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Research Paper

Evaluation of bioactive properties and antioxidant capacity of native and imported pomegranate fruit bark (*Punica granatum, L)* cultivars found in Algeria

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Abstract

Pomegranate *(Punica granatum L.)* is an ancient fruit endowed with therapeutic properties that is widely consumed as fresh fruit. Several works have demonstrated how this fruit acts as antioxidant, antidiabetic. The aim of this review was to present an overview of the functional properties of this fruit, which is a contribution to the valorization in a medical, industrial and pharmaceutical context of pomegranate peel relying on the evaluation of natural compounds which can be beneficial for the consumer. Pomegranate peels varieties were chosen in this study. The morphological studies were conducted in different varieties according to the geographical location, such as Italy Wonderful (E1), France Provence (E2), Morocco Sefri (E3) and two local varieties Algerian (L1) and (L2) respectively, Skikda and Sidi Bel Abbés local variety, were subjected to extraction using the solvent (water and ethanol). The extraction yield, antioxidant activity (DPPH and FRAP test) and total phenolic contents, flavonoids and tanins were evaluated. Highest yield was obtained from 70 % ethanol: in local Skikda variety (L1) (33.7 %). The results also showed that the extract of pomegranate skin *Punica granatum L.* is very rich in total polyphenols, flavonoids and tannins respectively obtained for the variety of France with values of (270,14 ± 11,04 mg EAG / g) and (30.84 ± 4.66 mg EC / g) (22.67 ± 2.51 mg EAG / g). Our results reveal that all the extracts have a good antioxidant activity whose IC50 varies between 35,90 ± 26,62 and 121,63 ± 0,77 µg / ml compared to the different varieties studied. The pomegranate bark is rich in potassium, an important source of polyphenols, which are nutritional components found in plants that actively fight against free radicals.

Keywords: Antioxidant activity, Polyphenols, Pomegranate bark*, Punica granatum.l*

Introduction

For thousands of years, the pomegranate, *Punica granatum L,* its fruits, as well as its seeds, its bark and its flowers, are used, in the Middle East and in Asia, regions from which this shrub is native, for their medicinal properties. Prescribed empirically in traditional medicines to treat gastro-intestinal diseases and parasitic diseases. This food and its products are rich sources of bioactive compounds such as total phenolic, total anthocyanin and total tannin contents and antioxidant activity assay with different methods have been published in literature[1,2,3] and minerals, mainly potassium, nitrogen,

calcium, phosphorus, magnesium, and sodium[4], and complex polysaccharides[5]. There has been a virtual explosion of interest in the pomegranate as a medicinal and nutritional product because of its multifunctionality and its great benefit in the human diet. Since ancient times, the pomegranate has been regarded as ahealing foo with numerous beneficial effects in several diseases[6]. Indeed, the pomegranate was commonly used in folk medicine, for eliminating parasites, and to treat and cure aphtae, ulcers, diarrhea, acidosis, dysentery, hemorrhage, microbial infections, and respiratory pathologies. It was also used as an antipyretic[7]. On the will be some components of the Pomegranate like to the polyphénols with antioxydants, anti-inflammatoires and potontiel anti-cancérigènes[8,9] In addition, In vitro studies reported that *Punica granatum L*. peel and seed extracts have antimicrobial activity as they are rich in phenolic compounds[10,11]. Chemical studies on pomegranate suggested that it contains different phytochemicals. While ellagitannins (punicalin and punicalagin) and numerous piperidine alkaloids are formed in roots and bark[12].

Recently, a study was done by[13] on the Pharmacological Effects and Therapeutic/Medicinal Applications of *Punica granatum L.* (Pomegranate) as a functional food in humans and animals, he demonstrated the fruit is rich in phenolic compounds with strong antioxidant activity and Ellagic acid is one of the main components of pomegranate with potent antioxidant activity. Naturally occurring polyphenols in a pomegranate can be a potential alternative medicine for the prevention of avian Colibacillosis diseases and can also be used as an intestine astringent to relieve diarrhea and enteritis in chickens. The fruit and bark of pomegranate are used against intestinal parasites, dysentery, and diarrhea in different animals and human models. According to some studies, the *Pomegrenate bark* and rather thick (2-3mm) brilliant red, the weight of pomegranates generally varies according to the origin and the cultivar between 197 and 315 g, whose the proportion of the epidermis that is the outer part of the fruit represents (32.28-59.82 %), of the total weight of the fruit according to[14]. It is yellow in some varieties and is rich in water soluble tannins, mainly in punicalin, pedunculaine and punicalagin[15]. It can reform up to 28% of the tannins[16]. The bark, rich in antimicrobial and antioxidant substances, protects the fruit from predators and attacks from solar radiation[17]. Pomegranate bark has been used in many food products where it has been shown that pomegranate bark powder (PEG) extract can be considered as a natural preservative in meat products against various strains such as Listeria monocytogenes, Bacillus subtilis, Bacillus cereus, Escherichia coli and Staphylococcus aureus, with a proven bactericidal effect[18]. Another study published by[9], revealed the effectiveness of the pomegranate bark ethanoid extract in the treatment of fungal-infected wounds in rabbits, proving the antiseptic property of pomegranate bark. Also, the iso-flavonoid extract of pomegranate bark can have a significant effect on the improvement of reproductive parameters in rabbit males.

