

Impact of different solvents on extraction yield, phenolic composition, in vitro antioxidant and antibacterial activities of deseeded *Opuntia stricta* fruit

Ines El Mannoubi^{1,2} 

Received: 21 November 2022 / Accepted: 28 January 2023

Published online: 17 February 2023

© The Author(s) 2023 **OPEN**

Abstract

Opuntia stricta the shrubby plant is naturally occurring in all parts of the world used as popular ornamental and is known to have medicinal properties due to its phytochemical compositions. In this study, the effect of solvents (80% ethanol, 80% methanol and 80% acetone (v/v) in water) on the extraction yield, total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity and antibacterial activity of *Opuntia stricta* fruit were investigated. For the antioxidant activity assay, three different *in-vitro* methods DPPH, ABTS and FRAP were followed. The One-way ANOVA calculations showed that the extraction solvents influenced greatly and significantly the TPC, TFC and antioxidant capacity. Overall, 80% ethanol showed highest percentage of extraction yield while 80% acetone extract showed the lowest extraction yield but the highest TPC, TFC and antioxidant activity of *Opuntia stricta* fruit. In addition, Pearson's correlation analysis proved a strong correlation between TPC, TFC and all antioxidant methods used. The antibacterial tests demonstrated that 80% methanol and 80% acetone extracts exhibited the highest zones of inhibition (11.66 and 11.33 mm respectively, $p > 0.05$) against *Salmonella thyphimurium*. Likewise, the 80% acetone extract revealed the best inhibitory effect contra *Escherichia coli* ($p > 0.05$) with an inhibition zone of 11 cm. Unfortunately, it was observed that gram-positive bacteria were less sensitive to all *Opuntia stricta* extracts compared to the gram-negative bacteria.

Keywords *Opuntia stricta* · Solvent extraction · Polyphenols · Antioxidant activity · Bacterial activity

1 Introduction

Opuntia stricta is a member of *Cactaceae* family and has been grown widely around the world in arid and semi-arid zones owing to their low water requirements [1, 2]. *O. stricta* is very famous in Tunisia and used as an ornamental plant in parks, botanical gardens and as potted plant. *O. stricta* is a spiny perennial succulent shrub of evergreen low-growing plant (nearly up to 200 cm tall) with relatively

large-flattened and elongated stem segments, and red-dish-purple fruits [3].

The *Opuntia* species are prominent and have been used in conventional medicine earlier for treatment of a range of ailments and it is a promising source of bioactive compounds [4]. The phytochemical composition and different bioactivities of different plant parts have been reported. Phytochemical screening of *O. stricta* fruits and cladodes are reported to contain flavonoids glycosides such as

✉ Ines El Mannoubi, ielmannoubi@bu.edu.sa | ¹Department of Chemistry, Faculty of Science, University of Gabes, Gabes, Tunisia. ²Chemistry Department, Faculty of Science, Albaha University, Al-Baha, Saudi Arabia.



isorhamnetin-3-O-rutinoside, Kaempferol-3-O-rutinoside and phenolic acids as well as vitamins A, C and E [5–7]. It was proven that *O. stricta* is an excellent source of betacyanin pigments with high concentrations of betanin, isobetanin and betanidin as well as betanidin-5-O-glucoside and betanidin-6-O-glucoside [2, 6]. *O. stricta* was utilized for the remedy of chronic diseases for many centuries and the fruits are said to possess a value in treatment of diabetes [4]. Moreover, *O. stricta* extracts obtained from flowers and whole fruits or peels exhibited many pharmacological activities such as anti-spermatogenic, hypotensive, anti-oxidant, antibacterial, anti-inflammatory, antiviral, analgesic and anti-cancer [8–16]. These beneficial medicinal features are as a result of their chemical contents of antioxidants, pigments, and phenolic acids [17, 18].

Kharat et al. study revealed that the natural extract of red prickly pear (*O. stricta*) was rich in polysaccharides, polyphenols and flavonoids, which displayed strong antioxidant and antibacterial activities due to the synergistic effect of these natural ingredients [19]. In a recent study, aqueous extracts of fruits peel exhibited higher antioxidant and anti-bacterial powers compared to cladodes. These effects have been linked to the presence of phenolic compounds and flavonoids especially quinic acid and hyperoside [20]. Moreover, it was found that the essential oil of *O. stricta* F had strong antibacterial and antifungal effectiveness against standard strains of *P. aeruginosa*, *B. cerus*, *E. colli*, *C. Albicans* and *B. licheniformis*. This antimicrobial efficacy was linked with the presence of thymol as a dominant constituent [21].

Koubaa et al. have investigated solvent extraction of *O. stricta* peels grown in Tunisia and noticed that the extract exhibited high in vitro antioxidant and antibacterial potentials [12]. The physicochemical features of the Tunisian *O. stricta* seed oils were investigated and the compositions of fatty acids, triacylglycerols, phytosterols, and tocopherols were studied. The compliance of fatty acid methyl esters indicators with the American Society for Testing and Materials, European Union and the Indian Standards, for biodiesel properties was proved [22]. Recently, Surup et al. have detected two-14-ring-membered cyclopeptide alkaloids by fractionation of the extracts of *O. stricta* var. *dillenii* fruits using HPLC [23].

To the best of our knowledge, there is no previous published papers investigated the effect of solvent on extraction yield, phenolic composition, anti-oxidant and antibacterial activities of deseeded *Opuntia stricta* fruit. In this context, this work aimed at examining, for the first time, the impact of various solvent concentrations on extraction yield, TPC, TFC and antioxidant activities using DPPH, ABTS and FRAP assays as well as antibacterial activities of deseeded *Opuntia stricta* Haw. fruit extracts.

