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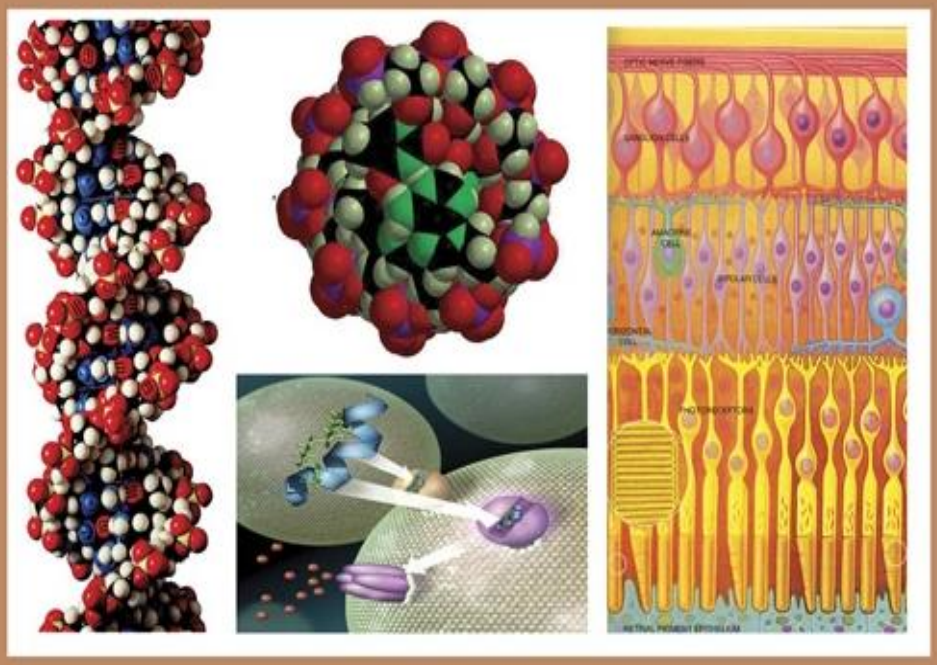

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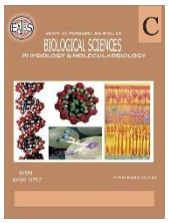

Polyphenol Content, Antioxidant and Antibacterial Activity of The Aqueous Extract of *Opuntia ficus-indica* Cladodes.

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ABSTRACT

In this study, the total phenolic levels, antioxidant and antibacterial activities of Claladods from *Opuntia ficus indica* extracts originating from Saida in Algeria were determined. The phenolic components of these extracts were also determined using HPLC. Total phenolic levels ranged from 10.75 to 1.80 mg g EC/mg E; the total flavonoid components of the extract ranged from 7.99 to 1.31 g EC/mg E. Antioxidant activity of the aqueous extract was increased according to FRAPP, CE50 scored 750 2.1 (g/ml) and free radical DPPH antiradicular activity was scored CI50 (18724.3 g/ml). The phenolic compounds identified by HPLC were acid cinapine ascorbic acid nicotamide tannic acid caffein vanillin kaempherol quercetin myirecetinepicatechin/catechin and five unknown compounds. All of the plants studied contain kaempherol (41%), epicatechin/catechin (14.5%), quercetin (12.9%), myrecetin (8, 9%), Vaniline (7.8%), Ascorbic Acid (3.1%), Nicotamide (2.7%), Tannic Acid (0.4%), Caffeine (0.1%), Cinapic Acid (0.1 %). the aqueous extract has significant antioxidant activities in vitro, the results indicated that the OFI could be attributed to a potential source of natural antioxidant for food applications, The antibacterial activity of *Opuntia ficus* were estimated towards multiple types of bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Bacillus Subtilis*). The plant shows high activity against all types of bacteria. The obtained results of the antibacterial application inhibitors seem to be quite promising material in the antibacterial fields.

