International Journal of Agricultural Policy and Research Vol.8 (1), pp. 1-10, February 2020
Available online at https://www.journalissues.org/IJAPR/
https://doi.org/10.15739/IJAPR.20.001i

ISSN 2350-1561

Original Research Article

Nutritive value and chemical composition of prickly pear seeds (*Opuntia ficus indica L.*) growing in Egypt

Received 17 October, 2019 | Revised 27 November,2019 | Accepted 8 December,2019 | Published 20 January, 2020

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The objective of this study was to determine Antimicrobial activity of methanolic extract of prickly pear seeds powder against (*Salmonella, Escherichia coli, Bacillus subtilis, Bacillus cereus,* yeast and mycotoxins metabolites).Prickly pear seed *Opuntia ficus-indica* (L.) was analyzed for its chemical and nutritional content. The analysis included those for: moisture, crude lipid, crude protein, ash, and crude fiber, phenols, antioxidants, saponine, flavonoids) and mineral contents (Fe, Cu, Zn and Se).Results illustrated that, mannan and β- glucan, content 153.44 and 69.20g/ Kg, respectively. Minerals determined of Fe, Zn, Cu and Se were: 127.20, 152.90 and 1.29 mg/kg and 24.76 ppb, respectively. Linoleic acid was established as the major fatty acid (54.03%), followed by oleic (22.41%) and palmitic (17.11%) acids. Both docosahexaenoic (DH) and docosenoic acids were detected in prickly pear seeds oil in low amounts. It could be concluded that, prickly pear seeds powder are an important source of natural fiber and, given its high linoleic acid content, its oil can be used as a nutraceutical agent.

Keywords: Nutritional composition, prickly pear *(Opuntia ficus-indica* (L.) seeds, fatty acids, fiber, minerals, protein, amino acids, antimicrobial activity.

INTRODUCTION

The prickly pear (cactus) is a fruit of the genus *Opuntia*, which belongs to the Cactaceae family. It is one of the most representative fruits in Egyptian culture. It is a fruit, which presents a thick pericarp with small prickles, enclosing a pulp, which is intermixed with a number of small seeds (Kossori et al., 1998). El-Samahy et al. (2006) showed that, seeds ratios between 11.14% to 13.80% in cactus pear fruit of Egypt, whereas it account for 3-7% on a weight basis in cactus pear fruit of Latin American countries (Felker et al., 2002 and Matsuhiro et al., 2006). Under optimal conditions, annual production of the aerial parts of the plant can reach 50 tons of dry matter per hectare. Fresh fruit production from cacti is nearly 40 t/ha/year (Nobel et al., 1992). About 1500 species of cactus are in the genus Opuntia and are distributed in Europe, Mediterranean countries, Africa, southwestern United States, northern Mexico and other areas (Hegwood, 1990). The prickly pear fruit has an oval, elongated shape, like an oval apple or pear and is technically a fleshy berry (Anderson, 2001). Its weight ranges from 67 to 216g (Mohammer et al., 2006). In the market, these fruits are available (peeled or unpeeled) in several attractive colors, such as white, green, yellow, orange, red, and purple, which vary in relation to the amount of betalain pigment content (Anderson, 2001; Stintzing et al., 2005). The thick pericarp, covered with minute, barbed spines (also known as glochids), encloses a juicy pulp with 150–300 non edible seeds. When eating the fruit, it is necessary to be very careful because the tiny glochids especially can easily pierce the skin, causing pain and irritation. Betalain pigments contained in these cactus pears have shown beneficial effects on the redox-regulated pathways involved in cell growth and inflammation

(Siriwardhana et al., 2006). Betalains are water-soluble pigments. Two betalain derivatives are present in cactus pears: betacyanin, which gives the red-purple color, and betaxanthin, which gives a yellow-orange color. These pigments show important antioxidant activities without toxic effects in humans (Livrea and Tesoriere, 2009).