2 Materials and methods

2.1 Plant material

The plant (*Opuntia stricta* Haw.) was identified by Dr Haidar Abdalgadir Mohamed an experienced taxonomist (Biology Department, Faculty of Science, Albaha University). The fruits were washed, air-dried and crushed by an electric mixer. The seeds were segregated from the purple juice which was frozen and then lyophilized (yield = 10.32 w/w%). After this, 3 gm of lyophilized samples were defatted by vigorous agitation with n-hexane twice. After discarding of the n-hexane layer, the phenolic compounds were extracted twice from the defatted residue by maceration extraction technique using 15 mL different solvents (acetone/ water (80/20 v/v), methanol/ water (80/20 v/v) and ethanol/ water (80/20 v/v). The obtained extracts were filtered and concentrated yielding to solids of different colors ranging from orange-red (hydro acetone) to red (hydro methanol) and wine (hydro ethanol). The freeze-dried extracts were stowed for further analysis. The extraction percent yield (w/w) was calculated as follows.

$$\% \text{Yield} = \frac{\text{The mass of the extract after solvent evaporation}}{\text{The mass of the lyophilized plant material}} \times 100$$

2.2 Solvents and chemicals

All solvents and chemicals were of analytical grade. Folin–Ciocalteu, quercetin, Butylated hydroxytoluene (BHT), gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), FeCl₃, 2,4,6-tripyridyl-5-triazine (TPTZ), potassium ferricyanide [K₃Fe(CN)₆] and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) were from Sigma–Aldrich Inc. (Steinheim, Germany).

2.3 Total polyphenol content (TPC)

Total polyphenol content was measured spectrophotometrically using Folin–Ciocalteu reagent adapted from Pourmorad et al. [24]. Briefly, 500 µL aliquot of extract solution was blended with Folin–Ciocalteu aqueous solution (2.5 mL, 10% v/v) reagent and of Na₂CO₃ (2 mL, 1 M). The obtained solution was kept for 2 h. The optical density of the obtained dark blue solution was read at 760 nm. TPC was presented as microgram gallic acid equivalents (µg GAE/mg dry weight).

2.4 Total flavonoid content (TFC)

TFC was assessed according to Pourmorad et al. slightly modified method [24]. An aliquot of extract solution (500 µL) was blended with 1.5 mL methanol, 0.1 mL of AlCl₃ (10%), 0.1 mL of Na₂CO₃ (1 M) and 2.8 mL of water and kept for half an hour. The optical density was recorded at 430 nm. TFC was assessed as microgram of quercetin equivalents (µg QE/mg dry weight).

2.5 Antioxidant activity

2.5.1 DPPH assay

The scavenging activity of extracts against DPPH[•] was established as described by Brand-Williams et al. slightly modified [25]. Different concentrations of extracts (1 mL) were mixed with a methanolic solution of DPPH (2 mL, 10⁻⁴ M). The obtained solution was shaken gently and left to stand for 1 h. The optical density was recorded against a blank at 515 nm. High free radical scavenging activities is indicated by low absorbance values. The scavenging activity was estimated utilizing the DPPH inhibition percentage as follows:

$$\% \text{DPPH radical scavenging} = \frac{A_0 - A_s}{A_0} \times 100$$

where A₀: control absorbance and A_s: the sample absorbance. The sample concentration providing 50% of inhibition (IC₅₀) expressed as mg/mL, was determined from the plotted curve of inhibition using several extract concentrations and compared to quercetin, BHT and trolox as positive controls.

2.5.2 ABTS radical scavenging assay

The ABTS free radical-scavenging assay was determined following the method reported by Re et al. in which 7 mM ABTS solution was mixed with 2.45 mM potassium persulfate and allowed to stand for 12–16 h in order to produce ABTS^{•+} [26]. The blue-green ABTS solution was diluted to obtain an absorbance of 0.7 at 734 nm prior to assay. 150 µL of appropriately diluted samples was mixed with 2850 µL of ABTS solution and kept for half an hour. Then, the absorbance was read at 430 nm. The ABTS radical scavenging potential of samples was presented as IC₅₀ (the concentration necessary to reduce 50% of ABTS^{•+}) and compared to Trolox as positive control. The procedure was like the DPPH scavenging assay.

2.5.3 Ferric reducing antioxidant power (FRAP) assay

The FRAP of extracts was studied using the method reported by Binsan et al. with minor modifications [27]. Briefly, the FRAP solution was made by adding 0.3 M acetate buffer (pH 3.6), 0.01 M TPTZ to 0.02 M FeCl₃ in a 10:1:1 ratio. The mixture was kept at 37 °C for half an hour. Different concentrations of extracts (150 µL) were added to the FRAP solution and the absorbance was read at 593 nm. Increased reaction mixture absorbance indicated increased reducing power. The sample concentration providing 0.5 of absorbance (EC₅₀) was calculated by plotting absorbance against the corresponding sample concentration and compared to Trolox.

2.6 Antibacterial activity

2.6.1 Bacterial strains and growth conditions

Four bacterial strains were used to evaluate the antibacterial capacity of the plant extracts: two gram-negative namely *Escherichia coli* (ATCC 8739) and *Salmonella typhimurium* ATCC 14,028 and two gram-positive namely *Enterococcus faecium* (ATCC 19,434) and *Streptococcus agalactiae* (ATCC 13,813). Apart from *Streptococcus agalactiae*, which was isolated in the National Institute of Applied Sciences (Tunis), the remaining strains were provided by the Pasteur Institute (Paris, France). Bacterial strains were cultured overnight at 37 °C in Mueller–Hinton (MH) agar nutrient.

