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Preliminary phytochemical screening, total phenolic, flavonoids and polysaccharides contents and antioxidant capacity of aqueous and hydroalcoholic extracts of *Opuntia ficus-barbarica* flowers

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- ✓ Flower,
- ✓ *Opuntia ficus-barbarica*.

Abstract

The impact of the extraction method under different conditions on preliminary phytochemical screening, total phenol, flavonoid and polysaccharide contents and antioxidant activity of *Opuntia ficus-barbarica* flowers extracts was investigated and discussed. The antioxidant activity was tested using DPPH radical scavenging, β-carotene bleaching and reducing power assays. Results indicate that phenolic compounds are higher in hydroalcoholic extracts. Whereas The impact of the extraction method under different conditions on preliminary phytochemical screening, total phenol, flavonoid and polysaccharide contents and antioxidant activity of *Opuntia ficus-barbarica* flowers extracts was investigated and discussed. The antioxidant activity was tested using DPPH radical scavenging, β-carotene bleaching and reducing power assays. Results indicate that phenolic compounds are higher in hydroalcoholic extracts. Whereas, aqueous extracts are richer in polysaccharides which increase significantly at high temperatures. However, the extraction at elevated temperatures could lead to the degradation of phenolic constituents, especially for a long duration. For the evaluation of antioxidant activity, hydroalcoholic extracts present the highest activities. Whereas aqueous extracts, obtained at high temperatures for short periods of time, shows important antioxidant activities. This study showed the rich content and the wide variety of chemical compounds of *Opuntia ficus-barbarica* flowers natural extracts responsible for its different therapeutic properties and reveals their interest in the development of functional food and nutraceuticals.

1. Introduction

Morocco is one of the African countries characterized by high biodiversity and important plant resources. Its rich flora contains more than 4200 vascular plant species. However, only 800 of them are used as aromatic and medicinal plants [1]. In this context, more scientific studies are needed for the valorization of these crucial natural resources. This valorization helps not only for scientific and economic progress, but also to prevent or reduce the use of synthetic chemicals that many studies have shown to be toxic and carcinogenic [2]. In recent years, an increasing interest has been observed in the use of medicinal plants owing to their beneficial and therapeutic effects. The exploitation of these resources contributes to the development of new safe substances and active components as well as natural antioxidants which have numerous emerging applications in food, cosmetic and nutraceutical [3; 4].

Opuntia ficus-barbarica of the Cactaceae family grows in arid and semi-arid climates with a geographical distribution encompassing Mexico, Latin America, South Africa, and Mediterranean countries [5]. *Opuntia ficus-barbarica* is a plant that contains many bioactive natural products such as betalains, phenolic acids, flavonoids [5; 6; 7], mineral composition [8], amino acide [9] and polysaccharide [10] which may serve as a biological source of phytonutrients. *Opuntia ficus-barbarica* flowers have been used in folk medicine, especially, against kidney disease, diabetes, diuretic, and prostate dysfunction since ancient time [11]. In Morocco, *Opuntia ficus-barbarica* flowers decoction is widely used for its diuretic, antidiarrheal and anti-hemorrhoids effects [12]. Also, the species is very much appreciated in Sicilian traditional medicine, cactus flower infusion is considered as depurative and particularly used for its diuretic and relaxant action on the renal excretory tract [13]. Therefore, it is stipulated that cactus flowers extracts may help the expulsion of renal calculus which are prepared by infusion or decoction as traditional methods [14].

The present study aims to determine the efficiency of different *Opuntia ficus-barbarica* flowers extracts prepared via different techniques in order to validate the traditional remedies of this flowers used in folk medicine and to reveal their interest in the framework of biotechnology exploitation. We mainly investigated the impact of the extraction solvent in the case of the maceration approach, widely used in medicinal plants research, and also the influence of the extraction time and temperature for the infusion and decoction methods, commonly used for tea preparation. For this purpose, qualitative and quantitative analysis of polyphenols, flavonoids and polysaccharides contents of various crude extracts of this flowers have been carried out in order to evaluate in vitro their antioxidant activity using three complementary methods; DPPH radical scavenging activity, β-carotene bleaching and reducing power assays.

2. Material and Methods

1. Chemicals

The chemical reagents namely 2,2-diphenyl-2-picrylhydrazyl (DPPH), ferric chloride (FeCl3), Potassium ferricyanide (K3[Fe(CN)6]), trichloroacetic acid (TCA), sodium hydroxide (NaOH), Folin–Ciocalteu's reagent, aluminum chloride (AlCl3), sodium carbonate (Na2CO3), acid sulfuric, phenol, β-carotene, linoleic acid, as well as standards; gallic acid, Rutin, 6-Hydroxy-2,5,7,8-tetramethyl-3,4-dihydrochromene-2-carboxylic acid (Trolox) and D-glucose, were purchased from Sigma-Aldrich Chemie GmbH (Germany). Deionized water was used. Ethanol of analytical grade was used in the part of extraction and analyses. Other reagents and solvents used in this study were of analytic grade.

2.1.Plant material

The flowers of Opuntia ficus-barbarica were collected, at their post flowering stage, in June 2016, from wild populations growing in Skhour Rhamna region, Morocco (latitude 32° 28' 53" N; longitude 7° 58' 38.2" E; elevation: 452m) which is characterized by a semi-arid climate and a mean rainfall of 312 mm/year. The moisture content of the collected plant material was determined by drying at 105 °C to constant weight [15] and it was found to be 9,53% ± 0,18. The flowers samples were used directly without any further drying process for extracts preparation. Plants were identified at the laboratory of Ecology and Environment Regional Herbarium 'MARK' of Cadi Ayyad University (Morocco).

2.2.Extraction procedure

To prepare the different extracts, flowers were ground to a fine powder, and then mixed with water or ethanol depending upon the extraction method. The liquid-solid extraction techniques used are: infusion, decoction and maceration. We performed all methods using the same sample to solvent ratio. 1 g of Opuntia ficus-barbarica flowers was soaked in 100 mL of solvent. The extraction yield (%) was calculated according to the following formula:

$$\textbf{Yield}\ (\%) = \frac{\boldsymbol{M_{lyoph}}}{\boldsymbol{M_p}}\boldsymbol{X100}$$

Where M_{lyoph} is the mass of lyophiliate and M_p is the mass of the plant.