The seeds contained significant amount of protein (4.13%), oil (11.5%), fibre (12.3%), β-carotene (56 µg/100g) and total carotenoids (289 µg/100 g). The seeds oil contained high levels of linoleic (70%), palmitic (12.5%) and stearic (12.3%) acids. The main fatty acids were linoleic, oleic, palmitic and stearic acids with high unsaturation level (83%) (Kunyanga et al.,2007).Prickly pear fruit seeds is an important source of sugars, minerals, aminoacids (Kossori et al., 1998 and Díaz-Medina et al., 2007), phenolic compounds (Díaz-Medina et al., 2007 and Stintzing et al., 2005), betalains (Stintzing et al., 2005 and Butera et al., 2002), and vitamin C (Fernandez-López et al., 2010 and Kuti, 2004). Phenolic compounds are localized in the cellular vacuoles. They play an important role in the growth and reproduction of plants, and also in protection against pathogens and predators (Balasundram et al., 2006 and Kuda et al., 2005). Phenolic compounds have anti-inflammatory, anti-allergenic and cardioprotective effects (Balasundram et al., 2006; Kuda et al., 2005 and Ndhlala et al., 2007). The seeds of the prickly pear fruit are highly variable in form, size, structure, and testa color. Seeds are normally discarded (Feugang et al., 2006) but often eaten as part of the fresh fruit.The principal objective of the present work was to study the nutritive value of the *Opuntia ficus indica* seeds oil and its potential utilization as antimicrobial agent as a part of an integrated project for the utilization of the *Opuntia ficus- indica* that are abundant in many parts of the world.

MATERIALS AND METHODS

Seeds collection and pre-treatment

The ripened prickly pear fruit were collected from local markets of Cairo, Giza and Kalyobia Governorates during summer months July and august 2017. The fruits were peeled, cut into thin slices dried using the solar dryer system in the energy department of National Research Center and indirectly dried by solar drying system using forced circulation as described by (Ibrahim, 2003). Some dried seeds have been separated from the dried flesh and the remainder seeds were removed from dried slices by hand and powdered using mixer grinder, kept in plastic bags and preserved at -20°C.

Chemical analysis of dry prickly pear seeds powder

Chemical and Reagent

All chemicals and reagents used in this study were purchased from Merck (Darmstadt, Germany) and Sigma Chemical (St. Louis, MO, USA). All assays were reported and documented in different Laboratories of Regional Center for Food and Feed "RCFF", Agriculture Research Center "ARC", Egypt; which has been gained the international accreditation ISO17025.

Chemical analysis

Moisture content, crude protein, fiber, fat and ash were estimated for the dried prickly pear seeds powder according to (AOAC, 2000), while, nitrogen free extract (NFE) content was estimated by difference.(Iron, copper and zinc) were determined according to the method of (Shahidi et al.,1999) with slight modification. Selenium content was determined according to (Levesque and Vendette, 1971) method.

Phytochemical analysis

The dried prickly pear seeds powder was obeyed to estimate some phytochemical compounds as total flavonoids as adapted by (Boham and Kocipai, 1994); total phenols content according to (Obadoni and Ochuko, 2001), evaluation of the total antioxidant capacity by the method of (Prieto et al., 1999); Saponin content of the sample was determined by double solvent extraction gravimetric method (Harbone, 1973) and (Obadoni and Ochuko, 2001).Glycosides were identified by the method of (Harbone, 1973).

Determination of flavonoids

About 10g of the plant samples were extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The extract was filtered through Whatman filter paper no. 42 (125mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed (Boham and Kocipai,1994).

Determination of total phenols

For total phenols, the fat free sample was boiled with 50ml of ether for 15 min. 5ml of the extract was pipette into 50ml volumetric flask, then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of pentanol were added. The samples were made up to mark and left to react for 30min for color development. The absorbance of the solution was read using spectrophotometer at 505 nm wavelength (Harborne, 1973; Obadoni and Ochuko,2001).

Determination of Aflatoxin and Ochratoxin

Extraction of aflatoxins was performed according to the (Shanon et al.,1983) method, whileOchratoxin A in samples was done according to (Trenk and Chu, 1971) method. Stock solutions were prepared by dissolving the toxin in the appropriate solvent at concentration of 1 mg/mL.Aflatoxin was dissolved in toluene: acetonitrile (99:1) and ochratoxin

in toluene: acetic acid (99:1). HPLC technique (Agillent 1200) series USA were used for aflatoxin and ochratoxins determination column C18, Lichrospher 100 RP-18, 5µm × 25cm was used. The mobile phase consisted of water: methanol: acetonitrite (54:29:17, v/v/v) at flow rate of 1ml/min. The excitation and emission wave lengths were 362 and 460 nm (Flourences detector).Ochratoxin was determined using column Nova- Pak C18 4µm, 3.9 × 150mm. The mobile phase consisted of acetonitril: acetic acid: water (495:10:495 v/v/v) at flow rate of 0.8ml/min. The excitation and emission wave lengths were 333 and 477 nm.

