Journal of Fundamental and Applied Sciences

ISSN 1112-9867

Available online at http://www.jfas.info

Research Article

COMPARATIVE STUDY OF PHYTOCHEMICAL COMPOSITION AND IN VITRO ANTIOXIDANT ACTIVITY FROM SWEET AND SOUR POMEGRANATE EXTRACT (*PUNICA GRANATUM* L)

M. Zidane[1], M. R. Ouahrani[2], S. E. Laouini[3]

[1]Department of Chemistry, Faculty of sciences of matter and mathematics, University of Kasdi Merbah Ouargla, 30000, Algeria

[2]Department of process engineering and petrochemical, Faculty of technology, Hamma Lakhdar University, El-Oued, 39000, Algeria

Received: 12 May 2018 / Accepted: 18 June 2018 / Published online: 01 September 2018

ABSTRACT

In this paper, to investigate the phenolic content and in *vitro* antioxidant of peel extracts from sour and sweet pomegranate (*Punica Granatum* L). The assessment of the antioxidant capacity using 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), DPPH, and metal chelating radical scavenging capacity assays. Furthermore, the total phenolic content measured by Folin-ciocalteu method as well conducted. The sour peel ethanolic extract was found to contain a high content of total phenolic content, for the flavonoids sweet ethanolic extract flavonoids contained the high amount. All extracts exhibited a higher antioxidant activity and the inhibitory effect of radicals scavenging activity against ABTS, DPPH, and metal chelating. The results suggest that the sour peel ethanolic extract and sweet peel ethanolic extract can be considered as a good source of natural antioxidant.

Keywords: *Punica Granatum* L; Phenolic content; DPPH; ABTS; Peel extract.

Author Correspondence, e-mail: medd.zidane@yahoo.fr

doi: http://dx.doi.org/10.4314/jfas.v10i3.28

Journal of Fundamental and Applied Sciences is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License. Libraries Resource Directory. We are listed under Research Associations category.

1. INTRODUCTION

The *Punica granatum* L is an important tree in North Africa, was diversified to Morocco, Algeria, Tunisia, Libya, and Egypt from the semi-tropics of Persia [1]. It is a fruit-bearing deciduous shrub or a small tree that belongs to *Punicaceae* family. The pomegranate tree is considered as a medicinal plant and used in against many diseases as the fruit is better known as nature's power fruit recognized worldwide for a pleasant taste and good health benefits [2]. The production and consumption of pomegranate have sharply increased with the increasing awareness of people about its superior therapeutic properties [3].

In addition to its ancient historical uses, pomegranate is used in several systems of traditional medicine. The synergistic action of the pomegranate constituents appears to be superior to that of single constituents. Recent studies on the scavenging of free radicals, anticarcinogenic, and anti-inflammatory properties of pomegranate constituents have been published, anticancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, bacterial infections and antibiotic resistance, and ultraviolet radiation-induced skin damage. Other potential applications include infant brain ischemia, male infertility, Alzheimer's disease, arthritis, and obesity [4]. The main antioxidant capacity of pomegranate is due to phenolic content in the peels [5]. Since the whole fruit is pressed to prepare juices, a large number of bioactive compounds would be expected to be extracted from the peels, and consequently commercial juices would have a strong antioxidant property [6]. Many publishers recently reported that pomegranate peel extract has an antibacterial activity [7]. Antimicrobial activity against some bacteria of extracts of fruit peels was tested and finding confirmed that various extract of peels was a forceful inhibitor for Listeria monocytogenes, S. aureus, Escherichia coli and Yersinia enterocolitica [8]. Extracts of pomegranate such as aqueous and methanolic demonstrated that good antibacterial activity against S. aureus and P. aeruginosa [9,10]. The fruits of pomegranate fruit include high amounts of organic acids, sugars, minerals, vitamins [11]. In addition, flavonoids are chief polyphenols of fruit, condensed tannins and hydrolyzable tannins [12]. Hydrolyzable tannins including ellagitannins and gallotannins consist of the common constituents present in an pomegranate, and punicalagin is the principal hydrolyzable tannin available in pomegranates [13]. The juice of fruits rich in sugars such as glucose [14], sucrose, and fructose, pectin, organic acids including citric, malic,