2.2.1. Infusion and decoction

The extraction was elaborated via infusion at 40˚C, infusion at 80˚C, and decoction at 100˚C at different durations.

For infusion preparation, 1 g of flowers sample was added to 100 mL of distilled water at different temperatures (40 ˚C or 80 ˚C). The mixture was allowed to infuse at various times. The infusion at 40 ˚C was conducted for 15 min (I40˚15'), 30 min (I40˚30'), and 120 min (I40˚120'). Infusion at 80 ˚C was performed for 5 min (I80˚5'), 30 min (I80˚30'), and 120 min (I80˚120').

In the case of decoction, sample preparation was carried out by adding 1g of plant material to 100 mL of distilled water. The mixture was heated at 100 ˚C (heating plate, VELP scientific) for 5 min (D5'), 15 min (D15') and 30 min (D30').

The obtained infusion and decoction extracts were filtered and frozen to be lyophilized (Martin Christ Alpha 1-2 LD plus, Germany).

2.2.2. Maceration

The flowers powder (1 g) was mixed with 100 mL of solvent. The maceration extraction was performed for 24 h at room temperature using different solvents namely; water (M.H2O), 50% EtOH (M.EtOH50) and 80% EtOH (M.EtOH80) under continuous stirring and obscure conditions. After maceration, the extracts are filtered through Whatman filter paper then centrifuged at 4000 × g for 10 min in order to remove any floating matters. The obtained extracts were evaporated at 40 °C under reduced pressure (Laborota 4001-efficient, Heidolph, Germany) and then further lyophilized.

2.3.Samples preparation

Analytical samples for phytochemicals analyses and antioxidant activity evaluation were prepared by re-dissolving the different extracts lyophilized obtained by the different extraction methods in an alcohol/water mixture containing 80% of ethanol. The final concentration was 5mg/mL. The resulting suspension was immediately cooled at 4 °C overnight and centrifuged at 4000 ×g for 15 min.

The supernatant was further diluted at different concentrations then submitted to the determination of total polyphenol and flavonoid content and then to the evaluation of their antioxidant activities.

The precipitate was washed three times with ethanol (75%, v/v) then desolvated by lyophilization at −55 °C for 12 h. The solid residues were dissolved in 20 mL of distilled water and then deproteinised by Sevag reagent (chloroform/butanol 4:1, v/v) as described by Navarini et al. [16]. The resulting aqueous fraction was filtered through a 0.45 µm Whatman Nylon Filter and precipitated again by adding four-fold volume of ethanol. After centrifugation, the precipitate was washed with anhydrous ethanol, dissolved in distilled water to be lyophilized. The extract yield of polysaccharides was determined for each freeze dried sample.

2.4.Phytochemical studies

2.4.1. Qualitative phytochemical analysis

The crude extracts from *Opuntia ficus-barbarica* flowers were phytochemically evaluated to qualitatively identify the presence of flavonoids, phenols, proteins, saponins, tannins, alkaloids, terpenoids, steroids and mucilage compounds according to standard methods described by Harborne [17]. The precipitate formation, or any color change was used as indicative of positive response.

2.4.2. Quantitative determinations of phytochemicals

2.4.2.1.Total phenolic contents

Total phenolic content was determined using Folin-Ciocalteu micro-method [18]. Briefly, 20 µL of each diluted extract solution were mixed with 1.58 mL ultra-pure water. Then, 100µL of Folin-Ciocalteu reagent was added and mixed well. After 30 sec to 8 min, 300 µL of sodium carbonate solution was added and shaked to mix. After incubation at 40 °C for 30 min in dark, the absorbance was measured at 765 nm. Gallic acid was used for

the calibration curve. The results were expressed as milligram Gallic acid equivalents mg GAE/g dry weight of flowers extracts and calculated as mean values ± SD (n = 3).

2.4.2.2.Total flavonoid contents

Flavonoid contents of each extract were determined by the following colorimetric method [19]. Briefly, 1 ml of *Opuntia ficus-barbarica* flowers sample was mixed with 1 ml of 2 % aluminium chloride prepared in methanol. The mixture was vigorously agitated. After 10 min of incubation in dark, the absorbance was measured at 415 nm. Rutin was used as a standard and the results were expressed as milligram rutin equivalents per gram of dried extract (mg RE/g).

2.4.2.3.Estimation of polysaccharide contents

The amount of polysaccharides in different *Opuntia ficus-barbarica* flower extracts was determined using the phenol-sulphuric acid method [20]. Briefly, 0.2 mL of polysaccharides solution was mixed with 0.2 ml of 5% phenol solution, followed by adding 1 mL of concentrated sulphuric acid and shaking the mixture for 30 min. The absorbance was measured at 490 nm and used to quantify polysaccharide contents, based on the standard curve of glucose, which was prepared by plotting six concentrations (10–100 µg/ml) against their absorbance. Polysaccharides extracts were further diluted to adjust concentration within the linear range of the standard curve.

2.5.Evaluation of antioxidant activity

2.5.1. Scavenging of DPPH free radical

DPPH radical-scavenging activity of *Opuntia ficus-barbarica* flowers extracts were determined according to the method of Krishnaiah et al. [21] with slight modifications. Briefly, 500 µL of each extract at different concentrations was added to 375 µL of 99% methanol and 125 µL of DPPH solution (0.2 mM in methanol) as free radical source. The mixtures were incubated for 60 min at room temperature under obscure conditions.

Scavenging capacity was measured spectrophotometrically by monitoring the decrease in absorbance. In its radical form, DPPH has an absorption band at 517 nm, but upon reduction by an antiradical compound, its absorption decreases. The lower absorbance of the reaction mixture indicated higher activity. Trolox was used as positive control. DPPH radical-scavenging activity was calculated as:

$$\textbf{DPPH radical scavenging activity} = \frac{\mathbf{A_{DPPH}} - \mathbf{A_{sample}}}{\mathbf{A_{DPPH}}} \times \mathbf{100}$$

Where A_{DPPH} is the absorbance of DPPH solution and A_{sample} is the absorbance of flowers extracts samples.

The extract concentration providing 50% radicals scavenging activity (EC_{50}) was calculated from the graph of inhibition percentage against extract concentration.