Estimation of carbohydrate profile

Mannan was detected according to (Moreiral and Filho, 2008). HPLC method for the determination of beta-glucan was conducted according to (Pérez-Vendrell et al., 1995). The beta-glucan was hydrolysed with lichenase [endo-beta-(1-3), (1-4)-D-glucan-4-glucanhydrolase from *Bacillus subtilis*] to oligosaccharides, which were analysed by reversed-phase HPLC using water as the mobile phase at a flow-rate of 0.7 ml/min. The separation of the oligosaccharides was performed in a C18 stainless-steel column (Spherisorb ODS-2) with 5-microns particles in less than 10 min, with refractive index detection.Sucrose content in dried prickly pear seeds powder was estimated according to (Dubois et al.,1956).

Determination of amino acids profile

Amino acids determination in prickly pear seeds powder was performed according to method of the AOAC, (2012). Oxidation with performic acid to protect methionine and cystine from distraction during acid hydrolysis with (6 M HCl) were carried out in a closed conical flask for determination of all amino acid other than tryptophan. Sample of 20-30 mg weighted in conical flask and 5 ml of performic acid was added. The flask was closed and inserted in ice water bath for 16 hr. Sodium metabisulfate (1.0~1.5 g) and 25 ml of HCl (6 N) were added to the oxidizing mixture. The flask was closed and placed; the mixture was then subjected to high temperature (110 °C) for 24 h in oven. The flask was then applied to concentration *in vacuo*till dryness using rotary evaporator. A suitable volume of sodium citrate puffer (pH 2.20) was added to hydrolyzed sample. After all soluble material completely dissolved, the sample is ready for analysis. The system used for the analysis was High Performance Amino Acid Analyser (Biochrom 30). The results were calculated in percentage compared to the total crudeprotein.

Fatty acids composition and characterization of the extracted oils

Fatty acid composition of extracted oils was trans esterified into their corresponding fatty acid methyl esters (FAMEs) using methanolic NaOH and boron tri-flouride (BF3) with methanol as described by (Ichihara and Fukubayashi, 2010). The FAMEs were quantified by Shematizu Gas Chromatograph Series 662010 equipped with a 2010 + auto sampler (Japan,) and interfaced with a flame ionizing detector (FID). The GC was equipped with a temperature programmable column.

Preparation of methanolic extract of dried prickly pear seeds powder

Two hundred grams of the dried seeds powder were extracted with 1 liter methanol in a soxhelet apparatus for several hours. The extracts were filtered using Whatman No.1 filter paper. The methanolic extract was concentrated at 45°C under reduced pressure using rotary evaporator to give yield of 32.4 g of dark brown methanolic extract.
The extracts of seeds was stored in airtight screw- capped bottles at 4°C and subjected to the upcoming biological and chemical studies.

Gas chromatography-mass spectrometry (GC/MS) analysis

GC/MS determination of the methanolic extract was performed at the Regional Center for Food and Feed (RCFF) using GC (Agilent Technologies 7890A) equipped with a mass-selective detector operating by HP-5ms capillary column **(**30 µm x 0.25 mm i.d. and 0.25 µm film thickness). The temperature was increased from 80 ^{o}C to 230^{o}C with rate of 3 ^{o}C min^{-1}. The carrier gas was helium at a flow rate of 1ml min^{-1}. The identification of bioactive compounds was performed by comparing their mass spectra and retention time with those of authentic standards and by computer matching with the database of National Institute Standard and Technique.

Antimicrobial assay

Bacterial strains

Salmonella, Escherichia coli, Bacillus subtilis, Bacillus cereus and yeast were kindly supplied by Food Safety laboratory, Regional Center for Food and Feed, Agricultural Research Center. The strains were maintained on slants of Nutrient Agar (NA) at 4 $^{\circ}$C in the laboratory. The microorganisms were cultured in Brain Heart Infusion broth at 37 $^{\circ}$C for 24 h.