These fruits have been studied at different levels, especially in phytochemistry, but until now, little research has been done on the determination of their biochemical compositions and particularly on the nutritional fractions of different varieties on the bark Pomegranate cultivated from Algeria more particularly in the area of sidi bel abbes and skikda (North and East of Algeria). This study has led us to undertake this work, given the availability of this fruit in our country, to put more value. This work is a contribution to the valorization in a medical, industrial and pharmaceutical context of pomegranate peel relying on the evaluation of natural compounds that can be beneficial for the consumer.

Geographic origin of pomegranate cultivars (*Punica granatun L*)

Five varieties of pomegranate, *Punica granatum L,* were chosen. Morphological studies of pomegranates were conducted in different countries according to geographical location (two local varieties and three foreign varieties) based on morphological characteristics to assess biochemical diversity. Varieties such as Italy (Wonderful E1), France (Provence E2), Morocco (Zehri E3) and Algeria (Yellow Grenada L1, L2) were collected, his last are harvested in October 2017 were healthy and had no lesions. For this, this experimental part was realized within laboratory of applied biochemistry, Department of Biology, Faculty of Sciences of Nature and Life, University of Sidi Bel Abbes. Algeria. Examples of the different varieties structures are illustrated in Figure 1.

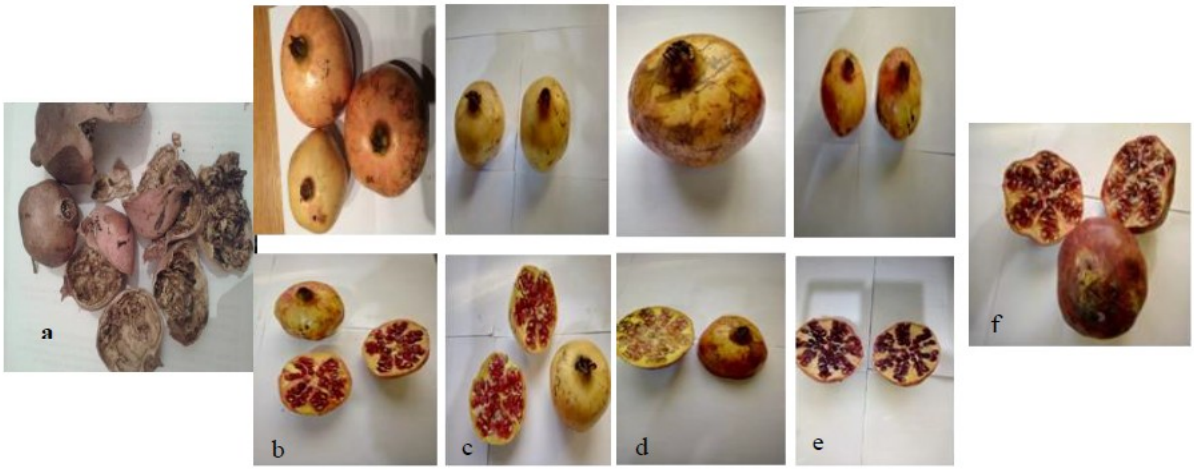

Figure 1: Morphological aspects of five grenadier fruits

(a) Dry Pomegranate (Personal view) **(b)** SBA variety L1 **(c)** Skikda variety L2
(d) France variety (Provence E2) **(e)** Morocco variety (Zehri E3) **(f)** Italy variety (Wonderful E1)

Preparation of extracts

There are a large number of pomegranate varieties that differ not only in their morphology but also in their physicochemical composition. According to this composition, the varieties are often divided into sour, sweet-sour and sweet pomegranates according to the work of [19,20]. For this study we selected the intact and intact fruits during the month of October until mid-January 2018 in the locality of Sidi Bel Abbes and Skikda, the foreign Pomegranate were bought from the market of 3 countries (Italy, France and Morocco), the barks were photographed for authentication. The whole fruit is weighed using a precision scale. After the manual separation of the bark, we have determined the percentage in bark. The measurement of the thickness of the bark, the hardness of the seeds, the taste and the color of the seeds are also evaluated (Table 1). In the first place, we proceeded to the different stages of preparation of our samples in order to obtain the fruit bark. The preparation of our extracts requires first a Peeling, Ginning, once dried for a period of 2 months in the sun, is followed by crushing the barks using the mortar, then the use of grinder using Moulinex is useful to obtain a fine powder that must be collected by sieving to 1mm. The powders obtained are stored in airtight containers until the day of use. Physical characterization of pomegranate fruit whose weight, thickness, color and taste were grouped in the Table 1. The preparation of the extracts is carried out by the choice of distilled water as a solvent, 10 g of the sample powder (dry weight of pomegranate bark) are taken in a mixture (of 30 ml of distilled water + 70 ml of ethanol) and then stirred for 24 hours with the aid of a stirrer. After maceration for 24h the solution was filtered using a cotton funnel and using a rotary evaporator (rota vapor) at a temperature between 35 to 45°C, in order to obtain a dry extract.

