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ORIGINAL PAPER

Phytochemical screening, antioxidant and antimicrobial activities of *Opuntia streptacantha* fruit skin

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Abstract

Opuntia species are utilized as local medicinal interventions for chronic diseases and as food sources mainly because they possess nutritional properties and biological activities. This study aimed to disclose the phytochemical composition, antioxidant potential, and antimicrobial activity of two extracts recovered from *Opuntia streptacantha* fruit skin collected from Kasserine region in Tunisia using ethanol (EFSE) and water (AFSE). The results revealed that the phytochemical contents are higher in the EFSE. The major phenolic compounds of this extract were quinic acid, trans ferrelic acid and hyperoside. Also, EFSE was shown to exhibit the highest free radical scavenging by DPPH assay with a half-maximally effective concentration (IC_{50}) of 0.22 ± 0.006 mg/ml, while AFSE was less active and it's IC_{50} (effective concentration at which DPPH radical was scavenged by 50%) were above 0.61 ± 0.002 mg/ml. Moreover, the extracts were screened for antimicrobial activity against 7 bacteria and 3 fungal strains and the results showed that the extracts exhibited the strongest activity against *Staphylococcus aureus* and that the *Micrococcus luteus* strain was the most sensitive to the EFSE, with minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations values of 4.75 mg/ml and 36.5 mg/ml respectively. For fungal strains, *Fusarium oxysporum* was the most sensitive for both extracts and exhibited the lowest MIC and minimum fungicidal concentrations compared to other strains. These findings reveal that the EFSE have strong bioactive compounds and hence support its ethnomedicinal application.

Keywords *Opuntia streptacantha* · Phytochemicals · LC–ESI–MS analysis · DPPH assay · Antimicrobial activity · Antioxidant compounds

Abbreviations

TPC	Total polyphenolic contents
TFC	Total flavonoid contents
GAE	Gallic acid equivalent
AAE	Ascorbic acid equivalent
CAT	Catechin
TAC	Total antioxidant capacity
DPPH	2,2-Diphenyl-1-picrylhydrazyl
Trolox	6-Hydroxy-2,5,7,8-tetra methyl-chroman-2-carboxylic acid
ABTS	2,20-Azinobis-3-ethylbenzthiazoline-6-sulphonate
FRAP	Ferric reducing antioxidant power
TEAC	Trolox equivalent antioxidant capacity
DMSO	Dimethyl sulfoxide
TCA	Trichloroacetic acid
MHA	Mueller Hinton agar
EFSE	Ethanol fruit skin extract
AFSE	Aqueous fruit skin extract
MTT	3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2 H-tetrazolium bromide
MBC	Minimum bactericidal concentration
MFC	Minimal fungicidal concentration
MIC	Minimal inhibitory concentration
PDA	Potatoes dextrose agar

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Introduction

Free radicals have been reported to be implicated in the human pathogenesis of at least 50 diseases [1, 2]. Accordingly, there has been a growing interest in plant-based dietary components to counteract oxidative stress since it is involved in various diseases and may exacerbate their symptoms [3]. Nowadays, much attention has been paid to health promotion related to the activity of phytochemical, and increasing attention has been given to the isolation of novel bioactive compounds from medicinal plant as an effective strategy for the treatment of different diseases [4, 5]. In order to find new natural sources of medicinal plants, it have been interested to study for the first time the efficiency of *Opuntia streptacantha*, which is belongs to the dicotyledonous angiosperm, *Cactaceae* family and the *Centrospermae* order. It is originating from Mexico is now spread in all the American hemispheres, South African countries and all over the Mediterranean basin [6, 7].This plant have commercial value in the production of juices, alcoholic bevrages and natural liquid sweeteners [8, 9]. It is also used in agrochemicals, cosmetics, chemical industries, and in wastewater treatments [10, 11].

The therapeutic properties of the green parts of the plant, the cladodes, have very long been known in the traditional medicine [12], however potential activities of the fruit, beyond nutritional benefits, have just been explored recently. But, certain fruit belonging to *Opuntia* specie like *Opuntia ficus indica* fruit, have attracted the greatest attention of researches due to their commercial value whereas, others were less documented. Few researches have been reported on the antioxidant activity of *O. streptacantha* fruit extracts.

The fruit of *Opuntia* is a fleshy berry with a number of hard seeds, which are consumed as fresh fruit or used for preparing a traditional jam. Diet supplementation with *Opuntia* pear fruit in healthy humans has shown to decrease the oxidative stress and, therefore, improves their overall antioxidant status. It have also been studied for ovarian cancer prevention. Their ability in suppressing carcinogenesis of in vitro and in vivo models has been already assessed. The nutraceutical benefits of fruit are believed to their antioxidant properties related to ascorbic acid, phenolics, and a mixture of betaxanthin and betacyanin pigments [13]. Cladodes of this plant named "Nopalitos" are consumed mainly as staple food, but according to Mexican popular medicine, some diseases like diabetes mellitus, blood glucose levels, hyperlipidemy, obesity and gastrointestinal disorders can be alleviated by eating this vegetable [14]. Experiments concerning the antiviral action of *Opuntia* cladode extract have been conducted against viruses such as herpes, HIV-1 virus, and influenza A [15]. Other studies demonstrate that cladode of this plant are used in folk medicine for their cicatrising activities [16].