Determination of Antimicrobial activity of methanolic extract of prickly pear seeds powder

Antimicrobial activity was determined according to the method described by (Cleidson Valgas et al., 2007). Briefly, the bacterial suspensions prepared from the overnight broth cultures were counted according to (NMKL reports No. 86, 2013) and were diluted to the required microbial density (about 10^{7} CFU/ mL). Tested materials were

Table 1. Proximate composition and minerals analysis of prickly pear seeds.

Components	Mean + SD [a]
Moisture, %	06.50
Protein, %	10.70
Fat, %	04.88
Crude fiber, %	46.31
Ash, %	03.39
Total carbohydrate, %	28.22
Mannan, g / Kg	153.44
β- glucan, g / Kg	069.20
Sucrose, g / Kg	001.73
Fe^{++} (ppm)	127.20
Zn^{++} (ppm)	152.90
Cu^{++} (ppm)	001.29
Se^{++} (ppb)	024.76

[a] Represents a minimum of the determinations

dissolved in methanol (v/v) and concentrations of 50%, 33% and 25% were prepared in methanol solution. A 100µL of the prepared concentrations was added into the bores of inoculated nutrient agar plates contained the selected microorganisms under study (*Salmonella, Escherichia coli, Bacillus subtilis, Bacillus cereus and* yeast). The plates were incubated for 24h. At 37 °C. Antimicrobial activity was determined by measuring the diameter of the inhibition zone around every bore.

Statistical Analysis

All values were obtained by triplicate and expressed as means ± standard deviations (SD). Data were analyzed using the SPSS V.15 software (SPSS Institute Inc., Cary, NC). An ANOVA was carried out to determine differences between oils extracted as well as its antimicrobial activity that were significant at the 5%level of probability and a Tukey test was used for comparison of data.

RESULTS AND DISCUSSION

Proximate analysis

Values for percent moisture, protein, fat, ash and fiber for prickly pear seeds are presented in Table 1. Results showed that, contents of protein (10.70%), fat (4.88%), fiber (46.31%) and ash (3.39%). In this respect, (Kunyanga et al.,2007) postulated that seeds of prickly pear contained significant amount of protein (4.13%), oil (11.5%), fibre (12.3%). Rabab and Maher, (2012) mentioned that, prickly pear powder content was 16.6% CP, 17.21% crud fat, 49.6% crud fiber and 3.14% ash. When compared these results with other seeds oil (Bemis et al., 1975), the protein content of prickly pear seeds is near to that of sunflower seeds (16.8 %) but is lower than sesame (22.3%), cotton seed (23.1%), soybean (37.1%) and buffalo gourd (31%). The fiber content of prickly pear seeds is considerably higher than that of the commonly consumed seeds oil such as soybean, peanuts and cottonseeds (Bemis et al., 1975 and Khalil and Chughtai, 1978), while the ash content is comparable to other plant materials (Watt and Merrill, 1963). The fat content is close to that of soybean and was previously reported to be of a very good quality when compared with most commonly consumed vegetable oils (Sawaya and Khan, 1982).

Nutritionally valuable micro elements minerals analysis for prickly pear seeds is shown in Table 1. Results shown that the seeds powder are rich sources of Fe^{++}, Zn^{++}, Cu^{++} and Se^{++}, where Fe^{++} having (127.20 ppm), and Zn^{++} 152.90 ppm), Cu^{++} (001.29 ppm) and Se^{++} (024.76 ppb). Saway et al. (1983) mentioned that, Fe^{++} content (9.45 mg| 100g, Zn (1.45 mg|100g), while Cu content (0.32 mg|100g). The amounts of zinc and copper, although lower than that of soybean, peanut and cowpea by 2 – 10 times, were significant from a nutritional standpoint. Trace elements such as iron, copper and zinc are essential in enzymes metabolism. The concentrations of these elements in the seeds are quiet important. The importance of iron in maintaining the good health has been recognized (Vaughan and Judd, 2003).