Table 1: The physicals characteristics of Pomegranate and the extraction yield in %.

Variety*	Fruit weight	Thickness of the bark (mm)	External color	Color of the arils	Taste of arils	Yield** in %
L1	275g	3,5	Pink/Yellow	Dark red	Soft	29.2
L2	500g	4,0	Yellow	Dark red	Sweet / Slight Acid	33.7
E1	350g	4,1	Dark red	Dark red	Acid	14.8
E2	474g	2,5	Red Yellow	Light RED	Acid ++	33.7
E3	400g	3,5	Dark Red	Light RED	Sweet/acid	30.7

***The different varieties of Pomegranate bark**
L1: SBA variety **L2:** Skikda variety
E1: Italy variety (Wonderful) **E2:** France variety (Provence) **E3:** Morocco variety (Zehri)
****Yield of hydro-ethanolic extracts in percentage of the varieties of pomegranate bark

Extraction of soluble free phenolic compounds in different pomegranate peels

Determination of total polyphenols
The determination of the total polyphenols of the extract is determined by the use of the Folin-Ciocalteu reagent and which is described by the method[21]. After addition of the Folin-Ciocalteu reagent (0.25 ml) and 20% aqueous sodium carbonate solution (1.25 ml), tubes were vortexed. After 40 min the absorbance of the resulting blue colored mixtures was recorded at 765 nm against a blank containing only an extraction solvent (0.2 ml). During the oxidation of phenols, the blue coloration produced has a maximum absorption around, with reference to a standard range obtained with a phenolic acid (gallic acid), to determine the amount of total polyphenols present in an extract. It is expressed in mg of gallic acid equivalent per g of dry matter.

Determination of flavonoid content
Flavonoid content was determined using a colorimetric method described previously[22]. Briefly, 0.5 ml of the ethanol extract was diluted with 1 mL of distilled water. Then, 0.075 mL of a 5% $NaNO_2$ solution was added to the mixture. After 6 min, 0.15 ml of a10% $AlCl_3 \times 6H_2O$ solution was added, and the mixture was allowed to stand for another 5 min. Half of a milliliter of 1 M NaOH was added, and the volume was made up to 2.5 ml with distilled water. The solution was well mixed, and the absorbance was measured immediately against the blank (containing the extraction solvent instead of a sample) at 510 nm. The flavonoids are quantified using a calibration curve obtained by measuring the absorbance of the known concentrations of the quercitrin spread solutions and the results are expressed in microgram equivalents of quercitrin per milligram of dry extract (MgEQ / g).

Determination of total tannins
Tannins are polyphenolic compounds found in the plant kingdom. Their main characteristic is the capability of binding and precipitating proteins. Tannins have molecular weights ranging from 500 to 3,000 for stufy[23]. Tannins are usually divided into hydrolysable tannins and condensed tannins (proanthocyanidins).

a/ Determination of hydrolysable tannins
They are polyesters of carbohydrates and phenolic acids; they are easily hydrolyzed by acids and enzymes (tannase) in ose (usually glucose) and in phenol acids. The Taupe method and the[24] was based on a reaction with ferric chloride. The mixture of tannic extract with the ferric chloride reagent results in the formation of purple-colored complex with Fe^{3+} ion formation.

b/ Determination of condensed tannins
The tannins are hardly soluble in cold water but soluble in hot water (colloidal solutions), they are soluble in alcohol and acetone, insoluble in organic apolar solvents (ether). Their solubility varies according to their degree of polymerization. 0.1 ml of each extract was placed in tubes to which 3 ml of 4% (w / v) vanillin methanol solution was added. Absorbance was measured at 500 nm after incubation for 20 minutes. The calibration curve was prepared under the same conditions using catechin as the standard and the results were expressed in catechin mg equivalent / g dry matter (mg EC / g MS[25].

c/ Determination of antioxidant activity
In order to evaluate the antioxidant activity of the pomegranate skin extract, two tests were carried out: reducing power, radical scavenging DPPH reducing power: The ability of total phenols to reduce ferric iron Fe (III) to iron (II) is evaluated by the FRAP method[26]

DPPH radical scavenging activity
Diphenyl picrylhydrazyl (DPPH), a violet stable free radical in solution and having an absorbance at 517 nm, this color rapidly disappear when the DPPH is reduced to diphenyl picryl-hydrazine by a compound with anti-radical property, also resulting in a declaration. (The intensity of the staining is inversely proportional to the capacity of the antioxidants present in the medium to give protons[15]. The DPPH scavenging activity was determined according to the[27] assay. Briefly, an aliquot of extract (0.3 ml) was mixed with the DPPH reagent (0.5 mM in methanol, 0.25 ml) and the acetate buffer (100 mM, pH 5.5, 0.5 ml). After standing for 30 min in the dark, the absorbance was measured at 517 nm against a blank containing absolute methanol instead of a sample aliquot. The results obtained for each extract tested are compared with ascorbic acid as standard antioxidant. The results as well were

expressed as an IC50 value that represents the amount of powder (in mg of dry matter) providing 50% inhibition of DPPH.

