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ANTIOXIDANT ACTIVITY OF PHENOLS AND FLAVONOIDS CONTENTS OF AQUEOUS EXTRACT OF *PELARGONIUM GRAVEOLENS* ORGIN IN THE NORTH-EAST MOROCCO

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ABSTRACT

The objective of this work is to characterize the phenols and flavonoids compounds of extracts of ***Pelargonium Graveolens*** from North-East Morocco (TAZA) in terms of antioxidant activity. The antioxidant activity of this aromatic plant was determined according to the DPPH radical scavenging assay to suggest it as a new potential source of natural antioxidants. The quantification of phenolics and flavonoids compounds of solvent extracts (diethyl ether and ethyl acetate) were determined spectrometrically. The DPPH scavenging activity of extracts increased in the order diethyl ether extract < ethyl acetate extract < ascorbic acid. Based on these results, we suggest that the phenols and flavonoids compounds of ***Pelargonium Graveolens*** have significant potential as a natural antioxidant.

Keywords: *Pelargonium Graveolens*, Phenols, Flavonoids, DPPH method, Antioxidant activity

INTRODUCTION

The oxidative degradation of food by free radicals, causes a real problem to the health of the consumer, so it is necessary to use food additives, called "antioxidants" to fight against this phenomenon. Many substances from aromatic and medicinal plants have been shown to contain antioxidants like flavonoid compounds, these Compounds are the main agents that can donate hydrogen to free radicals and thus break the chain reaction of lipid oxidation at the first initiation step (**Agraval, 1989**). This high potential of phenolic constituents to scavenge radicals may be explained by their phenolic hydroxyl groups (**Havsteen, 2002**). Several studies exhibited a strong relationship between total phenolic content and antioxidant activity in fruits, vegetables, and medicinal plants (**Ouariachi *et al*., 2004**).
The antioxidant activity of a compound is its ability to resist oxidation. The best known antioxidants are the β-carotene (provitamin A), ascorbic acid (vitamin C), tocopherol (vitamin E) as well as phenolic compounds. Indeed, the most synthetic antioxidants or naturally occurring groups have phenolic hydroxyl in structure and antioxidant properties are attributed in part, to the ability of these natural compounds to scavenge free radicals such as hydroxyl radicals (OH•) and superoxide (O_2•) **(Rice-Evans *et al*., 1995; Ouadi *et al*., 2015; Ghazi *et al*., 2015; Hazi *et al*., 2015; Saidi *et al*., 2016).** Pelargonium Graveolens is a shrub of the family Geraniaceae, this Moroccan medicinal plant, known locally as "LAATARCHA", is used for the treatment of various inflammatory disorders such as: Antispasmodic, Relaxing and Anti-inflammatory. The aim of this study is to investigate the antioxidative properties of solvent extracts of Pelargonium Graveolens from North East of Morocco (TAZA). Additionally, the total phenolic and flavonoid contents of diethyl ether and ethyl acetate extracts have been determined.

MATERIAL AND METHODS

Plant material

The aerial part of *Pelargonium Graveolens* was harvested in April 2013 in TAZA at the North-East of Morocco. A voucher specimen was deposited in the Herbarium of Faculty of Sciences, Oujda, Morocco. The dried plant material is stored in the laboratory at room temperature (298 K) and in the shade before the extraction. The different proprieties of *Pelargonium Graveolens* are:
Scientific name: Pelargonium Graveolens; Common name: Géranium-Rose; Local Name: Laâtarcha; Order: Geraniales; Family : Geraniaceae; Genre : Pelargonium; Kingdom : plantae; Division : Magnoliophyta; Class :Magnoliopsida and Flowering: August-January with a peak in September-October (**Demarne, 2002; Van der Walt and Andri. 1992**) (figure 1).

