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Research Article

Phenolic Composition and Antioxidant Activities of *Opuntia ficus Indica* L. Cladodes Related to Extraction Method

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ABSTRACT

We studied the richness of phenolic compounds and the antioxidant activities of Opuntia ficus indica L. cladodes by comparing two types of extraction, maceration and soxhlet made with methanol / water (8 : 2, v/v). The results showed that the extraction by maceration is better than that by soxhlet with a total polyphenol concentration of 36.7 mg GAE/ g DW, a total antioxidant activity of 18.5 mg GAE/ g DW and an IC50 of 77.5 µg/ml against DPPH radical scavenging. These results are very vaforable for a sustainable valorisation of the prickly pear cladodes for medicinal, pharmacological and alimentary fields.

Keyword: Opuntia, cladodes, phenol, DPPH, extraction.

INTRODUCTION

For thousands of years, medicinal plants have been for man the main source of remedies against various diseases and are used as drug[1]. In fact, their therapeutic properties are due to the presence of hundreds, even thousands of bioactive natural compound, including secondary metabolites. These latter are subsequently accumulated in different organs and sometimes in specialized cells of the plants.

Currently, the development of microbial resistance to antibiotics and the toxicity of synthetic antioxidants and their harmful effect have incite researchers to develop studies on plants, especially medicinal and culinary plants in search of natural molecules effective and devoid of any adverse effect[2]. Many studies have demonstrated the presence of secondary metabolites endowed with biological activities such as polyphenols, alkaloids and terpenes[3].

Phenolic compounds are molecules derived from the vegetable kingdom and produced exclusively by plants[4]. These compounds are synthesized by plants both during normal development and under stress conditions. In plants, they are involved in development, reproduction, cell growth, differentiation, organogenesis, flowering and lignification[5]. Moreover, content of these compounds in plant varies greatly depending on genetic, physiological and environmental parameters. These compounds are involved in different physiological stage of plant (organogenesis and growth) and in their relation with the physicochemical and biological environment[6]. Moreover, they contribute strongly to organoleptic qualities such as the color and astringency of plants. These compounds are known for their important antioxidant power. This property depends on the hydroxylation state of their aromatic rings, this activity is due to their redox property allowing them to adsorb and neutralize free radicals and scavenge reactive oxygen species[7,8]. In addition to these antioxidant activities, polyphenols have a great ability to chelate metal ions (especially iron and copper cations) and these capacities are related to the structures of these molecules[5]. Through these activities, phenolic compounds have several vital roles in plants. Phenolic acids, for example, are included in several processes necessary for plant life such as protein biosynthesis, enzymatic activities, and photosynthesis[9].

The diversity of natural biotopes resulting from the variability of geographical, edaphic and climatic conditions is the origin of the diversity of the plant.

Algeria has a rich and diverse plant flora. Among the medicinal plants that make up the vegetation cover is the genus Opuntia, which is widely distributed mainly in semi-arid regions and dry and rocky desert areas. It is a perennial plant of 2 to 3m of height, very ramified. Many species of this genus are used in traditional medicine because they contain many molecules endowed with therapeutic activities. However, the choice of the extraction method remains a determining factor in obtaining these interested biomolecules.

Through this work, we will study the phenolic composition of *Opuntia ficus indica* L. cladodes as well as the evaluation of its antioxidant activity by comparing the result of two extraction methods, maceration and Soxhlet system in order to highlight the importance of this plant and determine the most suitable means of extraction.

MATERIALS AND METHODS

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Sampling preparation and extraction

In this study, the collected plant material consists to *Opuntia ficus indica* cladodes from Batna region (northeastern Algeria). The sampling was conducted in December 2014. The samples were dried then grounded into fine powder which is extracted by two methods:

Extraction by maceration

This extraction was carried out according to the method of[10], by mixing 20 g of dry plant material with 200 ml of 80% methanol. The mixture is stirred for 30 min and kept at rest for 24 hours in dark room. The obtained extract is filtered and stored at 4° C until analysis.

Extraction by soxhlet system

Extraction of 30 g of plant powder is effected by 300 ml of 80% methanol. The extraction lasts 24 hours at the end of which the extract is recovered and stored in the dark at 4° C until analysis.