Despite this large flow of data on the promising properties and attributes of *Opuntia* plant, no studies have so far been performed to explore the antioxidant and antimicrobial prosperities of *O. streptacantha* fruit skin in various extracts. In this respect, the aim of this study was undertaken to evaluate and compare, for the first time, phytochemical composition as well as the antioxidant and antimicrobial activities of two different *O. streptacantha* fruit skin extracts (EFSE and AFSE).

Materials and methods

Solvents and chemicals

Ethanol, methanol, acetic acid, acetonitrile, helium, DPPH, Trolox, ABTS, gallic Acid, ascorbic acid, catechin, Folin-Ciocalteu, Sodium carbonate ($Na_2CO_{3)}$, aluminum chloride ($AlCl_3$), Sodium nitrite (NaNO2), Sodium hydroxide (NaOH), sulfuric acid, sodium phosphate, ammonium molybdate, potassium persulfate ($K_2S_2O_8$), potassium ferricyanide (*K3[Fe(CN)6]*), trichloroacetic acid (TCA), ferric chloride ($FeCl_3$), sodium nitroprusside ($Na_2[Fe(CN)_5NO]\cdot 2H_2O$), sulfanilamide, phosphoric acid (H_3PO_4), N-(1-naphthyl) ethylenediamine dihydrochloride, Tween 80, DMSO and MTT were purchased from Sigma Aldrich®. Mueller Hinton broth, Mueller Hinton Agar (MHA), Sabouraud medium agar and PDA were purchased from Bio-Rad (Bio-Rad France).

Plant material

Fresh fruits of *O. streptacantha* were collected from Kasserine region in Tunisia, in September 2015. Their identity was confirmed by Pr. Rachid CHEMLI, Professor at the University of Pharmacy, Laboratory of Pharmacognosy—Phytotherapy, Monastir, Tunisia. The spines were removed manually and the fruits skin were washed with distilled water and were cut into small pieces then were dehydrated by heating at 50 °C in the oven for 2 days. After drying, the pieces were ground into powder using a Nima electric grinder apparatus (nima®, Japan).

Preparation of plant extracts

The fruit skin powdered (200 g) was extracted twice (800 ml) for 24 h each using ethanol and distilled water as solvents respectively for EFSE was filtered through filter paper (Whatman) and the filtered solution was evaporated in a rotary evaporator at 45 °C, while the AFSE, by its filtration through a filter paper (Whatman), the extract was

freeze-dried in a freeze dryer at 4 °C. The two dry extracts were collected and maintained at 4 °C until further analysis.

Phytochemical investigation of *O. streptacantha* fruit skin

Total polyphenolic contents

TPC were determined by the modified method described by Cicco et al. [17] using Folin-Ciocalteu reagent. In a test tube, 125 µl of *Opuntia* extract, 125 µl of Folin-Ciocalteu reagent and 500 µl distilled water were combined and then mixed. After 3 min, 1250 µl of 7% Na_2CO_3 were added and then 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 in mg of gallic acid equivalent (GAE)/g of dry plant extract. The results were carried out in triplicate.

Total flavonoids contents

TFC were estimated by the aluminum chloride method. Briefly, 250 µl of *Opuntia* extracts were mixed with 75 µl of $NaNO_2$ (5%) and after 6 min of incubation at ambient temperature, 150 µl of $AlCl_3$, $6H_2O$ were added. 500 µl of NaOH (1 M) were added to the mixture after 5 min of incubation. The volume of solution was adjusted by distilled water until 2500 µl. Total flavonoids contents were quantified spectrophotometrically at 430 nm and the results were expressed in mg of catechin equivalent/g of dry extract [18]. The test was carried out in triplicate.

Tannin contents

Tannin contents were estimated according to the methods described by Abdessemed et al. [19] with minor modifications. 300 µl of extract were mixed with 3 ml of vanillin (4% in methanol) and 1.5 ml of HCl. After 15 min of incubation, the absorbance was measured at 500 nm. The results were expressed in mg of catechin/g of dry extract. The results were carried out in triplicate.

Liquid chromatography/electrospray ionization/mass spectroscopy (LC–ESI–MS) analysis

Phenolic acids and flavonoids were extracted according to the modified methods described by Ayaz et al. [20]. Briefly, 0.5 g of the powder was dissolved with 10 ml of ultra pure water and ethanol for AFSE and EFSE respectively. The samples were then shaken for 24 h at room temperature. Before being analyzed 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. LC–ESI–MS analysis was performed using a LCMS-2020 quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an electrospray ionisation source and operated in negative ionization mode. The mass spectrometer was coupled online with an ultra-fast liquid chromatography system, which consisted of a LC-20AD XR binary pump system, SIL-20AC XR autosampler, CTO-20AC column oven, and DGU-20A 3R degasser (Shimadzu). An Aquasil C18 column (Thermo Electron, Dreieich, Germany) (150 mm × 3 mm, 3 µm) preceded by an Aquasil C18 guard column (10 mm × 3 mm, 3 µm, Thermo Electron) were applied for analysis. The mobile phase comprised A (0.1% formic acid in H_2O, *v*/*v*) and B (0.1% formic acid in methanol, *v*/*v*) with a linear gradient elution: 0–45 min, 10%–100% B; 45–55 min, 100% B. Re-equilibration duration was 5 min between individual runs. The flow rate of the mobile phase was 0.4 ml/min, the column temperature was maintained at 40 °C, and the injection volume was 5 µl. Spectra were monitored in mode Selected Ion Monitoring and processed using Shimadzu Lab Solutions LC–MS software. High-purity nitrogen was used as the nebulizer and auxiliary gas. The mass spectrometer was operated in negative ion mode with a capillary voltage of − 3.5 V, a nebulizing gas flow of 1.5 l/min, a dry gas flow rate of 12 l/min, a dissolving line temperature of 250 °C, a block source temperature of 400 °C, a voltage detector of 1.2 V, and the full scan spectra from 50 to 2000 Da.

