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PHYTOCHEMICAL COMPOSITION AND EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF TUNISIAN *OPUNTIA DILLENII* PEEL FRUITS

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ABSTRACT

Considering that the chemical additives are generally toxic, *Opuntia dillenii* is known by their natural bioactive compounds useful as an added ingredient in drug and cosmetic product preservatives. The purpose of this study was to analyze the phytochemical composition, antioxidant and antimicrobial activities of peel fruit extracts (aqueous and ethanolic) from *O.dillenii* growing in southern Tunisia, especially in Djerba Island. The effect of two solvents (water and ethanol) on the phenolic composition of *O.dillenii* peel fruits and their biological activities were studied. The results showed that the ethanolic extract was the most effective solvent for the polyphenolic extraction, as confirmed by HPLC-MS. The quinic acid was the major compound in both extracts. GC-MS analysis revealed the presence of 9 bioactive compounds identified in the ethanolic extract. The little mean values of IC_{50} were recorded by ethanolic extract (IC_{50} DPPH= 0.32 ±0.001 mg/mL and IC_{50} NO=0.1±0.0006 mg/mL) and the highest mean values of FRAP (optical density= 1.84±0.002) and TAC (91.81±0.92 mg EAA/g) were observed also in this extract ($p<0.05$). *S. aureus, M. luteus, and P. Catenulatum* were found to be the most sensitive strains. The Pearson's coefficient revealed a strong correlation between the TPC, TFC and the parameters of antioxidant activity. The finding suggested that *Opuntia dillenii* is an edible plant that could improve the proprieties of drug and cosmetic products as a natural additive.

KEYWORDS: *Opuntia dillenii*, peel extracts, HPLC-ESI-MS, GC-MS, antioxidant activity, antimicrobial activity.

INTRODUCTION

Nowadays, the one of the current trends of drug industry is the production of healthy product thanks to the presence of natural compounds. Product spoilage caused by oxidation and microbial contamination is a major problem of consumers.[1] The natural compounds are bioactive antioxidants and preservatives, which are found in different plant sources (vegetables, fruits, medicinal plants, herbs, spices…). Herbal medicines are highly required by these industries thanks to their effectiveness, safety and reduced costs when compared to chemical preservatives that are associated with side-effects and toxicity.

Plant extracts, purified secondary metabolites (phenolic compounds, flavonoids), essential oils, vitamin, fibers and fatty acids are a potential source of natural products,[2] firstly, for the prevention of product spoilage caused by oxidation,[3] and secondly, for the prevention of many diseases that the oxidative stress is the principal cause. Oxidative stress is defined by the imbalance between antioxidant and oxidant molecules. This imbalance is one of the major causes of several disorders and diseases. Protection the body against these diseases needs to ingest antioxidant natural molecules that can reduce or inhibit the level of free radical and to avoid the oxidation process.

Opuntia dillenii (Ker Gawl) Haw is a succulent plant belonging to the Cactaceae family. The fruits or prickly pear of *O. dillenii* are rich in phenolic compounds, flavonoids (gallic acid, catechin, epicatechin, quercetin and derivatives of kaempferol, isorhamnetin and quercetin), betalains (betanin, isobetanin), polysaccharides and a high content of ascorbic acid in peel and pulp.[4,5,6] The color of the fruit ranged from purple to yellow orange that are the color of two combined betalains which are betanin and indicaxantin.[7] This plant was utilized as animal fodder, natural colorants, and a vegetable.[8] The cladodes of *O.dillenii* are a source of antioxidant compounds, vitamins, dietary fiber and minerals.[9] The fruits are oval, rich of seeds and have an acidic taste that is rarely consumed fresh and used as a food colorant agent.[10]

Numerous studies have indicated that all parts of *Opuntia dillenii* [fruits (peels, pulps, seeds), cladodes, flowers) have shown various biological activities such as antiproliferative and antigenotoxic (from peels, pulps and seeds),[6] anti-inflammatory (from cladodes and flowers),[5,8] hypotensive (from cladodes),[11] anti-asthmatic (from peels, pulps and seeds),[4] radical scavenging (from stems and fruits),[12,13] antiviral and cytotoxic activity (from flowers).[14] Furthermore, fruit extracts showed a protective effect in the term of LDL peroxidation inhibition due to the highest level of betalains.[4] Moreover, in folk medicine, *Opuntia dillenii* fruits were used to treat ulcers, gastrointestinal disorders, inflamed wound, diabetes.[15] Other activities such as anti-fertility,[16] antimicrobial,[17] anti-Alzheimer's and anti-Parkinson's disease by the protection of neuronal cells,[11] were identified in *Opuntia dillenii* fruit extracts.

To the best of our knowledge, there are few systemic researches on the evaluation of the effect of organic and aqueous solvents on antioxidant and antimicrobial activities of *O. dillenii* peel extracts. In the present study, total phenolic, total flavonoid, tannin content, the antioxidant and antimicrobial activities of absolute ethanol and aqueous extracts from *Opuntia dillenii* peel fruits were investigated. HPLC-ESI-MS and GC-MS were applied to identify the bioactive compounds related to these extracts.

MATERIALS AND METHODS

Solvents and Chemicals

All reagents and standard chemicals for TPC, TFC, tannins, HPLC-MS, GC-MS and antioxidant activity were purchased from Sigma Aldrich ®. The reagents for antimicrobial activity were purchased from Bio-Rad (Bio-Rad France).

Plant collection and cactus extract preparation

Matured fruits of *Opuntia dillenii* were obtained from Djerba Island, Tunisia in February 2015. The plant was identified by Pr. Rachid CHEMLI, Professor at the University of Pharmacy, Monastir, Tunisia. A voucher specimen was deposited at Higher Institute of Applied Biology of Medenine (Medenine, Tunisia) under the number Od02. The fresh fruits were peeled manually and the peels were washed with distilled water and were cut into small pieces then were dried during two days at 50 °C. After drying, the pieces were ground into powder using a Nima electric grinder apparatus, nima (Japan). Powder plant materials (200 g) were extracted successively with 800 mL of two solvents with increasing polarity (water and ethanol) for 24 h at room temperature (20-27 °C). The macerates were filtered and the filtered solution was evaporated at 45 °C under vacuum in a rotatory evaporator (Cole-Parmer Rotary Evaporator System, US) for ethanol peel extract (EPE). However, the aqueous peel extract (APE) was freeze-dried using a freeze-dryer (Bioblock Scientific Christ ALPHA 1–2, IllKrich-Cedex, France). The dry residues were collected and kept at +4 °C until further analysis.

Phytochemical composition

Total polyphenolic content (TPC)

The TPC was determined by the modified method described by Cicco et al.[18] using Folin-Ciocalteu reagent. In a test tube, 125 µL of cactus extract, 125 µL of Folin-Ciocalteu reagent and 500 µL distilled water were mixed then agitated with a vortex. After 3 min, 1250 µL of 7% sodium carbonate were added and adjusted with 3 mL of distilled water. This mixture was incubated in the dark at room temperature for 3 h. The absorbance was then measured at 760 nm. The results were expressed as mg of gallic acid equivalent/g of dry extract (mg GAE/g) using the calibration curve prepared for gallic acid (y=0.021x0.0132; R=0.9985).

