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ASSESSMENT OF ULTRASOUND EXTRACTION FOR THE RECOVERY OF PHENOLIC COMPOUNDS FROM *OPUNTIA STRICTA* PEEL: EVALUATION OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES

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ABSTRACT

By-products generated at high quantities by food industries are generally discarded, giving rise to environmental pollution worldwide. However, these could serve as abundant source of valuable bioactive compounds. In the present work, extracts of Moroccan *Opuntia stricta* peel was subjected to evaluation as a potential source of antioxidants. For this purpose, different extraction techniques (conventional maceration, Soxhlet extraction and ultrasonic extraction) were compared in terms of process global yield, total phenolic (TPC) and flavonoids contents (TFC). Moreover, the extract obtained by the most effective extraction method was then tested for its antioxidant and antimicrobial activities. The high total phenolic content (76.29 mg of gallic acid equivalent/100 g), flavanoids concentration (24.97 mg of quercetin equivalent /100 g) and extraction yield (12.26%) obtained from UAE proved its efficiency when compared with the other tested methods. Antioxidant activity of the UAE extract (IC_{50}=150µg/mL) was very high, similar to that of butylated hydroxyanisole (BHA). Antibacterial study showed that *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* were more sensitive to the extract then the tested bacterial strains with a Minimal Bactericidal Concentration (MBC) of 160 µg/mL. Overall; these results showed promising prospects for future exploitation of *Opuntia stricta* peel as a functional food ingredient and/or supplement.

KEYWORDS: *Opuntia stricta, Streptococcus pneumoniae* and *Pseudomonas aeruginosa.*

1. INTRODUCTION

Food processing industries generates substantial quantities of by-products which are discarded as natural waste. This type of waste is becoming a worldwide "hot" topic especially in developing countries and is increasingly problematic as demonstrated by the publication of several reports about this subject. According to a FAO study, around 1.3 billion tons of food wastes per year are produced by human consumption.[1] During the last years, pressure on natural resources has increased making them scarce. Thus, efficient valorization of these natural by-products for further production of high-value products may be of considerable economic benefit to food industries. It is well established that agro-food by-products may be an abundant source of bioactive compounds such as vitamins, minerals, amino acids and antioxidants (i.e. polyphenols).[2,3] Phenolic compounds are strong antioxidants and their positive effects on human health are well documented. They have been associated with protection against the development of diabetes, inflammation, cardiovascular and neurodegenerative diseases. In addition, phenolic compounds can prevent the development of several forms of cancer by inhibiting oxidative reactions induced by reactive oxygen species.[4,5] As natural components, these antioxidants are suggested to be a good substitute of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) which are suspected of being carcinogenic.[6]

Opuntia stricta species belong to the *Cactaceae* family which is distributed across arid and semiarid regions of the world and comprises about 300 species.[7] This purple prickly pear is widely cultivated in the south of Morocco and used as a traditional medicinal plant. Biological activities of *Opuntia stricta* fruit constituents (peel and edible portion) are well reported in the literature. These include their antioxidant activity, anti-diabetic and antimicrobial activity.[8-10] Beside its biological effects, *Opuntia stricta* contains a great amount of potential food additives such as food colorants (i.e. betanin/isobetanin).[11,12] The protective effect of *Opuntia stricta* fruit has generally been associated with the

presence of bioactive compounds notably phenolics, flavonoids, tannins and betalains.[9,13]

Cactus fruits and their by-products (peels and seeds) have attracted attention of numerous researchers due to its nutraceutical and healthy value.[13-18] However, little attention has been given to the phytochemical properties and biological activities of *Opuntia stricta* peel.[19,20] Furthermore, physicochemical and biological proprieties of plant extract can be highly affected by the genotype and the environmental conditions. In addition, these proprieties could be influenced by the choice of extraction method. Thus, the most commonly used extraction procedures are conventional solvent and Soxhlet methods, which have lower efficiency, lower extraction yield, longer extraction times and use larger quantity of solvents. Recently, novel extraction techniques have been developed, including ultrasound which reported to be an environment-friendly technology because of its high efficiency and low energy requirement.