Biological activities

Antioxidant activities

DPPH free radical-scavenging essay The DPPH free radical-scavenging was determined according to Sánchez-Moreno et al. [21]. 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. Ascorbic acid was used as positive control. The control tube contained only DPPH solution. The percentage inhibition of DPPH radical scavenging was calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = \left[(\text{Ac} + \text{Ab} - \text{As}) / \text{Ac}\right] * 100$$

where Ac, Ab and As 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 test was carried out in triplicate.

Antioxidant capacity by phosphomolybdenum method The TAC of the extracts was measured as described by Prieto et al. [22] 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 in mg equivalent of ascorbic acid.

Reducing power essay The ability of *Opuntia* extract to reduce the iron (III) was determined according to Yildirim et al. [23]. 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) $K_3[Fe(CN)_6]$. 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) $FeCl_3$ were added to 2.5 ml of supernatant and finally the absorbance was measured at 700 nm. In contrast to the DPPH test, a higher absorbance of the reaction mixture indicated a higher reducing power. The values are the mean of triplicate analyzes.

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. [24] with minor modifications. The radical $ABTS^+$ 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 ($K_2S_2O_8$) 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 is expressed as the mM concentration of Trolox solution. A lower absorbance in this test indicated a higher TEAC value of the extracts and a stronger antioxidant activity. The test was performed in triplicate.

Nitric oxide scavenging activity Nitric oxide (NO) scavenging activity was determined according to the method described by Jagetia and Baliga [25]. This activity was evaluated indirectly by generating nitrite ions from sodium nitroprusside ($Na_2[Fe(CN)_5NO]\cdot 2H_2O$) in aqueous solution and 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% H_3PO_4, and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added to 150 µl of extracts and incubated for 30 min. The absorbance was measured at 546 nm and the percentage of NO scavenging activity was calculated as follows:

$$\text{NO scavenging activity (\%)} = \left[(\text{Ac} - \text{As}) / \text{Ac}\right] * 100$$

where Ac and As are the absorbance of the control and the samples respectively. The test was conducted in triplicate.

Antimicrobial screening

Microbial strains and growth conditions The antibacterial activities of AFSE and EFSE were tested against 7 strains of bacteria. These included Gram-positive bacteria: *Bacillus subtilis* JN 934392, *Bacillus cereus* JN 934390, *Staphylococcus aureus* ATCC 6538, *Micrococcus luteus* and Gram-negative bacteria: *Salmonella enteric serotype* Enteritidis ATCC43972, *Salmonella enteric serotype* Typhimurium, *Escherichia coli* ATCC 25922, and *Klebsiella pneumoniae*. Antifungal activities were tested using three fungal strains: *Fusarium sp.* JX391934, *Fusarium oxysporum* AB586994 and *Pythium catenulatum* AY598675.

The test bacteria were cultured on Petri dishes containing Mueller Hinton Agar (MHA) and incubated for 18–24 h. From these dishes, a bacterial culture was prepared in 3 ml MH broth with agitation (200 rpm) for 24 h at 37 °C, except for *Bacillus* species, which were incubated at 30 °C. For the test (MIC), final inoculum concentrations of 10^7 UFC/ml bacteria were used [26].

For fungal strains, growth was carried out at 30 °C for 4 days on Sabouraud agar until mycelia growth covered the entire dishes, from which, a spore suspension was obtained in 10 ml sterile water containing 0.1% tween 80 to obtain spore suspension. For the test (MFC), final inoculum concentrations of 10^6 spores/ml were used [27].

Antimicrobial activity detection by agar diffusion method Antibacterial and antifungal activities were detected by the agar well diffusion test using a slightly modified version of the method described by Ben Hsouna et al. [27]. In brief, a cavity (wells) of 6 mm was created in the MHA using a sterile Pasteur pipette. A freshly prepared bacterial suspension or spore solution (100 µl) adjusted to 107 CFU/ml for bacteria and 106 spores/ml for fungus were inoculated onto the surface of agar plates using a sterile swab. Each well was then filled with 80 µl of each extract (125 mg/ml DMSO). A negative control was carried out simultaneously with the DMSO. The plate was left at 4 °C for 2 h to facilitate the diffusion of the extracts in the agar [28], and then incubated at 37 °C for 24 h for bacterial

strains and at 30 °C for 4–7 h for fungal strains. Antimicrobial activity was determined by measuring the diameter of inhibition zone around the well.

Determination of MIC and MFC by micro-dilution well method The MIC values, representing the lowest extract concentrations that prevented the visible growth of microorganisms, were determined by the method of Gulluce et al. [29] in a sterile 96-well microplate, with a final volume of 200 µl per well. A stock solution of each extract (125 mg/ml) was prepared in DMSO, which is known to have no strong antimicrobial activity [30]. Two-fold serial dilutions of the extracts were prepared in the microplate wells over the range 0.98 to 125 mg of extract/ml DMSO. Each well was supplemented with 100 µl of each extract dilution, and 10 µl of cell suspension to a final inoculum concentration of 107 CFU/ml and 106 spores/ml for bacteria or 90 µl of MH broth and PDB broth for fungi. The last well, which contained only bacteria or fungi in the adequate medium without the addition of extract, was considered as a positive growth control. The one containing DMSO without extract was used as a negative control. After content homogenization, the plates were covered with sterile plate covers and incubated for 24 h at 37 °C for bacterial strains and for 3 days at 30 °C for fungal strains.