2.6.2 Disk diffusion method

For the determination of the antibacterial activity, the disk diffusion method was used according to the National Committee for Clinical Laboratory Standards (NCCLS) [28]. Briefly, the bacterial suspension (100 µL/10⁸ CFU/mL) was spread on the MH solid media plate. Sterile filter paper disk (6 mm in diameter) was soaked with 15 µL of the extracts (dissolved in dimethylsulfoxide) and placed on the inoculated plates and kept at 4 °C for 2 h. Then, they were kept at 37 °C for 1 day. Amoxicillin antibacterial drug was used as standard reference for comparison. The definite inhibition zone surrounding the paper disk was measured accurately in mm.

2.7 Statistical analysis

All assays were repeated thrice and the data was statistically reported as average ± standard deviation (SD).

One-Way Analysis of Variance (ANOVA) using the SPSS 22.0 software. Differences among means were analyzed using Tukey test ($p < 0.05$). Pearson's correlation coefficients were calculated for TPC, TFC, and antioxidant activities.

3 Results and discussion

3.1 Extraction yield

The extraction yield of the active components from plant materials is well-known to be affected by the ratio of water to raw material, which is an important factor. Also, the polarity of the extractant play a key role in increasing the recovery of phenolic and flavonoid compounds [29]. In this work, the experiments were designed to evaluate the impact of extraction solvents on the response variables: extraction yield, TPC and TFC. The selected solvents were 80% ethanol, 80% methanol and 80% acetone and their polarity was in the order 80% methanol > 80% ethanol > 80% acetone. The obtained results presented in Table 1 indicated that polar protic solvents gave better extraction yields and among all used solvents the hydro-ethanol achieved significantly higher extraction yield, and this may tell us that 80% ethanol is a suitable solvent for optimizing extraction yield from *O. stricta* fruit. High extraction yield of hydro-alcohol solvents especially (20:80) hydro-ethanol can be attributed to their capability to dissolve polar along with non-polar molecules [30]. Indeed, the polarity of ethanol system is more consistent for extraction of polar components like, phospholipids, polysaccharides and lipoproteins, which may be the most dominant components of the *O. stricta* fruit material additionally to flavonoid and betacyanin glycosides like isorhamnetin-3-O-rutinoside, betanin, isobetanin and betanidin [5, 31–33]. On the other side, acetone or its combinations with water can be considered as common extractant for tannins and proanthocyanidins [7, 34, 35]. Brglez Mojzer et al. have announced that aqueous acetone is a suitable extractant for flavanols [36].

It is very clear from our experimentation that 80% acetone as extractant system is particularly a good and suitable solvent for recovery of the antioxidant phenolic

constituents from the plant material, while 80% ethanol is a suitable solvent for increasing extraction yield as a whole. This undoubtedly proves that selection of proper extraction solvent depends on the type and polarity of the plant material constituents [30–32, 37, 38].

3.2 TPC and TFC

Polyphenols are natural product molecules that are present in plants with different structures and chemical nature and result in mutability of the physico-chemical properties effecting their extraction [39]. It is also known that the antioxidant behavior of fruits is linked to the total polyphenols content which is due to their capacity to block free radicals. The physico-chemical characteristics of the polyphenols, the presence of other interfering chemicals and the nature of solvent can affect their extraction.

Table 1 presents the TPC and TFC of the extracts measured using the Folin Ciocalteu method. The data indicated that 80% acetone was the most effective solvent with a TPC of 29.223 $\mu\text{g GAE/mg}$ dry weight extract whereas 80% methanol and 80% ethanol were the least effective for phenols extraction (17.511 and 16.349 $\mu\text{g GAE/mg}$) respectively, ($p > 0.05$). It was seen that %80 acetone had enough potential for maximum extraction of *O. stricta* polyphenols.

It was observed that the impact of solvents on TFC is like that on TPC. Indeed, the ranking of increasing TFC was 80% acetone < 80% ethanol and 80% methanol ($p > 0.05$). The highest TFC was obtained in 80% acetone (6.016 $\mu\text{g QE/mg}$) followed by 80% methanol and 80% ethanol (4.825 and 4.905 $\mu\text{g QE/mg}$) respectively ($p > 0.05$).

As it can be noticed, 80% acetone solvent, which has the least polarity among the used solvents results in higher extraction percentage of TPC and TFC. This may be attributed to the polarity properties of lipophilic polyphenols and flavonoids like isoflavones, flavanones, methylated flavones, and flavonols which are usually more dissoluble in less polar organic solvents [40].