INTRODUCTION

Nature has long served as a crucial source of therapeutics. Plant-derived substances with biomedical relevance have gained great popularity and access to the international market as safer and more effective synthetic ingredients of pharmaceutical and cosmetic products, which are considered fraught with side effects and possible toxic interactions. Currently, a third to almost half of all available drugs are derived from plants or other natural sources (Al Adawi *et al*, 2013); Polyphenols have received enormous attention as pharmaceutical and cosmetic active ingredients. It is a very diverse group of natural phenolic compounds that includes several subgroups: phenolic acids, flavonoids (isoflavones, neoflavonoids, chalcones, flavones, flavanols, flavanones, flavanonols, flavanols, proanthocyanidins, anthocyanidins), polyphenolic amides, and non-flavonoid polyphenols (e.g., resveratrol, curcumin) (Tsao, 2010).

Numerous recent studies illustrate their relevance in the avoidance of the degenerative diseases (cancer, cardiovascular and other diseases, etc.) related to oxidative stress triggered by reactive Properties and Biomedical Relevance of Phytosome … 23 oxygen and nitrogen species (Semalty Et Al 2007; Khan Et El, 2013; Quiñones, 2013; Higdon, 2010). The potent antioxidant activity of polyphenols has been demonstrated in vitro and in vivo and has been attributed to the neutralization of free radicals by donating an electron or a hydrogen atom, thereby suppressing free radical production and reducing the frequency of oxidation by inhibiting free radical formation or deactivation reducing active species and precursors of free radicals or by neutralizing the free radicals, stopping the chain reactions(Tangney, 2013).In traditional medicine, *Opuntia ficus indica* cladodes extract is used for its healing and antiulcer properties (Park, H., 2002; Galati, E. M.,2009). In recent years, *Opuntia ficus cladodes indica* has attracted attention in the scientific community for its phytochemicals, phenols and flavonoids, which exhibit antioxidant (Santos-Zea,2011) and anti-inflammatory properties (Schepetkin, 2003; Galati, 2008), neuroprotective (Dok-Go, 2003) antiviral (Ahmad,1996) antiulcer (Galati, E. M.,2007), antigenotoxic (Zaied,2009) And hypotensive (Saleem, 2005). Cladodes are traditionally used as popular medicines due to their antioxidant, apoptotic, antibacterial and antifungal effects (López-Romero, 2014; Laura, A., 2014; Miller,2014), and as antibacterial inhibitors. The extract has antibacterial property and is a natural antibacterial agent.

MATERIALS AND METHODS

1 Sample Preparation:

Prickly Pear Samples (*Opuntia ficus-indica (L.) Mill*.):

The rackets and roots of the prickly pear variety Aissainerme were taken from a field located at Djabara, a small commune of Djbara Wilaya of Saida de (Algeria). These samples were placed in plastic bags and immediately placed in a cooler. Brought back to the laboratory, these samples were then rinsed and dried with blotting paper and put in a refrigerator until use. A 200 g quantity of each fresh segment of the prickly pear cladode specimens was washed with drinking water, then cleaned with a 10% sodium hypochlorite solution and with distilled water. The samples were cut into minute pieces and dried in an oven at a temperature of 50°C for 72 hours, then ground using a grinder. The different samples were macerated separately, with stirring, in four different solvents: 100% ethanol, 100% methanol, distilled water and in a hydroalcoholic solution (ethanol-water; v/v) at 10 g of dry substance type with 100 ml of each solvent (10th g/100ml). After 48 hours the macerates were filtered with a 0.75 mm sieve. The homogenates obtained were then centrifuged at 12,000 g for 20 min at a temperature of 4°C. The supernatants of the subsequently obtained extracts were evaporated at a temperature of 40°C using a rotary evaporator to remove ethanol and methanol. The extracts thus obtained were stored at -4°C until used.

2. Determination of Total Phenols:

Quantitation of total phenolic compounds was performed utilising the Folin-Ciocalteau method, this is the typical protocol followed in such an experiment. To measure phenolic acids, 1 ml of Folin-Ciocalteau's reagent diluted 10-fold in distilled water was added to 1 ml of each extract. After 4 minutes, 8 ml of a sodium carbonate solution (75 g/l) is added. After shaking and incubation for 2 hours protected from light, the absorbance was quantified with a UV spectrophotometer at a length of 765 nm. Total phenol levels are determined using the linear regression equation ($y=0.003X+0.08$ with $r2=0.998$) established with gallic acid calibration ranges (0-200 mg/L) and are expressed in micrograms of gallic acid equivalent per milligram of extract (mg EAG/g).