Microorganism viability assays involved the addition of 25 µl of MTT (3-(4,5-dimethyl-2-oxidation reduction indicator, to each well and subsequent incubation of the mixture for 30 min at 37 °C. In this assay, the wells involving microbial growth inhibition stay clear after incubation with MTT [27]. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of the microorganisms. The MBC values were interpreted as the highest dilution (lowest concentration) of the sample, which showed clear fluid with no turbidity thiazolyl)-2,5-diphenyl-2 H-tetrazolium bromide) (0.5 mg/ml sterile distilled water), as an development and without visible growth of microorganisms after incubation for 48 h at 37 °C [31, 32]. Minimum fungicidal concentrations (MFC) were defined by the first wells with no visible growth and determined by serial subcultivation of 10 µl in PDA (Potatoes dextrose agar) plates and incubation for 3–4 days at 30 °C. The MFC was considered as the lowest concentration that prevented mycelium growth [33].

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. 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 test to estimate the significance among the main effects at the 5% probability level.

Results

Phytochemical studies

Total phenols, flavonoids and tannins contents

The total phenolic (TPC), flavonoids (TFC) and tannins contents of AFSE and EFSE were investigated according to the Folin-Ciocalteu, aluminum chloride and vanillin method respectively. The results presented in Table 1 revealed that the AFSE yield was higher than that of EFSE (10.25 ± 1.23% and 7.75 ± 1.16% respectively). On the other hand, the TPC, TFC and tannins contents in EFSE were higher than those of AFSE (24.65 ± 0.5 and 12.78 ± 0.25 mg GAE/g dried extract for TPC; 14.08 ± 0.03 and 8.95 ± 0.51 mg CAT/g dried extract for TFC, 2.65 ± 0.05 and 1.98 ± 1.8 mg CAT/g dried extract for tannin contents respectively).

Liquid chromatography/electrospray ionization/mass spectroscopy (LC–ESI–MS) analysis

The results of total phenols obtained by the Folin–Ciocalteu method needed to be further complemented by LC-ESI-MS analysis so as to further qualify and quantify the phenolic

Table 1 Extractions yields, TPC, TFC and tannins content in AFSE and EFSE

	Yields (%)	TPC (mg GAE/g)*	TFC (mg CAT/g)**	Tannins content (mg CAT/g)**
AFSE	10.25 ± 1.23^{a}	12.78 ± 0.25^{b}	8.95 ± 0.51^{b}	1.98 ± 1.8^{a}
EFSE	7.75 ± 1.16^{b}	24.65 ± 0.5^{a}	14.08 ± 0.03^{a}	2.65 ± 0.05^{a}

The data are expressed as mean ± SEM (n = 3); Data in the same column with the different letters are significantly different at $p < 0.05$

TPC Total phenolics content, *TFC* total flavonoids content, *AFSE* aqueous fruit skin extract, *EFSE* ethanol fruit skin extract

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

**(mg CAT/g): mg of catechin equivalent/g of dry extract

constituents in the AFSE and EFSE extracts under investigation. Table 2 showed the phytochemical analysis of *O. streptacantha* fruit skin in both extracts, expressed as ppm of content. Our findings revealed the presence of 19 compounds, classified into 7 phenolic acids (7 compounds for EFSE and 5 for AFSE) and 12 flavonoids (12 molecules for EFSE and 3 compound for AFSE). Among these phenolic acids, quinic acid was the most abundant compound in both extracts with the highest concentration in AFSE (456.07 ppm vs 17.54 ppm registered in EFSE). Caffeic acid and 3,4-di-O-caffeoyquinic acid were not found in AFSE. In flavonoids contents, hyperoside exhibited a high concentration in ethanol extract (51.50 ppm) followed by cinnamic acid, luteolin and quercetin with concentrations of 10.12, 5.92 and 5.35 ppm respectively. The other polyphenolic compounds exhibited a low concentration. AFSE is poor in flavonoids compounds except for the presence of naringin with a small amount (0.96 ppm). According to these results, EFSE is rich in antioxidant molecules which are essentially in flavonoids compounds (Table 2).

Biological activities

Antioxidant activities

The antioxidant activities of the AFSE and EFSE were investigated by five complementary colorimetric methods, namely the DPPH, reducing power (FRAP), nitric oxide (NO), total antioxidant capacity (TAC) and ABTS radical scavenging activity (TEAC) scavenging assays and compared to ascorbic acid (AA) and Trolox used as references standards. The results of DPPH and NO activities presented in Table 3 are expressed in IC_{50} values. However, a lower IC_{50} value reflects an elevated DPPH radical scavenging assay and a greater antioxidant activity. Findings revealed that the standard antioxidant, ascorbic acid and Trolox have the lowers IC_{50} values (0.014 ± 0.01 and 0.012 ± 0.00 mg/ml respectively) followed by EFSE (0.22 ± 0.006 mg/ml), then AFSE (0.61 ± 0.002 mg/ml). It was observed that EFSE presented a radical scavenging ability higher ($p < 0.05$) than AFSE.

