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QUANTIFICATION OF PHENOLIC, FLAVONIODS COMPOUNDS, IN VITRO ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF FLOWERS EXTRACTS FROM *PUNICA GRANATUM* L

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ABSTRACT

This study was aimed at examining total phenolic content, flavonoids, antioxidant and antibacterial analysis of flowers sweet and sour of tow system extracts of *Punica Granatum* L. Antioxidant efficacies of extracts were evaluated by phosphomolybdenum method. The antimicrobial activity of extracts of was determined against three. The phenolic contents of ethanolic 80% sour extract (EASE) was founded to have the highest value 480 ± 0.02 mg AGE.g^{-1} DW and the lowest value in ethanoic sweet extract (ES) 118 ± 0.02 mg AGE.g^{-1} DW. The total antioxidant activity was also found to be highest with EASE extracts. For the antibacterial activity the extracts showed moderate activity. The most sensitive microorganism was *Staphylococcus aureus* (diameter of inhibition is 23 mm) showed in ethanolic 80% sweet extract at 20 mg/ml.

Keywords: *Punica Granatum* L; phenolic content; flavonoids; antioxidant; antibacterial.

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1. NTRODUCTION

There is much mark that feeding of fruits, vegetables and other derived plant products is favorable for human health for of the existence of bioactive molecules [1]. These compounds are secondary metabolites, biosynthesised by plants to inhibit pathogen dose, UV stress or to interest pollinator insects. The polyphenols counting flavonoids, phenolic acid and tannin are a main group of phytochemicals which presented robust antioxidant andantibacterial activities [2,3].

Free radicals are chemically unstable atoms that reason destruction to lipid cells and proteins as a result of disparity between the generation of reactive oxygen species (ROS) and the antioxidant enzymes [4]. They are known to be the fundamental cause of oxidative stress which is totally concerned in the pathogenesis of numerous infections such as cancer, diabetes, cardiovascular diseases, aging and metabolic syndrome [5]. Examples of these radicals comprise superoxide anions, hydroxyl, nitric oxide and hydrogen peroxide radicals. These radicals can be scavenged by the protective character of natural and synthetic antioxidant agents. In the meantime, the incorporation of numerous synthetic antioxidants as has been described toxic to man [6]. The use of natural antioxidant has expanded much courtesy from patrons because they are measured safer than synthetic antioxidants.

Propyl gallate (PG), butylatedhydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ) and butylatedhydroxy-toluene (BHT) are particular of the usually used synthetic antioxidants to check oxidation and to extend shelf life of foods. Conversely, use of synthetic antioxidants has been partial in many countries because of their probable toxic properties for human health and the environment; opposing side properties of their use [7]; use of such substances has been questioned due to their potential health dangers and toxicity. While synthetic antioxidants used level thought at low concentrations necessity for having antioxidants without side properties for the difficulties subsequent from the long-term use of these complexes can’t be disregarded. Extraction of phenolics from botanical sources is the first important step to exploit their industrial applications. Due to the chemical nature of these compounds in several plants, the extraction productivity of phenolics mainly rest on the polarity of solvent as well as the class of plant materials used [8].

This paper reports the evaluation of phenolic content, flavanoïds, antioxidant and antibacterial capacity of ethanolic and ethanolic and ethanolic 80% extract of flowers from *Punica Granatum* L for different methodical.

2. RESULTS AND DISCUSSION

2.1. Total phenolic content and flavonoids

The extracts of flower of sour and sweet obtained by to solvents were found to be rich in phenolic compounds. The total phenolic content is given in table 1. Ethanolic 80% sour extract (EASE) was founded to have the highest value 480 ± 0.02 mg AGE.g^{-1} DW, following by the ethanolic sour extract (ESE) 202 ± 0.04 mg AGE.g^{-1} DW, ethanolic aqueous sweet extract (EAS) 145 ± 0.02 mg AGE.g^{-1} DW and the lowest value in ethanoic sweet extract (ES) 118 ± 0.02 mg AGE.g^{-1} DW. Similar results were observed in quantification of total flavonoids and content ranged from 60.87±0.015 mg RE/g DW to 28.2± 0.015 mg CE/g DW. The Total flavonoids in increasing order were: EASE > ESE > ESE > EA.

Table 1. Total phenolic content and flavonoid *Punica Granatum* L

	Extract			
	EASE	**ESE**	**EAS**	**ES**
Extraction yield	26.96%	7.91%	30.6%	13.7%
Total phenolic content mg. GAE.g^{-1} DW	480 ± 0.02	202 ± 0.04	145 ± 0.02	118 ± 0.02
Total flavonoid mg RE.g^{-1} DW	60.87±0.015	32.93± 0.03	28.93± 0.02	28.2± 0.015