tartaric, succinic, fumaric and ascorbic acid. Formore has contained seeds hold constituents such as protein, crude fibers, vitamins, minerals, the phytoestrogen coumestrol estrone [15,16]. These activities can be related to antioxidant and antibacterial capacity. Several biological activities have been attributed in part to the presence of phenolic compounds [17]. Free radicals are chemically unstable atoms that cause damage to lipid cells and proteins as a result of an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant enzymes [18]. They are known to be the underlying cause of oxidative stress which is grossly implicated in the pathogenesis of various diseases such as cancer, diabetes, cardiovascular diseases, aging and metabolic syndrome [19]. Examples of these radicals include superoxide anions, hydroxyl, nitric oxide and hydrogen peroxide radicals. This research paper to estimate the phenolic compounds, flavonoids, and flavonols from the ethanolic and water solvent of sweet and sour pomegranate extract (*Punica Granatum* L). In vitro antioxidant properties, DPPH and FRAP radical scavenging activity were investigated.

2. RESULTS AND DISCUSSION

2.1. Phytochemical screening

Extraction is the main phase for obtaining extracts with acceptable phytochemical concentration and high antioxidant capacity [20]. The extract of sweet and sour pomegranate was found to be rich in phenolics constituent. The total phenolic content is given in table 1. sour ethanolic extract was founded to have the highest value 643.69 ± 14.75 mg GE.g-1 DW, following by the sour aqueous extract 452.22 ± 12.25 mg GE.g^{-1} DW, sweet ethanolic extract 352.97 ± 10.15 mg GE.g-1 DW and the lowest value in sweet aqueous extract 97.20 ± 4.18 mg GE.g^{-1} DW. For the quantification of total flavonoids, the content of total flavonoids was also found to vary significantly ($p < 0.05$) and content ranged from 9.72±0.6 mg CE/g DW to 116.30 ± 0.5 mg CE/g DW. The Total flavonoids in increasing order were: sweet ethanolic extract> sour ethanolic extract > sour aqueous extract > sweet aqueous extract. The content of total flavonols (Table 1) and was also found to vary significantly ($p < 0.05$) and the similar results obtained in flavonoids. The total flavonols in increasing order were: sweet ethanolic extract> sour ethanolic extract > sour aqueous extract > sweet aqueous extract. The values of phytochemical composition found in these extracts are comparable to those found in other

vegetal plants considered as important sources of phenolic compounds.

Table 1. Total phenolic, flavonoid and flavonols content of different extract from sweet and sour pomegranate

Extracts	Phenolic content mg GAE /g	Flavanoid content mg CE/g	Flavonols content mg QE/g
Sour ethanolic extract	643.69 ± 14.75	42.59 ± 0.8	05.57 ± 0.2
Sour aqueous extract	452.28 ± 12.25	07.87 ± 0.2	2.89 ± 0.05
Sweet ethanolic extract	352.97 ± 10.15	116.30 ± 0.5	15.42 ± 0.3
Sweet aqueous extract	97.20 ± 4.18	09.72 ± 0.6	3.18 ± 0.08

2.2. Scavenging inhibition of DPPH

The DPPH method is recommended as a simple and rapid screening method for estimated basic and information about the antioxidant capacity of the extracts. It is a commonly and widely used method despite some disadvantages. The sour ethanolic extract exhibited the strongest antioxidant activities against DPPH radicals IC_{50}= 1.14 ± 0.02 µg/ml, followed by sweet ethanolic extract IC_{50}= 12.90 ± 0.2 µg/ml, sour aqueous extract 16.61± 0.3 µg/ml and the lowest value in sweet aqueous extract IC_{50}= 30.65± 1.75 µg/ml (Fig.1).