Based on these statements, it is very important to further investigate the chemical composition and the bioactivity of Moroccan *Opuntia stricta* peel extract obtained by ultrasound-assisted extraction. Thus, the aim of this study is to compare the ability of classical and advanced green extraction technology to extract phenolic compounds from *Opuntia stricta* peels grown in Morocco. Three extraction methods; conventional maceration, Soxhlet extraction and ultrasonic extraction were studied. Each technique's performance was assessed by quantifying the mass, total phenols content, and flavonoids concentration in the extracts. Moreover, extracts obtained by the best performing technique, were screened further for their antiradical capacity and antibacterial activity. The ultimate goal of these studies is to promote *Opuntia stricta* peel as a source of natural antioxidant for an application in food industries.

2 . MATERIALS AND METHODS

2.1. Plant material, substrate preparation and chemical analysis

Opuntia stricta fruits were collected from Sidi Ifni region located in south of Morocco kingdom and characterized by a semi-arid climate. The peels were manually separated from the pulps and washed with distilled water to remove dust and organic impurities, then dried in hot air at 40 ◦C for 48 h. The dried peels were grounded in a grinding mill to obtain a fine powder with a size of 0.5 mm. They were stored in the dark at 4 °C until further analysis to prevent microbial spoilage and oxidation. The chemical composition of the raw was assessed in triplicate by analyzing ashes, moisture, °Brix (total soluble sugar) and the mineral content; the results were expressed as dry weight. Moisture was immediately determined by drying 5 g of each sample at 105 ° C until a constant mass.[21] The residue of moisture determination was maintained at 550 °C for 24 h to determine ash.[21] A total soluble solid (Brix degree) was obtained by refractometric measurement at 20 °C in the juice obtained by filtration.[21] Minerals and trace elements (Ca, Cu, Fe, K, P, Pb and Zn) were determined using ICP-AES (Inductively Coupled Plasma/ Atomic Emission Spectrometry).

2.2. Extraction experiments

Fresh and dried *Opuntia stricta* peel powder was extracted using three different extraction methods: Maceration extraction (MAE), Soxhlet extraction (SE) and Ultrasound assisted extraction (UAE). Solid/Liquid ratio (1:10 w/v) was kept constant for the three extraction methods.

2.2.1. Maceration extraction (ME)

The powdered *Opuntia stricta* peel samples were left to macerate for 24 h under stirring in contact with the solvent. The maceration was carried out in an Erlenmeyer flask on a shaker at a constant agitation speed (200 rpm) at room temperature and in the dark for 24h.

2.2.2. Soxhlet extraction (SE)

Classical Soxhlet equipment was used in this study. Briefly, the powder of *Opuntia stricta* peel samples were placed inside a thimble loaded into the Soxhlet extractor. Then, the solvent was added to the round bottom flask. The solvent was refluxed for 3-4 h at a maximum temperature of 45°C until the completion of five extraction cycles. Previously, we have found that the yield of extractive substances did not increase after the fifth cycle (unpublished data).

2.2.3. Ultrasound-assisted extraction (UAE)

For the UAE experiments, extraction was carried out in an ultrasonic device (CY-500 sonicator, JP Selecta S.A. Spain) at 500 W and at a frequency of 20 Hz. The dried powder sample was extracted in a 200 mL beaker at room temperature. Ultrasonic probe was directly inserted into the beaker about 4 cm under the surface of the mixture to provide direct contact with the sample for 60 min.

2.2.4. Solvent–solute separation

After each extraction, the extracts were then centrifuged for 10 min at 5000 rpm to remove any floating matters and the supernatant collected was filtered using a filter paper (Whatman n°1). The filtrates were evaporated under reduced pressure (below 40 °C) to remove the solvent. The extracts were kept in the dark at 4 °C until further analyses.

2.2.5. Extraction yield

The extraction yield is a measure of the extraction method efficiency to extract specific components from the original material. It was defined as the amount of extract recovered in mass compared with the initial amount of dry skins.[22] It is presented in % and was determined for each one of techniques tested.

2.3. Preliminary phytochemicals screening

Phytochemical screening was performed using standard procedures according to procedures described by Kaur and Arora[23] with slight modifications. The crude extracts were screened for flavonoids, saponins, tannins and triterpenoids using appropriate spray reagents and UV absorbance.

2.4. Total phenol content(TPC)

Total phenolic content of the ethanolic extracts was estimated by the Folin–Ciocalteu method described by Singleton et al[24] and Siddhuraju and Becker.[25] In brief, 100 µL of extracts (1 mg/mL in ethanol) or a standard solution of gallic acid was taken in test tubes and the volume was made up to 1 mL with distilled water. To this solution, 0.5 mL of Foline Ciocalteu reagent (1:1 v/v) was added, and the test tubes were thoroughly shaken. After 1 min, 2.5 mL of Na_2CO_3 (20%) were added sequentially in each tube. The mixture was incubated for 40 min in the dark at room temperature (25 °C). The absorbance against an ethanol blank was recorded at 725 nm. Gallic acid was used as a standard and TPC of extracts was expressed as gallic acid equivalents (mg GAE/100g extract).