Determination of minerals (dosage of Potassium and Sodium)
For the screening of the mineral salts, 0.5 g of the sample powder (dry weight of pomegranate bark) is taken in 10 ml of distilled water, shaken for 1 hour and then centrifuged for 10 min. 2.43 g of KCl are taken in 100 ml of distilled water for the calibration curve of k^+, but for the determination of sodium, 1.95 g of NACL are taken in 100 ml of distilled water. Absorbance was measured by flame photometry.

Results and Discussion

There has been a virtual explosion of interest in the pomegranate as a medicinal and nutritional product because of its multifunctionality and its great benefit in the human diet as it contains several groups of substances that are useful in disease risk reduction. As a result, the field of pomegranate research has experienced tremendous growth[28]. The aim of this review was to present an overview of the functionnal and physiological properties of the pomegranate. The chemical composition of the fruits differs depending on the cultivar, growing region, climate, maturity, cultivation practice, and storage conditions[29,30]. Significant variations in organic acids, phenolic compounds, sugars, water-soluble vitamins, and minerals of pomegranates have been reported over the years by various researchers[31,4]. About 50% of the total fruit weight corresponds to the peel, which is an important source of bioactive compounds such as phenolics, flavonoids, ellagitannins (ETs), and proanthocyanidin compounds[32], minerals, mainly potassium, nitrogen, calcium, phosphorus, magnesium, and sodium[4].

Herbal antioxidants are extracted from raw materials by organic solvents such as ethanol, methanol, acetone and diethyl ether. Ethanol is an effective extractor for a wide range of polyphenols so it is a frequently used solvent on a laboratory scale and on an industrial scale[33]. Extraction of the phenolic compounds from our samples was done by maceration with 70% ethanol.

The extracts obtained have a caramelized appearance of brown to dark brown. The extraction yields were determined based on the weight of the dried plant material and made into powder, the results were expressed as a percentage and all chemical analyses were performed in two replicates per plot and the results were analyzed are presented as means ± standard deviation (SD). According to the results obtained in Table 1, we find that the yields differ from one region to another. The hydro-ethanolic extract of *P.granatum* bark from Skikda has the highest yield with a percentage of 33.7%, while the extract from Italy has the lowest percentage (14, 8%). Previous studies report that plants with high yields of extracts contain a high content of phenolic compounds[34]. According to some studies[32], this method of extraction appears to be very effective in the extraction of antioxidants from pomegranate bark[35], note that for the organic solvent (ethanol 70 %), the extraction yield of *P. granatum* bark is of the order of 37% which is superior to our results. It is found that the yield is variable, although the extraction technique is the same; this variability is probably due to the variation of the following factors: growth stage, pedoclimatic conditions, period of harvest, drying.

Total polyphenol contents
Natural polyphenols can range from simple molecules (phenolic acids, phenylpropanoids, flavonoids) to highly polymerized compounds (lignins, melanins, tannins), with flavonoids representing the most common and widely distributed subgroup[36]. Chemically, phenolic acids can be defined as substances that possess an aromatic ring bound to one or more hydrogenated substituents, including their functional derivatives[37]. Flavonoids are low-molecular-weight compounds consisting of 15 carbon atoms, arranged in a C_6-C_3-C_6 configuration. Essentially, the structure consists of 2 aromatic rings joined by a 3-carbon bridge, usually in the form of a heterocyclic ring[38]. The content of the phenolic compounds of the hydroethanolic extracts from the bark of the five pomegranate varieties *P.granatum*, was determined by the method of Folin-ciocalteu[21] using the calibration curve made with the acid Gallic. Polyphenol levels can serve as important indicators of antoxidant capacity and can be used as a preliminary screening for any product when it is determined to be a natural source of antioxidants in foods[39].

The results of the quantitative spectrophotometric analyzes extracted from the *Punica granatum* pomegranate peel of the five varieties studied (Figure 2), show that the Provence variety (E2) is richer

in polyphenols than the other varieties with a content of 270.14 ± 11.104 mg EAG / g compared with Yellow variety (L1) extract (193,473 ± 45,515 mg EAG / g), Wonderful variety (E1) (169,73 ± 9,65 mg EAG / g), Zehri variety (E3) (55,3 ± 3,179 mg EAG / g) and Yellow variety (L1) (35 ± 6.928 mg EAG / g). These results are inferior to the several works in contrast to the variety of France (Provence) which shows a content consistent with the work reported by[40] with a content of 216.9 ± 7.3 mg EAG / g and a study[32] found a level of 216.9 ± 7.3 mg EAG / g, thus the study conducted by[41] indicate a total polyphenol content of 274 ± 17 mg EAG / g in the 80% methanolic extract of *P. granatum* bark. A study of *P.granatum* bark using 70% ethanolic extract revealed a significant amount of total polyphenols equal to 301.77 mg EAG / g[42]. Similarly, the[43] studies reported a rate of 449.60 ± 4.40 µg GAE / mg for the same species. According to the bibliography, the content of phenolic compounds depends on several parameters, such as the climatic conditions, the variety considered, the extraction processes, the cultural techniques, the genetic factors and the storage conditions[19,29.] These variations can also be explained by the fact that the Folin-Ciocalteu test overestimates total phenol levels due to interference with other compounds such as proteins, sugars, and organic acids (ascorbic acid, citric acid) that also reduce Folin's reagent[32,43].