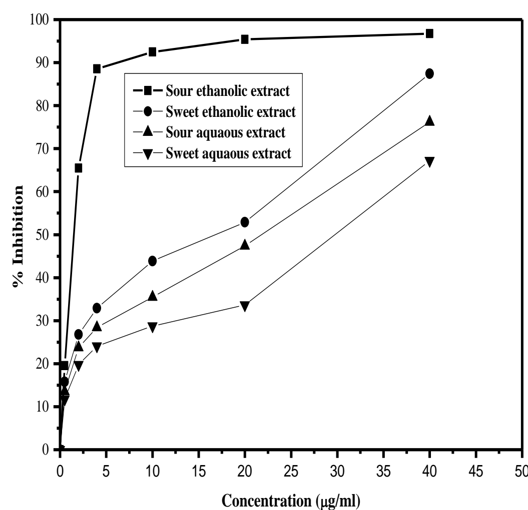

Fig.1. DPPH scavenging activity of peel sour and sweet extracts from pomegranate

2.3. ABTS radical scavenging assay

The capacity of extracts to inhibit ABTS free radical were also measured and compared for. Figure 2 shows that all extracts used in this study had significant ABTS radical scavenging activities. The IC_{50} values ABTS radical scavenging activities of sweet ethanolic extract, sour ethanolic extract, sour aqueous extract, and sweet aqueous extract were ranged of 34.25 ± 1.2 ± µg/ml at 69.84 ±1.3 µg/ml. The highest ABTS radical scavenging activities were found in sweet ethanolic extract IC_{50}=69.84±1.3 µg/ml, followed by sweet ethanolic extract 64.84 ± 1.33 µg/ml, sour aqueous extract 42.66 ± 1.25 µg/ml and the lowest value in sweet aqueous extract IC_{50}=34.25 ± 1.12 ± µg/ml.

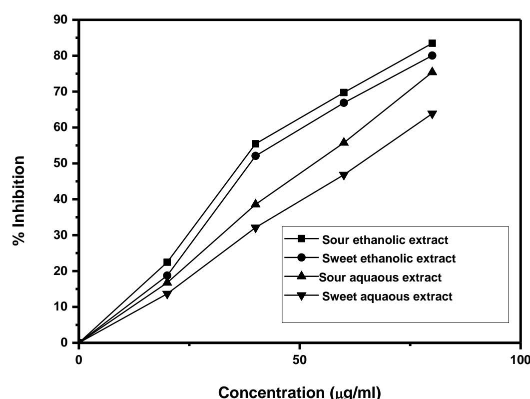

Fig.2. ABTS scavenging activity of peel sour and sweet extracts from pomegranate

2.4. Metal chelating activity

The metal ion chelating property of extracts was estimated and shown in figure 3. The decolorization of the red color of the reaction mixture depends upon the reduction of ferrous ions by the phenolic compounds. The results were expressed as mg EDTA equivalents/ g DW. The metal chelating activity of ethanolic and aqueous peel extract from sour and sweet pomegranate was 5.29±1.55, 43.78±1.45, 35.95±1.12 and 26.47±0.82 mg EDTA equivalents/ g DW for sweet ethanolic extract, sour ethanolic extract, sour aqueous extract and sweet aqueous extract respectively. Among the different extracts exhibited better metal ion chelating property.

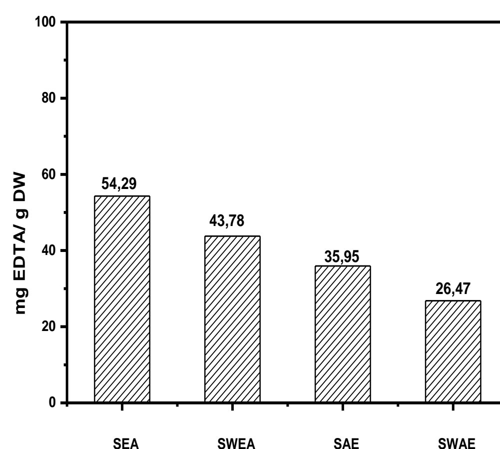

Fig.3. Metal chelating scavenging activity of peel sour and sweet extracts from pomegranate