2.5.2. β-Carotene bleaching by linoleic acid assay

The antioxidant activity of flowers extracts was performed by β-carotene linoleic acid assay as described by Koleva et al. [22]. β-carotene solution was prepared by dissolving 0.5 mg of β-carotene in 25 µL of linoleic acid. Afterward, 200 µL of Tween 40 and 1 mL of chloroform were added. Chloroform was then evaporated and 100 mL of distilled water was added to the mixture under vigorous shaking. 2.5 mL of freshly prepared emulsion was mixed with 300 µL of extracts at different concentrations and then incubated in a water bath at 50 °C for 2h. Trolox and deionised water were used to prepare the positive standard and the control tube, respectively. The absorbance was measured at 470 nm immediately and after 2 h of incubation. The antioxidant activity (AA%) values were calculated using the following equation:

$$\mathbf{AA\%} = \mathbf{1} - \frac{\mathbf{Ab_{0\ Sample}} - \mathbf{Ab_{120\ Sample}}}{\mathbf{Ab_{0\ control}} - \mathbf{Ab_{120\ control}}} \times \mathbf{100}$$

The extract concentration providing 50% of antioxidant activity (EC_{50}) was calculated by interpolation from the graph of β-carotene bleaching inhibition percentage against extract concentration.

2.5.3. Reducing power

The reducing power was determined according to the method of Oyaizu [23]. 0.5 mL of samples at different concentrations were mixed with 0.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 0.5 mL of 1% potassium ferricyanide (III) and incubated at 50 °C for 20 min. After that, 0.5 mL of 10% trichloroacetic acid, 0.5 mL of distilled water and 0.1 mL of iron (III) chloride were added and the mixture was incubated for 10 min at 50 °C. The absorbance was measured at 700 nm against a blank of distilled water. Trolox was used as a standard antioxidant.

2.6. Statistical analysis

All the samples of *Opuntia ficus barbarica* flowers were prepared and analyzed in triplicate. Results of phytochemical contents and antioxidant activity were statistically analyzed and expressed as means ± standard deviation (SD) using SPSS (version 21.0). Variance Analysis (ANOVA) was performed for multiple comparisons and the significance of the difference between treatments was accepted at $p < 0.05$. The linear Pearson correlation test was employed to determine the correlation coefficients among phenolic compounds and different antioxidant activities.

3. Results and discussion

3.1.Extraction yield

The extraction method choice is a key parameter that significantly affects the process efficacy. Also, the reaction temperature, extraction time and solvent nature affect significantly the extraction efficiency.

In this study, *Opuntia ficus-barbarica* flowers extracts were prepared via infusion, decoction using water. These reactions were performed at different temperatures at various extraction times. The maceration route was also conducted using different water/ethanol ratios. For aqueous extracts, the extraction yields ranged from 7.67% to 22.54% and were about 20.36% for hydroalcoholic extracts (Table 1). The extraction yields of the different methods decreased in the following order: M.H2O > D30' > M.EtOH50 > I80°120' > D15' > MEtOH80 > I80°30' > D5' > I40°120' > I80°5' > I40°30' > I40°15'. It can be seen that water extract yield (M.H_2O 22.54%) was higher than that of the hydroalcoholic extract (M.EtOH50 20.36% and M.EtOH80 18.17%). This shows that the extraction yield increases when increasing the polarity of the solvent used for phytochemicals extraction. Similar results reported that methanolic extract of *Opuntia ficus-barbarica* flowers present a yield of about 23.64% [24], whereas for aqueous extract, it was 29.74 % [19]. Also, aqueous extracts yields obtained by infusion at 80°C that lasted 120 min (19.05%) and decoction for 30min (22.14%) were close to that extracted by maceration using water as solvent for 24h (22.54%). The yield of infusion at 80°C ranged from 12.44% for 5min to 19.05 % for 120min and was higher in comparison to infusion at 40°C which ranged from 7.67% for 15 min to 12.69% for 120min. These results showed that increasing the extraction time and temperature improves the extraction yield. Also, the increasing of water concentration in the solvent enhances the extraction yield. The higher yield observed in the case of aqueous extractions may be due to the presence of compounds other than phenolic constituents. This may be attributable to the higher solubility of proteins and carbohydrates in water.

3.2. Qualitative phytochemical screening

Phytochemical qualitative analysis of aqueous and hydroalcoholic extracts, obtained via different extraction methods of *Opuntia ficus barbarica* flowers, revealed the presence of flavonoids, phenols, proteins, saponins, glycosides and mucilage. Alkaloids and terpenoids were absent in all aqueous extracts, mucilage were not detected in the hydroalcoholic extracts for maceration EtOH 80%. In addition, no sterols were found in any of the tested extracts.

3.3. Phytochemicals compounds

Regarding a general comparison among all preparation methods, the main conclusions are subsequently described and presented in Table 1. Total phenolic content (TPC) of *Opuntia ficus-barbarica* flowers extracts ranged from 113.94 ± 12.39 (M.H_2O) to 184.15 ± 3.06 mg GAE/g (I40°C120') for aqueous extracts and from 189.40 ± 4.23 (M.EtOH50) to 226.66 ± 7.77 mg GAE/g (M.EtOH80) for hydroalcoholic extracts.

Table 1: Yields, total polyphenols and flavonoids of *Opuntia ficus-barbarica* flowers extracts.

Method, T(°C), Solvent	Extraction time	% Yield [a]	Polyphenol content (mg GAE / g extract)	Flavonoid content (mg RE / g extract)
Infusion, 40°C, H_2O	15 min	7.67±1.49 ^{f}	169.74 ± 4.74 $^{c\,d}$	54.99 ± 1.71 ^{c}
	30 min	10.65±0.38 ^{ef}	176.69 ± 3.68 $^{b\,c}$	56.57 ± 2.26 ^{c}
	120 min	12.69±0.86 ^{e}	184.15 ± 3.06 ^{b}	58.56 ± 2.59 ^{c}
Infusion, 80°C, H_2O	5 min	12.44±0.27 ^{e}	158.96 ± 8.07 ^{c}	54.85± 1.27 ^{c}
	30 min	14.67±2.75 ^{cde}	177.92 ± 5.74 $^{b\,c}$	54.27 ± 1.51 ^{c}
	120 min	19.05±1.26 ^{ab}	169.59 ± 8.26 $^{c\,d}$	49.02 ± 1.87 ^{d}
Decoction, 100°C, H_2O	5 min	14.50±0,13 ^{de}	165.65 ± 7.04 $^{c\,d}$	58.27 ± 0.38 ^{c}
	15 min	18.80±1.73 ^{abc}	138.16 ± 1.18 ^{e}	47.68 ± 1.33 ^{d}
	30 min	22.14±1.21 ^{ab}	129.81 ± 6.09 ^{e}	47.19 ± 1.30 ^{d}
Maceration, 25°C, H_2O	24 h	22.54±1.54 ^{a}	113.94 ± 12.39 ^{f}	33.24 ± 4.65 ^{e}
Maceration, 25°C, EtOH50%	24 h	20.36±0.49 ^{ab}	189.40 ± 4.23 ^{b}	69.71 ± 3.73 ^{b}
Maceration, 25°C, EtOH80%	24 h	18.17±0.44 ^{bcd}	226.66 ± 7.77 ^{a}	89.65 ± 1.92 ^{a}

Each value is expressed as mean means ± stand deviation (n = 6×2).