Total flavonoid content (TFC)

The TFC was estimated by the aluminum chloride method.[19] Briefly, 250 µL of cactus extracts were mixed with 75 µL of sodium nitrite (5%). After 6 min of incubation at room temperature, 150 µL of aluminum chloride were added. An aliquot of 500 µL of sodium hydroxide (1M) were added to the mixture after 5 min of incubation. The volume of the solution was adjusted by distilled water until 2500 µL. TFC was quantified spectrophotometrically at 430 nm and the results were expressed as mg of catechin equivalent/g of dry extract (mg CATE/g) using the calibration curve prepared for catechin (y=0.0097x+0.0096; R=0.9927).

Tannin content

The tannin content was estimated according to the method described by Price et al.[20] with modifications. Briefly, 300 µL of extract were mixed with 3 mL of vanillin (4% in methanol) and 1.5 mL of hydrochloric acid. After 15 min of incubation, the absorbance was measured at 500 nm. The results were expressed as mg CATE/g using the calibration curve prepared for catechin (y=0.002x-0.0086; R= 0.998).

HPLC-MS analysis

Phenolic acids and flavonoids were extracted according to the modified method described by Ayaz et al. [21]. Briefly, 0.5 g of the powder was extracted with 10 mL of ultra pure water and ethanol for ACE and ECE, respectively. The samples were then shaken for 24 h at room temperature. Before being analyzed by the HPLC-MS system, the samples were centrifuged for 25 min at 4000 rpm and then filtered by a millipore filter (0.45 µm). Finally, 5 µL of the samples were injected.

HPLC analysis of compounds was carried out in a Shimadzu LC-20ADXR pump with a SIL-20AXR autosampler (40 °C). The separations were done at 75 °C in a Disco Very BIO Wide Pore C18 (250 mm x 4 mm, 5 µm) column. They were implemented with a flow rate of 0.4 mL/min and the mobile phase was combined with acified methanol/ H_2O (5%:95%, 0.15% acetic acid) (eluent A) and acetonitrile/ water (50%:50%, 0.15% acetic acid) (eluent B). The gradient program was used as follows: 0-14 min from 10 to 20% eluent B; 14-27

min with 20% eluent B; 27-37 min from 20 to 55% eluent B; 37- 45 min with 55% eluent B; 45- 52 min from 55 to 100% eluent B.

MS analysis was conducted on Shimadzu UFLC XR–2020 single-quadrupole mass spectrometer equipped with an electrospray interface (ESI: electrospray ionization**).** ESI conditions are: an electrospray source (source block temperature, 450 °C; desolvation temperature, 280 °C; capillary voltage, 2.7 kV; cone voltage, 35 V), a nebulizing gas flows 1.5 L/min, and a drying gas flow 15 L/ min. The identity of these compounds was confirmed by the comparison of standard molecules.

GC-MS analysis

The identification of volatile compounds was performed using the method cited in the work of Kohoude et al.[22] The analysis was done on a GC 7890A coupled to MS 5975C (Agilent technology, USA), fitted with a fused silica capillary HP-5MS column (5% phenylmethylpolysyloxane, 30 x 0.25 mm, film thickness 0.25 µm). Chromatographic conditions were 1 min at 50 °C, 50 °C to 250 °C at 15 °C/min. A second gradient was applied for 19 min at 250 °C, 250 °C to 300 °C at 50 °C /min and finally 300 °C for 7.5 min. The entire chromatographic program lasted 40 min. For analyzes, each sample was dissolved in its solvent and 1 µL was injected in the split mode ratio of 1:10. Helium was used as a carrier gas with a linear velocity of 1 mL/min. An emission current of 10 µA and an electron multiplier voltage between 1400 and 1500 V. 250 °C and 270 °C were the trap temperature and that of the transfer line respectively. Data were acquired over the range of 40 to 650 amu. The compounds were identified by comparing their mass spectra with those recorded in the Nist08 library database (National Institute of Standards and Technology, USA).

Antioxidant activity

Total antioxidant capacity (TAC) by phosphomolybdenum method

The TAC of the extracts was measured as described by Prieto et al.[23] with a slight modification. Briefly, 300 µL of each extract (1 mg/mL) were combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95 °C for 90 min. The absorbance was measured at 695 nm against a reagent blank. The TAC was expressed as mg equivalent of ascorbic acid/g dry extract using the calibration curve prepared for ascorbic acid (y=5.553x-0.065; R=0.999).

DPPH free radical-scavenging essay

The DPPH free radical-scavenging was determined according to Sánchez-Moreno et al.[24] An aliquot of 1 mL of extract at different concentrations was mixed with 1.5 mL of DPPH solution (2.5 mg in 100 mL methanol). The reaction was incubated 30 min in dark at room temperature and the absorbance was measured at 517 nm. The Blank was prepared for each concentration without DPPH solution. Standard of Ascorbic acid was used as positive control at concentrations that were the same as those in the samples. The control tube contained only DPPH solution. The percentage inhibition of DPPH radical scavenging was calculated:

DPPH radical scavenging activity (%) = $[(A_c + A_b - A_s) / A_c] * 100$

Where Ac, A_b and A_s are the absorbance of control, blank and sample, respectively. A higher DPPH radical scavenging activity corresponded to a lower absorbance of the reaction mixture. The experiment was performed in triplicate.

Reducing power (FRAP) essay

The ability of cactus extract to reduce the iron (III) was determined according to Yildirim et al.[25] An aliquot of 1 mL of extract was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide. The mixture was incubated for 30 min at 50 °C. After incubation, 2.5 mL of 10% (w/v) TCA were added and then the reaction mixture was centrifuged for 10 min at 5000 rpm. After centrifugation, 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) ferric chloride were added to 2.5 mL of supernatant. Standard of Ascorbic acid was used as positive control at the same conditions and at concentration that were the same as those in the samples. Finally the absorbance was measured at 700 nm.

ABTS radical scavenging activity

The antioxidant capacity of samples against ABTS radical was realized using the Trolox equivalent antioxidant capacity (TEAC) assays as described by Chang et al.[4] with minor modifications. The radical $ABTS^{\cdot+}$ was generated by mixing 5 mL of ABTS stock solution (7 mM; 36 mg in 10 mL distilled water) with 88 µL of 2.456 mM potassium persulfate and the mixture was kept in the dark at room temperature for 12-16 h. To obtain an absorbance of 0.70±0.02 at 734 nm, the ABTS radical cation solution was diluted in ethanol. The antioxidant activity of extracts was evaluated by adding 200 µL of extracts (1 mg/mL) in 2 mL of ABTS radical solution. The absorbance was measured after 6 min and the TEAC value was expressed as the mM concentration of Trolox solution using the calibration curve prepared for Trolox (y=-0.664x+0.65; R=0.999). A lower absorbance in this test indicated a higher TEAC value of the extracts and a stronger antioxidant activity.