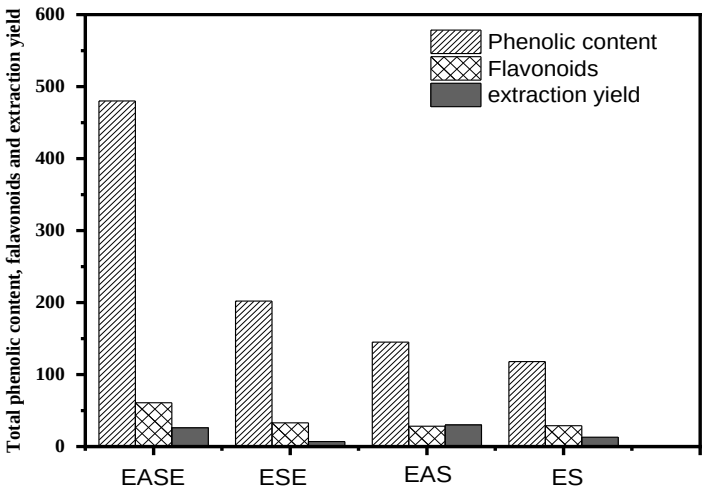

Fig.1. Total phenolic content, flavonoid and extraction yield of *Punica Granatum* L

2.2. Total antioxidant activity

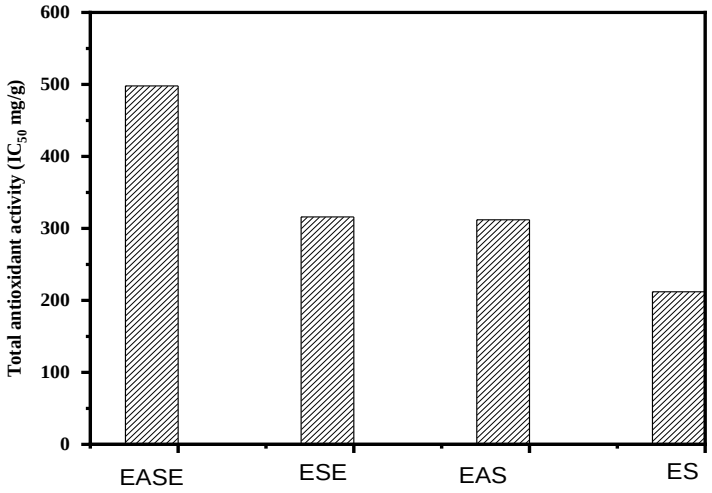

Fig.2. Total antioxidant activity of different extract from of *Punica Granatum* L

The results for total antioxidant activity of flowers from *Punica Granatum* L obtained by ethanolic aqueous and ethanolic solvents are presented in figure 2 and compared with the

standard querecetin. The results for figure 2 are expressed in terms of IC_{50}. The EASE show the best results in antioxidant potential with values of 498.44 ± 0.005, higher than ESE IC_{50}= 316.89±0.002 mg/g, followed by the EAS IC_{50}= 312.83 ± 0.02 mg/g. The ES shows a moderate antioxidant potential IC_{50}=212.57± 0.03 mg/g, the querecetin show an antioxidant activity lower than different extracts IC_{50}=125.71±3.57 mg/g.

2.3. Antibacterial activity

The results of antibacterial effect of flowers extracts of *Punica Granatum* L in terms of zone of inhibition (mm) were presented in Table 2. The flowers extract showed a high antibacterial activity against all the three bacterial strains tested against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The strongest antimicrobial activity of registered in EASE against *Staphylococcus aureus* 26 mm. For the extracts, the general order of their antibacterial activity would be EASE> ESE > EAS> ES. Previous study obtained similar results for the antibacterial activity of *Punica Granatum* L extract and reported that the this plant exhibit excellent antimicrobial activity against several bacteria [26,27]. Polyphenols comprise a wide variety of molecules with polyphenol structure, potentially useful structures for the development of new chemotherapeutic agents [28].

Table 2. Antimicrobial activity of flowers *Punica Granatum* L extract expressed as zone of inhibition (mm)

Bacteria	**Diameter of inhibition zone (mm)**							
	EASE		**ESE**		**EAS**		**ES**	
	15 mg. ml^{-1}	**20 mg. ml^{-1}**	**15 mg. ml^{-1}**	**20 mg. ml^{-1}**	**15 mg. ml^{-1}**	**20 mg. ml^{-1}**	**15 mg. ml^{-1}**	**20 mg. ml^{-1}**
Escherichia coli	13	15	11	13	15	15	7	22
Staphylococcus aureus	22	26	8	20	13	17	16	23
Pseudomonas aeruginosa	7	12	10	15	14	16	12	13

