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Evaluation of Antioxidant Activities of Two Different Solvent Extracts of Green Prickly Pear (*Opuntia ficus indica* L. Mill.).

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ABSTRACT

Investigating green prickly pear for their seed phenolic contents is of high importance due its potential use for food and nutraceutical applications. These phenolic compounds have an important content in bioactive substances; with have been associated with the prevention of some chronic diseases. Prickly pear possesses important antioxidant activities. The aim of this work was to compare the antioxidant activities of methanol and ethyl acetate extracts for phenolic compounds recovery from seeds.The extract yield of both solvent extracts was determined by maceration method. The antioxidant activities (FRAP and Phosphomolybdate assays, respectively) of prickly pear seeds extracts were evaluated using the spectrophotometer. The solvent extract yields of *O. ficus indica* seeds were 1.35 and 4.49 %, methanol and ethyl acetate respectively. The extract obtained using ethyl acetate was enriched in polyphenols (26.39 mg GAE/ 100 g DW) than that extract using methanol (12.05 mg QE/ 100 g DW). The extraction by maceration using ethyl acetate as solvent gave a high polyphenol contents and dominant antioxidant properties. The green variety could be an interesting source of nutrients compounds which not only have nutritional potential, but are also a source of dietary antioxidant components which may have beneficial effects on consumer's health.

Keywords: Prickly pear, Antioxidant, Seeds, FRAP, PPM.

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INTRODUCTION

There is an increasing evidence for the participation of free radicals, chemical reactions, and others redox reactions in the etiology of various diseases like cancer, diabetes, cardiovascular diseases, autoimmune disorders, neurodegenerative diseases and aging [1, 2].

In recent years, there has been a lot of scientific works on prickly pear as a source of bioactive compounds for nutrition, health and disease[3-5]. In the literature, family Cactaceae is reported to contain about 130 genera and nearly 1500 all well adapted to arid lands and to diversity of climates and are naturalized in several areas all other the world. Prickly pear is native to Mexico and was then through to Europe, America, Mediterranean basin, Autralia, India, Africa and Middle East, showing adaptation to arid and semi-arid climates in tropical and sub-tropical regions of the world [6]. In South Africa, Mediterranean areas and South America this species is cultivated for its edible fruit (prickly pear), although in some countries different parts of the plant are utilized in the food and cosmetic industry (e.g., fruits, flower, roots or leaves) [7, 8]. Cactus (*Opuntia ficus indica*) commonly known as prickly pear or cactus pear, have been traditionally used in folk medicine in several countries for several medicinal purposes [9]. In Morocco, the cactus ripening period is between July and October. There is a strong market demand by consumers. The fruit is consumed fresh, during this period.Prickly pear fruit has attracted attention due its nutritional and health impact (phenolic compounds). Phenolic compounds are plant secondary metabolites that constitute one of the most common and widespread groups of constituents in plants [10]. Fruits and vegetables are very important in human nutrition as sources of nutrients and non-nutritive food constituents as well as for the reduction in disease risks. The possible health benefits of phenolic compounds have been suggested to derive from their antioxidant properties for their role in the prevention of emerging degenerative diseases. There are several methods developed to estimate the antioxidant activity of different plant materials [11].Phenolic substances are ubiquitous in plants, and when plant food is consumed, these constituents contribute to the intake of natural antioxidants in the human diets. Currently, the research has been focused towards natural antioxidants in the seeds.In addition to the literature, the previous study had showed that the seed was rich in phenolic compounds [12, 13].The objective of this work was to compare the antioxidant activities of two different extracts of prickly pear seeds, and food-promoting benefit.

MATERIALS AND METHODS

Plant material

Prickly pear fruits [Opuntiaficusindica (L.) Mill.] were collected in October 2015 at full maturity (Rabat, Morocco). After washing and separation of the pulp from theseed, fruits of green color were homogenized, and used to measure physico-chemical parameters. The seeds were air dried and reduced into powder through an electric mill.

Preparation of extracts by using maceration extracting methods

The solid-liquid extraction method reported by Apraj and Pandita[14]was used with a few modification to extract the phenolic compounds from the seed powder. Seventy grams of dry matterwere added to 140 ml of extraction solvents: methanol and ethyl acetate respectively. The mixture was subjected to an agitation during 3 hours at room temperature in darkness and then filtered through filter paper (Whatman N°1). Second and third extractions were performed following the same procedure as first. The three filtrates were combined, concentrated under vacuum using a rotary evaporator. Dried extracts were kept in refrigerator and used for further tests.

