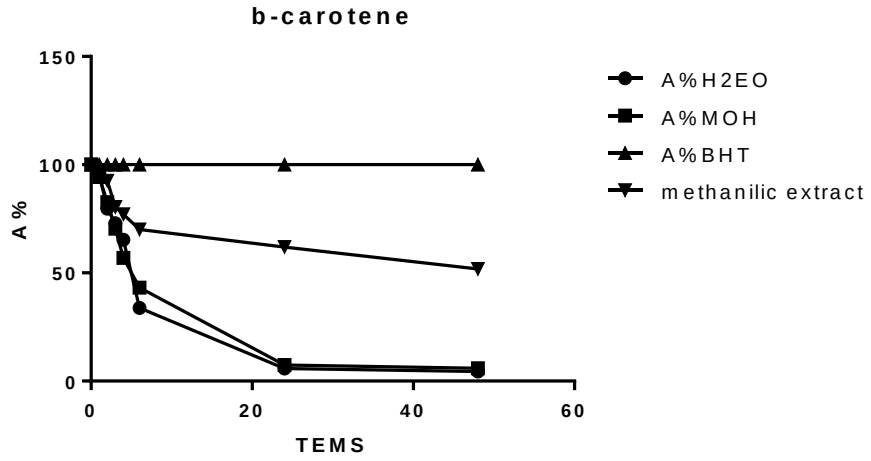

Fig-2: Antioxidant activities of tannin from *Paeonia mascula* by the β-carotene bleaching method

The potential antioxidant abilities of these plant extracts might be due to their phenolics contents, which can significantly inhibit oxidation of linoleic acid. Several studies have exhibited that the antioxidant activity is essentially correlated to phenolic and flavonoid contents [18].

Results of Anti-bacterial activity

Results obtained in the present study relieved that the tested metabolite group possess a low potential microbial activity against Escherichia coli, Shigella flexheri, Klebsiella pneumoniae, Proteus mirabilis, and Staphylococcus aureus (Tables1).

Table-2: Inhibition zones in millimeter for positive and negative control

	Escherichia coli ATCC 25922	*Salmonella typhimurium ATCC 13311*	*klebsela pneumoniae ATCC700603*	*Shigella flexheri NCCB1406*	*Proteus mirabilis* ATCC35659	*Staphylococcus aureus* ATCC25923
Positive Control	18,50± 0,41	19,17±0,24	24,67±0,47	24,33±0.14	35,33±0,47	27,67±0,47
Negative Control	NI	NI	NI	NI	NI	NI

Table-3: Inhibition zones in millimeter for methanolic extract

	4mg	8mg	Inhibition type
Escherichia coli ATCC 25922	10.66± 1.24	9.33±0,94	bactericidal
Salmonella typhimurium ATCC 13311	10.661,75	11,69±1,18	NI
Shigella flexheri NCCB1406	12.1±0,63	13.4±0,89	bactericidal
Proteus mirabilis ATCC35659	7,25±0,64	11.0±51.08	NI
Staphylococcus aureus ATCC25923	9,85±0,87	10.,7±0,84	bactericidal

There is a need for the discovery of new substances from natural sources, including plants. Although several studies are available on the chemical composition, antifungal, antiradical, antioxidant and antibacterial activities of different Paeonia species. The antimicrobial activity of *P. mascula* methanol extract against a panel of 5 microorganisms was examined and their potencies were assessed both qualitatively and quantitatively by the presence or absence of inhibition zones, zone diameters. The results are given in (Table 3).

Antifungal activity

Antifungal activities of methanolic extract again *Candida albican* , *A. flavius*, and *A. niger* are presented in Table 4, where the results of the last day of growing, applying three parallel measurements, are given.

Table-4: Inhibition zones in millimeter for positive and negative control

	Nystatin	Clotrimazon	Amphotericin.
Aspergillus niger 2CA936	9,40±0,22	15,53±0,79	9,29±0,19
Aspergillus flavius NRRL3357	15,85±0,32	23,86±1,15	44,28±0,49
Candida albicans ATCC1024	17,55±0,14	16,20±1,19	15,58±0,12

Table-5: Inhibition zones in millimeter for methanolic extract

	Aspergillus niger 2CA936	Aspergillus flavius NRRL391	Candida albicans ATCC1024.
concentration 50mg/ml	8.9, 58±1,18	11,58±0,89	13,24±1,24
Concentration 20mg/ml	7,78±0,68	NI	NI

CONCLUSION

Many bioactive compounds including monoterpene glycosides, triterpenoids, phenols, and tannins have been identified from different species of *Paeonia.* This study also successfully identified this plant with very high antioxidant capacities, which are a potentially rich source of natural antioxidants. Consolidated research with advanced molecular, pharmaceutical linkage is required to investigate the mechanism of antioxidant activity of these stress tolerant plants for their potential applications in therapeutics.

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