In general, a 100 g portion of prickly pear seeds meal can furnish approximately 10-20 of the requirements for the above minerals in terms of the Recommended Dietary Allowances (RDAs) or suggested daily intakes of the Food and Nutrition Board, NRC/NAS (Anonymous, 1974) except iron which is present in amounts representing approximately 100% of the RDA for this element.

Phytochemical compounds

Data in Table (2) shown that, Prickly pear seeds powder contain high amounts of total phenols compounds (504.825 mg/100g (Gallic acids equivalent)), total flavonoids (35.875 mg/100g (Quercetin equivalent)) and total antioxidant capacity (303.950 mg / 100g (Ascorbic acid equivalent),

Table 2. Total phenols, total flavonoids, total antioxidant and Saponin of prickly pear seeds

Compounds	Concentrations
Total phenols	504.825 mg/100g (Gallic acids equivalent)
Total flavonoids	035.875 mg/100g (Quercetin equivalent)
Total antioxidant capacity	303.950 mg/100g (Ascorbic acid equivalent)
Saponin	3.74 mg| 100 g

Table 3. Fatty acid composition of prickly pear seed oil (g| 100 g of total fatty acid [a])

Oleic acid Fatty acids	Name	Relative distribution(%)
C14:0	Myristic acid	0.32
C16:0	Palmitic acid	17.11
C16:1ω9	Palmitoleic acid	0.71
C16:2ω4		0.13
C18:0	Stearic acid	3.49
C18:1n9	Oleic acid	22.41
C182n6	Linoleic acid	54.03
C183n3	Linoleinc acid	0.63
C20:0	Arachidic acid	0.38
C20:1ω7	9-eicosaenoic acid	0.21
C22:1ω11	Docosenoic acid	0.16
C22:6ω3	Docosahexaenoic acid (DHA)	0.13
Non Identified fatty acids		0.29
Free Fatty Acids		0.23

+ [a] Each value is the mean of three observation ± standard error.

while Saponin contain (3.74 mg/100 g).

Fruit seeds contain high amounts of phenolic compounds ranging from 48 to 89 mg/100 g and including feruloyl derivatives, total flavonoids 1.5–2.6 and total tannins 4.1–6.6 mg|g powder sinapoyl D-glucoside(Chougui et al.,2013).

The growing interest in polyphenols results from their antioxidant potential, which is involved in health benefits such as the prevention of inflammation (Laughton et al., 1991),cardiovascular dysregulation and neurodegenerative diseases. Polyphenols have also proven anticancer activity. Kunyanga et al. (2007) reported that the cactus whole fruits exhibited remarkable levels of total phenols (1.6 g/100g), flavonoids (197 mg/100g), tannins (1.5 g/100g) and phytates (2.6 g/100g). The phytochemical extracts demonstrated high antioxidant activity in terms of FRAP assay (1.2-6.9 µg/m M Fe (II) reducing power) and DPPH assay (73-86%). The anti-diabetic effect of the extracts showed strong inhibition (> 50%) of α-glucosidase as compared to the α-amylase inhibition.

Fatty acids contents

Results in Table (3) shown that, major fatty acids and sterols are Linoleic acid (54.03%), Oleic acid (22.41%) and Palmitic acid (17.11%). These acids accounted (93.55%), while, myristic acid was detected in small amounts. Prickly pear seed oil was found to be highly unsaturated fatty acids(78.41%). Whereas, Docosahexaenoic acid accounted (0.13%).The profile of the seed oil indicates that the lipids from the prickly pear seeds are a good source of the nutritionally essential linoleic acid and the unsaturated oleic acid. The ratio of linoleic acid to oleic acid was about 2.41:1. According to (Ramadan and Mörsel, 2003 a, b) total seed lipids amount to 98.8 g/kg dry weight. Also, Major lipid acids and sterols are linoleic, palmitic as well as oleic acids.