Nitric oxide (NO) scavenging activity

NO scavenging activity was determined according to the method described by Jagetia and Baliga.[26] This activity was evaluated indirectly by generating nitrite ions from sodium nitroprusside in aqueous solution, under aerobic conditions and can be estimated by the Griess reagent. In fact, NO scavenging activity of extracts was performed by adding sodium nitroprusside (10 mM in phosphate buffer (0.5 M), pH=7.4) to 250 µL of samples with

different concentrations and then incubated at 25 °C for 150 min. After that, 150 µL of Griess reagent (1% sulfanilamide, 2% phosphoric acid, and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added to 150 µL of extracts and incubated for 30 min. Standard of vitamin C was used as positive control at concentration that were the same as those in the samples The absorbance was measured at 546 nm and the percentage of NO scavenging activity was calculated as follows:

NO scavenging activity (%) = $[(A_c - A_s) / A_c] * 100$
Where A_c and A_s are the absorbance of the control and the samples, respectively.

Antimicrobial activity

Microbial strains and growth conditions

Bacteria and fungi were obtained from international culture collections (ATCC) and the local culture collection of the Centre of Biotechnology of Sfax, Tunisia. The antibacterial activity of *Opuntia dillenii* cladodes were tested against 7 strains of bacteria. These include Gram-positive bacteria: *Bacillus cereus* JN 934390, *Bacillus subtilis* JN 934392, *Staphylococcus aureus* ATCC 6538, *Micrococcus luteus*, and Gram-negative bacteria: *Salmonella enteric* serotype *Enteritidis* ATCC 43972, *Escherichia coli* ATCC 25922 and *Klebsiella pneumonia*. A bacterial suspension of each bacterium was prepared in 3 mL of Mueller Hinton broth (agitation 200 rpm, for 18-24 h at 37 °C) by inoculation of a colony initially cultured on Petri dishes containing Mueller Hinton agar (MHA) (37 °C, 24 h; except for *Bacillus* species at 30 °C).[27] For the test, final inoculum concentrations of 10^7 UFC/mL bacteria were used.[28] by adjustment of optical density ranged to 0.08-0.10.

Three fungal strains were used: *Fusaruim oxysporum* AB586994, *Fusarium sp* JX391934 and *Pythium Catenulatum* JX391934. Firstly, the fungal strains were cultured on Sabouraud agar at 30 °C for 3 days until the appearance of mycelium and secondly were inoculated in 10 mL of sterile water containing 0.1% Tween 80 to obtain spore suspension. For the test, final inoculum concentrations of 10^6 spores/ mL were used by adjustment of optical density ranged to 0.08-0.10 [29]

Antimicrobial test assay by agar diffusion method

Antibacterial and antifungal activities were determined according to the modified method described by Ben Hsouna et al.[27] Briefly, the quarter of the sterile Petri dish is filled with the culture medium (45 °C) specific to each strain (MHA for bacteria and Sabouraud agar for fungi). After solidification, 100µL for each suspension (10^7 UFC/mL bacteria and 10^6 spores/ mL) were spread on the surface of a specific culture medium. After 1 min (once the plate was aseptically dried), wells of 6 mm were created in each of the seeded agar plates, using the diameter of a sterile pipette Pasteur. Sixty (60) µL of each extract (150 mg/mL DMSO) were placed into the wells. Chloramphenicol (15 µg/wells) and cycloheximide (20 µg/wells) were used as a positive control for the antibacterial test and antifungal activity, respectively. DMSO (10%) was used as a negative control.[29] The Petri dishes were left at +4 °C for 2 h to facilitate the diffusion of the extracts in agar and then incubated at 37 °C for 24 h for bacterial strain and at 30°C for 3 days for fungal strain. The antimicrobial activity was determined by measuring the diameter of inhibition zone around the wells with calipers. The test was carried out in triplicate.

Determination of MIC, MBC and MFC

The MIC is evaluated for the microbial strains, which were sensitive and presented a clear inhibition zone against the cactus extract and positive control in the test assay by agar diffusion method. This concentration was determined according to the modified methods described by Abdallah et al.[30] The determination of MIC was performed in a sterile 96-well microplate based on micro-dilution well assay, with a final volume of 200 µL per well. Briefly, a stock solution of each extract (150 mg/mL) was prepared in DMSO (10%) and then two fold serial dilutions were carried out to obtain a final concentration ranged between 1.17 and 150 mg. In the well test, 100 µL of each concentration supplemented with 90 µL of MH broth (for bacteria) or Sabouraud broth (for fungi) and 10 µL of cell suspension of 10^7 UFC/mL bacteria and 10^6 spores /mL. The positive control well contained only a cell suspension with adequate culture medium. The well contained DMSO without extract was used as a negative control. The 96-well plates were homogenized, covered with sterile cover plates, and incubated at 37 °C for 24 h for bacterial strains and at 30 °C for 3 days for fungal strains. The MIC was determined by adding 25 µL of MTT (0.5 mg/mL sterile distilled water) as an indicator of microorganism growth and the plates were then incubated at 37 °C for 30 min. The MIC was defined as the lowest concentration (mg/mL) of the extract at which the microorganism does not demonstrate visible growth after incubation (Ben Hsouna et al. (2011).

MBC is determined by the lowest extract concentration (mg/mL) which shows no visible growth of bacteria after incubating at 37 °C for 48 h.[31] The culture in the well that showed no visible fungal growth in the minimum inhibitory concentration was recultured in potatoes dextrose agar (PDA) plates and incubated at 30 °C for 48-72 h. MFC is determined by the lowest concentration of extract at which no growth occurred on the plates.

Statistical analysis

The experimental results were performed in triplicate. Results were expressed as means ± SEM (Standard Error Mean) and statistically analyzed using IBM SPSS statistics version 22 (USA). The correlation coefficient of Pearson and P-value were determined by the correlation test. A one-way analysis of variance (ANOVA) was then performed and followed by the Tukey's test to estimate the significance among the main effects at the 5% probability level.

RESULTS

Polyphenols, flavonoids and tannin contents

Table 1 shows the extraction yields, amount of polyphenols, flavonoids and tannins. The yield extraction varied considerably, from 8.33±0.24% in EPE to the highest yield in APE (15.07±0.11%). As shown in this table, the TPC and TFC depended on the solvent used during extraction. In fact, significant differences were observed between the two extracts and the highest content of TPC and TFC were obtained in EPE (18.40±0.22 mg EAG/g and 7.13±0.34 mg CATE/g, respectively), followed by APE (15.00±0.09 mg GAE /g and 4.6±0.01 mg CATE/g, respectively) ($p<0.05$). Tannin contents were similar in both extracts (1.99±0.08 mg CATE/g for EPE and 1.94±0.08 mg CATE/g for APE; $p> 0.05$).

HPLC-MS

Quantitative analysis of phenolic compounds from *O. dillenii* peel extracts showed that the EPE contained higher amounts of identified phenolics than APE (Table 2). The findings revealed 17 compounds identified (phenolic acids and flavonoids) in EPE and 7 compounds in APE. Among the all phenolic acids, the major identified phenolic acids were quinic acid and cinnamic acid. This later was, however, absent in APE and the amount was of 40.19 µg/g in EPE. Quinic acid is identified in both extracts but the amount is more noticeable in EPE (1437.03 µg/g) than in the APE (118.95 µg/g). In addition, caffeic acid, p-coumaric acid and trans-ferulic acid were present in trace in the two extracts but the amount of trans-ferulic acid is more noticeable in EPE (2.4 µg/g).