Determination of total polyphenol contents

The total phenolicsfor each extract was measured by Folin-Ciocalteu's (FC) method based on modified method by Hashemi *et al.* [15]. A volume of 250µl of each extract was mixed with 1.25 ml of Folin-Ciocalteu's reagent (which was diluted ten times) and 1 ml of sodium carbonate (7.5 g/100 ml) in a test tube. The mixture was then vortexed and incubated in dark for 30 min at room temperature. After that, the absorbance was read at 765 nm using UV/Vis spectrophotometer.The absorbance of samples was compared with that of a gallic acid

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standard curve [y= 0.011x + 0.013 (R^2= 0.999)] (Figure 1); and FC reducing capacity was expressed as gallic acid equivalents (GAE) mg/100 g dry weight (DW).

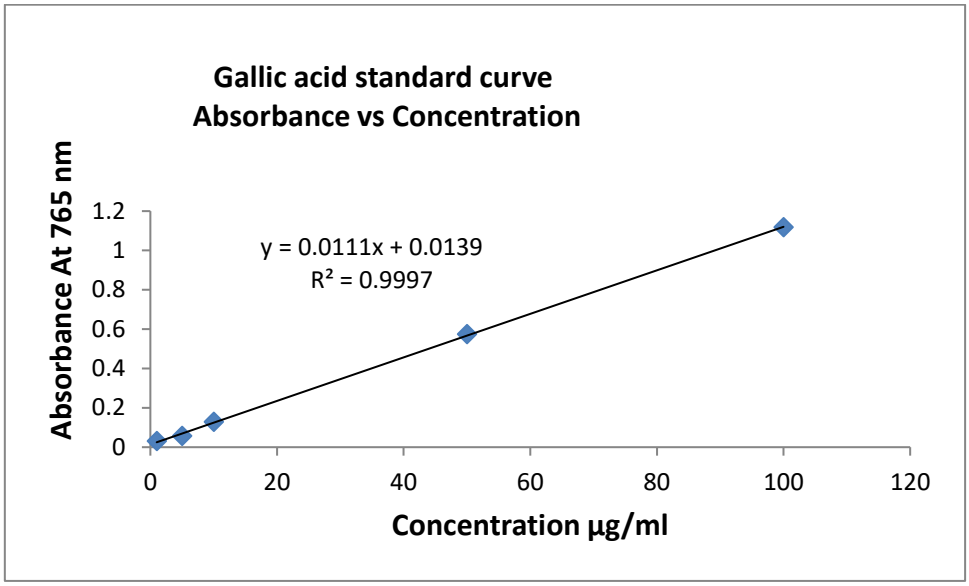

Figure 1: Gallic acid standard curve

Determination of total flavonoid contents

The total flavonoids were determined by spectrophotometric method by Chougui*et al.*with slight modifications[16]. To the aliquot of 1.5 ml was added 1.5 ml of $AlCl_3$ reagent (20 g/L). After 1H of incubation, the absorbance was measured at 430 nm against a blank. The absorbance of samples was compared with that of a quercetin standard curve [y= 0.028x + 0.060 (R^2 = 0.982)] (Figure 2); and Aluminium chloride reducing capacity was expressed as quercetin equivalents (QE) mg/100 g DW.