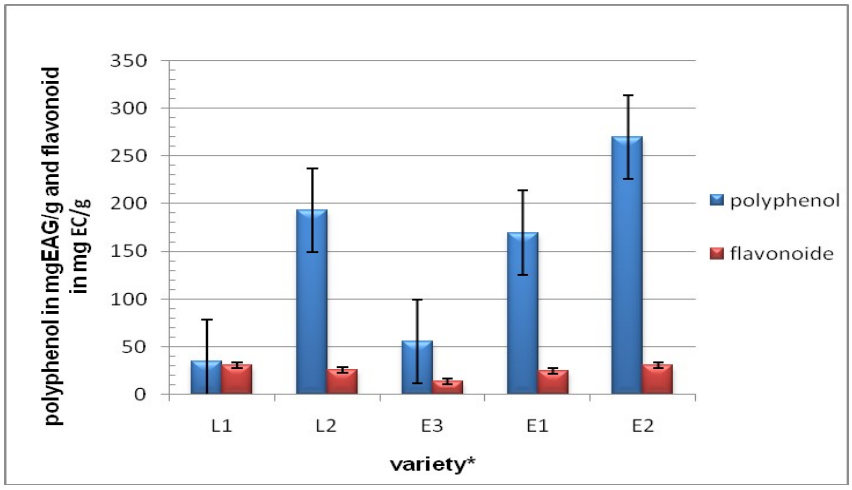

Figure 2: Concentration of polyphenols (in mg EAG/g) of *pomegranate bark*

*** The different varieties of Pomegranate bark**
L1: SBA variety **L2:** Skikda variety
E1: Italy variety (Wonderful) **E2:** France variety (Provence) **E3:** Morocco variety (Zehri)

It emerges that the variety E2 provence of the region of France presents a more acidic which is due to a significant value in polyphenol, followed by the local variety of the East Algerian region of Skikda, this study conferring us after a work[44] that acidic varieties are the richest in phenolic compounds and the most important antioxidant activity. The edaphoclimatic parameters, the genetic factors, the extraction method can play a role in the evaluation of total polyhenols. since two different regions such as that the variety of provence comes from France and the variety of Skikda of the east of Algeria adapted to the time, the middle and the climate of this coastal region which give it a flavor and a particular aspect, which can be competitive internationally and its export remains a very feasible option.

Flavonoids content
The main reason for choosing this class of polyphenols is that flavonoids are the most important polyphenolic class, with more than 5000 compounds[45]. The spectrophotometric determination of flavonoids was carried out by the $AlCl_3$ method in standard catechin. The flavonoid content is estimated from a quercetin calibration curve. According to the results obtained (Figure 2), it appears that it is the variety of Provence (E2) (30.84 ± 4.660 mg EC / g) and Yellow variety (L1) (30.75 ± 0.473 mg E / g) which present the contents the highest compares with the other varieties, Wonderful variety (E1) (25.93 ± 6.532 mg EC / g), Zehri variety (E3) (24.62 ± 2.470 mg EC / g) and Yellow variety (L2) (14.11 ± 1.406 mg EC / g).

Our results seem important compared to those found by[43], who note that the ethanolic extract of *P.granatum* bark contains a quantity of flavonoids of 38.44 ± 1.44 µg EQ / mg and are lower than

those reported. By[32] with a content of 59.1 ± 4.8 mg ER / g. According[46], the pomegranate bark is very rich in flavonoids, the presence of this saccharide fraction makes the flavonoids very soluble in water, so it is recommended to use for extraction a mixture of (organic solvent / water). In *P. granatum* bark, levels of 56.4 ± 2.7 mg ER / g and 30.37 mg EQ / g were recorded respectively[41]. The slight difference can also be said that the spectrometric method of the total flavonoid assay underestimates the actual content of total flavonoids the fact that certain compounds phenolics may not react with aluminum trichloride ($AlCl_3$) which may make values slightly low. Or another explanation, the varietal factor or by the method of determination or by the nature of the extraction solvent used. According to[46], the bark of the pomegranate is very rich in glycosylated flavonoids, the presence of this saccharide fraction makes the flavonoids very soluble in water, it is therefore recommended to use for their extraction a mixture of solvents organic / water.