[a] Expressed as 100 × (g dry extract/g dry flowers).

In each column different letters mean significant differences with a $p < 0.05$. They refer to Tukey's post-hoc test; therefore, significant different values were classified using letters in alphabetic order.

Concerning total flavonoids content (TFC), the highest amount was 89.65 ± 1.92 mg RE/g obtained in the case of hydroalcoholic extract using the maceration EtOH 80%. However, TFC for extract prepared by maceration using water was about 33.24 ± 4.65 mg RE/g. In comparison, aqueous extracts obtained by infusion at 40°C for 15, 30 and 120min, infusion at 80 °C for 5 and 30 min and decoction for 5 min showed higher TFC which ranged from 56.57 ± 2.26 mg RE/g to 58.27 ± 0.38 mg RE/g. Similar results were reported by Alimi et al. [18] where phytochemical contents of *Opuntia ficus-barbarica* flowers extracted using 50% methanol solution were 159.76 ± 0.32 mg GAE / g of total phenolic content and 79.51 ± 0.57 mgRE/g of flavonoids. In addition, Ammar et al. [25] reported that methanolic extract (100% of methanol) prepared by soxhlet method presented a greater content of total phenolic than aqueous and hydroalcoholic extracts (270.9 ± 7.2 mg GAE / g of extract). Whereas flavonoids were nearby to aqueous extract but lower in comparison to hydroalcoholic extract M.EtOH80 (60.81 ± 1.3 mgRE/g of extract).

The means of total phenolic and flavonoid contents for hydroalcoholic extracts were found to be comparable to those of aqueous extracts ($P < 0.05$). For infusion assisted extraction at 40˚C that lasted 15min and 30min, total polyphenol and flavonoid contents were approximately equivalent to the phytochemical contents obtained in the case of infusion at 80˚C for 5min and decoction at 100°C for 5min. For decoction assisted extraction at 100˚C, TFC decreased when increasing the reaction time from 5min to 30min. Accelerated autoxidation processes result of temperature can translate this [26]. TFC were lower in extracts obtained at elevated temperatures (80°C and 100°C). It would be advantageous to consume preparations of *Opuntia ficus-barbarica* flowers owing to their rich content in flavonoid compounds which are beneficial to human health. Flavonoids, a class of polyphenol compounds, are responsible for various pharmacological activities [27], such as antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory, and anticancer activities [28].

We also measured polysaccharides content of *Opuntia ficus-barbarica* flowers extracts (fig 1). Results of aqueous extracts showed higher content of polysaccharides compared to hydroalcoholic extracts. Total polysaccharides content varied from 395.5 ± 5.9 mg/g to 888.9 ± 9.1 mg/g. The best results were marked in aqueous extracts prepared at highest temperatures. Polysaccharides are usually present in plants and are well known for their therapeutic benefits [29]. In recent years, polysaccharides have received widespread attention owing to its remarkable pharmacological activities including Anti-colon-cancer [30], anti-tumor [31], immune-regulation [32], hypoglycemic activity [33], antibacterial effect [34] and antioxidant activity [35]. This suggests that extracts therapeutic properties does not depend only on its total phenolic content, but also on the presence of polysaccharides. In addition, crude extracts contain other components that may have positive or negative effects due to their interaction with phenolic compounds which influence biological activities [36].