Table 2 Chemical composition of EFSE and AFSE

Sample	Group of the compound	ID	Name of the compound	Retention time	Concentration (ppm)
EFSE	Phenolic acids	1	Quinic acid	2.07	17.54
		2	Protocatchuic acid	6.87	9.61
		3	Caffeic acid	14.27	0.54
		4	Syringic acid	15.65	0.14
		5	3,4-di-O-caffeoyquinic acid	16.55	0.42
		6	p-coumaric acid	20..85	3.91
		7	trans Ferrelic acid	22.80	15.15
	Flavonoids	8	Hyperoside	23.33	51.50
		9	Naringin	25.60	1.39
		10	Apegenin-7-O-glucoside	26.19	0.66
		11	1,3-di-O-caffeoyquinic acid	26.56	0.24
		12	Salviolonic acid	27.72	0.14
		13	Cinnamic acid	31.72	10.12
		14	Quercetin	33.49	5.35
		15	Apegenin	34.11	0.11
		16	Luteolin	34.11	5.92
		17	Hyperoside	35.11	28.26
		18	Cirsilineol	38.10	0.64
		19	Acacetin	39.66	0.14
AFSE	Phenolic acids	1	Quinic acid	2.48	456.07
		2	Protocatchuic acid	8.16	1.34
		3	Syringic acid	20.62	0.11
		4	p-coumaric acid	24.73	0.69
	Flavonoids	5	trans Ferrelic acid	27.13	3.17
		6	Naringin	31.16	0.96
		7	Cinnamic acid	36.01	0.344
		8	Luteolin	39.43	0.028

Table 3 Antioxidant profile of AFSE, EFSE and standards (Trolox and ascorbic acid)

	IC_{50} (mg/mL)		FRAP* (700 nm)	TEAC* (mM Trolox/g)	TAC* (mg AAE/g)
	DPPH	NO			
AFSE	0.61 ± 0.002^{c}	0.12 ± 0.005^{c}	1.12 ± 0.01^{d}	0.52 ± 0.15^{a}	55.40 ± 2.65^{a}
EFSE	0.22 ± 0.006^{d}	0.02 ± 0.003^{b}	1.05 ± 0.03^{b}	0.45 ± 0.75^{a}	65.25 ± 1.25^{a}
Ascorbic acid	0.014 ± 0.01^{b}	0.03 ± 0.001^{a}	2.41 ± 0.01^{a}	nd	nd
Trolox	0.012 ± 0.00^{a}	nd	$1.86^{\neq} \pm 0.01^{c}$	nd	nd

FRAP Ferric reducing antioxidant power, *TEAC* Trolox equivalent antioxidant capacity, *TAC* Total antioxidant capacity, *NO* Nitric oxide, *AAE* Ascorbic acid equivalent

^{a,b,c,d} Different letters in the same column indicate significant differences ($p < 0.05$)

*The concentration of fruit skin extracts used in antioxidant activities (FRAP, TEAC and TAC) assays was 1 mg/mL

Research has revealed that there is a direct correlation between antioxidant activities and reducing power [23]. To measure the reducing power of *O. sterptacantha,* we investigated the transformation of Fe^{3+} to Fe^{2+}; ascorbic acid and Trolox were used as reference materials. As in the DPPH test the positives controls, ascorbic acid and Trolox have the best reducing power followed by AFSE and then EFSE. At the concentration of 1 mg/ml, the reducing power activities (FRAP) of the ascorbic acid, Trolox, AFSE and EFSE were 2.41 ± 0.01, 1.86 ± 0.01, 1.12 ± 0.01, and 1.05 ± 0.03, respectively (Table 3).

The results of the NO radical scavenging by both extracts and the positives controls are expressed in IC_{50} values in Table 3 Our results revealed that the IC_{50} values were about 0.12 ± 0.005 mg/ml for AFSE followed by 0.03 ± 0.001 mg/ml for ascorbic acid, then 0.02 ± 0.003 mg/ml for EFSE. It was observed that EFSE presented a nitric oxide scavenging activity higher than the standard antioxidant ($p < 0.05$) and the latter had the higher activity than AFSE ($p < 0.05$).

Finally, with regard to total antioxidant capacity (TAC) and ABTS radical scavenging activity (TEAC), Table 3 presents the results of both parameters. The TAC was measured by the phosphomolybdate method and is expressed as equivalent number of ascorbic acid (AAE) per gram of dry extract. Interestingly, EFSE demonstrated remarkable antioxidant activity (65.25 ± 1.25 mg AAE/g dried extract) than AFSE (55.40 ± 2.65 mg AAE/g dried extract). The TEAC values of both extracts in the ABTS radical scavenging test were 0.52 ± 0.15 mM Trolox/g dried extract for AFSE and 0.45 ± 0.75 mM Trolox/g dried extract for EFSE ($P > 0.05$).

Antimicrobial activities

In the present study, the antimicrobial activities of AFSE and EFSE extracts were screened by the agar diffusion method against 10 microorganisms, including 7 bacteria and 3 fungi, and their potency were qualitatively and quantitatively analyzed by the diameters of the inhibition zones, minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC), and minimum fungicidal concentrations (MFC). The observed antibacterial activities were classified as follows: sensitive-inhibition zone, > 18 mm; intermediate-inhibition zone, 13–17 mm; and resistance-inhibition zone, < 13 mm [46], and then compared to the growth inhibition results obtained for the controls (chloramphenicol for bacteria and cycloheximide for fungi).