Figure 1 Pelargonium; Kingdom

Fractionating the aqueous extract

Polyphenols present in the aqueous extract, are extracted after filtration by two solvents of different polarity namely, ethyl acetate and diethyl ether, polarity indices respectively, 0.58 and 0.38. A sample of 100 ml of the extract recovered by steam distillation is hydrolyzed with 40 ml 2N HCl bath at 100 °C for one hour, at the end of this treatment, plant debris clusters are formed and extract aqueous recovered is filtered and then mixed in a separating funnel and shaken thoroughly with diethyl ether or ethyl acetate. After settling in an ampoule, the upper organic phase is collected in an Erlenmeyer flask, the extraction is repeated 3 times with solvent renewal. The latter is due evaporated after drying the organic phase with anhydrous sodium sulfate, and the resulting extract is considered as the fraction of diethyl ether or ethyl acetate. The fractions thus obtained were stored in glass vials and then kept at a temperature of 4 to 5 °C prior to analysis.

Determination of total phenolics contents

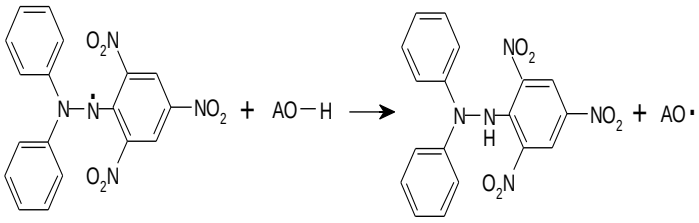
The ployphénols are estimated by various methods such as the method of Prussian blue (**Graham,1992**), but the most used is the Folin-Ciocalteu. This consists of a mixture of phosphotungstic acid (H3PW12O40) and phosphomolybdic acid (H3PMo12O40); it is reduced by the phenols in a mixture of the blue oxides of tungsten and molybdenum (**Boizot and Charpentier, 2006**). Moreover, 1ml of Folin reagent (diluted 10 times in distilled water) was added to 200 µl of sample or standard (gallic acid) with suitable dilutions in distilled water, after 4 min, 800 µl of a solution sodium carbonate (75 mg/ml) are added to the reaction medium. After 45 minutes incubation at room temperature, the absorbance of the resulting solution is measured at 760 nm. The same procedure was also applied to the standard solutions of gallic acid, and a standard curve was obtained. The concentrations of phenolic compounds expressed as µg gallic acid equivalent per mg of extract were calculated according to the standard gallic acid graph. All experiments were performed in triplicate assays, and gallic acid equivalent values were reported as X (average) ± SD (standard deviation) of triplicates (**El Ouadi *et al.*, 2015**).

Determination of total flavonoids contents

Quantification of flavonoids in extracts of *Pelargonium Graveolens* were performed by the method of aluminum trichloride (**Bahorun *et al.*, 1996; Arvouet-Grand *et al.*, 1994**). In addition, 1 ml of sample or standard (dissolved in methanol) was added to 1 ml of the solution of $AlCl_3$ (2% in methanol). After 30 minutes of reaction, the absorbance is read at 415 nm. The concentrations of flavonoid compounds expressed as µg rutin equivalent per mg of extract were calculated according to the standard rutin graph. All experiments were performed in triplicate assays and rutin equivalent values were reported as X ± SD of triplicates.

Antioxidant activity

The free radical-scavenging activities of solvent extracts were measured using 1,1-diphenyl-2-picrylhydrazyl(DPPH) as described by researchers (**Hatano *et al.*, 1985**); antioxidants react with the stable free radical DPPH (deep violet color) and convert it to 1,1-diphenyl-2-picrylhydrazine with discoloration.



DPPH DPPHH

Figure 2 antioxidant activity of DDPH

Where: (AO-H) represents a compound capable of yielding hydrogen to DPPH radical (violet) to transform it into picryl diphenyl hydrazine (yellow) [16]. Various different concentrations prepared in ethanol for the different samples and standard studied are between 0.2 to 2 µg/ml were added to 3.9 ml of a DPPH radical solution in ethanol. The mixture was strongly shaken and left to stand at room temperature for 30 min in the dark. The absorbance was measured at 517 nm against a blank. The radical-scavenging activity was expressed as percentage of inhibition (I%) according to the following formula [17]:

$$I\ (\%) = 100^* (A_{control} - A_{sample})/A_{control}$$

Where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the test compound. Thesample concentration providing 50% inhibition (IC50) was calculated from the graph of inhibition percentage against sample concentration. Tests were carried out in triplicate. Ascorbic acid was used as a positive control.