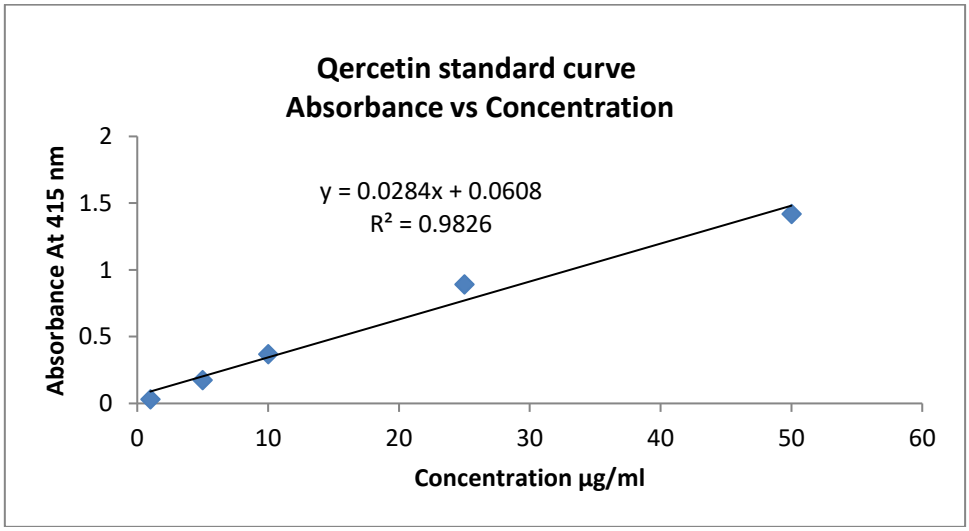

Figure 2: Quercetin standard curve

Antioxidant assays

Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) of the different extracts was determined by using the potassium ferricyanide –ferric chloride method, as described byLicayan*et al.*[17] with few modification.The absorbance was measured at 700 nm against a blank. The absorbance of samples was compared with that of a

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butyl hydroxyl anisole (BHA) standard curve [y= 0.003x + 0.002 (R^2 = 0.996)] (Figure 3); and FRAP reducing capacity was expressed as BHA equivalents (BHAE) mg/100 g DW.

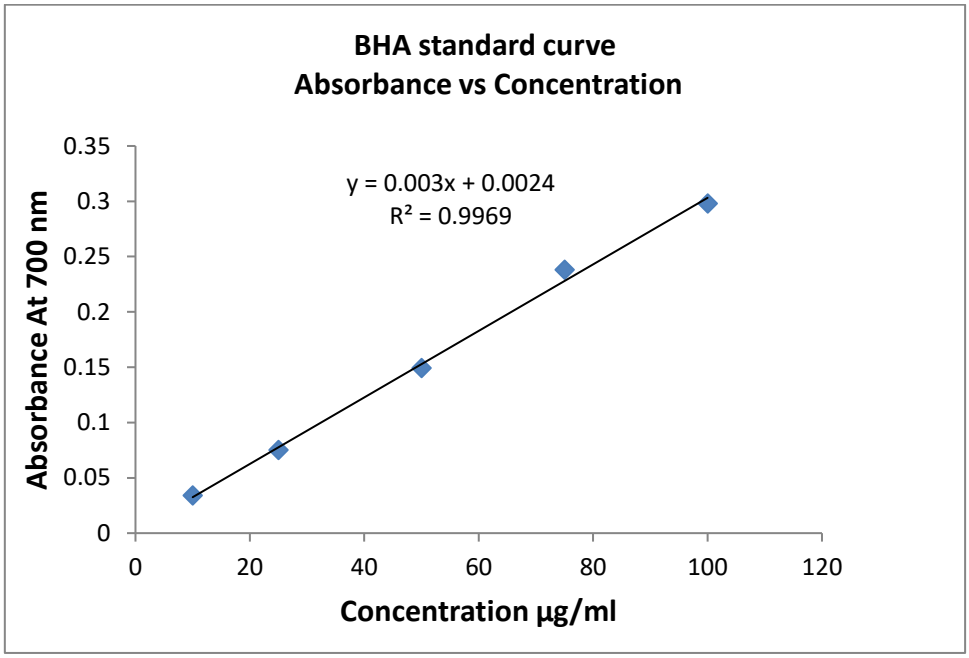

Figure 3 : BHA standard curve

Phosphomolybdate assay

The antioxidant capacity of the solvent extracts was determined by phosphomolybdate method[18] using ascorbic acid as a standard. An aliquot of 300 µl of sample solution was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM solution phosphate and 4mM ammonium molybdate). The mixture was vortexed and incubated in a water bath at 95°C for 90 min. After cooling at room temperature, the absorbance was measured at 695 nm against a blank.The absorbance of samples was compared with that of a gallic acid standard curve [y= 0.004x – 0.026 (R^2 = 0.998)] (Figure 4); and phosphomolybdate reducing capacity was expressed as ascorbic acid equivalents (AAE) mg/100 g DW.