2.5. Total flavonoids content (TFC)

Total flavonoid content was determined measured according to the aluminium chloride colorimetric method developed by Zhishen et al[26] using quercetin as standard.[27] Ethanol extracts (1 mL) or quercetin standard solution, was mixed with 0.5 mL of sodium nitrite solution (5%) and distilled water (4 mL). Then, 0.3 mL of 10% aluminium chloride and 2 mL of NaOH (1M) were added to the mixture. Both reagent additions were spaced by 6 and 5 min, respectively. Immediately after, the solution was diluted to a final volume of 5 mL with distilled water. After incubation at room temperature for 15 min, the absorbance was measured at 510 nm using spectrophotometer. The flavonoid content was expressed as quercetin equivalent (mg QE/100g extract).

2.6. Evaluation of the antioxidant activity via DPPH radical scavenging assay

The antioxidant activity of *Opuntia stricta* peel was measured in terms of hydrogen donating or radical scavenging ability, using the DPPH method[28] with some modifications.[29] Briefly, 0.1 mM of DPPH radical ethanolic solution was prepared and 2 mL of this solution was mixed with 50µl of the ethanolic extract solutions at various concentrations or the standard (BHA). The reaction mixture was mixed thoroughly and left in the dark at room temperature for 30 min. The decrease in absorbance at 517 nm was determined with a spectrophotometer. BHA was used as a positive control and the ability of sample to scavenge DPPH radical was calculated by the following equation.

$$scavenging\ activity(\%) = [(Acontrol - Asample)/Acontrol] \times 100$$

Where, $A_{control}$ is the control absorbance and A_{sample} is theabsorbance of the extract or standard. The radical scavenging activity (RSA) was defined by inhibition concentration (IC_{50}), which is the concentration of extract necessary to decrease the initial DPPH concentration by 50%. The lower IC_{50} value indicates higher radical scavenging capacity and vice versa.

2.7. Antimicrobial activity

The antibacterial assays were carried out by two methods; disc-diffusion[30] and microdilution method[31] against five pathogenic bacterial strains (*Staphyloccocus aureus* ATCC 29213, *Bacillus subtilis* ATCC 3366, *Streptococcus pneumoniae* ATCC 49619, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922).The cell suspensions were adjusted to approximately 10^8 CFU/mL using a 0.5 McFarland turbidity standard.

2.7.1. Disc-diffusion test

Disk diffusion test was used as a preliminary screening method for the antimicrobial potential of the extract using 100 µL of standardized inoculum suspension previously prepared. The crude extract was dissolved in the dimethyl sulphoxide (DMSO) to achieve the required concentration (160 mg/mL), while 10 µL were spotted onto the sterile filter and placed on the inoculated Muller–Hinton agar. Finally, the inoculated plates were incubated at 37 °C for 24 h and the inhibition zones were observed including the diameter of the disc (6 mm). Cefotaxime, novobiocin, penicillin, chloramphenicol and oxacillin were used as a standard and DMSO as a negative control.

2.7.2. Microdilution assay

The minimal inhibitory concentrations (MICs) were determined according to the method described by Gomez et al.[31] Briefly, the extract was first dissolved in dimethylsulfoxide (DMSO) with Tween 80 (0.01% v/v for easy diffusion), then diluted to the highest concentration in 5 mL sterile glass test tubes and a series of two-fold dilution of the extract was prepared ranging from 2.5 to 320 mg/mL. In each 96-well plates, 145 µL of Mueller-Hinton broth and 5 µL of the correspondent inoculum strain were added. Then, 150 µL from the stock solution and its serial dilutions were transferred into eleven consecutive wells. The last well containing 295 µL of the correspondent Mueller-Hinton broth without extract and 5 µL of the inoculum on each strip was used as the negative control. The final volume in each well was 300 µL. Plates were incubated at 37◦C for 24 h. The lowest concentrations of the sample, which did not show any visual growth of test organisms after macroscopic evaluation, were determined as MICs, which were expressed in µg/mL.