The analysis revealed also that detected flavonoids and their concentrations depend on the solvent used. Indeed, 11 flavonoids (flavones, flavonols, flavanones and flavanols) detected in EPE verses 3 detected in APE. Rutin, naringin and luteolin were present in both extracts but the amounts are more noticeable in EPE (11.42; 0.34 and 6.16 µg/g, respectively) than in APE (0.046; 0.007 and 0.014 µg/g, respectively). Rutin (11.42 µg/g) and quercetin (9.27 µg/g) were the dominant flavonols in the present study. The other flavonoids were present only in trace amounts.

GC-MS analysis of ethanolic extract

The major volatile compounds and area % of EPE are shown in Table 3. Total of 9 compounds (alkaloid, fatty acid, sterols…) were identified. The extract had a higher content of 2-furancarboxaldehyde-5-(hydroxymethyl)-, β-sitosterol, and n-hexadecanoic acid, which were estimated at about 32.91%; 9.57%; 3.52%, respectively. Furthermore, this extract showed the presence of a low quantity of α-D-Glucopyranoside, α-D-glucopyranosyl (2.36%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (2.21%), Phenol, 2-methyl-5-(1-methylethyl)- (1.36%), Ethyl iso-allocholate (1.13%), Vitamin E (1.11%), and Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-(0.20%).

Antioxidant effect

The results of free radicals DPPH• scavenging of ascorbic acid, APE, and EPE of *O. dillenii* peel fruits are given in Fig. 1A and Table 4. As shown this figure, it was found that the scavenging effect increased with the concentration and a maximum scavenging activity was recorded at 1 mg/mL for both extracts (80% of inhibition for APE and 95% for EPE) and at 0.4 mg/mL (100% of inhibition) for ascorbic acid. The results presented in Table 4 are expressed in IC_{50} values. A lower IC_{50} value corresponded to a higher scavenging activity. As shown in this table, there is a difference between the DPPH scavenging activity of the both extracts and the two positive controls, ascorbic acid and trolox ($p < 0.05$). The IC_{50} of APE, EPE compared with ascorbic acid and trolox were in the order of ascorbic acid (0.15 mg/mL) < EPE (0.32 mg/mL) < Trolox (0.33 mg/mL) < APE (0.37 mg/mL).

The reducing power of ascorbic acid, EPE and APE at different concentrations are shown in Fig. 1B and Table 4. Based in this figure, the values are expressed as absorbance. A high absorbance indicates a high reducing power capacity. The ferric reducing power of samples increased with their increasing concentrations. As presented in Table 4, the maximum absorbances were about 1.84, and 1.59 for the concentration of 1 mg/mL for EPE and APE, respectively, and 2.41 for the concentration of 0.4 mg/mL for ascorbic acid. ($p < 0.05$).

The antioxidant activity of *O. dillenii* extracts was determined also as % of inhibition of the NO scavenging activity (Fig. 1C). This activity exhibited a concentration-dependent rise up to a concentration of 1 mg/mL (100% of inhibition) for vitamin C, at the concentration of 0.5 mg/mL (55% of inhibition) for APE, and 0.5 mg/mL (53% of inhibition) for EPE. The CI_{50} values present in Table 4 were about 0.18±0.007, 0.1±0.006, and 0.04±0.001 mg/mL for APE, EPE and vitamin C, respectively ($p <0.05$).

Regarding scavenging effects of the extracts on ABTS radical cation and the results of total antioxidant activity (TAC) of *O. dillenii* peel extracts (Table 4), the TEAC values were in the following order compared to betanin determined by Chang et al.[4] betanin > APE and EPE ($p<0.05$). APE and EPE had the similar TEAC values ($p> 0.05$). The ethanolic extract had the highest total antioxidant capacity (TAC) (91.81±0.92 mg AAE/g for EPE verses 64.18±0.67 mg AAE/g for APE) ($p<0.05$).

According to the Pearson's correlation between TPC, TFC and antioxidant activity (Table 5), the highest correlation was found for DPPH, FRAP, NO and TAC ($p<0.01$), while for tannin content, the highest correlation was found for ABTS.

Antimicrobial activity

The results of antimicrobial activity of O. dillenii peel extracts are reported in Table 6. The antibacterial activity

of the tested extracts was evaluated on a panel of 7 bacterial strains, four Gram-positive (B. cereus, B. subtilis, S. aureus, M. luteus) and three Gram-negative (S. enteritidis, K. pneumonia and E. coli) strains. The inhibition zones were in the range of 08-20 mm. Compared to chloramphenicol, the standard antibiotic, EPE exhibited the highest inhibition zones against Staphylococcus aureus (20.33 mm) ($p<0.05$) and this inhibition is close to that of the standard against Micrococcus luteus (20.5 mm) ($p>0.05$).The MIC and MBC results varied between 2.34 - 9.37 mg/mL and 37.5-75 mg/ mL, respectively. However, in the case of Escherichia coli, the lowest MIC (2.34 mg/mL) and MBC (37.2 mg/mL) values were measured for EPE.

The antifungal activity of the tested extracts was evaluated on 3 strains (*Fusarium oxysporum, Fusarium sp* and *Pythium Catenulatum)* (Table 6).The diameters of inhibition zones varied between 9 - 23.5 mm, and the lowest MIC and MFC values were observed in the case of *P. Catenulatum* (4.68 and 37.2 mg/mL, respectively) for EPE. Compared to the inhibition zone values against *P. Catenulatum*, EPE showed significantly higher potential than cycloheximide (standard antibiotic) ($p<0.05$).

Table 1: Extraction yields, TPC, TFC and tannin contents in *Opuntia dillenii* peel extracts.

	Yields (%)	TPC (mg GAE/g)*	TFC (mg CAT/g)**	Tannin contents (mg CAT/g) **
APE	15.07 ± 0.11^{a}	15.00 ± 0.22^{b}	4.6 ± 0.01^{b}	1.94 ± 0.08^{a}
EPE	8.33 ± 0.24^{b}	18.40 ± 0.22^{a}	7.13 ± 0.01^{a}	1.99 ± 0.08^{a}

APE: Aqueous Peel Extract; EPE: Ethanolic Peel Extract*;* TPC: Total phenolic content; TFC: Total flavonoid content.
The data are expressed as mean ± S.D. (n=3); Data in the same column with the different letters are significantly different at $p < 0.05$.

* (mg GAE/g): mg of gallic acid equivalent/g of dry extract.
** (mg CATE/g): mg of catechin equivalent/g of dry extract.

Table 2: HPLC-MS parameters and concentrations of phenolic compounds in APE and EPE.

No.	Compounds	m/z	CC (µg/g) APE	Rt (min)	CC (µg/g) EPE	Rt (min)
	Phenolic acids					
1	Quinic acid	191	118.95	2.45	1437.03	2.17
2	Protocatechuic acid	153	nd	-	1	6.87
3	Caffeic acid	179	0.041	18.36	0.5	14.31
4	P-coumaric acid	163	0.046	24.69	1.27	20.83
5	Trans ferulic acid	193	0.042	27.13	2.4	22.84
6	Cinnamic acid	147	nd	-	40.19	31.46
	Flavonoids					
7	Catechin	289	nd	-	0.36	10.91
8	Rutin	609	0.046	30.63	11.42	23.57
9	Hyperoside	463	nd	-	0.92	24.15
10	Naringin	579	0.007	31.18	0.34	25.29
11	Quercetrin	447	nd	-	0.36	26.21
12	Quercetin	301	nd	-	9.27	31.46
13	Naringenin	271	nd	-	0.29	33.45
14	Apeginin	269	nd	-	0.095	34.18
15	Luteolin	285	0.014	39.44	6.16	34.45
16	Cirsiliol	329	nd	-	7.18	35.08
17	Acacetin	283	nd	-	0.19	39.5

CC: Concentration; Rt: Retention time; APE: Aqueous peel extract; EPE: Ethanolic peel extract*;* nd: not detected.