The findings revealed that EFSE exhibited higher antimicrobial behavior than AFSE, with Gram-positive bacteria being more susceptible than Gram-negative bacteria (Table 4). In fact, the largest inhibition zone recorded for *O. Sterptacantha* fruit skin was produced by EFSE, with values ranging from 11.6 ± 0.6 (*Bacillus subtilis*) to 18.5 ± 1.8 mm (*Micrococcus luteus*). Ethanol and water extraction were found to have the same antibacterial performance for *Staphylococcus aureus* (14.7 ± 0.6 and 12.1 ± 0.5 mm respectively) ($p > 0.05$) and the EFSE was found to be similar to Chloramphenicol (15 ± 0.6 mm) ($p > 0.05$).

For Gram− bacteria, none of the two extracts have a good antimicrobial activity against *Salmonella enteritidis*, *Escherichia coli* and *Klebsiella pneumonia.*

For fungal strains, there is no inhibition against *Fusarium sp* for both extracts. A similar antifungal effect of EFSE and AFSE was observed against *Fusarium oxysporum* (17.25 ± 0.5 and 18.63 ± 0.5 mm respectively) and it is not different from the positive control, Cycloheximide (20.2 ± 0.4) ($p > 0.05$).

The results obtained in terms of MIC, MBC and MFC values against all tested microorganisms were noted to depend on the extraction solvents (ethanol and water) and were generally consistent with those recorded for the diameters of the inhibition zones, with some slight irregularities (Table 5). According to our results, the lowest observed MIC and MBC were for *Micrococcus luteus* in the case of the EFSE (4.75 mg/ml and 36.5 mg/ml respectively). This is in agreement with the results found in the well diffusion assay where *Micrococcus luteus* is the most sensitive bacterium than the others (18.5 ± 1.8 mm) (Table 4). That is to say, when the strain is more sensitive, it needs a minimum

Table 4 Antimicrobial activities of AFSE and EFSE against bacterial and fungal strains

Strains/extracts	Inhibition zones diameters (mm)*		
	EFSE	AFSE	
Gram +			Chloramphenicol[x]
Bacillus cereus	–[β]	9.0 ± 0.0[b]	25.5 ± 0.7[a]
Bacillus subtilis	11.6 ± 0.6[b]	8.8 ± 0.2[b]	24.3 ± 0.2[a]
Staphylococcus aureus	14.7 ± 0.6[ab]	12.1 ± 0.5[b]	15 ± 0.6[a]
Micrococcus luteus	18.5 ± 1.8[b]	–	21.8 ± 1.5[a]
Gram –			
Salmonella enteritidis	11.6 ± 0.4[b]	–	16.9 ± 0.6[a]
Escherichia coli	–	–	23.8 ± 0.7
Klebsiella pneumonia	–	7.1 ± 0.2[b]	22.8 ± 0.5[a]
Fungal strains			Cycloheximide[y]
Fusarium oxysporum	17.25 ± 0.5[a]	18.63 ± 0.5[a]	20.2 ± 0.4[a]
Fusarium sp	–	–	19.5 ± 0.3
Pythium Catenulatum	12.5 ± 0.4[c]	11.15 ± 0.3[b]	15.8 ± 0.6[a]

The data are expressed as mean ± SEM (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

[β]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

Table 5 Determination of MIC, MBC and MFC (mg/mL) of AFSE and EFSE

Strains/extracts	Concentration (mg/mL)			
	EFSE		AFSE	
	MIC	MBC	MIC	MBC
Gram +				
Bacillus cereus	–	–	–	–
Bacillus subtilis	–	–	–	–
Staphylococcus aureus	8.25	65	10.07	73.5
Micrococcus luteus	4.75	36.5	–	–
Gram –				
Salmonella enteritidis	16.75	75	–	–
Escherichia coli	–	–	–	–
Klebsiella pneumonia	–	–	18.25	75
Strains/extracts	Concentration (mg/mL)			
	EFSE		AFSE	
	MIC	MFC	MIC	MFC
Fungal strains				
Fusarium oxysporum	2.25	16.25	4.68	65
Fusarium sp	–	–	–	–
Pythium Catenulatum	18.25	65	18.75	75

concentration for its inhibition/death. For AFSE, *Staphylococcus aureus* presented the lowest MIC (10.07 mg/ml) than *Klebsiella pneumonia* (128.25 mg/ml) and it exhibited the same MBC value (75 mg/ml). For fungal strains, *Fusarium oxysporum* showed the lowest MIC in EFSE and AFSE (2.25 mg/ml and 4.68 mg/ml respectively) and the lowest

MFC value was observed with the same strain in EFSE (16.25 mg/ml).

Discussion

Medicinal plants contain large amounts of antioxidants such as polyphenols, luteolin flavonoids, tannins, chlorogenic acid, and chrysoeriol, which can plays an important role in adsorbing and neutralizing free radicals [34]. Among these compounds, polyphenols, flavonoids, and tannins play diverse roles as antioxidants and can prevent from various diseases like the neurodegenerative diseases such as Alzheimer's and Parkinson's [35]. These compounds are commonly found in both edible and inedible plants, and they have been reported to have multiple biological effects, including antioxidant, antiviral and antimicrobial activities [36, 37].