The minimum bactericidal concentrations (MBCs) of the sample were determined by sub culturing the test dilution that showed no visible turbidity onto freshly prepared Mueller-Hinton agar media. The plates were incubated

further for 42 h at 37°C. The MBC was the lowest concentration of the test extract that showed no visible growth in the culture incubating at 37°C for 48 h.

2.8. Statistical analysis

All data represent the average of three tests. For results, the statistical significance was evaluated by one-way analysis of variance (ANOVA) and the p-value < 0.05 was considered to indicate statistical significance.

3. RESULTS AND DISCUSSION

3.1. Extraction yields

Extraction techniques strongly affect the extractive yields and chemical composition of the extract. For this reason, three different extraction techniques; maceration (ME), Soxhlet extraction (SE) and ultrasound-assisted extraction (UAE) were assayed for their capacity to recover bioactive compounds from *Opuntia stricta* peel. The technique that showed the highest high extraction yield was then applied for the rest of the investigation. The extraction yield obtained for each one of the three different techniques is presented in figure 1.

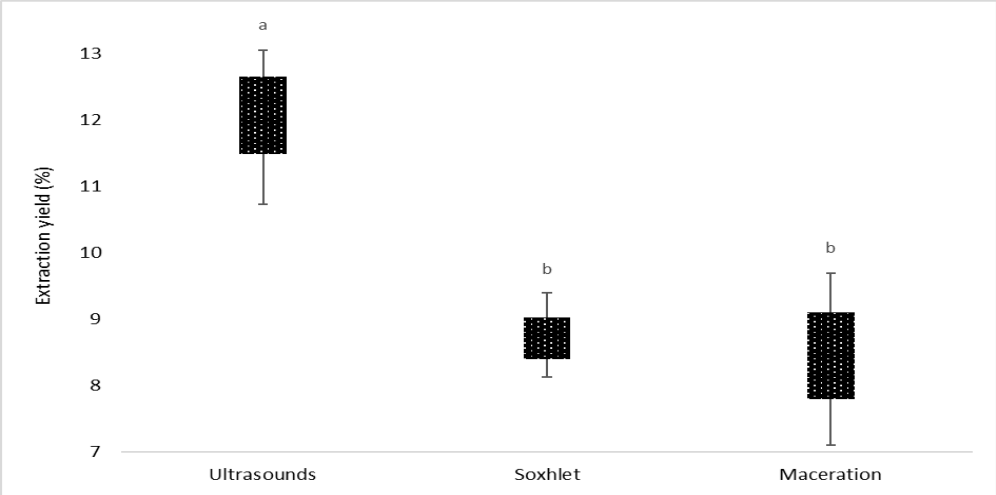

Figure 1: The extractive yield obtained by different extraction techniques from *Opuntia stricta* skin (mean ± SD, n = 3). Means with different letters differ significantly at p <0.05.

The highest yield was achieved by UAE (median value 12.26%) followed by SE (median value 8.66%) and ME (median value 8.5%). Extractive yield obtained by UAE was statistically significant ($p<0.05$) when compared with the two conventional extraction methods. However, no significant differences have been found between the yields obtained by ME (8.43 %) and the SE (8.73%). The highest yield obtained by UAE in extraction yield could be explained by the intensification of mass transfer and solvent penetration into plant material based on cavitational effects[32], when ultrasound waves cross the liquid solvent, a large amount of tiny gas bubbles (cavitation bubbles) implode plant particles, producing very high local temperatures, pressures and velocities of solvent microjets.[33] This generates several effects such as surface peeling, erosion and particle breakdown, which intensify mass transfer and consequently close interaction between the solvent and the plant tissues. Similar results were obtained by Smelcerovic et al[34] who noted that UAE achieved the highest extraction yield of phenolic compounds from *H. perforatum*, followed by Soxhlet and maceration. In addition,[35] reported that ultrasound-assisted extraction carried out for 30 min gave grape (*Vitis vinifera* L.) seed oil yield similar to Soxhlet extraction for 6h. The same findings were reported by Diouf et al[36] who observed that samples of yellow Brich treated with UAE for 30 min had an equal extraction yield compared with maceration for 24 h. Another study showed that corn carotenoids recovery after 20 min of ultrasonic assisted extraction was higher than that obtained with conventional extraction (with mechanical stirring) during 240 min.[37] In line with the present work, Mason et al[38] reported the application of ultrasound was very promising to obtain the highest yield and activities. In conclusion, UAE showed better extraction yield followed by SE and ME. Nevertheless, SE and ME required longer extraction time 24h and 3h respectively than UAE (30 min).