Tocopherols are considered effective antioxidants that prevent lipid oxidation. Thus, prickly pear seed oils should be quite stable. With regard to the lipid profile, Opuntia seed oil is comparable with grape seed or corn germ oil (Coskuner and Tekin, 2003; Krifa et al., 1993). Linoleic acid (60.69%) was the dominant fatty acid, followed by oleic (21.42%) and palmitic (12.76%) acids, respectively, while, myristic acid was detected in small amounts. Prickly pear seed oil was found to be highly unsaturated (83.12%) of prickly pear seed oil (El Mannoubi et al., 2009). Kunyanga et al., (2007) reported that the seed oil of prickly pear contained high levels of linoleic (70%), palmitic (12.5%) and stearic (12.3%) acids. The main fatty acids were linoleic, oleic, palmitic and stearic acids with high unsaturation level (83%). The ratio of linoleic acid to oleic acid was about 5:1. The predominant fatty acid was linoleic acid (69.5%), followed by palmitic acid (12.5%) and oleic acid (12.3%). According to (Matsuhiro et al.,2006), Hydrocolloids (endosperm) of prickly pear seed powder contain 98.8 mg/k (on dry weight basis) total lipids, main lipids (linoleic, oleic, palmitic acids) and main sterols (β-sitosterol,campesterol).

Table 4. Amino acids composition of prickly pear seed powder (g amino acid |100 g protein).

Amino acid protein	Cactus seeds(g) amino acids/100g protein	1985 FAO/WHO/UNU[a] (mg/g) protein[b]	2007 FAO/WHO/UNU mg/g protein[c]
Histidine	4.10	15	15
Isoleucine	4.68	15	30
Leucine	8.04	21	59
Lysine	5.98	18	45
Sulfur amino acids			
Methionine + cysteine	---	20	22
Methionine	3.74	----	16
Cysteine	3.56	----	6
Aromatic amino acids			
Phenylalanine + tyrosine	----	21	30
Phenylalanine	5.60	----	----
Tyrosine	5.98	----	----
Threonine	4.86	11	23
Tryptophan	----	5	6
Valine (VAL)	6.36	15	39
Total indispensable amino acids	**52.9**	**141**	**269**
Aspartic (ASP)	10.64	----	----
Serine (SER)	04.86	----	----
Glutamic (GLU)	20.74	----	----
Glycine (GLY)	07.66	----	----
Alanine (ALA)	07.66	----	----
Argnine (ARG)	14.76	----	----
Proline (PRO)	07.66	----	----
Total dispensable amino acids	**73.98**	**----**	**----**

aEnergy and protein requirements. Report of a Joint FAO/WHO/UNU Expert Consultation. Geneva, World Health Organization, 1985 (WHO Technical Report Series, No. 724).
bMean nitrogen requirement of 105 mg nitrogen/kg per day (0.66 g protein/kg per day).
cProtein and amino acids requirement in Human Nutrition Report of a Joint WHO/FAO/UNU Expert Consultation (2007). WHO Technical Report Series No 935.
---- are not defined.

High level of omega-6 linoleic acid was reported in cactus seed oil (53.5% to 70.29%) (El-Mostafa et al., 2014), and this level is higher than in sunflower oil (Filip et al. 2011), grape seeds oil or sesame oil. As a precursor of arachidonic acid, linoleic acid has long been accepted as having a hypocholesterolemic effect and inhibitory properties against colon cancer metastatic cells (Soel et al., 2007). Omega-3 linolenic acid is known to be beneficial for health, cardiovascular diseases, inflammatory conditions, autoimmune disorder and diabetes.

Amino acids composition

The amino acids profile of prickly pear seeds protein is shown in Table (4). Glutamic acid, arginine, aspartic acid, and leucine accounted for half of total amino acids content. These results agree with finding with (Sawaya et al., 1983) who, these amino acids accounted for half of the amino acid content of the protein. The prickly pear protein contained a significantly high amount of the sulfur-containing amino acids, methionine + cystine, which are generally the most limiting amino acids in seed proteins. In this respect, prickly pear seed protein is comparable to sesame protein which is high in the sulfur-containing amino acids containing about 6 g of methionine + cystine/16 gN(Brito and Nunez, 1982). According to the (FAO/WHO 1985) reference pattern, the amount of methionine + cystine in prickly pear protein represents nearly twice that required. Lysine was the most deficient amino acid in prickly pear seeds protein with a chemical score of 62, while all other essential amino acids were present in amounts exceeding 90 of the (FAO/ WHO 2007) reference pattern. Kunyanga et al., (2007) reported that the principal amino acids of prickly pear seed in the fruits were arginine, tyrosine, glutamic acid, proline and aspartic acid.