3. EXPERIMENTAL

3.1. Extraction of phenolic compounds

The peel of *Punica granatum* L was thoroughly washed and reduced into small pieces before being ground and powdered into particles (1 mm). Then the powder was put in a hot air oven at 55 °C until complete drying. Depending on the physical characteristics of the samples, the time environment 48 hours. The bioactive compounds were extracted using 50 g of the peel (sweet and sour) powder prepared above and was extracted with 500 ml of ethanol, water and ethanol 80% by maceration technique for 24 h at 35 °C. The extracts were filtered and evaporated under vacuum at 45°C before being dried and lyophilized for 4 h, the raw extract was stored for further investigation and analysis.

3.2. Estimation of total phenolic content

The total polyphenols were quantified by the Folin-Ciocalteau reagent with a small modification [21,22]. This method is based on the degradation of the Folin-Ciocalteau by the phenolic compounds. 0.2 ml of each three extracts were mixed with 1 ml of Folin-Ciocalteau solution diluted 10 times. The Mixture is agitated and incubated for 5 minutes at the ambient temperature. Then, 0.8 ml of sodium carbonate %7.5 was added and agitated for 30 min. The absorbance measured at 765 nm using spectrophotometer UV visible. The concentration of total polyphenols in the extracts was expressed as mg gallic acid equivalent (GAE) per g of dry weight using and the equation of calibration curve All results presented are means (±SD)

and were analyzed in three replications.

3.3. Estimation of total flavonoid content

The method described by Biglari et *al* using for determination of flavonoids content [23]. 0.5 ml of peel extract was mixed with 2 ml of distilled water. After, 0.15 ml of the $NaNO_2$ solution was added to the above reaction (0.5 %), come behind by 0.15 ml of $AlCl_3$ solution (10%). The reaction was incubated at ambient temperature for 5 min, and then 2 ml of 1 M NaOH were added to the above reaction. After, the above reaction was completed with 5 ml of distilled water. The reaction was thoroughly vortexed and the absorbance measured at 510 nm. A calibration curve was prepared with catechin and the results were expressed as mg catechin equivalents (CE)/g of dry weight using and the equation of calibration curve. All results presented are means (±SD) and were analyzed in triplicate.

3.4. Estimation of flavonols

The spectrophotometric method described by Daniels et *al* using for determination of flavonols content [24]. 25 µL HCl (0.1 %) in 95 % ethanol was mixed with 25 µL of extract and were added to 500 µl HCl (2 %) and incubated for 30 min at room temperature and then the absorbance was measured at 360 nm using UV-Visible spectrophotometer (Shimadzu UV-1800, Japan). The is prepared with the same procedure described above but we replace the simple extract by the quercetin. Total flavonol content was expressed as quercetin equivalent (QE)/ g of dry weight.

3.5. DPPH assay

1 ml aliquot of each extract was mixed with 0.5 ml of a DPPH ethanolic solution (7.8 mg DPPH in 100 ml ethanol). The mixture was vigorously shaken and left to stand in the dark for 30 min at room temperature. The antioxidant activity was then measured by the diminution in absorption at 517 nm using UV-Visible Shimadzu UV-1800 (Japan) and corresponds to the extractability to decrease the radical DPPH to the yellow-colored diphenilpicryldrazine [25]. The capacity of extract scavenger the DPPH radials was expressed as IC_{50} (µl/ml). The antiradical percentage inhibition calculated by the following equation:

DPPH scavenging activity = $(A_o - A_1) / A_o \times 100$. Where A_o is the absorbance of control test after 30 min, A_1 is the absorbance of the sample extract after 30 min.