Our data is in contradiction with Cha et al. findings who observed that the decreasing order of TPC and TFC in Korean *Opuntia humifusa* fruit was 80% ethanol > 80% methanol and 80% acetone ($p > 0.05$) whereas they are in

Table 1 Extraction yield, TPC and TFC of *Opuntia stricta* extracts

Extract	80% acetone	80% methanol	80% ethanol
Color and nature	Orange-red solid	Red solid	Red wine solid
Extraction yield (% w/w)	4.67 \pm 0.18 ^c	40.92 \pm 0.36 ^b	57.69 \pm 0.22 ^a
TPC ($\mu\text{g GAE/g}$)	29.223 \pm 1.894 ^a	17.511 \pm 1.737 ^b	16.349 \pm 1.719 ^b
TFC ($\mu\text{g QE/g}$)	6.016 \pm 0.069 ^a	4.825 \pm 0.069 ^b	4.905 \pm 0.119 ^b

Values are mean of three replicates \pm standard deviation ($n = 3$)

Different letters in the same row indicate a significant difference according to Tukey test ($p < 0.05$)

agreement with works of Do et al. and Xiong et al. where the acetone (70–80%) had the larger quantity of TPC and TFC in *Osmanthus fragrans*' seed and *Limnophila aromatica* compared to (70–80%) methanol and ethanol extracts [31, 41, 42]. Although alcoholic aqueous mixtures have been confirmed to be very effective for the extraction of high polyphenols and flavonoids, some recent studies proved that different combinations of acetone and water (50–80%) exhibited the highest values of TPC and/or TFC sometimes without any significance difference with their homologous alcoholic water mixtures [42–48].

3.3 Antioxidant activity

The antioxidant potential analysis can be performed with different methods; however, each method has its merits and demerits. In our study, *O. stricta* extracts have been subjected to three in vitro chemical assays to evaluate their antioxidant capacities which are DPPH, ABTS and FRAP.

Results, summarized in Table 2, exhibited that the values of all in vitro antioxidant activities vary significantly with the type of solvent ($p < 0.05$) and confirmed that the extracts' antioxidant properties are significantly impacted by the type of extraction solvents. All extracts exhibited excellent antioxidant activities but lower than used standards. In DPPH assay, 80% acetone extract exhibited the best scavenging radical activity with an IC_{50} equal to 0.732 mg/mL pursued by 80% ethanol and 80% methanol extracts ($IC_{50} = 1.223$ and 1.699 mg/mL) respectively. Similarly, the order of increasing FRAP was 80% acetone > 80%

ethanol > 80% methanol with EC_{50} equal to 1.860, 3.837 and 3.208 mg/mL respectively. On another hand, the highest quenching potential against ABTS was noticed for 80% acetone ($IC_{50} = 1.793$ mg/mL) followed by 80% methanol and finally 80% ethanol extracts ($IC_{50} = 3.768$ and 4.074 mg/mL) respectively.

As it can be observed, the ABTS IC_{50} and FRAP EC_{50} are higher than DPPH IC_{50} which can be explained by the fact that the same antioxidant molecule may act differently in the scavenging of various kinds of radicals especially that in vitro chemical assays had different reaction principles [49].

Our results were in accordance with the findings of Kobus-Cisowska et al. who proclaimed that hydro acetone (acetone: water, 75:25 v/v) was the strongest DPPH' radical and ABTS⁺⁺ scavengers compared to hydro ethanol and hydro methanol (75:25 v/v) [37] and in excellent agreement with works of Zhao et al. who showed that the greatest DPPH' and ABTS⁺⁺ scavenging activities and the best reducing power were detected in 80% acetone extract compared to 80% methanol and 80% ethanol extracts [50]. Overall, our results from the three in vitro assays suggested that antioxidant capacities of extracts are strongly reliant on the solvent. This may be associated with the chemical structure of phenolic compounds and the availability of phenolic hydroxyl groups which have the capacity to donate their electron or hydrogen, thereby forming stable end products [37]. Furthermore, the presence of flavonoid glycosides in ethanolic and methanolic deseeded fruit extracts may have contributed to their reduced antioxidant activities. In fact, glycosylated polyphenols have reduced ability as hydrogen donors and are less effective as antioxidants compared to their free aglycone forms [51].

Table 2 Antioxidant activities of *Opuntia stricta* extracts

DPPH assay (IC_{50} mg/mL)			
Extract	80% acetone	80% methanol	80% ethanol
	0.732 ± 0.017 ^c	1.699 ± 0.036 ^a	1.223 ± 0.011 ^b
Positive control	Quercetin	Trolox	BHT
	0.006 ± 0.000 ^d	0.010 ± 0.000 ^d	0.026 ± 0.000 ^d
ABTS assay (IC_{50} mg/mL)			
Extract	80% acetone	80% methanol	80% ethanol
	1.793 ± 0.019 ^c	3.768 ± 0.101 ^b	4.074 ± 0.111 ^a
Positive control	Trolox		
	0.040 ± 0.000 ^d		
FRAP (EC_{50} mg/mL)			
Extract	80% acetone	80% methanol	80% ethanol
	1.860 ± 0.018 ^c	3.837 ± 0.083 ^a	3.208 ± 0.078 ^b
Positive control	Trolox		
	0.204 ± 0.000 ^d		

Values are mean of three replicates ± standard deviation (n = 3)

Different letters in the same row indicate a significant difference according to Tukey test ($p < 0.05$)

3.4 Pearson's correlation analysis

It is recognized that the plant material antioxidant potential usually appears to correlate with the TPC [52]. In our investigation, a correlation analysis was executed on polyphenol contents (TPC and TFC) and in vitro antioxidant

Table 3 Pearson's correlation test (r-values) between TPC, TFC and antioxidant capacity (DPPH, ABTS and FRAP assays) for *Opuntia stricta* extracts

	TPC	TFC	DPPH	ABTS	FRAP
TPC	1	0.962**	-0.805**	-0.970**	-0.900**
TFC		1	-0.891**	-0.972**	-0.953**
DPPH			1	0.799**	0.978**
ABTS				1	0.902**
FRAP					1

**Correlation is significant at $p < 0.01$

potentials using three assays (DPPH, ABTS and FRAP) as summarized in Table 3. The correlation between TPC and TFC assay was strong ($r=0.962$ at $p<0.01$). This illustrates that flavonoids are the dominating phenolic group in *O. stricta* fruit. Some flavonoid glycosides were identified among relevant metabolites in *O. stricta* var. *dillenii* [6].