Aflatoxin and ochratoxin in the dried powder of prickly pear seeds

aflatoxin and ochratoxin determinations were under detection limits and considered free and revealed that, the dried prickly pear seeds powder was free of toxins, making it suitable for the preparation of extracts for further analysis.

Microbiological assay

Effects of different levels of methanolic extracts of prickly pear seeds on microbial activity are shown in Table (5). The best results of mehanolic concentration is 50% (v/v) have been shown to reduce the growth of selected microorganisms under study (*Salmonella, Escherichia coli, Bacillus subtilis, Bacillus cereus and* yeast) bores and fungal metabolites (aflatoxin and ochratoxin), while there is no

Table 5. Effects of different levels of methanolic extracts of prickly pear seeds on microorganism activity

Con.	*E.coli*	*Salmonella*	*Bacillus subtilis*	*Bacillus cereus*	Yeast
50%	2.0cm[a]	2.2cm[a]	2.3cm[a]	2.1cm[a]	1.7cm
33%	2.0cm[b]	2.0cm[b]	2.1cm[b]	1.9cm[b]	1.7cm
25%	No effect	2.0cm[b]	2.0cm[c]	1.8cm[c]	1.7cm

[a–c] Different letters in the same column indicate significant differences.

Table 6. The chromatogram of methanolic extract of the bioactive compounds, area (%) and retention time (RT) of prickly pear seed powder**.**

	R.T	Compounds	Area Sum %
1	8.7	Thymol	2.13
2	10.525	6,2'-Dimethylflavone	2.62
3	11.976	5,7,3',4',5'-Pentahydroxyflavone	0.38
4	12.232	Xanthine	0.4
5	12.33	Gulose	0.44
6	12.693	3-Hydroxy-7,8,2'-trimethoxyflavone	0.36
7	12.962	: Vitexin	0.38
8	13.22	(S)-(-)-Citronellic acid	0.57
9	13.373	4'-Benzyloxy-5,7-dimethoxyflavone	4.04
10	13.45	Oleic Acid	0.4
11	13.507	Lupanine	0.42
12	13.564	cis-Vaccenic acid	1.33
13	13.715	4',6-Dimethoxyisoflavone-7-O-β-D-glucopyranoside	13.23
14	14.024	7,4'-Dimethoxy-3-hydroxyflavone	0.41
15	14.155	Artesunate	1.08
16	14.31	7,3'-Dimethoxy-3-hydroxyflavone	0.63
17	14.448	7-Diethylamino-3-(4-methoxyphenyl)coumarin	11.38
18	14.506	Vincamine	9.96
19	14.656	2',4'-Dimethoxy-3-hydroxy-6-methylflavone	1.99
20	14.819	N-Glycolylneuraminic acid	32.19
21	14.966	6,4'-Dimethoxy-7-hydroxyisoflavone	1.81
22	15.805	Methylprednisolone succinate	1.02
23	16.033	5,7,3',4'-Tetramethoxyflavone	0.41
24	16.172	Ouabagenin	0.9
25	16.347	β-Citronellol	0.93
26	17.329	Linolic acid	1.94
27	18.013	Astilbin	2.12
29	18.84	Squalene	1.15
30	19.624	trans-Farnesol	0.43
31	20.372	4,8,12-Tetradecatrienal, 5,9,13-trimethyl-	2.2
32	20.837	5,6,7,3',4'-Pentamethoxyflavone	0.55
33	21.884	Dihydrosqualene	0.54
34	22.087	Acitretin	1.12
35	23.126	Janex 1	0.57

response to yeast bores. In this respect (Ramírez-Moreno et al.,2017 and Bajpai et al.,2012) mentioned that, methanolic extract of prickly pear seeds powder was used to determine the antimicrobial activity and prickly pear seeds oil produced a microbial inhibition zone in most of the microorganisms evaluated.