3.6. ABTS radical scavenging assay

The antioxidant activity of different two extracts was evaluated by ABTS scavenging assay radical [26]. ABTS reagent was prepared by 10 mL (7 mM ABTS solution and 178 µL of 140 mM potassium persulfate aqueous), the reaction was incubated at room temperature in darkness for 13 h before use. 2 µL of extracts or standard were added to 1.588 mL diluted ABTS solution to react in the dark at room temperature after 10 min. The absorbance was measured at 732 nm. The percentage inhibition of ABTS radical as calculated following the equation:

ABTS radical scavenging activity = $[(Abs_{control}-Abs_{sample})/ Abs_{control}]$ x100

3.7. Metal chelating activity

The chelating activity of extract from pomegranate was measured according to the method described by Lucci et *al* [27]. 0.5 ml of extract was added to 1.6 ml of deionized water. After, 0.05 ml of $FeCl_2$ (2 mM)0.1 ml and ferrozine (5 mM) was added. The absorbance of the Fe^{2+}-ferrozine complex was measured at 562 nm. EDTA was used as positive control. The metal chelation activity was calculated using the following equation: Metal chelating activity (%) =$[(A_{control} – A_{sample}) /A_{control}]$ x 100. where $A_{Control}$ is the absorbance of control devoid of sample and A_{Sample} is the absorbance of the sample in the presence of the chelator. The extract concentration providing 50% metal chelating activity was calculated from the graph of Fe^{2+} chelating effects percentage against extract concentration.

4. CONCLUSION

In this research paper, comparative study of sour and sweet extract from pomegranate growth in Southeast of Algeria (El Oued). In vitro antioxidant activities, phenolic content, flavonoids and flavonols ethanolic and aqueous extracts have been evaluated. The results indicated that sour ethanolic and sweet ethanolic extracts exhibited strongest antioxidant activities. The contents of polyphenols of tow extracts were significantly higher than those by sour and sweet aqueous extracts, which were possibly responsible for higher antioxidant activities of sour and sweet ethanolic extract. From the results we can draw the conclusion that not only the more bioactive components are obtained but also the extract has better free radical and metal chelating. These findings further illustrate that peel sour and sweet has a bright prospect for extracting of bioactive compounds.

6. REFERENCES

[1] Singh B, Singh JP, Kaur A, Singh N. Phenolic compounds as beneficial phytochemicals in pomegranate (Punica granatum L.) peel: a review. Food Chem. 261, 2018, 75-86.

[2] Kahramanoglu I, Usanmaz S. Pomegranate Production and Marketing. CRC Press.

[3] Karimi M, Sadeghi R, Kokini J. Pomegranate as a promising opportunity in medicine and nanotechnology. Trends Food Sci Techno. 69, 2017, 59-73.

[4] García-Ruiz A, Baenas N, Benítez-González AM, Stinco CM, Meléndez-Martínez A J, Moreno DA, Ruales J. Guayusa (Ilex guayusa L.) new tea: phenolic and carotenoid composition and antioxidant capacity. J Sci Food Agri. 97(12), 2017, 3929-3936.

[5] Jurenka J. Therapeutic applications of pomegranate (Punica granatum L.): a review. Alter med review. 13(2), 2008, 128.

[6] Gil MI, Tomas Barberan FA, Hess-Pierce B, Holcroft DM, Kader AA. Antioxidant capacity of pomegranate juice and its relationship with phenolic composition and processing. J. Agric. Food Chem. 48 (10), 2000, 4581–4589.

[7] Hmid I, Elothmani D, Hanine H, Oukabli A, Mehinagic E. Comparative study of phenolic compounds and their antioxidant attributes of eightee pomegranate (Punica granatum L.) cultivars grown in Morocco. Ara J Chem. 10, 2017, S2675-S2684.

[8] Rahmani AH, Alsahli MA, Almatroodi SA. Active Constituents of Pomegranates (Punica granatum) as Potential Candidates in the Management of Health through Modulation of Biological Activities. Pharmacognosy J, 9(5), 2017.

[9] Devatkal SK, Jaiswal P, Jha SN, Bharadwaj R, Viswas KN. Antibacterial activity of aqueous extract of pomegranate peel against Pseudomonas stutzeri isolated from poultry meat. J Food Sci Techno. 50(3), 2013, 555-560.

[10] Al-Zoreky NS. Antimicrobial activity of pomegranate (Punica granatum L.) fruit peels. Int J Food Microbiology. 134(3), 2009, 244-248.