2.4. Relation between total phenolic content and total antioxidant activity

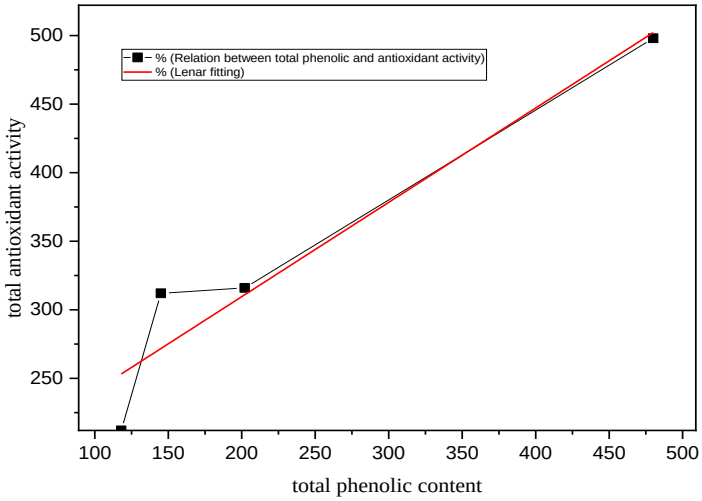

Fig. 3. Relation between total phenolic content and antioxidant activity of different extract from of *Punica Granatum* L

The antioxidant capacity of *Punica Granatum* L, similarly to other studies, is associated with numerous bioactive compounds that have antioxidant properties. Total phenolics and flavonoids were considered widely due to their possible correlation. However, the overall antioxidant capacity may be elucidated by understanding into the assembly of different sources of bioactive compounds. *Punica Granatum* L extracts with higher contents of phytochemicals showed higher antioxidant capacity.

3. EXPERIMENTAL

3.1. Plant material and extraction

The flower *Punica Granatum* L were collected from southeast of Algeria, state of El Oued on October 2018. The plant material were washed, reduced into small pieces before being ground and powdered into particles of small size. Then the powder was put in a hot air oven at 60 °C until complete drying. 20 g of plant material were extracted with 160 ml of ethanol and 80% ethanol/water for 48 h in maceration. The extracts were filtered and evaporated under vacuum at 55°C before being dried and lyophilized for 24 h. the raw extract was stored at -4 °C.

Fig.4. Flowers of *Punica Granatum* L

3.2. Determination of total phenolic content

The total phenolic contents of flower were determined by the Folin-Ciocalteu method [9]. Briefly, 0.25 mL of each extract and standard (gallic acid) of were mixed with 1.25 mL of 1N Folin-Ciocalteu reagent. After 5 min, 1 mL of sodium carbonate aqueous solution (7.5%, w/v) was added to the mixture and completed the reaction for 120 mn at room temperature. The absorbance was read at 765 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Japan). The results were expressed in equivalent milligrams of gallic acid per gram of dry weight of plant extract (mg GAE**.** g^{-1} DW).

3.3. Total flavonoids content

According to the colorimetric method assay we quantification the flavonoids content of different extracts [10]. 4 mL of distilled water was added to 1 mL of each extract. Then, 5% of sodium nitrite solution (0.3 mL) was added followed by 10% aluminum chloride solution (0.3 mL). After, the mixture was incubated at room temperature for 5 min, and then 2 ml of 1 M NaOH were added. Immediately, the volume of reaction mixture was made to 10 ml with distilled water. The absorbance of the pink color developed was determined at 510 nm by UV-Visible spectrophotometer. A calibration curve was prepared with rutin and the results were expressed as mg rutin equivalents (RE). g^{-1} of dry weight.

3.4. Determination of antioxidant capacity total

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method [11,12]. An aliquot of 0.1 ml of sample solution was combined with 1 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). In the case of the blank, 0.1 ml of methanol was used in place of sample. The tubes were capped and

incubated in water bath at 95 _C for 90 min. After the samples were cooled to RT, the absorbance of the aqueous solution of each was measured at 695 nm. The experiment was conducted in triplicate and the results were expressed as mean ±SD values

3.5. Antibacterial activity

Antibacterial activity of flower of *Punica Granatum* against: *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Sensitivity of different bacterial strains to the extract was measured in terms of zone of inhibition using agar-diffusion assay.

The bacterial suspension was adjusted with sterile saline to a concentration of approximately 1.0 10^7 cells.mL^{-1}. The plates containing Mueller–Hinton agar is spread with 0.2 mL of the inoculums. Wells (6 mm diameter) were cut out from agar plates using a sterilized stainless steel borer [13]. In this method, extracts were dissolved in a small quantity of ethanol and were then prepared to the different concentrations of 5, 10, 15 and 20 mg/mL. Each well was filled with 100 µL of solution and the plates inoculated with different bacteria were incubated at 37℃ for 24 h and diameter of resultant zone of inhibition was measured.

3.6. Statistical Analysis

Experimental values are given as means ± standard deviation (SD) of three replicates for antioxidant activity and antibacterial activity. Values of $p < 0.05$ were regarded as significant.

4. CONCLUSION

In this research paper, using different flowers extract from sour ans sweet *Punica Granatum* L, it was shown that the EASE extract as a polyphenolic-rich extract, possesses the potent inhibitory antioxidant effect against ESE and flowers sweet extracts. In addition, the antimicrobial activity found in EASE especially against *Staphylococcus aureus*. As well as Gram-negative *Echerichia coli*, is a capable finding to fight the most frequent causes of bacterial food-borne infections in developed countries. Concluded that EASE extract has a high antioxidant potency which might be attributed to its high quantity of polyphenols. Additional studies are needed to characterize the bioactive compounds responsible for the observed activities.

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