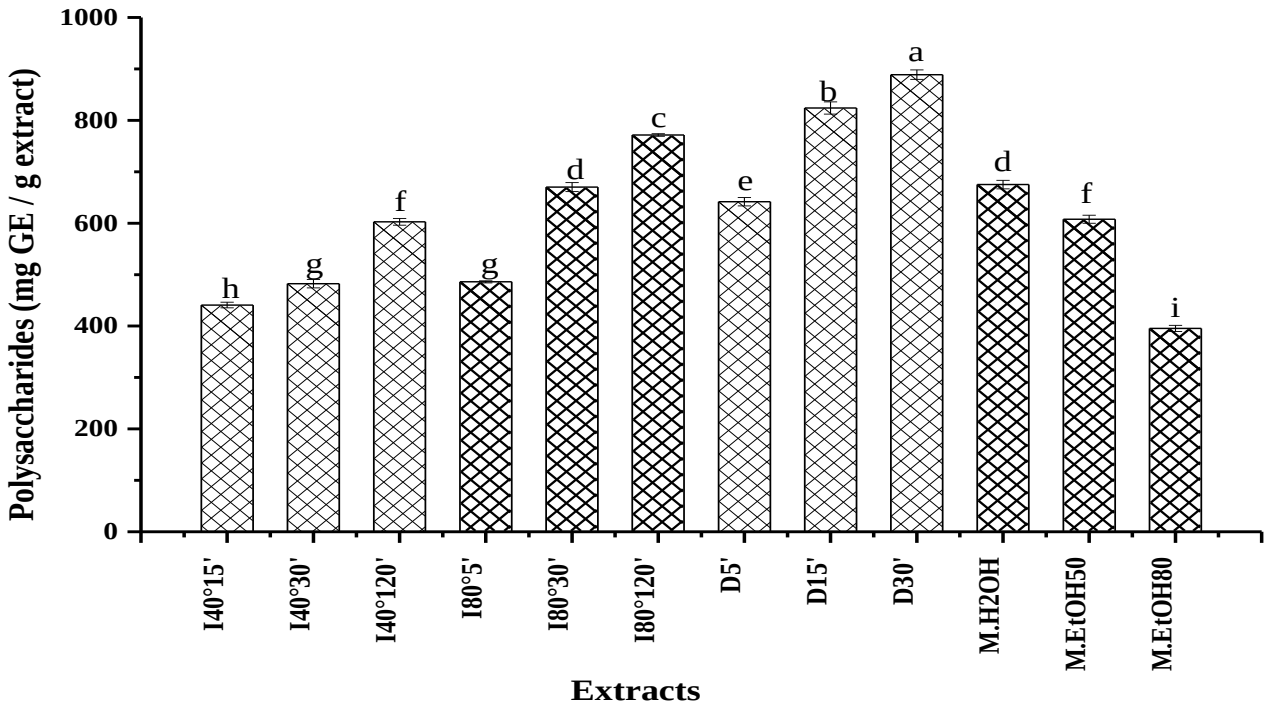

Fig1. Polysaccharide content of *Opuntia ficus-barbarica* flower extracts. Different letters indicate statistical differences according to Tukey's post-hoc test ($P < 0.05$). Each value is expressed as mean means ± stand deviation ($n = 6 \times 2$).

3.4. Antioxidant activity

Many investigations have reported the potent antioxidant properties of *Opuntia ficus-barbarica* flowers owing to its rich content in phenolic compounds and flavonoids that are well known for their antioxidant activities. In the present study, we evaluated the antioxidant activity of different extracts of *Opuntia ficus-barbarica* flowers, obtained via different traditional preparations. Herein, most of the extracts showed high antioxidant activity, including free radicals scavenging activity, β-carotene bleaching inhibition and reducing power. The hydroalcoholic extract prepared using the maceration EtOH 80% exhibited the most important activity. Results of EC_{50} values of antioxidant activities are presented in Table 2. The sequence for DPPH radical-scavenging capacities of *Opuntia ficus-barbarica* flowers extracts have been ranked as follows: hydroalcoholic extracts at 25°C > Hot water extracts > water extract at 25 °C. At 350 µg /ml, scavenging abilities of DPPH radicals were 89.9%, 83.5%, 75.6%, 74.6%, 60.4%, 43.9% for M.EtOH80, M.EtOH50, I40°120', I80°30', D5', M.H2O, respectively (Fig. 2). Results presented in Table 2 show that EC50 values for aqueous extracts ranged from 215.7 to 403.9 µg /mL and its values for hydroalcoholic extracts were 76.8 and 124.1 µg /mL for M.EtOH80 and M.EtOH50, respectively.

Table 2: EC_{50} values of antioxidant activities of *Opuntia ficus-barbarica* flowers extracts.

Method, T(°C), Solvent	Extraction time	EC_{50}[a] of DPPH radical-scavenging activity (µg /mL)	EC_{50}[b] of β-carotene bleaching inhibition (µg /mL)	EC_{50}[c] of reducing power (µg /mL)
Infusion, 40 °C, H_2O	15 min	277.0 ± 1.9 [f]	325.3 ± 3.2 [d]	475.7 ± 18.1 [c]
	30 min	254.5 ± 3.0 [e]	284.9 ± 13.5 [c]	468.4 ± 15.3 [c]
	120 min	215.7 ± 1.2 [c]	264.8 ± 1.4 [c]	466.1 ± 20,7 [c]
Infusion, 80 °C, H_2O	5 min	272.6 ± 2.3 [f]	327.6 ± 14.9 [d]	457.7 ± 5.4 [c]
	30 min	240.8 ± 4.1 [d]	274.1 ± 3.5 [c]	458.7 ± 27.2 [c]
	120 min	317.1 ± 7.8 [h]	384.4 ±8.6 [e]	468.7 ± 17.0 [c]
Decoction, 100 °C, H_2O	5 min	294.1± 1.8 [g]	345.7 ± 1.1 [d]	464.1 ± 13.3 [c]
	15 min	337.5 ± 6.1 [i]	484.6 ± 7.7 [f]	490.6 ± 8.7 [c]
	30 min	380.0 ± 3.2 [j]	487.7 ± 4.3 [f]	503.4 ± 5.3 [c]
Maceration, 25 °C, H_2O	24 h	403.9 ± 9.9 [k]	511.5 ± 16.1 [g]	567.6 ± 41.8 [d]
Maceration, 25 °C, EtOH50%	24 h	124.1 ± 4.8 [b]	209.5 ± 4.7 [b]	355.5 ± 29.9 [b]
Maceration, 25 °C, EtOH80%	24 h	76.8 ± 1.6 [a]	165.2 ± 1.7 [a]	321.6 ± 18.6 [a]
Trolox		3.2 ± 0.3	8.3 ± 0.3	34.6 ± 0.9

[a] EC50 means the effective concentration of sample that can decrease DPPH concentration by 50%.
[b] EC50 means the effective concentration of sample that can inhibit the peroxidation of β-carotene linoleic acid by 50%
[c] EC50 is the concentration for which the absorbance at 700 nm is 0.5.

In each column different letters mean significant differences with a $p < 0.05$. They refer to Tukey's post-hoc test, therefore, significant different values were classified using letters in alphabetic order.