On the other hand, the comparison between the two conventional methods used in this study, show that the Soxhlet method achieved a slightly better extraction yield than maceration ($p>0.05$). This difference could be explained by the different temperatures used in both processes: Soxhlet works at higher temperature (50°C) because it is necessary to boil the solvent which reduces its surface tension and viscosity. Therefore, the solvent can penetrate more easily to the active sites inside the matrixand solubilize the target compound. It had been reported also that the Soxhlet method achieved better extraction yield than maceration in *Opuntia ficus-indica* flowers.[39]

3.2. Chemical characteristics and nutrient profile

The physico-chemical parameters of *Opuntia stricta* peel were measured (Table 1). According to the obtained

results, these peels were characterised by a high humidity (70.5%). Table 1.

Table 1: Chemical composition of *Opuntia stricta* peel.

Parameter	Value
Humidité (%)	70.5
°Brix (g/100g)	2.4
Cendres (g/100g)	2.15
pH	4.22
Ca (mg/l)	6,12
Cu (mg/l)	0,025
Fe (mg/l)	0,153
K (mg/l)	18,26
P (mg/l)	< 0.01
Zn (mg/l)	0,038
Mg (mg/l)	3,884

This value is close to that obtained by Kunyanga at al[9] for *Opuntia stricta* fruits peel growing in Kenya (81.7%). The obtained total ash was 2.15% which is lower than those obtained for *Opuntia stricta* fruit peels grown in Tunisia (3.11%). This important difference might be due to genotype and environmental effects. The mean pH remained constant at 4.22. No data have been reported in literature describing the pH value of *Opuntia stricta* peels. In this study, the average Brix value was 2.4°. The total sugars content obtained in this study were lower than those obtained for cactus pears grown in other countries.[9,20]

The mineral analysis of *Opuntia stricta* peel (Table 1) showed that the most abundant elements were potassium (18.26 mg/L), calcium (6.12 mg/L) and magnesium (3.884 mg/L). These are followed by iron, zinc and phosphorus and iron, respectively. Similar results have been reported for other cactus fruit species. It was reported that *Opuntia ficus-indica* peel contains high levels of potassium (98 mg/100 g), calcium (15.7 mg/100 g) and magnesium (15.2 mg/100 g).[40]

3.3. Phytochemical screening

Phytochemical screening results (Table 2) have shown that all extracts contain the majority of secondary metabolites such as tannins, saponosides, terpenoids and flavonoids. Table 2.

Table 2: Qualitative phytochemical parameters of *Opuntia stricta* peel.

Phytochemicals	Extraction techniques		
	Maceration	Soxhlet	Ultrasounds
Flavonoids	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Triterpenoids	+	+	+

These classes of phytochemicals are known to possess a variety of biological activities including antifungal, antiviral, anti-inflammatory, antitumor, anticancer, enzyme inhibition.[41-45] Thus, the presence of these phytochemicals may be responsible for some of the anti-microbial and antioxidant activities of the extracts, and is in agreement with the customary use of the *Opuntia stricta* peel for the treatment of skin related diseases such as eczema.

3.4. Total phenol and total flavonoid contents

To validate the efficiency of ultrasound to extract antioxidants from *Opuntia stricta* peel, a comparison was made between the UAE method and two conventional extraction techniques (maceration and Soxhlet extraction).The quality of the extracts was estimated in terms of total phenol and total flavonoid contents. The results of the analyses demonstrated that *Opuntia stricta* peel contains high quantity of phenolic compound (Figure 2).