Colorimetric quantification of phenolics

Determination of total polyphenol content

Colorimetric quantification of total polyphenol was determined as described by[11]. Hundred and twenty five microliter of each sample extract suitably diluted was dissolved in 500 µl of distilled water and 125 µl of the Folin–Ciocalteu reagent. The mixture was shaken, before adding 1250 µl Na2CO3 (7%) and adjusting with distilled water to a final volume of 3 ml. After incubation for 90 min at 23°C in dark, the absorbance versus prepared blank was read at 760 nm. A standard curve of gallic acid was used. Total phenolic content of cladodes was expressed as mg gallic acid equivalents per gram of dry weight (mg GAE/g DW) through the calibration curve with gallic acid (0–400 µg/ml). All samples were analyzed in triplicates.

Estimation of total flavonoid content

Total flavonoids were measured by a colorimetric assay according to[11]**.** An aliquot of the samples was added to test tubes containing 75 µL of a 5% $NaNO_2$ solution, and mixed for 6 min. Then, 0.15 ml of a freshly prepared 10% $AlCl_3$ solution was added. After 5 min at room temperature, 0.5 ml of 1N NaOH was added. The final volume was adjusted to 2.5 ml with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm against the same mixture without the sample as a blank. The concentrations of flavonoid compounds were calculated according to the equation that was obtained from the standard (+)-catechin graph, and were expressed as mg catechin equiv/g DW (mg CE/g DW). All samples were analyzed in triplicates.

Total condensed tannins assay

The analysis of condensed tannins (proanthocyanidins) was carried out according to the method of[12]**.** To 50 µl of properly diluted sample, 3 ml of 4% methanol vanillin solution and 1.5 ml of concentrated hydrochloric acid were added. The mixture was allowed to stand for 15 min, and the absorbance was measured at 500 nm against methanol as a blank. The amount of total condensed tannins is expressed as mg CE/g DW. The calibration curve range was 0– 400 µg ml−1. All samples were analyzed in triplicates.

Determination of antioxidant activities

Evaluation of total antioxidant capacity

The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acidic pH[13]. An aliquot of sample extract was combined in an eppendorf tube with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated in a thermal block at 95°C for 90 min. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank in UV-Visible spectrophotometer (Anthelie Advanced 2, SECOMAN). The antioxidant capacity was expressed as mg gallic acid equivalent per gram of dry weight (mg GAE/g DW). All samples were analyzed in triplicates.

Scavenging ability on DPPH radical

DPPH is a free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH quenching ability of *Opuntia ficus indica* cladodes extracts was measured according to[14]**.** One milliliter of the extract at known concentrations was added to 0.5 ml of a DPPH methanolic solution. The mixture was shaken vigorously and left standing at room temperature in the dark for 30 min. The absorbance was then measured at 517 nm and corresponds to the extract ability to reduce the radical DPPH to the yellow-coloured diphenylpicrylhydrazine. BHT was a synthetic phenolic used as positive standard. The antiradical activity was expressed as IC50 (µg/ml), the antiradical dose required to cause a 50% inhibition. The ability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = [(A0 - A1)/A0]* 100

Where A0 is the absorbance of the control at 30 min, and A1 is the absorbance of the sample at 30 min. All samples were analyzed in triplicate.

Statistical analysis

Means were statistically compared using MINITAB 2000 program with Student's t-test at $P < 0.05$ significance level. One-way analysis of variance (ANOVA) and Newman-Keuls multiple range test were carried out to test any significant difference between means at $P < 0.05$.

RESULTS AND DISCUSSION

Methanolic extracts of *Opuntia ficus indica* L. cladodes were studied through assays of total polyphenols, flavonoids and condensed tannins as well as in vitro tests by evaluating total antioxidant activity and The DPPH free radical scavenging test by comparing the results of two types of extraction: maceration and soxhlet.

Total polyphenol, flavonoid, and condensed tannin contents

Estimation of total polyphenol content of methanolic extract using the Folin Ciocalteu reagent indicates that the prickly pear cladodes are rich in these compounds (Table 01).

The results obtained show that the total polyphenol contents vary considerably depending on the type of extraction. It was the maceration extraction which led to a rich methanolic extract as compared to the soxhlet where we noted a concentration of 36.7 mg GAE/g DW for the first and 22.73 mg GAE/g DW for the second method.

Analysis of variance according to the type of extraction revealed significant differences at 5% threshold in favor of maceration extraction.

Concerning flavonoid, we noticed a slight significant difference in the contents as function of extraction's type. As with polyphenols, maceration extraction gave the best result with 2.61 mg CE/g DW compared to that of soxhlet where we noted a concentration of 2.07 mg CE/g DW. The analysis of variance revealed the existence of significant differences between the two types of extraction at the 5% threshold.