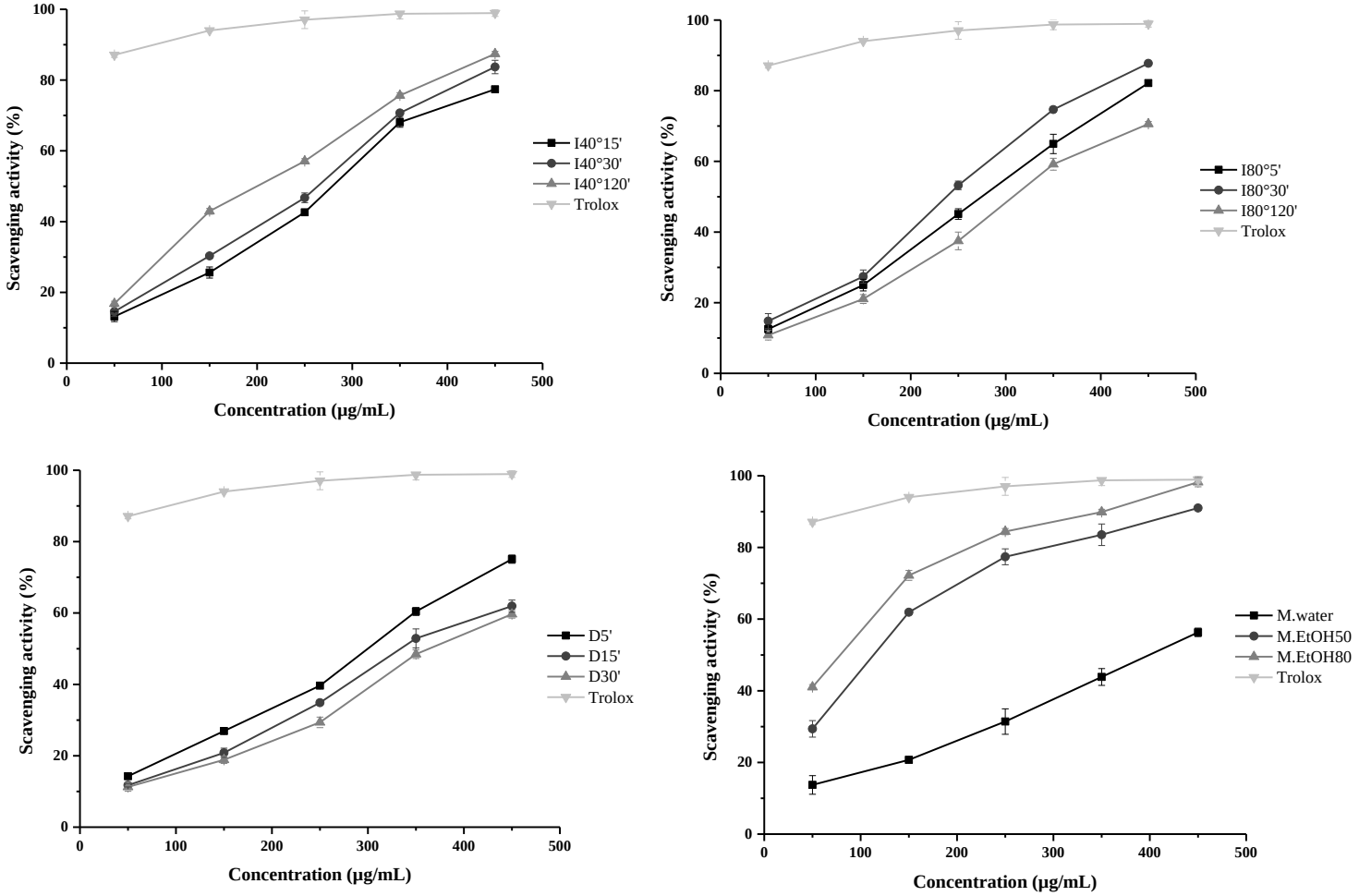

Fig 2. DPPH radicals scavenging activity of *Opuntia ficus-barbarica* flowers extracts prepared by various methods at different concentrations. Values are mean ± standard deviation (n = 3).

For β-carotene bleaching assay, Fig. 3 shows that the antioxidant activity of samples can be ranked as follow: M.EtOH80 > M.EtOH50 > I40°120' > I80°30' > I40°30' > I40°15' > I80°5' > D 5' > I80°120' > D15' > D30' > M.H_2O. At 350 µg /mL, β-carotene bleaching inhibitions were 35.7 %, 68.7 % and 81.3 % for M.H2O, M.EtOH50 and M.EtOH80, respectively, 53.1 %, 60.1 % and 65.4 % for I40°15', I40°30' and I40°120', respectively, 53.3%, 61.9% and 46.7% for I80°5', I80°30' and I80°120', respectively, and 50,5%, 38.32%, and 37.6% for D5', D15' and D30', respectively. For aqueous extractions, the best activities were obtained for samples prepared by infusion at 40°C for 120 min (I40°120'), infusion at 80°C for 30 min (I80°30') and decoction at 100 °C for 5min (D5'). At 450 µg /mL, the inhibition has increased to 85.8% and 96.4% for the hydroalcoholic extracts M.EtOH50 and M.EtOH80, respectively. For aqueous extracts, it was 80.3%, 76.9% and 61.7% for I40°120', I80°30' and D5', respectively. Results of β-carotene bleaching assay have shown that hydroalcoholic extracts have the highest activity with EC_{50} values of 165.2 and 209.5µg /mL for M.EtOH80 and M.EtOH50.