3. Determination of Total Flavonols (TF):

The TF values were quantified

using a modified colorimetric method. The extracts (0.5 ml) were placed in a test tube containing 0.5 ml of a 60% ethanol solution. Sodium nitrite solution (5%, 0.15 mL) was added to the mixture and reacted for 5 min, followed by the addition of 0.3 mL of 10% aluminum chloride. After 5 min, 1 ml of 1 mol/l sodium hydroxide was added. The absorbance of the mixture was quantified at 510 nm. Quercetin was adopted as the standard. The results were expressed as the equivalent of milligrams of rutin per 100 g dry weight (mg RE/100 g DW).

4. Analysis of Phenolic Compounds by HPLC:

The identification of cladode powder polyphenols was determined by HPLC analysis at the OpenLDAP laboratory in Tlemcen in 2021. After diluting the cladode powder in water and methanol and at the end we took the preparation 10 mg extract + 10 ml ultrapure water which gave the result shown in Table 3.

5. Ferric Reducing Ability Assay (FRAP):

The FRAP test was done according to the method described by Benzie and Strain (1996). First, three stock solutions were prepared acetate buffer (300 mM, pH 3.6), anhydrous FeCl3 solution (20 mM) and 10 mm TPTZ (2,4,6-tripyridyl-s-triazine) solution obtained by dissolving 46.85 mg of the TPTZ reagent in 15 ml of 40 mM hydrochloric acid. The working solution (FRAP reagent) was freshly prepared by mixing the acetate buffer, TPTZ solution and FeCl3 in 10:1:1 ratio, respectively and incubating for 20 min at 37 °C. A volume of 10 µl of extract or ascorbic acid was added to 240 µl of the FRAP reagent and incubated for the reaction at 37 °C for 30 min and in the dark, and the absorbance of the coloured product (tripyridyltriazine ferrous complex) was measured at 593 nm against the blank using a microplate reader. The results were expressed in mg ascorbic acid equivalent/g of dry extract (mg AAE/g extract).

Free Radical Scavenging by the DPP Test:

The assessment of the antiradical activity by the DPPH free radical was carried out according to the technique of (Brand-Williams, 1995) It is a method that uses DPPH as a relatively stable free radical, purple in solution and with a characteristic absorption maximum at 517 nm. The protocol applied routinely is based on the disappearance of this maximum when the DPPH is reduced by a compound with an antiradical property, thus causing discoloration from purple to yellow. A volume of 1mL of both extracts plus an equal volume of a methanolic solution of DPPH (0.1 mM) is incubated (30 min). The absorbance at 517 nm was recorded.

Antibacterial Activity:

The antibacterial activity of the extracts was evaluated towards different types of bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphy- lococcus aureus*, and *Bacillus subtilis*) using the very method presented by (Mokhtar, 2020). 0.5 ml of cultures were diluted in 20 ml of Muller Hinton liquid. The latter, apparently solid, is melted by heating and then cooled before putting in contact with the microbial suspension. The mixture was placed in Petri dishes of 90 mm. Using a Pasteur pipette, wells of 7 mm diameter are dug in Muller Hinton agar and placed into sterile Petri plates. 30 mg of the extract was put in the wells. The antibacterial activity is determined after incubation of the dishes in an oven at 37 °C for 24 h. The inhibition zone around the spots was observed visually.

RESULTS AND DISCUSSION

Total Phenolic Content (TP) and Total Flavonoids (TF) Content:

The polyphenolic compounds are part of a group of secondary metabolites with high antioxidant capacity and occur naturally in most edible and inedible plants. The TP of the four extracts from *Opuntiaficus indica* is shown in Table 1.

Table 1: Total polyphenol result in *Opuntia ficus indica*

Samples	Methanolic extract	aqueous extract	Ethanolic extract 100%	Ethanolic extract 50%
µg EC /mg E	3,55	10,75	1,80	3,51

The highest and lowest TP levels were achieved in aqueous extract and ethanolic extract 10,75%, 1,80% respectively, 3,55% in Methanolic extract and 3,51% in the hydro-ethanolic extract. In reality, the greatest value of TP has been observed in aqueous extract. In fact, our results indicate that the vast majority of polyphenols are water-soluble. In order, to obtain fractions rich in polyphenols, it is therefore used mixtures of the appropriate organic solvent with water such as e.g., Ethanol extract 50% (v/v). The addition of the water to organic solvents increases the solubility of polyphenols. This increase may be due to the weakening of hydrogen bonds in aqueous solutions. It could also be due to the increase in basicity and ionization of polyphenols in such solutions. The solubility of polyphenols depends mainly on the number of hydroxyl groups, the molecular weight, and the length of the carbon chain in the basic skeleton. The results we obtained are lower than those of (Boutakiout,2015). And approximate to those found by (Benattia, F, 2017).