Table 3: Compounds identified in the ethanolic peel extract of *Opuntia dillenii* by GC-MS.

No.	Tr (min)	Compound name	Mw (g/mol)	Area %	Formula
1	6.11	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144	2.21	$C_6H_8O_4$
2	6.75	2-Furancarboxaldehyde-5-(hydroxymethyl)-	126	32.91	$C_6H_6O_3$
3	7.37	Phenol, 2-methyl-5-(1-methylethyl)-	150	1.36	$C_{10}H_{14}O$
4	7.42	α-D-Glucopyranoside, α-D-glucopyranosyl	342	2.36	$C_{12}H_{22}O_{11}$
5	10.69	Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-	206	0.20	$C_9H_9F_3O_2$
6	11.08	n-Hexadecanoic acid	256	3.52	$C_{16}H_{32}O_2$

7	31.75	Ethyl iso-allocholate	436	1.13	$C_{26}H_{44}O_5$
8	32.20	Vitamin E	430	1.11	$C_{29}H_{50}O_2$
9	34.65	β-Sitosterol	414	9.57	$C_{29}H_{50}O$

Tr: retention time, Mw: Molecular weight

Table 4: Antioxidant profile of *Opuntia dillenii* peel extracts and standard compounds.

	IC_{50} (mg/mL)		FRAP* (700 nm)	TEAC* (mM Trolox/g)	TAC * (mg AAE/g)
	DPPH	NO			
APE	0.37 ± 0.00^{d}	0.18 ± 0.007^{c}	1.59 ± 0.004^{d}	0.68 ± 0.01^{b}	64.18 ± 0.67^{b}
EPE	0.32 ± 0.001^{b}	0.1 ± 0.006^{b}	1.84 ± 0.002^{c}	0.76 ± 0.06^{b}	91.81 ± 0.92^{a}
Ascorbic acid	0.015 ± 0.00^{a}	0.04 ± 0.001^{a}	2.41 ± 0.00^{a}	nd	nd
Trolox	$0.33\pm0.01^{\Omega c}$	nd	$1.86\pm0.00^{\neq b}$	nd	nd
Betanin	nd	nd	nd	$0.87 \pm 0.09^{\pi a}$	nd

^{a,b,c,} Different letters in the same column indicate significant differences (p<0.05). nd: not determined; :* The concentration of *Opuntia dillenii* extracts used in antioxidant activities (FRAP, TEAC and TAC) assays was 1mg/mL;

DPPH: 2, 2-diphenyl-1-picrylhydrazyl; FRAP (Ferric reducing antioxidant power); Trolox: 6-hydroxy-2,5,7,8 - tetra methyl-chroman-2-carboxylic acid; TEAC (Trolox equivalent antioxidant capacity); TAC (Total antioxidant capacity); NO: Nitric oxide; AAE: Ascorbic acid equivalent.

$^{\neq}$ From Köksal et al.[46]

$^{\pi}$ From Chang et al.[4]

$^{\Omega}$ From Yeddes et al.[44]

Table 5: Pearson's correlation between total phenolics and antioxidant activity of *O. dillenii* extracts.

	TPC	TFC	Tannin	DPPH	FRAP	NO	ABTS	TAC
TPC	1	0.99**	0.29	-0.99**	0.99**	-0.97**	0.71	0.99**
TFC		1	0.26	-0.99**	0.98**	-0.96	0.65	0.98**
Tannin			1	-0.32	0.34	-0.45	0.62	0.37
DPPH				1	-0.99**	0.98**	-0.72	-0.99**
FRAP					1	-0.99**	0.74	0.99**
NO						1	-0.78	-0.99**
ABTS							1	0.74
TAC								1

** indicate the significance levels at 0.01

Table 6: Antimicrobial activities, MIC, MBC, and MFC of cactus peel extracts against bacterial and fungal strains.

	Inhibition zone diameters (mm)*		Standards D (mm)	Concentration (mg/mL)	
Strains / Extracts	**Ethanol**	**Aqueous**		**Ethanol**	**Aqueous**
Gram +			Chloramphenicol ^{x}	**MIC / MBC**	**MIC / MBC**
Bacillus cereus	ni	8.0 ± 0.0^{b}	25.8 ± 0.7^{a}	- / -	- / -
Bacillus subtilis	$8,83 \pm 0.76^{b}$	8.46 ± 0.83^{b}	24.1 ± 0.2^{a}	- / -	- / -
Staphylococcus aureus	20.33 ± 1.2^{a}	13.53 ± 0.25^{c}	16 ± 0.5^{b}	9.37 / 37.5	9.37 / 75
Micrococcus luteus	20.5 ± 0.5^{a}	12.93 ± 1.36^{b}	20.8 ± 1.0^{a}	4.68 / 37.5	9.37/ 37.5
Gram -					
Salmonella enteritidis	ni	ni	15.9 ± 0.05^{a}	-/-	- / -
Escherichia coli	14.0 ± 1^{b}	11.0 ± 0.6^{c}	23.8 ± 0.7^{a}	2.34/ 37.2	4.68/ 37.5
Klebsiella pneumonia	ni	8.46 ± 1.30^{b}	21.8 ± 0.7^{a}	- /-	/
Fungal strains			Cycloheximide ^{y}	**MIC / MFC**	**MIC / MFC**
Fusarium oxysporum	9 ± 0^{c}	16.66 ± 2.08^{b}	20.5 ± 0.4^{a}	9.37 / 75	9.37 / 75
Fusarium sp	ni		18.5 ± 0.4^{a}	- / -	/-
Pythium Catenulatum	23.5 ± 0.5^{a}	ni	16.8 ± 0.7^{b}	4.68/ 37.2	18.75 / 75

The data are expressed as mean ± S.D (n=3); ^{a,b,c}: Different letters in the same rows indicate significant differences (p<0.05).

*Diameter of inhibition zones of extract including diameter of well 6 mm; D: Diameters ni: No inhibition

^{x}Chloramphenicol was used as a standard antibiotic at a concentration of 15 µg/well.

^{y}Cycloheximide was used as a standard antibiotic at a concentration of 20 µg/well.

MIC: Minimal inhibitory concentration; MBC: Minimum bactericidal concentration; MFC: Minimal fungicidal concentration.

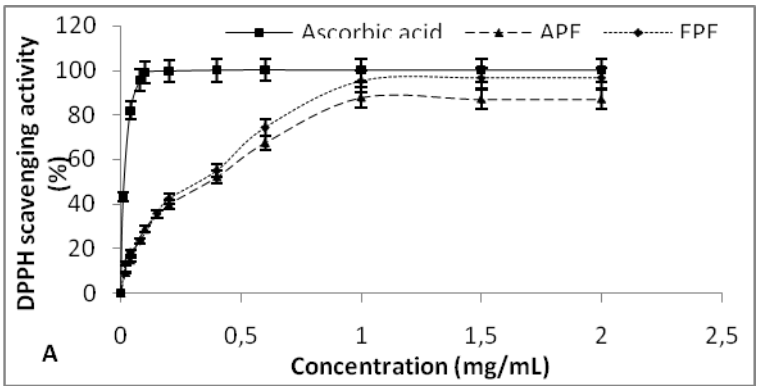

Fig. 1A.