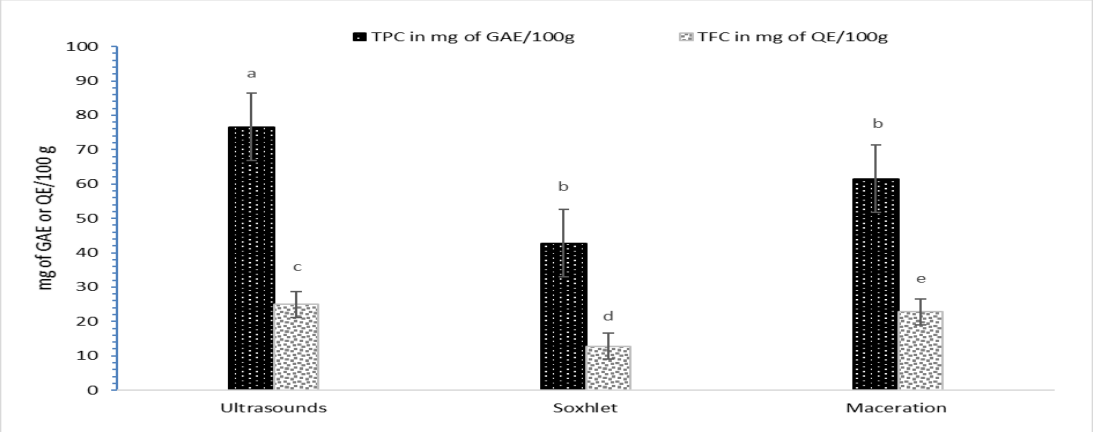

Figure 2: Total polyphenols (black) and total flavonoids (gray) yield for with different extraction techniques (mean ± SD, n = 3). Means with different letters differ significantly at p <0.05.

Among the studied methods ANOVA Test showed that ultrasound extracts had a high level of total phenol and flavonoid contents and showed the highest yield than those obtained using Soxhlet extraction and maceration ($p<0.05$). UAE proved to be the most efficient method to extract antioxidants (76.29 mg GAE/100g and 24.97 mgEQ/100g of TPC and TFC, respectively), followed by ME (61.53 mgGAE/100g and 22.8 mgEQ/100g of TPC and TFC, respectively). This could be attributed to cavitation and thermal effects of the ultrasound technique, which cause disruption of the cell wall and intensification of mass transfer.[46,47] Additionally, an increase in the rate of mass transfer by disruption of the cell wall and hydrophobic forces in the cell membrane can lead to a high permeability.[48] The contrary, extracts obtained by SE displayed a lowest total phenolic content (42.79 mg GAE/g) and flavonoid (12.8 mg EQ/100g). This could be due to antioxidants destruction under the high temperature of Soxhlet extraction. So far, this method is not always acceptable for industrial applications due to long extraction time. This result is in agreement with the reports of Ping Xu et al[49], which proved that ultrasound is the most suitable for extraction of phenolic compounds compared with conventional maceration and Soxhlet extraction methods. In addition, although statistically there is a slight difference in total phenolic and flavonoid contents between the ultrasonicated and macerated extracts, UAE (60 min) could greatly shorten the extraction time compared with Soxhlet extraction (3h) and maceration (24 h). Annegowda et al.[50] showed that 1 h of sonication extracted out a significant amount of phenolic content ($p<0.05$) from *Bauhinia purpurea* in comparison with 48 h of Soxhlet extraction and 72 h of maceration.

Similar results for a Tunisian variety of *Opuntia stricta* peel, reported the presence of TPC (1.35 mg GAE/g of extract) for which the peel was 1.66-fold higher than the pulp.[19] These authors reported a higher TFC value for *Opuntia stricta* peel than the one of this study. This difference in the value might be due to the number of cycle extractions used in each study, the origin of the plant material, type of the cultivar, degree of maturity, the storage conditions, and to the analytic assays. Guzmán-Maldonado et al[51], reported that *Opuntia matudae*, showed higher values of TPC in the peel ranging from 8.63 to 8.40 mg GAE/g DWB and lower values were reported for endocarp (1.68–1.28 mg GAE/g DWB). The highest value of total phenol content and flavonoid in cactus peel compared with pulp was also observed in *Opuntia joconostle* with 2.07 mg GAE/g fresh weight and 0.46 mg catechin equivalents /g FW respectively.[52] Ndhlala et al[53] reported that *Opuntia megacantha* peel contains 600 µg GAE/100g and 39µg Catechin/g of total phenolic content and flavonoid, respectively. This is consistent with the data reported by Tomas-Barberan et al[54], who found that peel tissues usually contain higher amount of phenolics, and flavonoids than flesh did tissues in nectarines, peaches and plums. Phenolic compounds are produced by plant to protect themselves against ultraviolet light, and act as defenses against pathogenic microorganisms in plants. This type of protection is necessary for the fruit and may explain the high phenolic content found in the peel, as compared to the content in the pulp.[52]

3.5. Antioxidant activity

The bioactivity of the extract was evaluated, as a measure of its anti-radical capacity, determining IC_{50} for DPPH scavenging effects. DPPH is scavenged by polyphenols through the donation of hydrogen, forming the reduced DPPH-H*.[55] The antioxidant compounds can react with DPPH, a deep-violet colored stable free radical, converting it into a yellow coloreda,a-diphenyl-b-picrylhydrazine. The color changes can be quantified by measuring the absorbance at 517 nm, which indicates the radical-scavenging ability of the antioxidant. The DPPH free radical-scavenging activity of the crude ultrasound extract was compared with reference antioxidant commonly used in the food industry (BHA), results are shown in Figure 3.