TPC and TFC had a negative tight correlation with antioxidant features of the extracts with r -values ranging from -0.805 to -0.972 at $p<0.01$. This strong correlation value proposes that the polyphenols and flavonoids present in our extracts are main antioxidants in *O. stricta* fruit. The negative correlation can be explained by the fact that the lower the IC_{50} or EC_{50} value, the higher the antioxidant capacity inhibition [32]. The smallest correlation was obtained between TPC/DPPH and TFC/DPPH while the correlation between TPC/ABTS and TFC/ABTS was the strongest. Previous studies noticed that the rung of correlation between polyphenols and in vitro antioxidant activities relies on the TPC and the composition of extracts [53]. It was announced that polyphenol and flavonoid contents correlated well with antioxidant potency in *Opuntia* genus [41, 43, 54–57].

All in vitro chemical assays showed great positive correlation ($p<0.05$) of DPPH/ABTS.

($r=0.799$), DPPH/FRAP ($r=0.978$) and ABTS/FRAP ($R=0.902$) which indicates that these methods had an identical response and can be used without discrimination to assess antioxidant potential in *O. stricta* fruit which is comparable to an earlier report [58].

3.5 Antibacterial activity

The results observed for antibacterial capacity of different *O. stricta* extracts against two gram-negative bacteria (*Escherichia coli*, *Salmonella thyphimurium*) and the two gram-positive bacteria (*Enterococcus faecium* and *Streptococcus agalactiae*) were summarized in Table 4 as inhibition zones (mm). It was clear that all extracts were active towards the four bacterial strains.

The obtained results showed that 80% acetone and 80% methanol extracts exhibited the largest inhibition zones (11.66 and 11.33 mm respectively, $p>0.05$) against *Salmonella thyphimurium*. These activities presented

approximately more than 67% of ampicillin activity. The 80% acetone extract exhibited the best inhibitory effect on *Escherichia coli* additionally to ampicillin ($p>0.05$) with an inhibition zone of 11 cm. This value is significantly larger than the inhibition zones caused by water-alcohol extracts ($p<0.05$). These findings are of great importance especially that the studied gram-negative bacteria can cause serious intestinal diseases. Gram-Positive bacterial strains were also inhibited to varying degrees by *O. stricta* extracts. As it can be seen, the type of extractants has no significant impact on antibacterial activity against *Enterococcus faecium* ($p>0.05$) although 80% ethanol had the strongest inhibition zone. On the other hand, 80% ethanol and 80% acetone extracts were more active towards *Streptococcus agalactiae* ($p>0.05$) than 80% methanol extract. Our extracts exhibited less than 30% of ampicillin activity against the gram-positive bacteria.

Overall, gram-negative bacterial strains were inhibited by 80% acetone while gram-positive bacteria were inhibited by 80% ethanol. These findings confirmed that gram-positive microorganisms were more susceptible to hydro-alcoholic extracts than gram-negative ones as announced by Aruwa et al. [51].

The inhibitory effect of plant extracts against bacterial pathogens was usually linked with the phenolic composition which can be explicated by adsorption to cell membranes, interaction with enzymes or deprivation of substrate and metal ions [59]. Many research works had successfully identified flavonoid glycosides and their aglycones in water-alcohol extracts of *Opuntia* fruit and cladodes [51, 58–60]. Moreover, Yeddes et al. had identified Isorhamnetin-3-O-rutinoside as marker flavonoid in *O. stricta* peel methanol extract. The existence of flavonoid derivatives in water-alcohol mixtures could explain their antibacterial activity [5].

Furthermore, the capability of 80% acetone extract to inhibit gram-negative bacteria could be expounded by its high amount of phenolic acids which have been revealed to be more inhibitory and toxic to microorganisms [51]. Indeed, a recent study demonstrated that phenolic acids were the prominent polyphenols of 80% (v/v) acetone extract of *Opuntia robusta* and *Opuntia ficus-barbarica*

Table 4 Antibacterial activity (inhibition zone measured in mm) of *Opuntia stricta* extracts

	80% acetone	80% methanol	80% ethanol	Ampicillin
<i>Escherichia coli</i>	11.00 ± 0.00 ^a	10.00 ± 0.00 ^b	9.50 ± 0.50 ^b	10.83 ± 0.29 ^a
<i>Salmonella thyphimurium</i>	11.33 ± 0.29 ^{bc}	11.66 ± 0.29 ^b	10.16 ± 0.58 ^c	16.67 ± 0.58 ^a
<i>Enterococcus faecium</i>	9.50 ± 0.50 ^b	10.50 ± 0.50 ^b	10.83 ± 0.76 ^b	45.67 ± 0.58 ^a
<i>Streptococcus agalactiae</i>	10.00 ± 0.00 ^{bc}	9.33 ± 0.29 ^c	10.50 ± 0.50 ^b	31.67 ± 0.58 ^a

Values are mean of three replicates ± SD (n = 3)

Different letters in the same row indicate a significant difference according to Tukey test ($p < 0.05$)

with high quantities of ferulic acid and p-hydroxy benzoic acid [61].