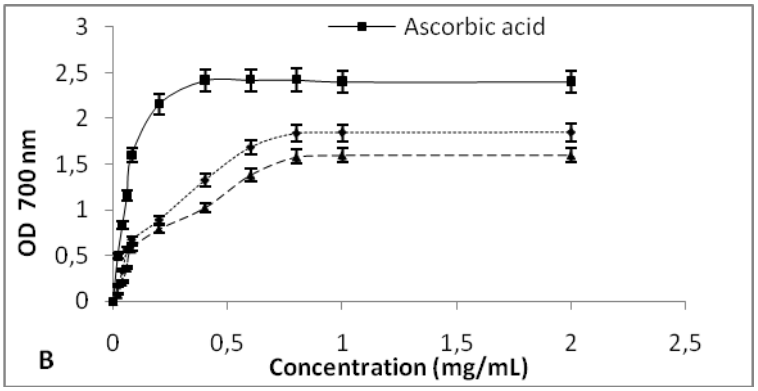

Fig. 1B.

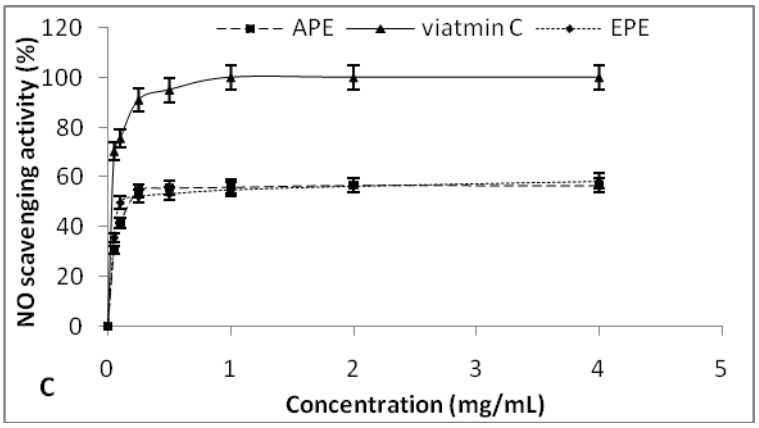

Fig. 1C.

Figure 1: DPPH scavenging activity (A), reducing power (B) and NO scavenging activity (C) of ascorbic acid (the standard), APE and EPE. (DPPH: 2, 2-diphenyl-1-picrylhydrazyl; NO: Nitric Oxid; APE: Aqueous peel extract of *Opuntia dillenii*; EPE: Ethanolic peel extract of *Opuntia dillenii.*

DISCUSSION

The extracts of *O. dillenii* peel, collected from Djerba Tunisia, contained several classes of bioactive chemical compounds, mainly phenolic compounds in ethanolic extract. The highest content of these compounds was inversely proportional to the yield extraction. The difference in yield extraction may be depended on the solvent with varying polarity and composition of the sample. This can also be attributed to other compounds such as proteins and carbohydrates, which are soluble in water and therefore increasing this yield. The results are in discordance with previous published data by several research groups. Chang et al.[4] reported that the peels of *Opuntia dillenii* fruits from Penghu (Taiwan) showed a low TPC and TFC (133 mg GAE/100 g fresh sample and 32 mg QE/100 g fresh sample, respectively). Recently, Katanić et al.[6] reported a high content of total phenolic compounds from different *Opuntia dillenii* extracts from

two localities from Morocco, among other aqueous and ethanolic extracts (120 mg GAE/g dry extract (from Nador region) and 130 mg GAE/g dry extract (from Essouiria region) for aqueous extract and 191 mg GAE/g (from Nador region) and 320 mg GAE/g (from Essouiria region) for ethanolic extract). Also, Betancourt et al.[13] reported a high content of total phenolics in the crude extract of *O. dillenii* fruits (312 mg GAE/100 g fresh fruit) from Colombia. Our results indicated that EPE contained more phenolic compounds. Thus, the high total phenolic content in EPE is mainly due to massive amount of phenolic compounds which can be more soluble in organic solvent than aqueous solvent.[32] This implies that the difference between species could arise from the different factors such as different climatic conditions, plant age, geographical origin, and among others to the solvent extraction.

The highest content of phenolic compounds in ethanolic extract of *O.dillenii* peels were confirmed also by HPLC-MS. Quinic acid was the dominant compound. In literature, this phenolic acid exhibited various biological activities such as antioxidant, anticancer,[33] antiviral and antimicrobial capacities.[34] Hydroxybenzoic acid (Quinic acid) and hydroxycinnamic acid (Cinnamic acid, caffeic acid, p-coumaric acid and trans-ferulic) are the major group of phenolic compounds found in plants.[35] Many researchers indicated that caffeic acid had various biological effects including anti-diabetic, cardio-protective and antioxidant proprieties.[36] Moreover, several studies showed that p-coumaric acid possesses various biological activities such as antioxidant, antimicrobial, antiviral, anticancer, and protective effect against oxidative stress *in vivo*.[37] Protocatechuic acid was present only in EPE and this compound presents anti-inflammatory as well as anti-hyperglycemic and antimicrobial activities. It has also a noticeable pharmacological propriety by inhibiting chemical carcinogenesis and exerting an anti-proliferative effect in cancerous tissues.[38] In addition, flavonoid compounds are an important class of antioxidant molecules which decreased the risk of the apparition of the chronic diseases.[39] The presence of phenolic compounds such as protocatechuic acid, caffeic acid, p-coumaric acid, trans-ferulic acid, catechin, and quercetin has been already reported in *O.dillenii* peel fruits.[4]

GC-MS analysis of ethanolic extract showed the dominant of 2-Furancarboxaldehyde-5-(hydroxymethyl) which exhibited an antioxidant activity and anti-proliferative agent against human melanoma A 375 cell lines. Some of the major compounds and many others have been found to possess several biological activities. N-hexadecanoic acid can be antioxidant, nematicide, pesticide, hypocholesterolemic activities.[40] Phenol, 2-methyl-5-(1-methylethyl)- has an antimicrobial activity. Furthermore, vitamin E possessed an antioxidant and anti cancer activities.[41]

It is known that the antioxidant activity depends on the amount of the phenolic compounds. In fact, these latter contributed to this activity by free radicals scavenging properties and metals ions chelating.[42] The ethanolic peel extract of *Opuntia dillenii* had stronger antioxidant activity by the DPPH scavenging capacity IC_{50}% than trolox. This can be associated with total phenolics content in this extract, as it is well known that phenolic compounds have a good antioxidant capacity.[42] In addition, the results publied by Katanić et al.[6] showed that the DPPH IC_{50}% of aqueous and ethanolic extracts of the peel fruits of *O.dillenii* were lower than our DPPH IC_{50}% values. Moreover, according to Rayan et al. [43] obtained results found in the seed powder of *Opuntia dillenii* showed a lower antioxidant effect. Furthermore, antioxidant activity by the DPPH scavenging capacity IC_{50}% of *Opuntia ficus indica* (spiny and thornless) and *Opuntia stricta* peels were lower than those of our *Opuntia dillenii*.[44] Moreover, the results of FRAP and NO scavenging capacity followed a similar trend. The antioxidant activity showed the advantage of the EPE. For the TAC essay, the EPE had the highest value (91.81±0.92 mg AAE/g) than the APE (64.18±0.67 mg AAE/g). The current finding is comparable with those described by Katanić et al.[6] in which the TAC values of ethanolic extracts of skin of *O.dillenii* where the extracts from Essouiria had much stronger antioxidant capacity. The results of ABTS scavenging capacity, the antioxidant capacity showed the advantage of the EPE and APE.