Concernig condensed tannin content of prickly pear cladodes, results for both types of extraction are summarized in Table 01.

Contrary to the resuts of total polyphenols and flavonoids, the two types of extraction do not influence the contents of condensed tannins contained in cladodes and this result was confirmed by the analysis of variance at the probability threshold 5%. The contents for the two types of extraction are identical with 0.013 mg CE/g DW.

Antioxidant activities of pickly pear cladodes extracts

As for the assays, we also evaluated antioxidant activities through two *in vitro* tests, total antioxidant capacity and DPPH radical scavenging test. The results are summarized in Table 02.

As for total polyphenols and flavonoids, the total antioxidant capacity of prickly pear cladodes is very variable depending on the type of extraction. We noted that extraction by maceration gives the best result with 18.5 mg GAE/g DW whereas extraction by soxhlet gives a lower value (3.25 mg GAE/g DW). The analysis of variance with a single controlled factor confirms the existence of significant differences between the two types of extraction at the threshold $\alpha = 0.05$.

As for total antioxidant activity, the free radical scavenging DPPH capacity was evaluated for both types of extraction (Table 02).

From Table 02, we noticed a variability of the IC_{50} as function of extraction's type. The lower IC_{50} value for the extract is obtained by maceration (77.5 µg/ml) which is the most active one, while for soxhlet extraction the value of IC_{50} is 97.65 µg/ml. The analysis of variance revealed the existence of significant differences at the threshold α=0.05 between the two methods in favor of maceration extraction.

DISCUSSION

The prickly pear cladodes (*Opuntia ficus indica* L.) were collected from Batna region in December 2014. This species is well known for its use in traditional medicine, agro-food and cosmetology. Through our study, we quantified the total polyphenol, flavonoid and condensed tannin contents, as well as the evaluation of total antioxidant capacity and scavenging free radical DPPH. Through these analysis, we compared the results of two extraction methods, the first by maceration and the second by soxhlet. The results revealed that the prickly pear cladodes are rich in these compounds and show good antioxidant activity. The results also showed that all these parameters are influenced by the type of extraction.

According to obtained results, both types of extraction have an interesting amount of total polyphenol content. However, the highest concentration was noticed for the methanol extract from maceration with 36.7 mg GAE/g DW.

The total polyphenol contents contained in prickly pear cladodes are important in comparison with other species, glycophytes and halophytes, known for their medicinal uses. Our results are better than those obtained by[15] who studied the glycophyte *Nigella sativa* and reported 10.04 mg GAE/g DW of total polyphenol contents. Our results are more important than those found by[16] who studied a halophyte *Tamarix gallica* and reported amount of 34.44 mg GAE/g DW. Similarly,[17] reported values of total polyphenols with 37.1 mg GAE/g DW in the halophyte plant *Suaeda fruticosa*.

As regards the influence of the type of extraction on the total polyphenol contents, our results show that the methanol extract resulting from the extraction by maceration gives a much better result than that obtained by soxhlet with respectively the contents of 36.7 and 22.73 mg GAE/g DW. These results corroborate those obtained by[18] who compared the total polyphenol contents in *Crithmum maritimum* from two types of extraction: maceration and soxhlet, from two different growing periods, and reported that for the vegetative period maceration gives a better result in comparison with that obtained by soxhlet with respectively 7.16 and 3.68 mg GAE/g DW. Similarly during the flowering period, the maceration gives a better result with 8.27 mg GAE/g DW compared to that obtained by soxhlet with 4.33 mg GAE/g DW. Many studies have shown that the extraction technique is an important factor influencing total polyphenol content[19,20]. These studies have proved the effectiveness of the soxhlet system[21]. also reported that solvent boiling causes a change in some of properties such as selectivity and extractant power to certain molecules. However, the high temperature used in this system can lead to the deterioration of thermosensitive molecules[22].

As with total polyphenols, flavonoids also follow the same tendency. We noticed 2.61 mg CE/g DW for maceration against 2.07 mg CE/g DW for the soxhlet system. Although this difference is minimal, it remains statistically significant. This result follows the same trend observed by[18] which reported flavonoid levels in *Crithmum maritimum* equal to 3.45 mg CE/g DW for maceration and 1.70 mg CE/g DW for soxhlet. These results can be explained by the work of[23] who mentioned that the structure of certain groups of phenolic compounds can be deteriorated and modified by the thermal effect of the soxhlet system. This is the case of isoflavones which are hydrolysable under the effect of high temperatures, this may explain the fact that the concentration of flavonoids has decreased in the soxhlet system in comparison with the maceration.