RESULTS AND DISCUSSION

Total phenolic and flavonoid contents of solvent extracts

The determination of levels of total phenols and flavonoids in two fractions of aqueous extract of *Pelargonium Graveolens* was made by using separately methods colorimetric (Folin-Ciocalteux and trichloride aluminum (AlCl3)).
The content of total phenols estimated by the Folin-Ciocalteu method for each extract fraction was reported in µg gallic acid equivalent / mg of extract. The results show that the fraction of ethyl acetate has a high content of total phenols (437± 25 µg/mg) compared to that of the fraction diethyl ether (400 ± 29 µg/mg) (Table 1).
The flavonoid content determined by the method trichloride alumiunm of each extract fraction was reported in µg equivalent rutin / mg of extract. The results reveal that ethyl acetate and diethyl ether fractions of aqueous extract of *Pelargonium Graveolens* have respectively moderate levels (Table 1) (29±1,6 and 12±0,2 µg equivalent of rutin per mg of extract). Figures 3 and 4, show the calibration curves of gallic acid and rutin.

Table 1 Determination of total polyphenols and flavonoids in both fractions extracts of *Pelargonium Graveolens*

	Extract	polyphenols in µg equivalent of gallic acid per mg of extract	flavonoids in µg equivalent of rutin per mg of extract
Pelargonium Graveolens	Fraction diethyl ether	400 ± 29	12± 0.2
	Fraction ethyl acetate	437± 25	29± 1.6

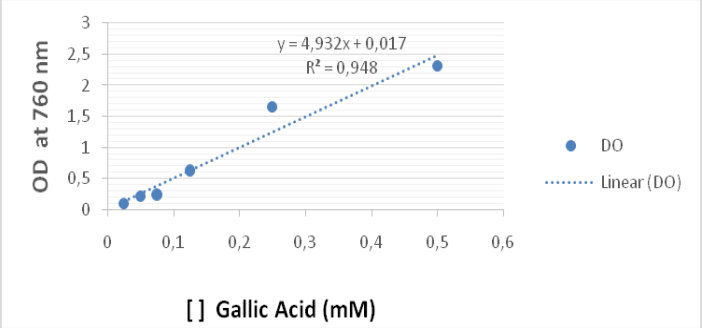


Figure 3 Calibration curve of Gallic Acid

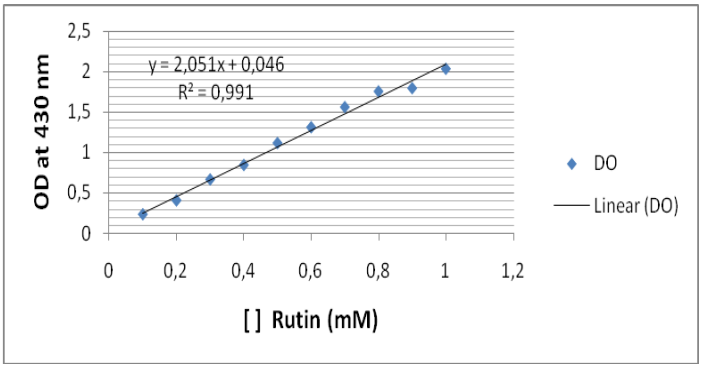


Figure 4 Calibration curve of Rutin

Antioxidant Activity

Results of free radical scavenging activity of fraction diethyl ether, fraction ethyl acetate and Acid Ascorbic (positive control) are given in Table 2. Moreover, the examination of Table 2, we spotted that the DPPH scavenging activities (%) were increased significantly with increasing the concentration of the studied samples from 0.2 to 2 µg/ml.