4 Conclusion

Our study findings proved that extractability of bioactive components has been greatly affected by the type and concentration of solvents. Overall, the highest amounts of polyphenols and flavonoids and the highest antioxidant activities were obtained with the less polar solvent (80% acetone) although it presents the least extraction yield. This could be linked to the type and polarity of extracted compounds which might be most likely phenolic acids. It should be also noted that TPC and TFC were highly significantly correlated with the antioxidant activity (DPPH, ABTS and FRAP assays) which make them TPC and TFC might be the major contributors to antioxidant potential. Besides, *O. stricta* extracts were capable to inhibit gram-negative and gram-positive bacterial strains to varying degrees without any observed correlation between polyphenol contents and antibacterial activity. Although, the gram-positive bacteria were less sensitive to all extracts than the gram-negative bacteria, the 80% acetone extract showed the best inhibitory effect on the gram-negative *Escherichia coli* which was slightly higher than ampicillin ($p > 0.05$) and on the gram-negative *Salmonella thyphimurium* in addition to 80% methanol ($p > 0.05$) with highest zones of inhibition.

Acknowledgements The author extends their appreciation to Dr Haidar Abdalgadir Mohamed (Biology Department, Faculty of Science, Al baha University) for the identification of the plant material.

Funding This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data availability The manuscript has no associated data.

Declarations

Conflict of interest The author declares that they have no relevant financial or non-financial interests to disclose.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

1. Stintzing FC, Carle R (2005) Cactus stems (*Opuntia* spp.): a review on their chemistry, technology, and uses. *Mol Nutr Food Res* 49:175–194
2. Castellar MR, Solano F, Obón JM (2012) Betacyanin and other antioxidants production during growth of *Opuntia stricta* (Haw). *Plant Foods Hum Nutr* 67:337–343
3. Shackleton RT, Witt ABR, Piroris FM, van Wilgen BW (2017) Distribution and socio-ecological impacts of the invasive alien cactus *Opuntia stricta* in eastern Africa. *Biol Invasions* 19:2427–2441
4. Kampamba M, Ezeala CC, Hikaambo CN, Bwalya AG (2022) *Opuntia stricta* (Haw): A review on its chemical composition, putative in-vitro antidiabetic mechanism of action and potential pharmacological uses in chronic disease. *Int J Pharmacogn Phytochem Res* 11(2):01–08
5. Yeddes N, Cherif JK, Ayadi MT (2014) Comparative study of antioxidant power, polyphenols, flavonoids and betacyanins of peel and pulp of three Tunisian *Opuntia* forms. *Pak J Biol Sci* 17:650–658
6. Tran TMT, Thanh BN, Moussa-Ayoub TE, Rohn S, Jerz G (2019) Profiling of polar metabolites in fruits of *Opuntia stricta* var. *dillenii* by ion-pair high-performance counter-current chromatography and off-line electrospray mass-spectrometry injection. *J Chromatogr A* 1601:274–287
7. Izuqbuna O, Otunola G, Bradley G (2019) Chemical composition, antioxidant, anti-inflammatory, and cytotoxic activities of *Opuntia stricta* cladodes. *PLoS ONE* 14(1):e0209682. <https://doi.org/10.1371/journal.pone.0209682>
8. Gupta RS, Sharma R, Sharma A, Chaudhury R, Bhatnager AK, Dobhal MP, Joshi YC, Sharma MC (2002) Antispermatic effect and chemical investigation of *Opuntia dillenii*. *Pharm Biol* 40(6):411–415
9. Saleem R, Ahmad M, Azmat A, Ahmad SI, Faizi Z, Abidi L, Faizi S (2005) Hypotensive activity, toxicology and histopathology of opuntioside-I and methanolic extract of *Opuntia dillenii*. *Biol Pharm Bull* 28(10):1844–1851
10. Kumar AS, Ganesh M, Peng MM, Jang HT (2014) Phytochemical, antioxidant, antiviral and cytotoxic evaluation of *Opuntia dillenii*. *Bangladesh J Pharmacol* 9:351–355
11. Prabhakaran D, Rajeshkanna A, Senthamilselvi MM (2019) In vitro antioxidant and anti-inflammatory activity of the flower extracts of *Opuntia stricta*. *Asian J Pharm Clin Res* 12(3):208–212
12. Koubaa M, Ktata A, Bouaziz F, Driss D, Ellouz Ghorbel R, Ellouz Chaabouni S (2015) Solvent extract from *Opuntia stricta* fruit peels: Chemical composition and Biological activities. *Free Radic Res* 5(2):52–59
13. Ahmed MS, El Tanbouly ND, Islam WT, Sleem AA, El Senousy AS (2005) Anti-inflammatory flavonoids from *Opuntia dillenii* (Ker-Gawl) Haw. flowers growing in Egypt. *Phytother Res* 19(9):807–809
14. Siddiqui F, Abidi L, Lubna PCF, Naqvi S, Faizi S, Dar Farooq A (2016) Analgesic potential of *Opuntia dillenii* and its compounds opuntiol and opuntioside against pain models in mice. *Rec Nat Prod* 10(6):721–734
15. Prabhakaran D, Senthamilselvi MM, Rajeshkanna A (2017) Anti-cancer activity of *Opuntia stricta* (Flowers) against human liver cancer (HEPG2) cell line. *J Pharm Res* 11(7):793–797
16. Shafei S, Kariminik A, Hasanabadi Z (2013) Antimicrobial activity of methanol extract of *Opuntia stricta* F. *Int res j appl basic sci* 7(12):907–910
17. Gómez-López I, Lobo-Rodrigo G, Portillo MP, Cano MP (2021) Ultrasound-assisted “green” extraction (UAE) of antioxidant compounds (Betalains and Phenolics) from *Opuntia stricta* var.