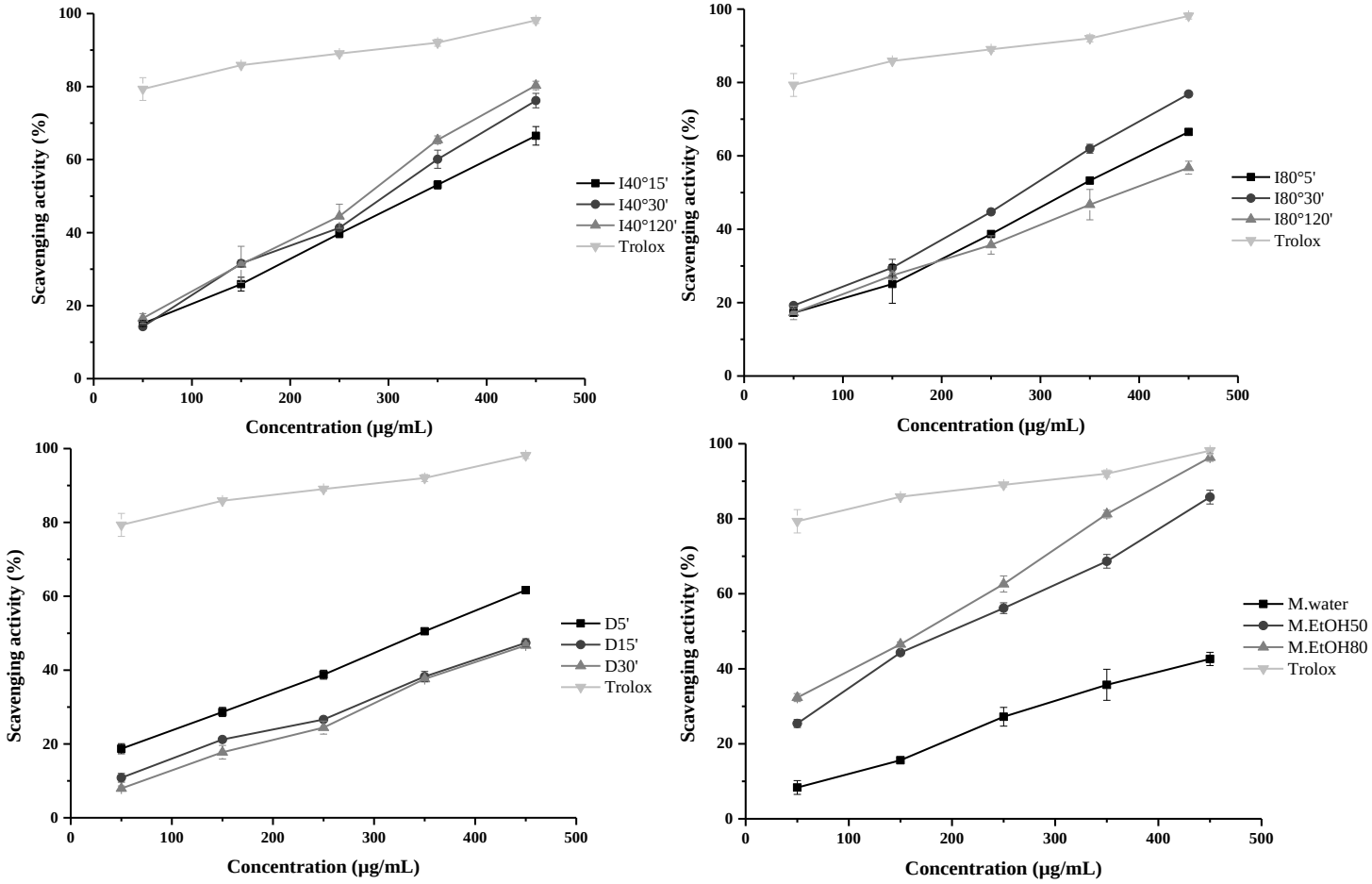

Fig 3. β-carotene bleaching inhibition activity of *Opuntia ficus-barbarica* flowers extracts prepared by various methods at different concentrations. Values are mean ± standard deviation (n = 3).

Fig. 4 illustrates that the absorbance increase when increasing extract concentration. In fact, higher absorbance values indicate stronger reducing capacity. M.EtOH80 extract exhibited the highest reducing power while M.H_2O extract exhibited the lowest. The sequence for reducing power was as follow: M.EtOH80 > M.EtOH50 > I80°5' > I80°30' > D 5' > I40°120' > I40°30' > I40°15' > I80°120' > D15' > D30' > M.H_2O.

For M.EtOH80 and M.EtOH50, EC_{50} values of reducing power were 321.6 and 355.5 µg/mL, respectively, and those for I80°5', I80°30', D 5', I40°120', I40°30', I40°15', I80°120', D15', D30' and M.H_2O EC_{50} values were 457.7, 458.5, 464.1, 466.1, 468.4, 468.7, 475.7, 490.6, 503.4, 567.6 µg/mL, respectively.

The results of antioxidant assays of the different extracts were compared and correlated to their phytochemicals content. The analysis showed a strong correlation between total phenolic contents and EC_{50} values of β–carotene blenching inhibition (r^2 = 0.910), EC_{50} values of DPPH radical-scavenging activity (r^2 = 0.885) and

EC_{50} values of reducing power (r^2 = 0.794). Likewise, total flavonoid contents showed a good correlation with EC_{50} values of reducing power (r^2 = 0.922) and EC_{50} values of DPPH radical-scavenging activity (r^2 = 0.884). However, total flavonoid contents were weakly correlated to EC_{50} values of β–carotene blenching inhibition (r^2 = 0.753). In addition, there was a close correlation between total phenolic and total flavonoid contents (r^2 = 0.850). Whereas, Ammar et al. [25] have found no direct correlation between total phenolic content and antioxidant activity of *Opuntia ficus-barbarica* flowers extracts which was attributed to the presence of many other components that also contribute to antioxidant activity. The different extracts obtained using different organic solvents and methods contain minor compounds that could have a major impact on antioxidant activity [25]. In our case, polysaccharides and phenolic extracts were separately extracted. Thus, our results showed that phenolic components play an important role in the antioxidant capacity of *Opuntia ficus-barbarica* flowers extracts.