Table 2 The antioxidant activity of the fractions (diethyl ether and ethyl acetate) of the aqueous extract of ***Pelargonium Graveolens*** at different concentrations

Echantillons	Antioxidant activity						
Fraction diethylether	Concentrations of the extract (µg/ml)	0.2	0.35	0.5	1	2	
	Effet of trapping on DPPH (%)	12.07	21	28.31	43	51.84	
	DPPH IC_{50} (µg/ml)						1.54
Fraction acetate d'ethyle	Concentrations of the extract (µg/ml)	0.2	0.35	0.5	1	2	
	Effet of trapping on DPPH (%)	13.24	22.70	30.01	45	53	
	DPPH IC_{50} (µg/ml)						1.49
ascorbic Acid	Concentrations of the extract (µg/ml)	0.2	0.35	0.5	1	2	
	Effet of trapping on DPPH (%)	20	28	32	55	83	
	DPPH IC_{50} (µg/ml)						0.90

From Table 2, the antioxidant activity of ethyl acetate fraction is greater than the diethyl ether fraction. This activity of the extracts increases with the concentration, this is explained by the fact that the studied samples give hydrogen to DPPH who then converted to the color violet in yellow and absorbs less light. When the concentration is high, more antioxidants DPPH is reduced, so less it absorbs light passing through it (**El Ouadi *et al*., 2015**)

Generally, *Pelargonium Graveolens* shows some good antioxidant activity at the concentration of 2 µg/ml reach until 53% and 51.84% for the ethyl acetate fraction and diethyl ether fraction respectively (Figure 5). The two fractions of *Pelargonium Graveolens* extracts exhibit a lower activity than that of ascorbic acid (83%). The ethyl acetate fraction had the highest radical scavenging activity with the lowest IC50 value (1.49 µg/ml). This value was higher with diethyl ether fraction (IC50 is 1.54 µg/ml). Additionally, the fraction of ethyl acetate has a lower scavenging capacity than ascorbic acid (IC50 is 0.90 µg/ml).

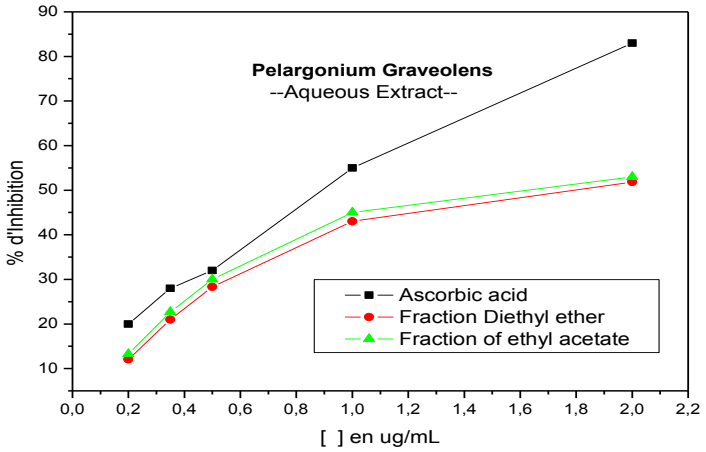


Figure 3 Antioxidant power of two fractions of the aqueous extract of *Pelargonium Graveolens*, OD reading after 30 min of incubation.

CONCLUSION

The test DPPH• is considered a simple, quick and easy to implement, experiments have shown some difficulties in measuring the reduction of state: a dynamic phenomenon at low concentrations and accompanied many compounds formed in some cases unstable. The DPPH• test is not quantitative, it compares different extracts then according to their ability to scavenge DPPH• and thus to appreciate the qualitative changes in phenolic compounds. Evaluation of the antiradical activity should be interpreted with caution, knowing that the absorbance of DPPH• at 515-520 nm decreases under the action of light, oxygen, depending on the pH and the type of the solvent added to the antioxidant. According to the results obtained, there is a relationship between the total polyphenol content and antioxidant activity. The both fractions of the *Pelargonium Graveolens* aqueous extract can reduce the radical 2,2-diphenyl-1-picrylhydrazyl, so they have an antioxidant effect in vitro, and can be proposed as new potential sources of natural additives in the food and pharmaceutical industries.

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