Content of hydrolysable tannins and condensed tannins

The results are shown in (Figure 3) .The determination of rate are hydrolyzable tannins, shows that the (E2) variety contains 22.67 ± 2.51 mg EAT / g, followed by (L2) variety with 21.29 ± 0.36mg EAG / g, (E1) variety is also richer in tannins with 21.43 ± 6.54 mg EAG / g, followed by (L1) with 17.60 ± 2.35 mg EAG / g and the (E3) with 13.18 ± 1.24 mg EAG / g. A slight significant difference was observed for all varieties of pomegranate peel at the reception of the Zehri variety from the region of Morocco.

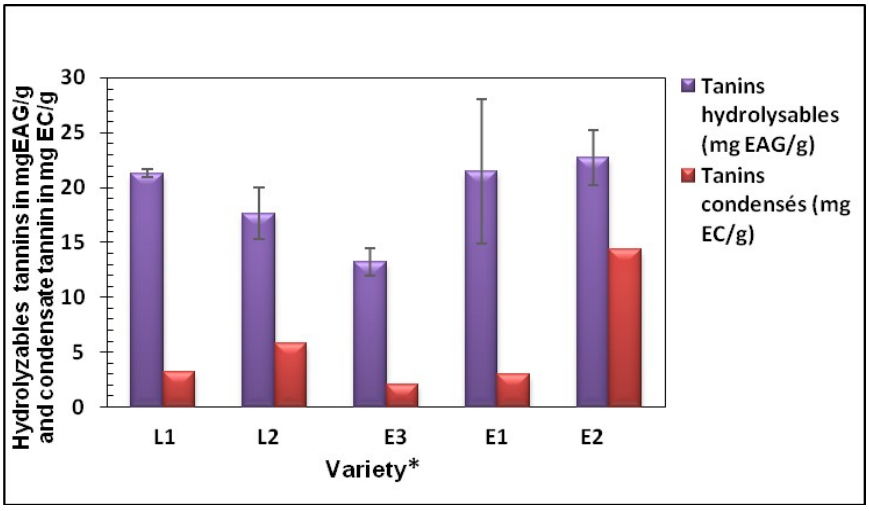

Figure 3: Concentrations of hydrolysable tannins and condensed tannins of different extracts of *P. granatum bark*

*** The different varieties of Pomegranate bark**

L1: SBA variety **L2:** Skikda variety
E1: Italy variety (Wonderful) **E2:** France variety (Provence) **E3:** Morocco variety (Zehri)

According to[47], hydrolysable tannins constitute 25% of the constituents of the bark of the pomegranate, including punicalines and punicalagines which are the major constituents of the bark and greatly contribute to color. In study[33], found that the hydroalcoholic extract of *P.granatum* fruit contains 81.66 ± 3.51 mg / g. It has been reported that pomegranate extract is rich in ellagitannins which are hydrolysable tannins[48]. Regarding the results of the condensed tannin contents are very low in the three following varieties (L1) (2.06 ± 0.28 mg CE / g), in (E1) (3.02 ± EC 1,22mg / g), in (E3) (3.25 ± 1.15 mg CE / g) and little higher in variety in (L1) (5.08 ± 0.34 mg CE / g) except that the variety (E2) revealed a high content (14.35 ± 0.23 mg EC / g) (Figure 4). The quantities are low compared to the quantities obtained by[32], which cite 10.9 ± 0.5 mgEC / g ES with ethanol as extraction solvent. the variations which are less important and which differ slightly between the varieties of the same species on the same product from one year to another and according to the varieties cultivated, the region of culture, the cultivated varieties the cultivation techniques.

Antioxidant activities

It is now known that the antioxidant properties of plant extracts cannot be evaluated by a single method due to the complex nature of phytochemicals, a complete antioxidant analysis of plant

extracts should involve several activity studies[49]. Among these different methods, the DPPH and FRAP tests were the two methods that were taken into consideration for the evaluation of the antioxidant activity of ethanolic extracts of *P.granatum* from different regions.

DPPH test

DPPH is a very stable organic free radical of dark violet color that gives a maximum absorption band around 515-528 nm, when accepting a proton from any hydrogen donor, mainly the compounds Phenolic, it loses its chromophore and becomes yellow. The DPPH test is a method frequently used to evaluate the antioxidant potential of various natural compounds for its speed, reliability and low cost[50]. Our results expressed as a percentage of the antioxidant activity (Figure 4), reveal that all extracts tested as well as ascorbic acid taken as reference are antioxidants. The (L1) variety had the highest antioxidant activity (91.55 ± 2.82%), followed by the (E2) variety (82.17 ± 3.92%) while with the (L2) variety the lowest percentage (70.36 ± 5.45%) was recorded at a concentration of 250 µg/ml.