The influence of phenolic compounds content was also confirmed using the Pearson's correlation between total phenolics and antioxidant activity of *O. dillenii* extracts. There was a strong correlation between TPC, TFC and the most antioxidative parameters (DPPH, FRAP, NO, and TAC) on contrary for tannin content. This suggests that the amount of tannin did not increase the antioxidant activity levels. The observed negative correlation between TPC, TFC and tannin content with DPPH and NO scavenging activity suggests that these phenolic compounds did not significantly were contributed to their antioxidant activity. These implicated that there is other compounds such as vitamins, ascorbate, betalains which are responsible for this antioxidant effect. Chang et al.[4] also showed a significant antioxidant activity of *O. dillenii* fruit extracts, where phenolic compounds and betalains had a significant role. The betalains are water-soluble pigments that are responsible for red (betacyanins) and yellow (betaxanthins) colors of the flowers and fruits of some plant species. It has been reported that the main betacyanins in *O. dillenii* fruits are betanin and isobetanin, while the principal detected betaxanthin is indicaxanthin.[45]

The results of antimicrobial test did not show a big difference between the both solvents, with the best effect was observed in EPE. The results are consistent with recently published data on the antimicrobial activity from fruit extracts of *Opuntia dillenii* from Morocco.[6]

Phenolic compounds, especially flavonoids, present in the peel extracts participate in the antimicrobial effect of *O. dillenii* on some microorganisms. These later are among the most important human and animal pathogens and causes food contamination and infection diseases. The present results indicated that the peel fruits of *O.dillenii* were active and may useful in drug preservation and in traditional medicine for the treatment of pathologies and encouraged their potential as alternative antimicrobial agents.

CONCLUSION

The *in vitro* antioxidant and antimicrobial activities of *Opuntia dillenii* peel extracts were shown. The ethanolic and aqueous extracts served as a source of phenolic bioactive compounds with interesting characteristics. Correlation analysis confirmed that phenolic compounds significantly contributed to the antioxidant activity. Overall, the finding suggested a potential use of *Opuntia dillenii* as an antioxidant and antimicrobial agent in food, drug and cosmetic product ingredients.

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REFERENCES

1. Panda SK, Mohanta YK, Padhi L, Luyten W. Antimicrobial activity of select edible plants from *Odisha*, India against food-borne pathogens. LWT - Food Sci., 2019; 113: 108246.
2. De Wit M, Gernot T, Arno O. Cactus pear antioxidants : a comparison between fruit pulp, fruit peel, fruit seeds and cladodes of eight different cactus pear cultivars (*Opuntia ficus* - i*ndica* and *Opuntia robusta*). J Food Meas Charact., 2019; (0123456789).
3. Ng KR, Lyu X, Mark R, Chen WN. Antimicrobial and antioxidant activities of phenolic metabolites from flavonoid-producing yeast : Potential as natural food preservatives. Food Chem., 2020; 270(July 2018): 123–129.
4. Chang SF, Hsieh CL, Yen GC. The protective effect of *Opuntia dillenii* Haw fruit against low-density lipoprotein peroxidation and its active compounds. Food Chem, 2008; 106(2): 569–575.
5. Ahmed MS, El Tanbouly ND, Islam WT, Sleem AA, El Senousy AS. Antiinflammatory flavonoids from *Opuntia dillenii* (Ker-Gawl) Haw. Flowers growing in Egypt. Phyther Res., 2005; 19(9): 807–809.
6. Katanić J, Yousfi F, Caruso MC, Matić S, Maria D, Loukili EH, Boroja T, Mihailović V, Galgano F, Imbimbo P, Petruk G, Bouhrim M, Bnouham M. Characterization of bioactivity and phytochemical composition with toxicity studies of different *Opuntia dillenii* extracts from Morocco. Food Biosci, 2019.
7. Tamba A, Servent A, Mertz C, Cissé M, Dornier M. Coupling of pressure-driven membrane technologies for concentrating, purifying and fractionizing betacyanins in cactus pear (*Opuntia dillenii* Haw.) juice. Innov Food Sci Emerg Technol., 2019; 52: 244–255.
8. Siddiqui F, Naqvi S, Abidi L, Faizi S, Lubna, Avesi L, Mirza T, Farooq AD. *Opuntia dillenii* cladode: Opuntiol and opuntioside attenuated cytokines and eicosanoids mediated inflammation. J Ethnopharmacol., 2016; 182: 221–234.
9. Kalegowda P, Chauhan AS, Nanjaraj Urs SM. *Opuntia dillenii* (Ker-Gawl) Haw cladode mucilage: Physico-chemical, rheological and functional behavior. Carbohydr Polym., 2017; 157: 1057–1064.
10. Kalegowda P, Singh Chauhan A, Mysore NanjarajUrs S. *Opuntia dillenii* (Ker-gawl) haw fruit peel pectin: Physicochemical, rheological, and functional behavior. J Food Process Preserv., 2017; 41(5): e13165.
11. Saleem R, Ahmad M, Azmat A, Ahmad SI, Faizi Z, Abidi L, Faizi, S. Hypotensive activity, toxicology and histopathology of opuntioside-I and methanolic extract of *Opuntia dillenii*. Biol Pharm Bull., 2005; 28(10): 1844–1851.
12. Qiu Y, Chen Y, Pei Y, Matsuda H, Yoshikawa M. Constituents with radical scavenging effect from *Opuntia dillenii*: structures of new alpha-pyrones and flavonol glycoside. Chem Pharm Bull (Tokyo), 2002; 50(11): 1507–1510.
13. Betancourt C, Cejudo-Bastante MJ, Heredia FJ, Hurtado N. Pigment composition and antioxidant capacity of betacyanins and betaxanthins fractions of *Opuntia dillenii* (Ker Gawl) Haw cactus fruit. Food Res Int., 2017; 101: 173–179.
14. Kumar AS, Ganesh M, Peng MM, Jang HT. Phytochemical, antioxidant, antiviral and cytotoxic evaluation of *Opuntia dillenii* flowers. Bangladesh J Pharmacol., 2014; 9(3): 351–355.
15. Babitha S, Bindu K, Nageena T, Veerapur VP. Fresh Fruit Juice of *Opuntia dillenii* Haw. Attenuates Acetic Acid–Induced Ulcerative Colitis in Rats. J Diet Suppl., 2018; 0211: 1–12.
16. Bajaj VK, Gupta RS. Fertility suppression in male albino rats by administration of methanolic extract of *Opuntia dillenii*. Andrologia., 2012; 44: 530–537.
17. Ratnaweera PB, de Silva ED, Williams DE, Andersen RJ. Antimicrobial activities of endophytic fungi obtained from the arid zone invasive plant *Opuntia dillenii* and the isolation of equisetin, from endophytic Fusarium sp. BMC Complement Altern Med., 2015; 15(1): 1–7.
18. Cicco N, Lanorte MT, Paraggio M, Viggiano M, Lattanzio V. A reproducible, rapid and inexpensive Folin-Ciocalteu micro-method in determining phenolics of plant methanol extracts. Microchem J., 2009; 91(1): 107–110.