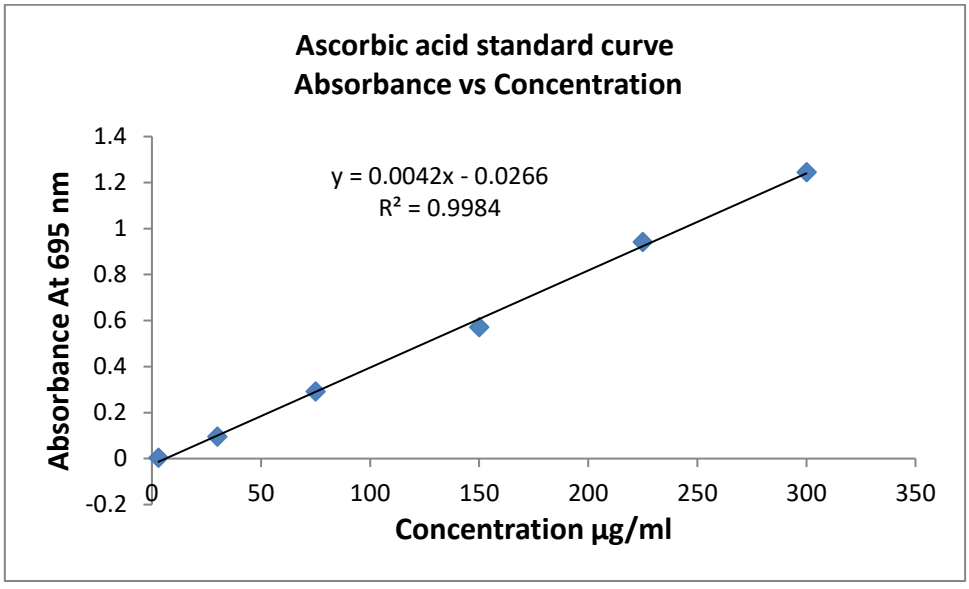

Figure 4: ascorbic acid standard curve

Statistical Analysis

The results were expressed as mean ± standard deviation (n=3). The significance of differences among the treatment means was determined using one-way analysis of variance (ANOVA), calculated by SPSS version 13.0, with a significance level of $p \leq 0.05$.

RESULTS

The methanol and ethyl acetate extracts were 12.05 and 26.39 mg GAE/100 g dried samples, respectively (Table 1). In the same table, the aluminum chloride metering method has not detected the presence of flavonoid in the ethyl acetate extract.

Table 1: Flavonoid and Polyphenol contents in the studied plant extracts

Sample	Extraction	Phenol compounds	
		Polyphenol (mg GAE/100g)	Flavonoid (mg QE/100g)
Seeds	Methanol	12.05±0.71	0.86±0.03
	Ethylacetate	26.39±5.66	Not detected

Each value in the table was obtained by calculating the average of three experiments ± standard deviation
The different value in column 3 means significant difference at $p < 0.05$.

According to the results in table 2, the ethyl acetate extract gave the highest antioxidant activities for both tests (FRAP and Phosphomolybdate tests, respectively).

Table 2: Comparison of FRAP and Phosphomolybdate tests of the plant extracts

Sample	Extraction	Antioxidant assay	
		FRAP (mg/100g)	Phosphomolybdate (mg/100g)
Seeds	Methanol	46.20±6.23	161.31±7.22
	Ethylacetate	114.58±11.99	369.97±14.16

Each value in the table was obtained by calculating the average of three experiments ± standard deviation
The different value in column (all) means significant difference at $p < 0.05$.

DISCUSSION

Maceration extracting

The polar contents (dry basis) of *Opuntia ficus indica* seeds was determined using maceration method. The 1.35 and 4.49 % seed yields obtained from Methanol and Ethyl acetate extracts, respectively, may be due to the polarity of the solvents used or the affinity of the compounds extracted in contact with the solvent**.**

Total polyphenol contents

The methanol extract had significantly lower total polyphenol contents than the ethyl acetate extract ($p < 0.05$). In particular,ethyl acetate extract had total polyphenol contents twice as high as those of methanol extract (Table 1). Albano *et al.*[6]reported that fruit pulp of orange color contains a total polyphenol content of 69.8 mg/100 g fresh weight. Chougui*et al.*[16] reported that typical seeds contains 61 mg GAE/100 g dry weight, while, Lee *et al. [19]*reported a phenolic content of 370 mg GAE/ 100 g (*Opuntiaficus-indica* var. saboten).Our results showed that the seed extracts used in this experiment were lower in polyphenol contents compared to results used in the literature. This variability may be due to solvents or the experienced method.