- Dilenii's fruits: optimization and biological activities. *Antioxidants* 10(11):1786
18. Zhu X, Athmouni K (2022) HPLC analysis and the antioxidant and preventive actions of *Opuntia stricta* juice extract against hepato-nephrotoxicity and testicular injury induced by cadmium exposure. *Molecules* 27:4972
 19. Kharrat N, Salem H, Mrabet A, Aloui F, Triki S, Fendri A, Gargouri Y (2018) Synergistic effect of polysaccharides, betalain pigment and phenolic compounds of red prickly pear (*Opuntia stricta*) in the stabilization of salami. *Int J Biol Macromol* 111:561–568
 20. Affi W, Zourgui MN, Lataief SB, Agil A, Zourgui L (2021) Comparative study of phenolic compound antioxidant and antimicrobial activities of fruits peel and cladodes from Tunisian *Opuntia Stricta*. *J Altern Complement Integr Med* 7:201
 21. Moosazadeh E, Akhgar MR, Kariminik A (2014) Chemical composition and antimicrobial activity of *Opuntia stricta* F. essential oil. *J biodivers environ sci* 4(5):94–101
 22. El Mannoubi I (2021) Tunisian *Opuntia stricta* seed oil: extraction, characterization, and prediction of fatty acid methyl ester properties as biodiesel fuel. *Chem Nat compd* 57:614–619
 23. Surup F, Tran TMT, Pfitze S, Jarmo Budde J, Moussa-Ayoub TE, Rohn S, Jerz G (2021) Opuntisines, 14-membered cyclopeptide alkaloids from fruits of *Opuntia stricta* var. *dillenii* isolated by high-performance countercurrent chromatography. *Food Chem* 334:127552
 24. Pourmorad F, Hosseinimehr SJ, Shahabimajd N (2006) Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr J Biotechnol* 5(11):1142–1145
 25. Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci Technol* 28:25–30
 26. Re R, Pellegrini N, Pannala A, Yang M, Rice-Evan C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization. *Free Radic Biol Med* 26:1231–1237
 27. Binsan W, Benjakul S, Visessanguan W, Roytrakul S, Tanak M, Kishimura H (2008) Antioxidative activity of Mungoong, an extract paste, from the cephalothorax of white shrimp (*Litopenaeus vannamei*). *Food Chem* 106(1):185–193
 28. NCCLS: National Committee for Clinical Laboratory Standards (1997) Performance Standards for Antimicrobial Disk Susceptibility Test 6th edn Wayne. NCCLS, PA
 29. Haminiuk CWI, Plata-Oviedo MSV, de Mattos G, Carpes ST, Branco IG (2014) Extraction and quantification of phenolic acids and flavonols from *Eugenia pyriformis* using different solvents. *J Food Sci Technol* 51(10):2862–2866
 30. Mujtaba A, Masud T, Ahmad A, Naqvi SMS, Qazalbash MA, Levin RE (2016) Effect of solvents on extraction yield, total flavonoid, total phenolic contents, DPPH scavenging activity and antibacterial potential of three apricot cultivars. *Transylvanian Rev XXIV* 10:1662–1676
 31. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju YH (2014) Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *J Food Drug Anal* 22:296–302
 32. Alara OR, Abdurahman NH, Abdul Mudalip SK, Olalere OA (2018) Characterization and effect of extraction solvents on the yield and total phenolic content from *Vernonia amygdalina* leaves. *J Food Meas Charact* 12:311–316
 33. Wang J-L, Yu Z-L, Yin F-W, Li D-Y, Liu H-L, Song L, Zhou D-Y (2021) Comparison of different solvents for extraction of oils from by-products of shrimps *Penaeus vannamei* and *Procambarus clarkia*. *J Food Process Preserv* 45:e15754
 34. Downey MO, Hanlin RL (2010) Comparison of ethanol and acetone mixtures for extraction of condensed tannin from grape skin. *South Afr J Enol Vitic* 31(2):154–159
 35. Liu X, S & White E, (2012) Extraction and characterization of proanthocyanidins from grape seeds. *The Open Food Sc J* 6(1):5–11
 36. Brglez Mojzer E, Knez Hrnčič M, Škerget M, Knez Ž, Bren U (2016) Polyphenols: extraction methods, antioxidative action, bioavailability and anticarcinogenic effects. *Molecules* 21(7):901
 37. Kobus-Cisowska J, Szczepaniak O, Szymanowska-Powałowska D, Piechocka J, Szulc P, Dziędziński M (2020) Antioxidant potential of various solvent extract from *Morus alba* fruits and its major polyphenols composition. *Cienc Rural* 50(1):1–12
 38. Vieito C, Fernandes É, Vaz Velho M, Pires P (2018) The effect of different solvents on extraction yield, total phenolic content and antioxidant activity of extracts from pine bark (*pinus pinaster* subsp. *atlantica*). *Chem Eng Trans* 64:127–132
 39. Koffi E, Sea T, Dodehe Y, Soro S (2010) Effect of solvent type on extraction of polyphenols from twenty three Ivorian plants. *J Anim Plant Sci* 5(3):550–558
 40. Ferreira O, Pinho SP (2012) Solubility of flavonoids in pure solvents. *Ind Eng Chem Res* 51(18):6586–6590
 41. Cha M, Jun H, Lee W, Kim M, Kim M, Kim Y (2013) Chemical composition and antioxidant activity of Korean cactus (*Opuntia humifusa*) Fruit. *Food Sci Biotechnol* 22(2):523–529
 42. Xiong Y, Rao L, Xiong L, Ai Q, Wu X (2012) Effects of extraction solvent on polyphenolic contents and antioxidant activities of *Osmanthus fragrans*' seed. *ICBEB* 2012:217–220
 43. Benayad Z, Martinez Villaluenga C, Frias J, Gomez Cordoves C, Es-Safi NE (2014) Phenolic composition, antioxidant and anti-inflammatory activities of extracts from Moroccan *Opuntia ficus-indica* flowers obtained by different extraction methods. *Ind Crops Prod* 62:412–420
 44. Dailey A, Vuong QV (2015) Effect of extraction solvents on recovery of bioactive compounds and antioxidant properties from macadamia (*Macadamia tetraphylla*) skin waste. *Cogent food agric*. <https://doi.org/10.1080/23311932.2015.1115646>
 45. Assefa AD, Keum YS (2017) Effect of extraction solvent and various drying methods on polyphenol content and antioxidant activities of yuzu (*Citrus junos Sieb ex Tanaka*). *J Food Meas Charact* 11:576–585
 46. Türkyılmaz M, Tağı Ş, Özkan M (2017) Effects of extraction solvents on polyphenol contents, antioxidant and antibacterial activities of pomegranate parts. *Akademik Gıda* 15(2):109–118
 47. Benchaachoua A, Bessam MH, Saidi I (2018) Effects of different extraction methods and solvents on the phenolic composition and antioxidant activity of *Silybum marianum* leaves extracts. *Int J Med Sci Clin Invent* 5:3641–3647
 48. Lasano NF, Ramli NS, Hamid AH, Karim-R, Pak Dek MS, Shukri, R, (2019) Effects of different extraction solvents on polyphenols and antioxidant capacity of peel, pulp and seed kernel of kuini (*Mangifera odorata*). *Orient Pharm Exp Med* 19:277–286
 49. Venkatesan T, Choi Y-W, Kim Y-K (2019) Impact of different extraction solvents on phenolic content and antioxidant potential of *Pinus densiflora* bark extract. *Biomed Res Int* 2019:1–14
 50. Zhao H, Dong J, Lu J, Chen J, Li Y, Shan L, Lin Y, Fan W, Gu G (2006) Effects of extraction solvent mixtures on antioxidant activity evaluation and their extraction capacity and selectivity for free phenolic compounds in barley (*Hordeum vulgare* L.). *J Agric Food Chem* 54:7277–7286
 51. Aruwa CE, Amoob S, Kudangaa T (2019) Phenolic compound profile and biological activities of Southern African *Opuntia ficus-indica* fruit pulp and peels. *LWT Food Sci Technol* 111:337–344
 52. Złotek U, Mikulska S, Nagajek M, Swieca M (2016) The effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves (*Ocimum basilicum* L.) extracts. *Saudi J Biol Sci* 23:628–633