19. Palacios I, Lozano M, Moro C, D'Arrigo M, Rostagno MA, Martínez JA, García-Lafuente A, Guillamón E, Villares A. Antioxidant properties of phenolic compounds occurring in edible mushrooms. Food Chem., 2011; 128(3): 674–678.
20. Price ML, Scoyoc S Van, Butler LG. A Critical Evaluation of the Vanillin Reaction as an Assay for Tannin in Sorghum Grain. J. Agric. Food Chem., 1978; 26 (5): 1214–1218.
21. Ayaz FA, Hayirlioglu-Ayaz S, Gruz J, Novak O, Strnad M. Separation, characterization, and quantitation of phenolic acids in a little-known blueberry (*Vaccinium arctostaphylos* L.) fruit by HPLC-MS. J Agric Food Chem, 2005; 53(21): 8116–8122.
22. Kohoude MJ, Gbaguidi F, Agbani P, Ayedoun MA, Cazaux S, Bouajila J. Chemical composition and biological activities of extracts and essential oil of *Boswellia dalzielii* leaves. Pharm Biol., 2017; 55(1): 33–42.
23. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem., 1999; 269(2): 337–341.
24. Sánchez-Moreno C, Larrauri J a., Saura-calixto F. A procedure to measure the antiradical efficienc y of polyphenols. J Sci Food Agric., 1998; 270(199802): 270–276.
25. Yildirim A, Mavi A, Kara AA. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. J Agric Food Chem., 2001; 49: 4083–4089.
26. Jagetia GC, Baliga MS. The evaluation of nitric oxide scavenging activity of certain Indian medicinal plants *in vitro*: A preliminary study. J Med Food, 2004; 7(3): 343–348.
27. Ben Hsouna A, Trigui M, Ben R, Mezghani R, Damak M, Jaoua S. Chemical composition, cytotoxicity effect and antimicrobial activity of *Ceratonia siliqua* essential oil with preservative effects against *Listeria* inoculated in minced beef meat. Int J Food Microbiol, 2011; 148(1): 66–72.
28. Trigui M, Ben Hsouna A, Tounsi S, Jaoua S. Chemical composition and evaluation of antioxidant and antimicrobial activities of Tunisian Thymelaea hirsuta with special reference to its mode of action. Ind Crop Prod., 2013; 41: 150–157.
29. Daoud A, Malika D, Bakari S, Hfaiedh N, Mnafgui K, Kadri A, Gharsallah N. Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of Date Palm Pollen (DPP) from two Tunisian cultivars. Arab J Chem., 2015.
30. Abdallah EM, Hsouna A Ben, Al-khalifa KS. Antimicrobial, antioxidant and phytochemical investigation of *Balanites aegyptiaca* (L.) Del. edible fruit from Sudan. Afr.J.biotech., 2012; 11(52): 11535–11542.
31. Diao WR, Hu QP, Zhang H, Xu JG. Chemical composition, antibacterial activity and mechanism of action of essential oil from seeds of fennel (Foeniculum vulgare Mill.). Food Control., 2014; 35(1): 109–116.
32. Hwang E, Thi N Do. Effects of extraction and processing methods on antioxidant compound contents and radical scavenging activities of laver (Porphyra tenera). Prev. Nutr. Food Sci., 2014; 19(1): 40–48.
33. He X, Rui HL. Cranberry phytochemicals: Isolation, structure elucidation, and their antiproliferative and antioxidant activities. J Agric Food Chem. 2006;54(19):7069–74.
34. Zanello PR, Koishi AC, Rezende Júnior C de O, Oliveira LA, Pereira AA, d'Almeida MV, Duarte dos Santos CN, Bordignon J. Quinic acid derivatives inhibit dengue virus replication *in vitro*. Virol J., 2015; 12(1): 223.
35. Olthof MR, Hollman PCH, Katan MB. Chlorogenic acid and caffeic acid are absorbed in Humans. J Nutr. 2018; 131(1): 66–71.
36. Agunloye OM, Oboh G, Ademiluyi AO, Ademosun AO, Akindahunsi AA, Oyagbemi AA, Omobowale TO, Ajibade TO, Adedapo AA. Cardio-protective and antioxidant properties of caffeic acid and chlorogenic acid: Mechanistic role of angiotensin converting enzyme, cholinesterase and arginase activities in cyclosporine induced hypertensive rats. Biomed Pharmacother, 2019; 109: 450–8.
37. Shen Y, Song X, Li L, Sun J, Jaiswal Y, Huang J, Liu C. Protective effects of p -coumaric acid against oxidant and hyperlipidemia-an *in vitro* and *in vivo* evaluation. Biomed Pharmacol, 2019; 111: 579–587.
38. Semaming Y, Pannengpetch P, Chattipakorn SC, Chattipakorn N. Pharmacological properties of protocatechuic acid and its potential roles as complementary medicine. Evid. Based Complement. Alternat. Med., 2015; 1-11.
39. Xiang J, Beta T, Apea-Bah FB, Ndolo VU, Katundu MC. Profile of phenolic compounds and antioxidant activity of finger millet varieties. Food Chem., 2018; 275: 361–368.
40. Chowdhury NS, Islam TB, Farjana F, Jamali S. Pharmacological values and phytochemical analysis of aquatic plant genus Aponogeton : A review. Int. J. Recent Innovat.Acad. Research, 2019; 3: 125–141.
41. Singariya P, Mourya KK, Kumar P. Gas chromatography-mass spectrometric analyses of acetone extract of marwar dhaman grass for bioactive compounds. Plant Arch., 2015; 15: 105-1074.
42. Mattera R, Benvenuto M, Giganti MG, Tresoldi I, Pluchinotta FR, Bergante S, Tettamanti G, Masuelli L, Manzari V, Modesti A, Bei R. Effects of polyphenols on oxidative stress-mediated injury in cardiomyocytes. Nutrient., 2015; 1–43.
43. Rayan AM, Morsy NE, Youssef KM. Enrichment of rice-based extrudates with Cactus *Opuntia dillenii*

seed powder : a novel source of fiber and antioxidants. J Food Sci Technol., 2017.
44. Yeddes N, Chérif JK, Ayadi MT. Comparative study of antioxidant power, polyphenols, flavonoids and betacyanins of peel and pulp of three Tunisian *Opuntia* forms. Pakistan J Biol Sci., 2014; 17(5): 650–8.
45. Böhm, H. *Opuntia dillenii* – an interesting and promising Cactaceae taxon. J. Prof. Assoc. Cactus Dev., 2008; 10: 148-170.
46. Köksal E, Bursal E, Gülçin İ, Korkmaz M, Gören AC, Alwasel SH. Antioxidant activity and polyphenol content of Turkish thyme (*Thymus vulgaris*) monitored by liquid chromatography and tandem mass spectrometry, 2017; 2912.