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PHYTOCHEMICAL SCREENING, ANTIMICROBIAL, ANTIAXIDANT, ANTICANCER ACTIVITIES AND NUTRITIONAL VALUES OF CACTUS (*OPUNTIA FICUS INDICIA*) PULP AND PEEL

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ABSTRACT

Opuntia ficus-indica (L.) Mill is belonging to the family Cactaceae. The chemical composition of the pulp and peel of cactus showed that pulp has high content of proteins, moisture and lipid but low content of total fiber and ash as compared to the peel. The peels of the cactus were considerably higher in fiber than the pulp. The ethanolic and ethyl acetate extracts of peel have high concentrations of total phenols, flavonoids, tannins, alkaloids than the pulp extracts. The ethanolic extract in both pulp and peel gave higher concentrations of phytochemical compounds than the ethyl acetate extract. Therefore, pulp and peel extract has antioxidant and antimicrobial activity against gram positive, negative bacteria and fungus. Also, the percentage of Liver cell line (HepG2), Colorectal adenocarcinoma (Caco-2) and Breast cell line (MCF-7) viability was decreased with increasing the concentrations of the ethanolic extract of the pulp and peel cactus (500, 1000, 1500, 2000, 3000 μg/ml). The most pronounced reduction in the viability of cancer cells was detected after treatment with pulp extract than peel extract. The high concentrations of ethanolic extract of the pulp and peel cactus caused the high reduction in the viability of cancer cells especially in Liver cell line (HepG2). In addition, the phytochemical compound screened by GC-MS method. In this GC-MS analysis, 31 bioactive phytochemical compounds were identified in the pulp of cactus and 27 bioactive compounds were detected in peel extract. These different active phytochemicals have been found to possess a wide range of activities, which may help in the protection against incurable diseases.

KEYWORDS:
Phenols, flavonoids, tannin, carotenoids, vitamins, DPPH, antibacterial, anticancer activity.

INTRODUCTION

Today the world appears to be increasingly interested in the health benefits of foods and has begun to look beyond the basic nutritional benefits of foodstuffs to disease prevention. It is generally accepted that the beneficial effects of herbal remedies can be obtained from active constituents present in the whole plant, parts of the plant (e.g., flowers, fruits, roots or leaves), or plant materials or combinations thereof, whether in crude or processed state [1-4]. Medicinal plants represent a rich source of antimicrobial agents. *Opuntia ficus-indica (L.) Mill.*, commonly called prickly pear or nopal cactus, belongs to the dicotyledonous angiosperm Cactaceae family, a family that includes about 1500 species of cactus. *O. ficus indica* is a tropical and subtropical plant. It can grow in arid and semi-arid climates with a geographical distribution encompassing Mexico, Latin America, South Africa and Mediterranean countries (Spain, Italy, Greece, Egypt, Turkey) [5, 6]. Prickly pear is in mature stage up to 2 m long, with plump, even, rounded segments, armed with spines are called pods. The fruits are yellow and orange color; barrel or egg shaped and up to 10 cm long. The stems are green, plump, flattened and can be very large which approximately 60 cm length. However, the plant can spread up to 40 m in diameter at from root region. The fruit pulp is disk-shaped and has numerous colors. An *Opuntia* fruit has high medicinal value and was established to display many pharmacological properties such as antiulcerogenic, neuroprotective, antioxidant, hepatoprotective and anticancer activities [7-11]. The use of prickly pear fruit is suggested for their beneficial and therapeutic properties and also used for treating diabetes, burns, bronchial, asthma and indigestion in many countries over the world [12, 13]. Chemical analysis showed that the prickly pear fruits contain 12–15% sugars, 0.6% protein, and 0.1% lipids; minerals including calcium, potassium, and magnesium (490, 2200, and 850 ppm, respectively [14]. The fruit pulp is rich in

vitamin C, free amino acids (proline, taurine, glutamine, serine), polysaccharides, polyphenolic compounds (Quercetin, kaempferol, isorhamnetin and their derivatives), pigments (betaxanthins and betacyanins responsible for yellow and red color, respectively) and flavor compounds [15]. Cactus pear fruit, usually consumed fresh or in processed form such as beverages, syrups, candies, jellies, marmalades, barbecue sauces, natural sweeteners, dehydrated sheets and nectars [16]. Being high in nutritional and bioactive phytochemicals, cactus pear fruit can be used both as a potential source of natural antioxidants and as a direct functional food [17].

The aim of this work is to study the chemical composition of *Opuntia ficus-indica* pulp and peels, to study its effect as an antioxidant, antimicrobial and anticancer activity and to spate active compounds by GC-mass analysis.