Flavonoids have emerged as an important class of secondary metabolites with a wide range of biological activities. Antioxidant, anti-mutagenic, anti-carcinogenic, anti-proliferative and anti-inflammatories are just to name a few. The highest value of FT was observed in the methanolic extract 7, 99% in Fig. 1 and decreased in the order of aqueous extract 7, 52%, 1, 32%ethanolic extract and finally 1,31 in the hydroethanolic extract.

The flavonoid content in prickly pear pulp has been reported to be 0.98 ± 3.0 mg/100g (KUTI, Joseph O,2004). . In grape juice, deemed to be the richest in flavonoids, its content reaches 7.24 mg / 100 mL (Dos Santos Lima, 2014). Therefore, our aqueous and hydroethanolic cladode extracts contain a higher amount of flavonoids.

Statistically, the difference between the flavonoid contents of the extracts is significant ($p < 0.001$). Our results are close to the values reported by (Chougui, 2013), for the different Opuntia extracts, this difference is probably explained by the difference in the standard used for the flavonoid assay. However, it is difficult to compare these results with those of the bibliography because the use of different extraction methods reduces the reliability of comparisons between studies. Table 2.

Table 2: Total flavonoids result in *Opuntia ficus indica*

Sample	Methanolic extract	aqueous extract	Ethanolic extract 100%	Ethanolic extract 50%
[ ] µg EC /mg E	7,99	7,52	1,32	1,31

1. DPPH and FRAP Test:

The antioxidant activity of certain foods is based on the content of a combination of antioxidants with various means of action, for this reason, it is vital to combine various methods to determine the antioxidant capacity in vitro. Table 2 shows the scavenging effect of *Opuntia ficus indica* aqueous extract on DPPH and FRAPS free radicals. The IC50 was used to compare the anti-radical activity of the aqueous extract. The IC50 value shows the IC50 value (0,187±0,024 mg/mL) and (187±24,3 µg/ml have the highest anti-free radical activity

against DPPH free radical. This value was significantly higher (p b 0.05). In the case of Ferric reducing ability assay (FRAP) the EC50 value varied significantly (p b 0.05) and was 0, 75 ± 0, 0021 mg/ml and750 ± 2, 1 µg/mL. We note that the FRAP test compared to the DPPH test gave more interesting results in this study, suggesting that the antioxidants present in this extract exert a strong antioxidant rather than scavenging activity. Similar weak scavenging activity of *Opuntiafius indica*juce was reported by (Boutakiout, 2015) due to the presence of a large amount of compounds for which no powerful antioxidant activities were established in the literature (Fig. 1).

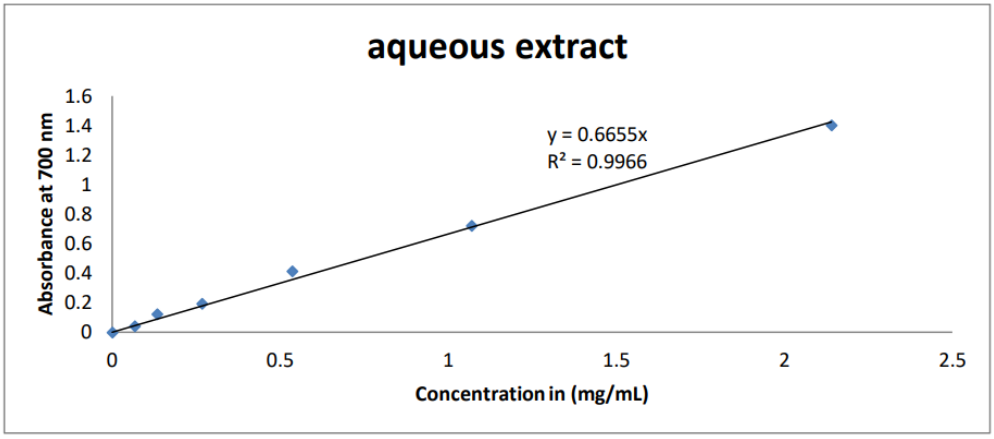

Fig 1. Antioxidant activity of aqueous extract from *Opuntiaficus indica* cladodes by Ferric reduction antioxidant power (FRAP).