In this study, total phenol, flavonoids, tannins contents, antioxidant activity, and antimicrobial activity were determined for two different extracts of, *O. streptacantha* fruit skin (AFSE and EFSE). These parameters usually depend on the type and polarity of solvent. In the present study, a significant difference was observed between the content of phenolic compounds of AFSE and EFSE. In fact, AFSE had the highest concentration of the yields (10.25 ± 1.23). In contrast, the TPC, TFC and tannins contents in EFSE were higher than those of AFSE (24.65 ± 0.5 and 12.78 ± 0.25 mg GAE/g dried extract for TPC; 14.08 ± 0.03 and 8.95 ± 0.51 mg CAT/g dried extract for TFC, 2.65 ± 0.05 and 1.98 ± 1.8 mg CAT/g dried extract for tannin contents respectively). This is similar to results of Unuofin et al. [38] obtained using *Vernonia mespilifolia*, where the ethanol extract gave the highest yield of total polyphenols, flavonoids, and tannins. In recent times, various scientists have been appraising the effects of extraction solvents on natural products. The polarity of solvent used for extraction has a great impact on the yield of different phytochemical classes present in the plant [37, 39]. According to De Wit et al. [40] ethanol extract contains more bioactive components with better antimicrobial potency. The amount of total phenolic compounds in the fruit extract detected in our study appears higher than the values reported by Mabrouki et al. [41] who found a concentrations between 1.04 ± 1.52 mg GAE/g extract for *O. streptacantha* and about 0.54 ± 2.51 mg GAE/g extract for *O. ficus-indica* species. Comparing our results with those of the other species of *Opuntia* similar levels of these natural antioxidants were also detected in the ethanolic extract by De Wit et al. [40].

LC-ESI-MS analysis of *O. streptacantha* fruit skin revealed the presence of phenolic acids and flavonoids. Retention time and percent area are represented in Table 2. A total of 7 phenolic acids were identified in *O. streptacantha* fruit skin extracts (7 compounds for EFSE and 5 for AFSE) which include quinic acid, trans ferrelic acid, caffeic acid, protocatchuic acid and hyperoside. The LC-ESI-MS elution profile of flavonoids showed 12 compounds (12 molecules for EFSE and 3 compound for AFSE), including four known flavonoids such as hyperoside, cinnamic acid, luteolin and quercetin. According to our results, EFSE is rich in antioxidant molecules which are essentially in flavonoids compounds. Among these phenolic acids, quinic acid was the most abundant compound in both extracts with the highest concentration found was in EFSE (456.07 ppm vs 17.54 ppm registered in AFSE). This non-toxic compound is a natural sugar found in many varieties of plants. It has been reported that quinic acid displayed several biological properties such as antioxidant, anticancer, antiviral and antimicrobial effects [42, 43].

According to Farasat et al. [44] the highest antioxidant activity in plants is attributed to polyphenols among other secondary metabolites. Phenolics have the ability of oxidizing a broad spectrum of free radicals to their stable radical intermediates [45]. They also serve as electron donors, metal chelators, and singlet and triplet oxygen quenchers [46]. Tannins (a polyphenol) bind and precipitate microbial proteins, thus starving bacteria of major nutrients [47].

Babaa and Malik [48] stated that flavonoids such as flavones, flavanols, and condensed tannins possess antioxidant potentials as a result of their free OH groups and thus they can suppress the formation of reactive oxygen species, mast cell histamine release, and antimicrobial activities. Antioxidants scavenge free radicals by different modes of action, which include using transition metal chelation, singlet oxygen quenchers, free radical scavenger (donate H), and peroxide stabilizers [49] Because of these numerous modes of action of scavenging free radicals, we decided to assay for 5 different antioxidant assays (DPPH, FRAP, NO, TAC and ABTS). DPPH radical shows a maximum absorption at 517 nm. On encountering proton radical scavengers, its purple color fades rapidly. Antioxidants with the capacity of donating a hydrogen atom or electrons can quench DPPH free radicals, thus converting them to colorless bleached products, which brings about the reduction in absorbance [50].

EFSE had the highest DPPH scavenging potential when compared with AFSE, and this may be due to its high phenolic content, which contribute to their electron transfer/hydrogen donating ability. Our results are expressed in IC50 values (extract concentrations required to scavenge DPPH radical by 50%), thus, a lower IC50 value would reflect greater antioxidant activity of the sample. As shown in Table 2, the DPPH-scavenging activity of EFSE (0.22 ± 0.006 mg/ml) and AFSE (0.61 ± 0.002 mg/ml) was lower than that of Trolox and ascorbic acid, a well-known antioxidants (0.012 mg/ml and 0.014 ± 0.01 mg/ml, respectively). EFSE, by its lower IC50 value, revealed the

highest antiradical activities compared to AFSE. We think that this activity was closely related to the large phenolics content detected in EFSE. Phenolics compound was able to liberate an electron from their hydroxyl group and could scavenge DPPH radical as function of concentration [51]. In addition to phenolics compounds, flavonoids and tannins are also detected and could in part contributing to the *O. streptacantha* fruit skin extracts radical-scavenging ability. Inal and Kahraman [52] previously reported that phenols and flavonoids compounds possess a radical-scavenging activity, respectively due to their electron and hydrogen donating ability. Results obtained by José et al. [53] demonstrated also the scavenging activity of *Opuntia* fruit extract on DPPH radicals. Moreover, earlier published data [54, 55] indicated positive DPPH test of *Opuntia ficus indica* roots and stems extracts with IC50 values of 118.65 ± 2.51 µg/ml and 9.30 µg/ml, respectively.