MATERIALS AND METHODS

Plant Materials. Prickly pear fruits (*Opuntia ficus indica*) in the ripe stage were obtained from the local market, Giza, Egypt, in August 2018. One kilogram of edible prickly pear pulps and peels were brushed for two minutes under distilled water with a nail brush. The pulps and peels were removed from each other and then dried in oven at 105°C for 24 h to determine water content. Dry matter of each fraction (pulps and peels) was ground separately and passed through a 100-mesh sieve before analysis. All analysis was done in the labs of Cairo University. Research Park (CURP), Faculty of Agriculture, Cairo University, Cairo, Egypt.

Microbial strain. Table (1) illustrated the microorganisms which were used in this study and were obtained from the American Type Culture Collection (ATCC) as well as the culture collection of the Microbiology Lab, Cairo University Research Park (CURP), Faculty of Agriculture, Cairo University.

Biochemical composition of Prickly pear. Moisture, lipids, total fibers and ash were determined according to AOAC methods [18]. Nitrogen was determined by a Kjeldahl procedure. The factor 6.25 was used to convert nitrogen to crude protein [19].

Sugars extraction and determination by HPLC. Samples were cut into small pieces, homogenized using commercial blender, then soaked in hot HPLC grade water for one hour and extracted in an ultrasound bath (Crest®, Malaysia) at 40±1 °C for 45 minutes. Solution was filtrated twice using filter paper and 0.45 µm Millipore membrane. Kept at -21°C until analysis. Sugars were determined by HPLC, column used was Phenomenex, Luna NH_2 250 x 4.6 mm, column temperature kept constant at 30°C, mobile phase was Acetonitrile: HPLC grade water 80: 20 (v/v), detection by RI detector and data integration by claritychrom@ software.

Determination of mineral composition. The Ca, Mg, P and Na contents were determined using the dry-ashing procedure [18]. The K, Mg, Na, Fe, Mn, Zn, Cu ions concentrations were measured with a double beam atomic absorption spectrometer, (Analyst 300, Perkin Elmer, USA). The organic components were previously eliminated at 550 °C for 24 h.

Phytochemical composition of Prickly pear. The total phenolic content was estimated by Folin Ciocalteu method as described by Singleton and Rossi [20]. The absorbance was measured at 765 nm using a spectrophotometer Thermo Scientific HERYIOS. The flavonoids content was determined by aluminium trichloride method as described by Zhishen et al. [21]. The absorbance was measured at 510 nm using a spectrophotometer. Tannin content in *Opuntia ficus indica* pulp and peels was determined by using Folin-Denis reagent as described by Saxena et al. [22]. The absorbance was read at 700 nm using spectrophotometer. Alkaloids was measured according to the method described by Harborne [23]. The absorbance was taken at 565 nm. The alkaloid concentration was calculated from the calibration curve of atropine used as standard and results expressed as g/100 g equivalent of atropine. The total anthocyanin contents were determined by the pH differential method using a spectrophotometer (Thermo Scientific HERYIOS) [24]. The absorbance of the fruit extract was measured at 515 and 700 nm in pH 1.0 and 4.5 buffers, respectively, using A = (A515 - A700) pH 1.0 - (A515 - A700) pH 4.5 with a molar extinction coefficient of 26,900. The results were expressed as mg of cyanidin-3-glucoside equivalent per 100 grams of fresh weight (g cy-3-glu kg^{-1} FW).

TABLE 1
Microbial strains used to test the antimicrobial activities of Prickly pear fruit and peel extracts

Microbial group	Indicator strain	Positive control	Cultivation conditions
Gram positive bacteria	*Staphylococcus aureus* (ATCC 25923) *Bacillus cereus* (ATCC 33018)	Kanamycin	Muller-Hinton broth, 37ºC/ 24 h Muller-Hinton broth, 30ºC / 24 h
Gram negative bacteria	*Escherichia coli* (ATCC 8739) *Salmonella typhimureum* (ATCC 14028)	Polymyxin	Muller-Hinton broth, 37ºC / 24 h Muller-Hinton broth, 37ºC / 24 h
Fungus	*Aspergillus niger* (nrrl 326) *Candida albicans* ATCC 10231	Nystatin	Sabouraud dextrose broth, 25 ºC / 3days Sabouraud dextrose broth, 25ºC / 24 h

Lipid Extraction. The pulp and peels of *Opuntia ficus-indica* oil content was determined using the Soxhlet extraction according to the official method [18]. 50 g of dried Fruits and peels were ground and then extracted with petroleum ether in a Soxhlet apparatus for 6 h. After extraction, the samples were ground again, but more finely, and extracted for 6 h (second extraction). Petroleum ether was evaporated under reduced pressure using a rotavapor. Lipid content was expressed as g/100 g of seed fresh weight.