2. Results of Chromatographic Analysis by HPLC:

Opuntia ficus-indica is an active medicinal plant and a natural remedy due to it remarkable chemical polymorphism, the extract analyzed by HPLC is the aqueous extract that represents a significant quantification of the phenolic compounds detected by the "colorimetric analysis. The moving phase used for the quantitative analysis of our extracts is a mixture of solvents. In the HPLC analysis, 15 pure phenolic compounds were used as standards for their retention times (Tr) which are given in Table (3).

Table 3: Result of the chromatographic HPLC analysis of *Opuntia ficus indica*.

	Reten. Time	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1,700	325,663	4,193	3,2	3,2	1,65	Unknown
2	2,967	9,227	0,781	0,1	0,6	0,26	Acidcinapic
3	4,143	300,882	7,845	3,0	6,0	0,48	Ascorbicacid
4	4,637	271,855	7,288	2,7	5,6	0,71	Nicotamide
5	5,290	282,006	6,151	2,8	4,7	0,68	Unknown
6	7,530	104,201	2,513	1,0	1,9	0,42	unknown
7	9,127	33,849	1,285	0,3	1,0	0,39	Unknown
8	10,847	41,501	0,557	0,4	0,4	0,66	Tannicacid
9	14,460	5,402	0,299	0,1	0,2	0,31	Cafein
10	14,930	7,314	0,311	0,1	0,2	0,43	Unknown
11	16,227	10,851	0,247	0,1	0,2	0,51	Unknown
12	18,113	789,574	15,499	7,8	11,9	0,83	Unknown
13	20,087	4263,627	18,505	41,9	14,2	4,28	Vanilline
14	27,900	79,126	0,860	0,8	0,7	1,63	Kaempherol
15	28,943	1308,478	22,579	12,9	17,4	1,12	Quercetine
16	30,007	858,068	16,778	8,4	12,9	0,91	Myirecetine
17	30,967	1486,317	24,348	14,6	18,7	0,73	Epicatechine/Catechine
	Total	10177,942	130,039	100,0	100,0		

In *Opuntia ficus-indica* cladode juice, 14 compounds have been quantified and are ranked in descending order as follows: Kaempherol(41%),epicatechine/catechine(14 ,5%), quercetine(12,9%), myrecetine(8,9%), vaniline(7,8%), acideascorbique(3,1%), nicotamide(2,7),tannic acid (0,4%), cafeine(0,1%),acid cinapic(0,1%).

Table 4: Calcule of CI_{50} et CE_{50}(*CI_{50}: Inhibitory concentration at 50%,*CE_{50}: Effective concentration at 50%, * Mean ± standard deviation)

	DPPH		FRAP	
Aqueous extract	CI_{50} (mg/mL)	CI_{50} (µg/mL)	CE_{50} (mg/mL)	CE_{50} (µg/mL)
	0,187±0,024	187±24,3	0,75 ± 0,0021	750 ± 2,1