As in the DPPH test, the positive controls (Trolox and ascorbic acid), have the best reducing power followed by EFSE and then AFSE. We think that EFSE phenolics and flavonoids content appear to function as good hydrogen donors and therefore should be able to reduce Fe^{3+} to Fe^{2+} form. Similar observation between phenolics, flavonoids and reducing power activity has been reported for several plant extracts [56, 57].

The antioxidant activities of *O. streptacantha* fruit skin monitored also in vitro by the ABTS (TEAC), TAC and NO scavenging essays. In the present study, AFSE and EFSE exhibited a good antioxidant activities with the highest observed in EFSE. According to Heim et al. [58] flavonoids and phenolic acids exhibit antiradical and antioxidant activities. The ethanol extract thus may have scavenged best due to its high phenolic acid. Our result corroborate with Mabrouki et al. [41] report, in which demonstrated that *O. streptacantha* fruit pulp, who contained a large amount of polyphenols contents, exhibited a high radical-scavenging activity in comparison with *O. ficus indica* extract.

As regards to the antioxidant activity of some plants, we demonstrated that the antioxidant activity of *O. streptacantha* fruit skin could be attributed to the differences in their chemical composition and primarily related to their hydroxylation and methylation patterns, to the presence of many phenolic compounds, such as flavonoids and polyphenols and others molecules such as proteins, ascorbate, β carotene, α-tocopherol and lycopene which enhance the antioxidant activity. Caffeic acid, catechin, and epicatechin have previously been reported for their abilities to provide stronger protective benefits against lipid oxidation, which may be helpful for oxidation-related disease prevention [59]. Overall, these results are of significant importance, given the scarcity of data on the antioxidant activity of *O. streptacantha* fruit skin in the literature.

These days, microbial resistance to the currently used antimicrobial agents is becoming a serious global health problem. A large number of bacterial species are becoming resistant to the currently used antibiotics and causing several infectious diseases. Hence, finding new antimicrobial agents with novel mechanism of action is one the alternatives to overcome these problems. Plants are among the potential sources of different phytochemicals that could be used for the prevention and treatment of various infectious diseases. *Opuntia* species is one the important medicinal plants that have been used traditionally for the treatment of different infectious diseases in several countries [60]. The results of the present study revealed the presence of phenolic substances, tannins, glycosides, alkaloids, flavonoids, saponins, steroids, alkaloids and amino acids in the *Opuntia* extracts. The observed considerable antibacterial activity of this plant extracts could be attributed to the presence of different phytochemicals.

On the basis of the result obtained in this present investigation, we conclude also that EFSE had significant in vitro antimicrobial activity higher than AFSE. The results of present research highlights, the fact that the organic solvent extracts exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted only through the organic solvent medium [61]. The present observation suggests that the organic solvent extraction was suitable to verify the antimicrobial properties of medicinal plants and they supported by many investigators [61, 62]. The weak activity of AFSE in this current study could be due to a very low concentration of the compounds present in the crude extracts that are active against the various organisms.

In this study, *O. streptacantha* fruit skin extracts have shown great antibacterial activity against both Gram-positive and Gram-negative bacteria. But the inhibitory activity of these extracts against Gram-positive bacteria was by far greater than the Gram-negative bacteria, which is in agreement with antibacterial activity of *O. ficus indica* reported by other studies [63]. This might be because of having only an outer peptidoglycan layer in Gram-positive bacteria, which is not an effective and strong permeable barrier. Our results agree with several other studies showing that the inhibitory effect of phenolic compounds from natural extracts are more effective to Gram-positive than Gram-negative bacteria [64]. The susceptibility of bacteria to drugs depends on the characteristics of the drug (hydrophobicity or hydrosolubility) and on the microbial membrane composition [65].

A few authors have reported the antimicrobial activity of *Opuntia* spicies. Ginestra and co-workers [66] reported that different phytochemical fractions of *O. ficus-indica* did not show antimicrobial activity against the tested bacterial strains. On the other hand, it has been reported the antimicrobial activity of EFSE and AFSE of *Opuntia* cladodes

against *Vibrio cholerae* and *Proteus mirabilis* [21]. Moreover, other authors [66] described the antimicrobial activity of *Opuntia* cladodes against *Escherichia coli* and *Staphylococcus aureus*, with a minimum bactericidal concentration (MBC) of 4 mg/ml and 1 mg/ml, respectively. The antimicrobial activity of *Opuntia* extracts may be related to its high content of polyphenols, especially isorhamnetin that has been already reported exerting antimicrobial activity [67]. In previous studies [68] it has been shown that the only cladodes have possessed the antimicrobial activity but in this investigation we have taken fruit skin and obtained results which indicate this part of plant have significant antimicrobial agent.

Conclusions

This study reveals that *O. streptacantha* fruit skin extracts have high amount of polyphenolic compounds. EFSE had the highest phytochemicals content, which could have contributed to the high antioxidant activities observed. Also, this extract was the most promising against Gram-positive and Gram-negative bacteria and exhibited higher antifungal ability. Thus, given the remarkable antioxidant effects of *O. streptacantha* fruit skin extracts, its consumption should be further exploited, as this plant may play an important role in preventing several health disorders involving free radicals' overproduction, carcinoma, cardiovascular diseases, and premature aging. However, more in-depth research is needed on the isolation and individual characterization of bioactive compounds for the development of promissory foods and/or cosmetic preservatives.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to disclose.

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