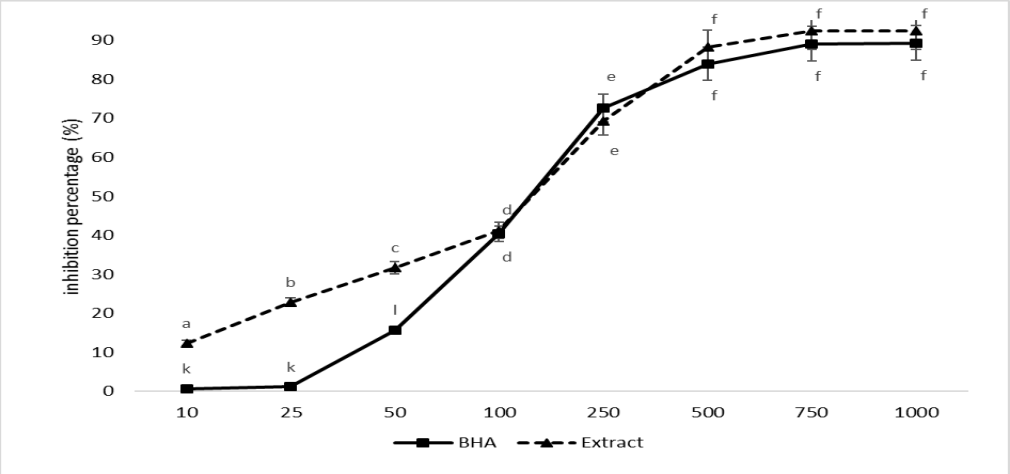

Figure 3: DPPH radical-scavenging effects of *Opuntia stricta* pee extract prepared by ultrasound extraction method at different concentrations (mean ± SD, n = 3). Means with different letters differ significantly at p <0.05.

Our results showed that the antioxidant activity increased with an increase in extract concentration. The highest percent scavenging (92.30%) was obtained at the concentration of 750 µg/mL. When compared to our extract, BHA showed relatively low DPPH radical-scavenging activity (89.03%). alThese results revealed that *Opuntia stricta* peel extract exhibits a higher antioxidant activity than those observed in previous studies. DPPH free radical scavenging activity obtained in this study was higher when compared to those obtained by Koubaa et al[20] using Hydrodistillation extraction during 3 h. In fact, those authors reported that lower scavenging activity required high concentration of extract (50 mg/mL) to inhibit 84% of DPPH free radicals. In addition, a study conducted by Yeddes et al[19] have compared the antioxidant activity of methanol extracts from peel and pulp belonging to two species of Tunisian prickly pears *Opuntia stricta* and *Opuntia ficus indica.* They found that *Opuntia stricta* fruit had higher antioxidant activity than the *Opuntia ficus indica,* for the two spieces the peels with high phenolic content showed higher antioxidant capacity than the pulps.

In order to further quantify the antioxidant activity, the concentration of sample required to scavenge 50% of DPPH (IC_{50}) was determined. The antioxidant activity of the sample is highest at lower IC_{50} value. The results showed that the IC_{50} value of the DPPH radical-scavenging activity were 150µg/mL and 145 µg/mL for the ultrasonic extract and BHA, respectively. The IC_{50} value of this sample was much lower than the *Opuntia stricta* peel of the Tunisian variety (400 µg/mL).