Concerning condensed tannins, we did not notice any difference between the two extraction systems. However, the amount of condensed tannins remains very low. This may be due to the choice of the extraction solvent itself which is not the most suitable for the extraction of this type

Table 1: Total polyphenol (mg GAE/ g DW), Flavonoid and Condensed tannin (mg CE/g DW) in *Opuntia ficus indica* L. for methanolic extract obtained by soxhlet and maceration.

	PT	Flav	TC
maceration	36.7 a	2.61 a	0.013 a
soxhlet	22.73 b	2.07 b	0.013 a

Table 2: Total antioxidant capacity (mg GAE/g DW) and DPPH radical scavenging (µg/ml) in *Opuntia ficus indica* L. for methanolic extract obtained by soxhlet and maceration.

	AAT	DPPH
maceration	18.5 a	77.5 b
soxhlet	3.25 b	97.65 c
BHT	-	11.6a

of molecules. The work of[24] showed that 80% acetone gave a good result in the extraction of condensed tannins in *Limoniastrum monopetalum* with a amount of 4.55 mg CE/g DW, while with methanol 80% they recorded a value of 2.4 mg CE/g DW.

In the evaluation of antioxidant activities, two tests were used: the first was total antioxidant capacity and the second was scavenging free radical DPPH.

The total antioxidant capacity of the prickly pear cladodes is expressed by the number of equivalents gallic acid in one gram of dry weight. The phosphomolybdenum process is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and formation of a green phosphate / Mo (V) complex with maximum absorption at 695 nm. The results showed that cladodes have an interesting total antioxidant activity with 18.5 mg GAE/g DW. Our results are significantly better than those mentioned by[25] who worked on *Mesembryanthemum crystallinum* and *Mesembryanthemum nodiflorum* in which total antioxidant activity was estimated at 3.46 and 0.20 mg GAE/g DW respectively. Similarly for other species such as *Diplotaxis harra* and *Diplotaxis simplex* where the authors recorded a total activity of 16.14 and 17.3 mg GAE/g DW respectively. The values obtained for the two types of extraction for this activity are very variable, where we noticed the best value for maceration extraction with 18.5 mg GAE/g DW compared to that obtained by the soxhlet system with 3.25 mg GAE/g DW.

This strong antioxidant activity of the pickly pear cladodes could be attributed to the presence of phytochemicals such as phenolic compounds and flavonoids[26,17]. Indeed, recent studies have shown that many flavonoids and polyphenols contribute significantly to the total antioxidant activity for many fruits and vegetables[27,28] and for medicinal plants[15]. Thus, the difference in total antioxidant activity between the two types of extraction is probably related to the difference in their phenolic content[29], which may be influenced by the extraction method[19,20] The relationship between the phenolic compounds and the total antioxidant activity explains this decrease in the extract resulting from the soxhlet system.

DPPH radical scavenging activity of the pickly pear cladodes is evaluated by the capacity to quenching the synthetic DPPH radical and this activity is compared with that of the standard compound BHT. DPPH is a stable free radical that accepts an electron or a hydrogen radical to become a stable diamagnetic molecule.

The scavenging effect of the methanolic extracts on the DPPH radical is expressed in IC_{50} values. In comparison with previous work, our results are better than those obtained by[30] on *Mesembryanthemum crystallinum* where they mentioned an IC_{50} of 160 µg/ml. Similarly, our extracts are more efficient than those obtained from two glycophytes, *Pisonia alba* and *Centella asiatica*, where the IC_{50} values are respectively 175 and 200 µg/ml[31].

The two extractions exhibit different values for the neutralization DPPH radical, more the extract is rich in phenolic compounds over expressing significant activity against the radical DPPH. In general, the antioxidant power is strongly correlated with the concentration of phenolic compounds[32]. These data are corroborated by the work of[24], which showed a significant and positive correlation between the amount of phenolic compounds and the antiradical activity. Similarly, significant relationships between phenol concentration and antioxidant efficacy have already been reported in *Suaeda maritima*[33] and *Cakile maritima*[34].

CONCLUSION

Our work has revealed, in a general, that *Opuntia ficus indica* L. cladodes are rich enough in phenolic compounds with good antioxidant anti-radical activities. The comparative study of two extraction methods indicated a signivicative variability, but the results remain important especially those obtained by maceration, which confers the plant a good place in the list of plants of interest and to be a good source of bioactive molecules that can be used in various fields: pharmacological, therapeutic, cosmetic and alimentary.

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