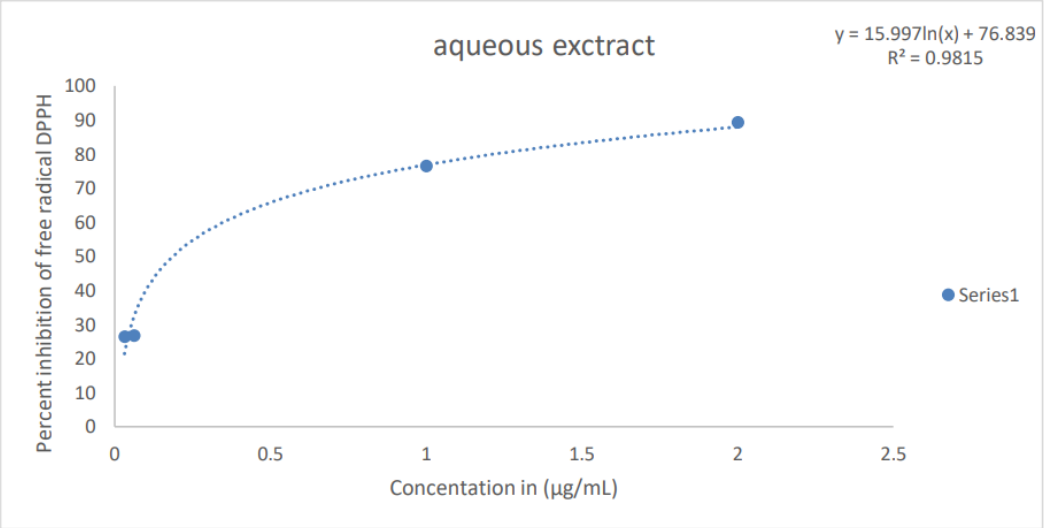

Fig 2. The concentration effect on the scavenging activity levels of aqueous extract from *Opuntia ficus indica* cladodes against DPPH radical.

Studies conducted on prickly pear cladodes have shown the presence of different ingredients and compounds: gallic acid ((0,64 – 2,37 µg /g), coumaric acid (14,08 – 16,18µg /g), -dihydroxybenzoic acid (0,06 – 5,02µg /g), 4-hydroxybenzoic (0,5 – 4,72 µg /g), ferulic acid (0,56 – 34,77 g /g), salicylic acid (0,58 – 3,54 µg /g), is quercetin (2,29 – 39,67 µ µg /g), isorhamnetin 3-O-glucoside (4,59 – 32,21 µg/g), nicotiflorin (2,89 – 146,5 µg/g), rutin (2,36 – 26,17 µg/g) and narcissin (14,69 – 137,1 µg/g) [29]Being in this cladode age, environment, soil type and climate could illustrate these discrepancies in phenolic content of cladode juice (Boutakiout,2015). Cladode juice extracted from spiny cladodes is richer in phenolic compounds compared to spineless cladodes.

Referring to kaempferol as an essential natural compound present in numerous plants and vegetables such as cabbage, broccoli, tea and beans (Agarwal,2019), kaempferol has anti-myloidogenic activity, chelates metals and controls oxidative stress (Simunkova , 2019) and has also been shown to inhibit platelet aggregation and thrombosis and have interesting effects on vascular forms of cognitive decline (Choi, 2015). How the resulting Kaeampherol is more protective against cognitive decline than other major flavonoid compounds (myricetin and quercetin) (Root, 2015) and have shown beneficial effects in experimental models of Alzheimer's disease (Beg, 2018).

In addition, several studies, such as (Ginestra,2009), have confirmed the presence of high concentrations of kaeampforol in Cladodes *Opuntia Ficus Indica*, while high-performance liquid chromatography (HPLC) analysis mainly showed the presence of kaempferol (38, 8%) and the quercetin (21,1%). another study showed the existence of Kaempferol and quercetin in the whole fruit, pulp and peel of 6 Mexican and Spanish prickly pear (*Opuntia ficus indica l. Mill*) phenolic acid, piscidic acid. The highest phenolic content was observed in Spanish Morada cultivar (49,012 µg/g dry peel); (García-Cayuela, 2019). The Antibacterial Activity of The Aqueus Extract:

The antibacterial activities of our extract were achieved by gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa)*, and gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis)* bacteria. The inhibition zones are shown in Table 5 .

Table 5: Antibacterial test results Inhibition zone size (in mm)

	E. coli (a)	*S. aureus* (b)	*E. faecalis* (c)	*B. Subtilis* (d)	*P. Aeruginosa* (e)
Aqueous Extract	17	16	19	24	32

Conclusions

In summary, due to the richness of *Opuntia ficus indica* extracts in flavonoids, phenolic compounds and antioxidant activity in vitro, these results using the prickly pear as an alternative forage crop can be exploited, given its interest and importance in both human and animal nutrition and, in relation to nutrition, agro-industrial conversion through the techniques for valorization, extraction and use of products based on this type in the fields of nutrition, medicine and cosmetics. In addition, studies are also underway to evaluate the unknown bioactive compounds *of Opuntia ficus indica*. our extract exhibited good antibacterial activity against both gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*) bacteria. it could be an efficacious antibacterial agent used in different fields.

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