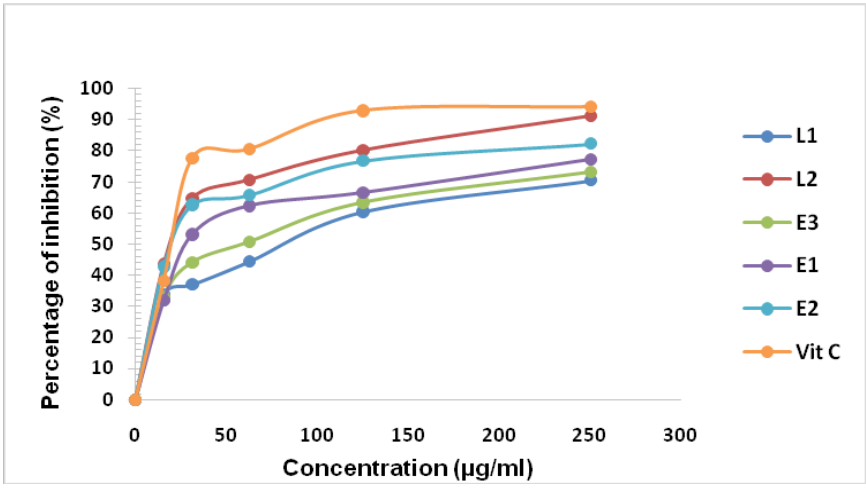

Figure 4: Antioxidant activity of different extracts of *P.granatum bark.*

*** The different varieties of Pomegranate bark**

L1: SBA variety **L2:** Skikda variety

E1: Italy variety (Wonderful) **E2:** France variety (Provence) **E3:** Morocco variety (Zehri)

The antioxidant activities of various extracts and bark of *P.granatum,* remains lower compared to the positive control Vit c (94.10 ± 1.22%). Cultivars are classified as follows according to the ability to trap the free radical DPPH. Yellow grenada (*L1) > Prvence (E2) > Wonderful (E1) > Zehri (E3) > Yellow grenada (L2).*

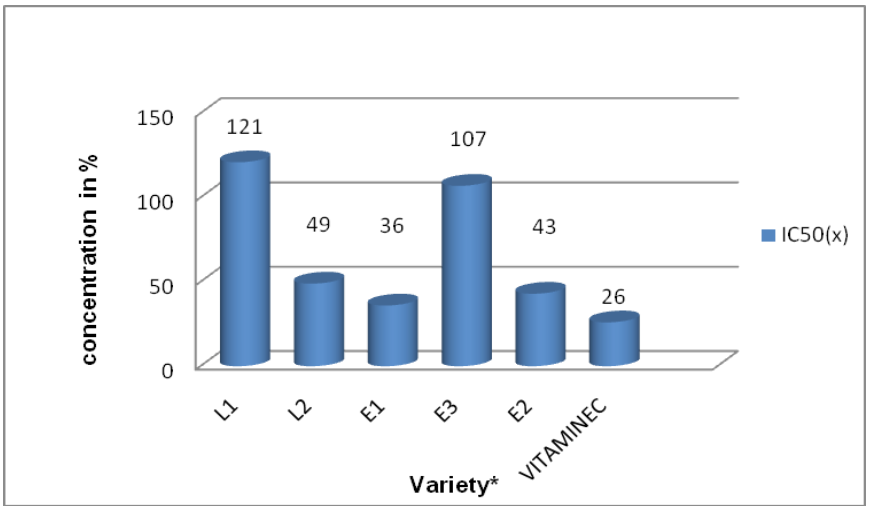

Figure 5: Inhibition concentration of different varietal extracts of *P. granatum*

*** The different varieties of Pomegranate bark**
L1: SBA variety **L2:** Skikda variety
E1: Italy variety (Wonderful) **E2:** France variety (Provence) **E3:** Morocco variety (Zehri)

In the study developed by[51], the sequential extraction of polyphenols from pomegranate bark resulted in percent inhibition of DPPH radical uptake of (90.53%, 86.4%, and 83.2%) for the methanol fraction of acetone, ethanol respectively. It should be noted that the active ingredients found in the extract of the pomegranate fruit bark are phenolic compounds, ellagic acid and flavonol as well as quercetin and kaempferol[52]. The IC50 of a compound is inversely related to its antioxidant capacity because it expresses the amount of antioxidant required to reduce the DPPH radical to 50%. A low IC50 indicates the highest antioxidant activity[50,53]. Our results reveal that all extracts have a good antioxidant activity whose IC50s vary between 35.90 ± 26.62 and 121.63 ± 0.77 µg / ml (Figure 5). The methanolic extract of the bark of *P.granatum* studied by[54] shows IC50 equal to 5.49 ± 0.039 µg / ml which are inferior to our results. These differences from our results can be explained by other factors such as the difference between grenadier cultivars or the extraction method used in these analyzes. The antioxidant activity of our samples can be dependent on their richness in phenolic compounds; the flavonoids have an ideal structure for the free radical scavenging, since they have a number of hydroxyls acting as hydrogen donors, which in turn makes important antioxidants[55].