3.6. Antimicrobial activity

The antimicrobial activity of *Opuntia stricta* peel was evaluated against Gram-positive bacteria (*Staphyloccocus aureus, Bacillus subtilis* and *Streptococcus pneumoniae*) and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). These specific microorganisms were selected due to the well-known implication of these pathogens in foodborne diseases.[56] The inhibitory potency of the extract on the tested bacterial is presented in Table 3. The preliminary screening-indicated that the antimicrobial activity differs depending on the strains. *Opuntia stricta* peel extract showed significant activity (>10 mm) against *S. aureus* (12 mm), *S. pneumoniae* (11 mm) and *P. aeruginosa* (10 mm); this inhibition remains lower than that of positive controls. Thus, *E. coli* and *B. subtilis* were resistant to 160 µg/mL of extract with an inhibition diameter equal to 7 mm. In this context, and in agreement with our results, Koubaa et al[20] showed that most prominent activity of the non-polar extract from *Opuntia stricta* peel was found against *S. aureus*. Ammar et al[57] evaluated the antibacterial potential of *Opuntia stricta* flowers extract at concentration of 100 mg/mL. The authors reported better inhibitory effects against *P. aeruginosa* and *E coli*, and a moderate antimicrobial activity against *S. aureus* (11 mm), but no activity was found against *B. subtilis.*

In addition, the inhibitory effect of peel extract against these pathogens may be due to its richness in antioxidant compounds, in particular, phenolic acids and flavonoids.[58] From the phytochemical screening (Table 3), it is observed that the extract contained flavonoids, tannins, terpenoids and saponosides, any one of these classes of compounds may be the active constituents responsible for the observed activity. **Table 3.**

Table 3: Antimicrobial activity of the *Opuntia stricta* skin extract prepared by ultrasound extraction method.

Microorganism	Inhibition zone (mm)	MIC (µg/ml)	MBC (µg/ml)
Gram positive			
S. aureus	12	40	-
S. pneumoniae	11	40	160
B. subtilis	7	>160	-
Gram negative			
E. coli	7	>160	-
P. aeruginosa	10	80	160

In fact, flavonoids can retard the growth of microorganisms by the destruction of the microbial membrane, inhibiting synthesis of nucleic acids and energy metabolism.[59] It has been reported that tannins affect bacterial growth through different mechanisms, such as inhibiting the action of extracellular microbial enzymes, chelating the substrates required for growth and metal ions or by direct action on microbial metabolism by oxidative phosphorylation.[60,61]

To further study the inhibitory effect of *Opuntia stricta* peel extract, the minimal inhibition concentrations (MICs) and the minimal bactericidal concentrations (MBCs) values were determined (Table 3). According to Kuete et al[62] materials can be classified as antimicrobial agents based on the MIC values of its extracts; the antimicrobial activity of a crude extract is considered significant when the MIC is below 100 µg/mL, moderate if 100<MIC<625 µg/mL and low when MIC>625 µg/mL. In our study, the tested extract displayed a significant activity against *S. aureus*; *S. pneumoniae* and *P. aeruginosa.* Consequently, this activity could be attributed to the presence of natural antimicrobial agents such as terpenes. It has been associated with the

bactericidal activity of terpenes by their lipophilic character.[63,64]

Moreover, the strains of *E. coli* and *B. subtilis* were not susceptible to the extract at the tested concentration range. Thus, higher concentration of the extract will be required for the inhibition of this bacterium. Regarding MBCs, it was observed that among all the tested bacteria *S. pneumoniae* and *P. Aeruginosa* were most sensitive to the tested extract. The comparison of MICs and MBCs values allows a better evaluation of antibacterial effect of the extract. According to Marmonier[65], an extract is bacteriostatic when MBC/MIC $\geq$ 4 and bactericidal if MBC/MIC $\leq$ 4. Based on these data, it can be advanced that *Opuntia stricta* peel exert bactericidal effects against *P. aeruginosa* and *S. pneumoniae* with a MBC/MIC = 2 and 4, respectively. Unfortunately, the MBC value of the extract for *S. aureus* strain has not been gained when the concentration of extract reached the maximum in method system tested.

4. CONCLUSION

In this study, phenolic compounds content in *Opuntia stricta* peel growing in Moroccan south region was investigated by the eco-friendly ultrasound assisted extraction. The results showed that the extracting techniques significantly affect the extractive yield and phenolic composition of the extracts. UAE appeared very effective in comparison to maceration and Soxhlet extraction. Thus, ultrasonic extract exhibited good antioxidant activity by effectively scavenging DPPH radical. In addition, these extract displayed strong antibacterial activity against *Streptococcus pneumoniae* and *Pseudomonas aeruginosa.* In summary, the use of *Opuntia stricta* peel as nutraceuticals supplement could provide considerable benefits for health especially as treatment of diseases related to reactive species and bacterial infections. Nevertheless, the characterization of the phenolic composition is needed in order to explore the mechanisms of action involved and predict their reaction on human health.

Conflict of Interest statement

The authors declare that there are no conflicts of interest.

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