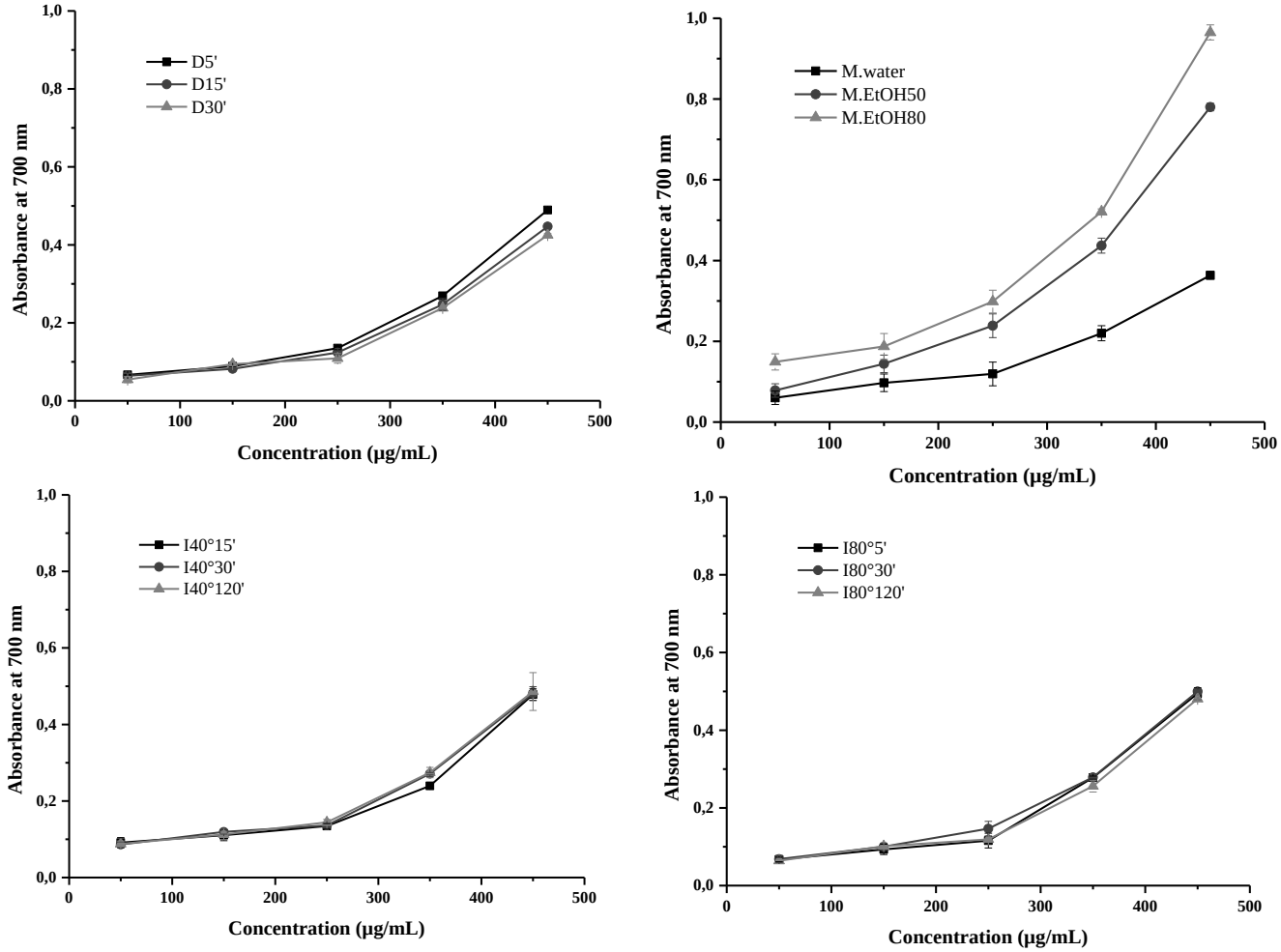

Fig 4. Reducing power of *Opuntia ficus-barbarica* flowers extracts prepared by various methods at different concentrations. Values are mean ± standard deviation (n = 3)

3.5. **Comparison between conventional extraction methods**

Many investigations have outlined the potent therapeutic properties of *opuntia ficus-barbarica* flowers. It is one of the most potential sources of natural bioactive molecules. The main objectives of this study were to compare phytochemicals extraction efficiency of various extraction procedures used to consume this flower and to evaluate its nutritional values. In this work, extracts prepared by infusion, decoction and maceration from *Opuntia ficus-barbarica* flowers were analyzed with respect to their content in phytochemicals and their antioxidant activities. Among all extract samples, hydroalcoholic extract prepared by maceration EtOH80% showed the highest content of phenolic compounds and the strongest antioxidant activities. For aqueous extracts, the extraction by infusion 40˚C for 120 min, infusion 80˚C for 30 min and decoction for 5 min were the best natural extraction techniques. Whereas, the maceration extraction using H2O at 25˚C that lasted 24 hours produces the lowest content of phenolic compounds and a weak antioxidant activity.

Aqueous extraction prepared by decoctions provides the highest polysaccharides content, which justify their viscose appearance. The choice of the extraction method depends on the types of metabolites expected to be extracted from plant materials. High contents of phenolic and flavonoid compounds were obtained for hydroalcoholic extractions at room temperature and also for aqueous extractions conducted at high temperatures in short durations. Whereas aqueous techniques, conducted at high temperatures and long duration, were characterized by a high content of polysaccharides.

In previous research, methanolic extract of Tunisian *Opuntia ficus-barbarica* flowers exhibited in vivo anti-inflammatory [37], anti-ulcerogenic [24] and wound healing effects [38] and in vitro antioxidant and anti-microbial activities [38; 39]. Similarly, Benayad et al. [40] reported the high antioxidant and anti-inflammatory activities of *Opuntia ficus-barbarica* flowers growing in the north of Morocco. *Opuntia ficus-barbarica* flowers extracts contain a significant amount of phytochemicals bioactive which include isorhamnetin glycosides as the major phenolic compounds, followed by quercetin and kaempferol glycosides in both methanolic and aqueous extracts of *Opuntia Ficus-barbarica* flowers grown in Italy, Tunisia and Morocco [41; 40; 42; 13]. In addition, a number of phytochemical studies have demonstrated that the flower contains several chemical constituents and is an important source of natural antioxidants and nutraceuticals. Recent studies highlight the presence of sugars (fructose, glucose, sucrose and trehalose), organic acids (Oxalic, quinic, malic, and citric acids), fatty acids (caprylic acid, capric acid, tridecanoic acid, myristoleic acid, eicosatrienoic acid plus heneicosanoic acid and tricosanoic acid) [43; 44] and also is a good source of minerals with the predominance of Sodium (Na) and potassium (K) [8; 41]. All these results indicate that natural extracts of *Opuntia ficus-barbarica* flowers contain a wide variety of chemical compounds to which antioxidant capacities could be attributed.

Conclusion

This work presented the impact of various extraction methods and conditions (maceration using different solvents, infusion at 40˚C or 80˚C and decoction at 100˚C) on extract yield, phytochemical constituents and antioxidant activity to determine the optimal extraction technique. Phytochemical screening revealed the presence of flavonoids, phenols, proteins, saponins, glycosides and mucilage condensed in our extracts. Quantitative study showed significant variation in polyphenols, flavonoids and polysaccharides composition according to their extraction methods. Results showed that phenolic compounds present higher content in hydroalcoholic extract. Whereas, aqueous extracts are richer in polysaccharides. The extraction at elevated temperatures enhances polysaccharides content but could lead to the degradation of phenolic constituents, especially, at long duration. The antioxidant activity of *Opuntia ficus-barbarica* flowers extracts, determined by free radicals scavenging activity, β-carotene bleaching inhibition and reducing power, showed that hydroalcoholic extracts present the highest activities. Aqueous extracts obtained at high temperatures and short durations show important antioxidant activities, suggesting that they are the best effective traditional methods for extraction of antioxidants from *Opuntia ficus-barbarica* flowers. Indeed, natural extracts of *Opuntia ficus-barbarica* flowers contain a wide variety of chemical compounds to which antioxidant capacities are attributed. Also, therapeutic quality of this flower is mainly related to the efficacy and selectivity of the extraction method. This study suggests that tisanes prepared via the infusion and the decoction from *Opuntia ficus-barbarica* flower could be further explored as beneficial health ingredients to be used, for example, in innovative herbal beverages.

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