FRAP test
The reducing power of an extract, which can serve as a reflection of its antioxidant activity, is determined by using the Fe^{+3} reduction test in Fe^{+2}, whereby the yellow color of the test solution changes to a blue-green color and this according to the reducing power of the sample. The presence of the antioxidants in the sample causes the reduction of the Fe^{+3} / ferricyanide complex to Fe^{+2}, Therefore, Fe^{+2} can be evaluated by measuring the increase in the density of the blue color in the reaction medium at 700 nm. The highest absorbance indicates the greatest reduction power[56]. The results of the reducing activity of our extracts are shown in (Figure 6).

It is noted that the increase in iron reduction is proportional to the used concentrations of our extracts ranging from 1 to 0.0625 mg / ml. Their reducing powers are lower than that of ascorbic acid, which is known to be a powerful reducing agent[57]. The reducing power of the extract increases with the increase of the amount of extract of each sample concerning the (L1) variety sample has a very high reduction power with an absorbance of 0.98 ± 0.16 to 1 mg / ml followed by sample from (E1) with significant iron reduction power with an absorbance of 0.89 ± 0.57 at the same concentration, however the low potency is observed in the (L2) extract (0.56 ± 0, 11). The reducing power can be attributed mainly to bioactive compounds associated with antioxidant activity such as total phenolic compounds, flavonoids and other hydrophilic and hydrophobic antioxidants which are good electron donors and can terminate the free radical reaction chain by conversion of free radicals to more stable products[58].

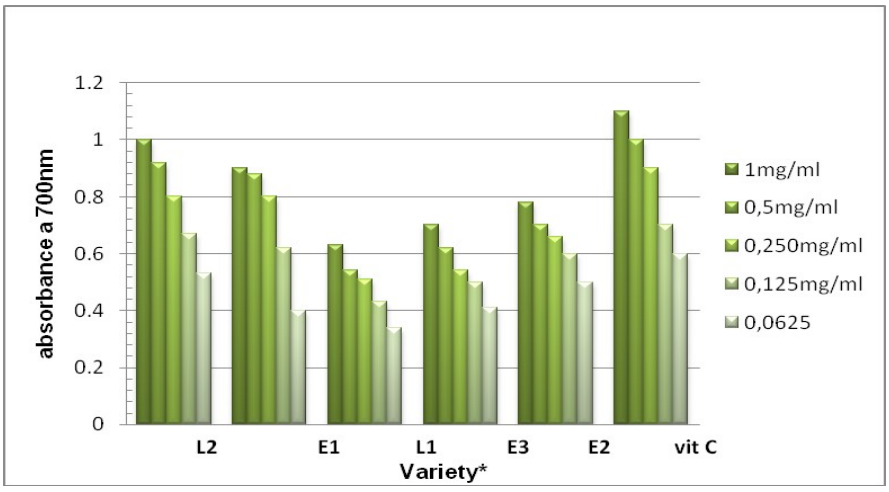

Figure 6: Antioxidant Activities of Ethanolic Extracts of *P.granatum bark* by Iron Reduction (FRAP Test)

*** The different varieties of Pomegranate bark**
L1: SBA variety **L2:** Skikda variety
E1: Italy variety (Wonderful) **E2:** France variety (Provence) **E3:** Morocco variety (Zehri)

Mineral salts
Minerals are essential for the proper functioning of the body; pomegranate bark contains varying concentrations of these minerals, he results obtained for the potassium and sodium content are illustrated in (Table 2). The results of the quantitative spectrophotometric analyzes (flame spectrophotometer) of the *Punica granatum L* fruit bark extracts of the five varieties studied, indicate that the variety of (L1), (L2), (A3) are richer in K which are of the order of 305.32 mg, while the sample of (E1) contains a quantity of 251,27mg that of (E2) a quantity of 197,21mg. On the other hand, sodium contents of 27.5 mg were recorded for the varieties of (E1), (E3) and France, and for (L1) and (L2) varieties 18.79 mg. Pomegranate is rich in potassium, an important source of polyphenols, which are nutritional components found in plants that actively fight against free radicals according to[59]. Sodium (Na) and potassium (K) are electrolytes that regulate the balance and play the role in the optimal maintenance of the acid-base balance in humans, are very essential extracellular cations, A potassium deficiency can cause muscle cramps and hypertension[60].

Table 2: Potassium and sodium content in the five *P. granatum pomegranate* bark cultivars

Variety*	Potassium (K) in (mg)	Sodium (NA) in (mg)
L1	305	18
L2	305	18
E1	251	27
E2	197	27
E3	305	27

*** The different varieties of Pomegranate bark**
L1: SBA variety **L2:** Skikda variety
E1: Italy variety (Wonderful) **E2:** France variety (Provence) **E3:** Morocco variety (Zehri)

Conclusion

In this study, shown that Algerian and imported pomegranate varieties particularly sour varieties (Yellow pomegranate and Provence), were rich in polyphenols as well as flavonoids and mineral components. This study provides a useful insight into production for new varieties presenting a considerable source of phenolic compounds and flavonoids as already proven in the two local Algerian variety of Skikda (Yellow pomegranate) and the French variety (Provence) imported which have a good antioxidant activity and have considered by-products can be used as natural foods, additives in human and animal nutrition to enhance immunity, microbial safety.

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