[11] Gil MI, Tomás-Barberán FA, Hess-Pierce B, Holcroft DM, Kader AA. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J Agri Food Chem. 48(10), 2000, 4581-4589.

[12] Van Elswijk DA, Schobel UP, Lansky EP, Irth H, van der Greef J. Rapid dereplication of estrogenic compounds in pomegranate (Punica granatum) using on-line biochemical detection

coupled to mass spectrometry. Phytochemistry. 65(2), 2004, 233-241.

[13] Seeram NP, Aviram M, Zhang Y, Henning SM, Feng L, et al. Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. J Agri Food Chem. 56(4), 2008, 1415-1422.

[14] Fischer UA, Carle R, Kammerer DR. Identification and quantification of phenolic compounds from pomegranate (Punica granatum L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD–ESI/MS n. Food Chemi. 127(2), 2011, 807-821.

[15] Mena P, García-Viguera C, Navarro-Rico J, Moreno DA, Bartual J, et al. Phytochemical characterisation for industrial use of pomegranate (Punica granatum L.) cultivars grown in Spain. J Sci Food Agri. 91(10), 2011, 1893-1906.

[16] Qu W, Breksa III AP, Pan Z, Ma H. Quantitative determination of major polyphenol constituents in pomegranate products. Food Chem. 132(3), 2012, 1585-1591.

[17] Lobo V, Patil A, Phatak, A, Chandra N. Free radicals, antioxidants and functional foods: impact on human health. Pharmaco Review. 4(8), 2010, 2968-2972.

[18] Nasira J, Moinuddin A, Syed SS. Interactive activity of *Asphodelus tenuifolius* on germination and growth of wheat (*Triticum aestivium* L.) and sorghum (*sorghum bicolor* l.). Pak J Botanic. 43(1), 2011, 325-331.

[19] Nadembeg P, Boussim JI, Nikiema JB, Poli F, Antognoni F. Medicinal plants in Baskoure, Kourittenga Province, Burkina Faso: An ethnobotanical study. J Ethnopharmacology. 133, 2011, 378–395.

[20] Sowndarya, S, Balasaraswathi K, Vidhya V, Sridevi J, Mandal, AB, Rose C. Evaluation of total antioxidant and free radical scavenging activities of Callistemon citrinus (Curtis) Skeels extracts by biochemical and Electron Paramagnetic Resonance analyses, Royal Soc Chem adv. 6,2016, 12382-12390.

[21] Liu S, Sun J, Yu L, Zhang C, Bi J, Zhu F, Qu M, Yang Q. Antioxidant activity and phenolic compounds of Holotrichia parallela Motschulsky extracts. Food Chem. 134, 2012, 1885-1891.

[22] Biglari F, AlKarkhi AFM, Easa AM. Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. Food Chem. 107, 2008, 1636–1641.

[23] Daniels CW, Rautenbach F, Mabusela WT. Comparative antioxidant-capacity and content

of leaves, bulbs, roots, flowers and fruit of Gethyllis multifolia L. Bolus and G. villosa Thunb species. S Afr J Botany. 77, 2011,711-717.

[24] Upadhyay NK, Yogendra Kumar MS, Gupta A. Antioxidant, cytoprotective and antibacterial effects of Sea buckthorn (Hippophae rhamnoides L.) leaves. Food Chem Toxico. 48, 2010, 3443–3448.

[25] Lucci P, Pacetti D, Loizzo MR, Frega NG. Punica granatum cv. Dente di Cavallo seed ethanolic extract: Antioxidant and antiproliferative activities. Food Chemistry 2015; 167: 475–483.

[26] Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J Agri Food Chem. 49(11), 2001, 5165-5170.

How to cite this article:
Zidane M, Ouahrani M R, Laouini S E. Comparative study of phytochemical composition and in vitro antioxidant activity from sweet and sour pomegranate extract (*punica granatum* l). J. Fundam. Appl. Sci., 2018, *10(3), 419-429*.