GC-MS analysis of methanolic extract

The chromatogram of methanolic extract is shown in Table (6) and Figure (1).The bioactive compounds, area (%) and retention time (RT) are presented of GC-MS analysis revealed the existence of many common compounds in the extract as N- Glycolylneuraminic acid (32.19%); 4',6-Dimethoxyisoflavone-7-O-β-D-glucopyranoside (13.23%) and 7- Diethylamino-3-(4-methoxyphenyl) coumarin (11.38%) which has antioxidant properties and reduces the risk of stomach cancer (Ferguson et al., 2005) by reducing the formation of carcinogenic nitrosamines (Kikugawa et al., 1983). N-Glycolylneuraminic acid is widely expressed on most mammalian tissues, but is not easily detectable on human cells (Varki, 2001). 4',6-Dimethoxyisoflavone-7-O-β-

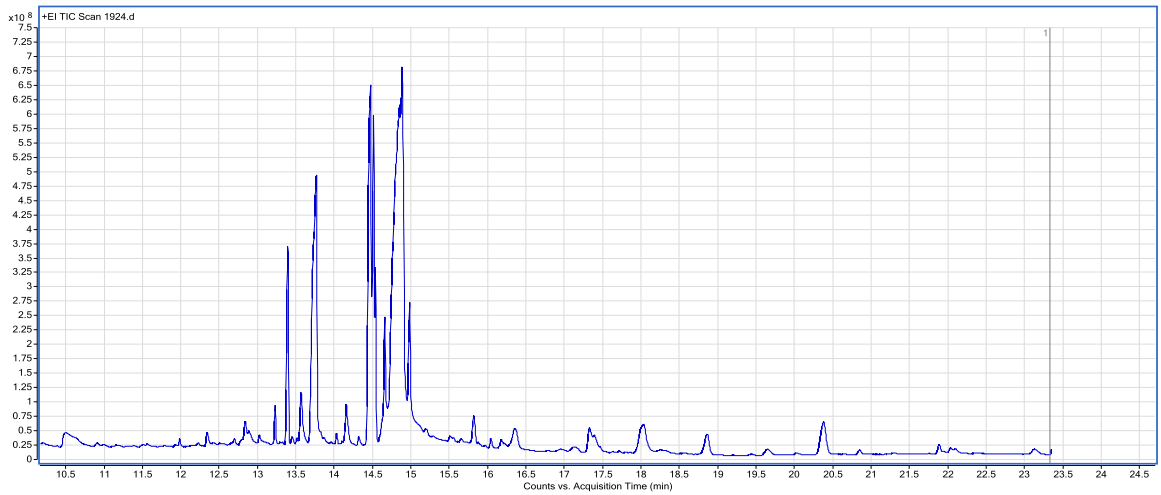

Figure 1: The chromatogram of methanolic extract of the bioactive compounds of prickly pear seed powder

D-glucopyranoside (13.23%) is an integral membrane protein that is located in the mitochondrial inner membrane of brown adipocytes (Krauss et al., 2005). Prickly pear oil is used as an anti-aging skin and is included in cosmetics (Chougui et al., 2013).

Conclusions

Prickly pear seeds powder have a good source of protein, oil, vitamins, phenols and antioxidants and free from fungal metabolites (Aflatoxin and Ochratoxin). The results demonstrated that oil extracts from seeds have a noticeable antimicrobial activity against *Salmonella, Escherichia coli, Bacillus subtilis and Bacillus cereus* bores. This research provides further incentives to develop additives for the food, cosmetic, and pharmaceutical sectors seeking natural compounds with antimicrobial activity.

These results give informative profile not only on the effect of variety, location and other parameters on the oil of prickly pear seeds quality of Opuntia species, but also for future work in a product development and value addition. Further studies are needed to determine the optimum levels of oil extract and the antimicrobial effectiveness in the food matrix.

Acknowledgments

This work was supported by the Action Intégrée of different Laboratories of Regional Center for Food and Feed "RCFF", Agriculture Research Center "ARC", Egypt; which has been gained the international accreditation ISO 17025 with Natural Products Research Unit & Chemistry Department in Faculty of Science, Helwan University.

Author Contributions

MS, SHB and AS: collected data from the literature and prepared tables and figure. AS involved in writing the manuscript and formatting the references. MS and SHB were deeply involved in the manuscript correction and revision as well as involved in general supervision of the review. AS and SHB Involved in writing several paragraphs and revision. MS, SHB and AS Designed the review, revised the manuscript and supervised. The collected data by MS, SHB and AS. All authors have read and approved the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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