Total flavonoid contents

The total flavonoid contents of green prickly pear varieties are shown in Table 1.The valueobtained from methanol extract through a standard curve (Figure 2)was 0.86quercetin equivalent (QE)/ 100 g dried sample. The ethyl acetate extract had not detected flavonoid contents. The same author [16]reported that the flavonoid contents of green seeds (1.5 mg QE/100 g dried weight) is slightly larger than our result.To sum up, we can say that the results are similar in methanol extract. The absence of flavonoid in ethyl acetate extract may be justified by the choice of solvent extract.

Ferric reducing antioxidant power

In reducing power assay, the yellow color of the solution changes to green depending on the reducing power of aliquot. The presence of the reductants in the mixture causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Furthermore, Fe^{2+} can be monitored by absorbance measurement at 700 nm. Previous works suggested that the reducing properties have been shown to use antioxidant effect by donating of a hydrogen atom to break the free radical chain [18]. Increasing optical density at 700 nm indicates an increase in reducing power. The antioxidants present in the solvent extracts of green prickly pear seeds (*Opuntiaficusindica*L.) caused their reduction of Fe^{3+}/ ferricyanide complex to the ferrous form, and thus proved the reducing action.

Plant materials rich in phenolic compounds are increasingly being used in the food industry by reason they retard oxidative degradation of lipids and improve the quality and nutritional value of food[20]. Phenolic substances are considered secondary metabolites and these phytochemical compounds derived from phenylalanine and tyrosine occur ubiquitously in plants and are diversified [21]. Phenolic substances of plants are very important by reason their hydroxyl groups confer scavenging action.It is known that different phenolic compounds have different responses in the FC test. The molecular antioxidant response of phenolic compounds varies, depending on their chemical structure [22].

The optical density values for the ferric reducing power of the prickly pear seeds extracts are shown in Table 2. With a sample concentration 1 mg/ ml, ethyl acetate extract had the highest reducing power of114.58; methanol extract had less reducing power of 46.20 ($p < 0.05$).Our results showed that green prickly pear seed had higher or lower reducing power effect than those of other varieties [3].

Phosphomolybdate assay

The phosphomolybdatemethod has been routinely used to evaluated the antioxidant activity of extracts[2]. After mixing the two phases, Mo (VI) is reduced to Mo (V) and forms a green coloredphosphomolybdenum V complex, which shows a maximum absorbance at 695 nm.

The results of phosphomolybdate test of the prickly pear seeds are shown in table 2. With a sample concentration of 1 mg/ml, antioxidant effects in ethyl acetate and methanol extracts were 369.97and 161.31, respectively but the different was significant ($p < 0.05$). Methanol extract had the lowest antioxidant effect. In particular, ethyl acetate extract had antioxidant activity twice as high as those of methanol extract.Abdel-Hameed *et al.*[3] reported that the phosphomolybdate effects were760.61, 735.35, 228.57 and 204.31 mg AAE/ 100 ml juice red peels, red pulps, yellow peels and yellow pulps, respectively. Comparing to our results, the antioxidant activity of red fruits (peels and pulps) is higher than that of the ethyl acetate extract but against yellow fruits have a lower effect antioxidant than the same extract. On the other hand, there is less data on the antioxidant activity study of green prickly pear.

CONCLUSION

Antioxidant activity was significantly higher in the ethyl acetate extract than in the methanol extract. The activity of these two solvents is attributed to the phenolic and flavonoid contents.Differences among the extracts in terms of polyphenol and flavonoid contents, FRAP and phosphomolybdate tests may be due to the green prickly pear variety. Ethyl acetate extract had the highest total polyphenol content and higher antioxidant activity (FRAP and Phosphomolybdate tests, respectively). Additional analyses are required to investigate polyphenol compound structures and mechanisms of pharmacological action. The use of natural and functional foods has increased in worldwide. As a result, this study may be useful in developing the green prickly pear cultivar which have highly antioxidants and can be used as functional food agents.

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