53. Librán CM, Mayor L, Garcia-Castello EM, Vidal-Brotons D (2013) Polyphenol extraction from grape wastes: solvent and pH effect. *Agric Sci* 4(9B):56–62
54. Alimi H, Hfaiedh N, Bouon Z, Hfaiedh M, Sakly M, Zourgui L, Ben Rhouma K (2010) Antioxidant and antiulcerogenic activities of *Opuntia ficus indica* f. *inermis* root extract in rats. *Phytomedicine* 17:1120–1126
55. Saravanakumar A, Ganesh M, Peng MM, Aziz AS, Jang HT (2015) Comparative antioxidant and antimycobacterial activities of *Opuntia ficus-indica* fruit extracts from summer and rainy seasons. *Front Life Sci* 8(2):182–191
56. Ouerghemmi I, Harbeou H, Aidi Wannes W, Bettaieb Rebey I, Hammami M, Marzouk B, Saidani Tounsi M (2017) Phytochemical composition and antioxidant activity of Tunisian cactus pear (*Opuntia ficus indica* L) flower. *J Food Biochem* 41(5):e12390
57. Minhaj M, El Jemli Y, Taourirte M, Bouyazzai L (2019) Preliminary phytochemical screening, total phenolic, flavonoids and polysaccharides contents and antioxidant capacity of aqueous and hydroalcoholic extracts of *Opuntia ficus-barbarica* flowers. *J Mater Environ Sci* 10(12):1369–1381
58. Allai L, Karym E, El Amiri B, Nasser B, Essamad A, Terzioğlu P, Ertas A, Öztürk M (2017) Evaluation of antioxidant activity and phenolic composition of *Opuntia ficus-indica* cladodes collected from moroccan settat region. *Eurasian J Anal Chem* 12(1):105–117
59. Abd El-Moaty HI, Sorour WA, Youssef AK, Goud HM (2020) Structural elucidation of phenolic compounds isolated from *Opuntia littoralis* and their antidiabetic, antimicrobial and cytotoxic activity. *S Afr J Bot* 131:320–327
60. Antunes-Ricardo M, Hernández-Reyes A, Uscanga-Palomeque AC, Rodríguez-Padilla C, Martínez-Torres AC (2019) Isorhamnetin glycoside isolated from *Opuntia ficus-indica* (L) Mill induces apoptosis in human colon cancer cells through mitochondrial damage. *Chem Biol Interact* 310:108734
61. Kıvrak Ş, Kıvrak İ, Karababa E (2018) Analytical evaluation of phenolic compounds and minerals of *Opuntia robusta* J.C. Wendl. and *Opuntia ficus-barbarica* A. Berger. *Int J Food Prop* 21(1):229–241

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.