Separation of fatty acids and unsaponifiables from lipid samples. Lipid material was saponified with methanolic KOH (40 %, w/v) for 24 h at room temperature according to Ahmed et al. [25]. The unsaponifiables were extracted three times with ether. The aqueous layer was acidified with HCl (1:1, v/v) and the liberated fatty acids were extracted three times with ether. The combined extracts of unsaponifiables and fatty acids were washed several times with distilled water and then dried over anhydrous sodium sulfate. The standard and the sample fatty acids were converted to methyl esters using an ethereal solution of diazomethane according to Vogel [26].

Determination of fatty acid composition by (GC–MS). The fatty acid methyl esters were determined by GC–MS using Trace GC Model 2000 series produced by Thermo equipped with Selective Detector Mass Spectroscopy Model SSQ 7000 produced by Finnigan. This equipment was interfaced via HP chemstation version A 02.12 software (Hewelwett-Packard. Avondale, PA). The gas chromatography was equipped with DB-23 (J & W 122-2362) 25µ capillary column, 60 m x 0.25 mm ID, 0.15 µ m. The operating conditions for gas chromatography were as follows: injector temperature 250°C, carrier gas: helium at 30 cm/sec, measured at 150°C, oven temperature 50°C for 4 min, 150°C for 4 min and held at 250°C until the chromatogram was completed. The detector temperature was 280°C. Mass spectroscopy operating parameters were electron ionization at 70 ev, accelerating voltage 10 kV and scan M/Z range from 50 to 500. National Institute of Standards and Technology (NIST) library according to Jiang et al. [27].

Determination of sterols profile by GC–MS. The unsaponifiable fractions were finally collected in ether and taken to dryness under vacuum. The residue was analyzed using the gas chromatograph HP 5890 (Hewlett Packard) equipped with the MS detector (MSD 5970), EI, 70 ev and fitted with a capillary column DB-1701 (12 m x 0.18 mm x 0.4 mm; J&W Scientific). The column temperature was programmed from 260 to 300°C while injection temperature was set at 280 °C. Helium was the carrier gas at a flow rate of 0.7 cm^3/min. Identification of peaks was based on the retention time of standard substances and MS spectra. Analyses were run in triplicate. Calculations of percent composition of demethylsterol fractions were based on the peak area.

Water soluble vitamins. Sample Preparation. Water soluble vitamin were determined by HPLC analysis after extraction from the sample according to Albala-Hurtado et al. [28]. Dry weighed 0.2 g of fruits and peels of *Opuntia ficus-indica* powder was placed into centrifuge tube and add 15 mL of deionized water. After 15 min of ultrasonic extraction, centrifuge at 4000 rpm for 5 minutes, then quantitively transfer to 25 mL volumetric flask, add water to the mark. Filter through 0.2um nylon membrane before injection.

Instrument Conditions. Agilent 1260 infinity HPLC Series (Agilent, USA), equipped with Quaternary pump, a Kinetex XB-C18 column 100 mm x 4.6 mm (Phenomenex, USA), operated at 35ºC. The separation is achieved using a binary linear elution gradient with (A) 25 mM NaH_2PO_4 pH = 2.5, (B) methanol. The injected volume was 20 µL. Detection: VWD detector set at 254 nm for ascorbic acids and 220nm for vitamins B3, B6, B9 and B12 (Jedlick and Klimes, 2005) [29].

Determination of phenolic and flavonoid compounds by HPLC. Extraction of phenolic and flavonoid compounds. 0.2g dry sample extracted with 20 ml ethanol 80%, soak in brawn bottle for 24 hr at room temperature, centrifuged for 5 min, volume adjusted to 25 ml by ethanol 80%, filtered through Whatman filter paper, 10 ml of the solution evaporated to dryness then dissolved in 5 ml HPLC grade methanol 50%, filtered through PTFE filter with pore size 0.2 µm.

Instrument Condition for phenolic compounds. Agilent 1260 infinity HPLC Series (Agilent, USA), equipped with Quaternary pump, a Zorbax Eclipse plusC18 column 100 mm x 4.6 mm i.d., (Agilent technologies, USA), operated at 30ºC. The separation is achieved using a ternary linear elution gradient with (A) HPLC grade water 0.2 % H_3PO_4 (v/v), (B) methanol and (C) acetonitrile. The injected volume was 20 µL. Detection: VWD detector set at 284 nm.

Instrument Condition for Flavonoids. HPLC, Smart line, Knauer, Germany., equipped with binary pump, a Zorbax Eclipse plusC18 column 150 mm x 4.6 mm i.d., (Agilent technologies, USA), operated at 35ºC. Eluent: methanol: H_2O with 0.5% H_3PO_4, 50:50 with flow rate 0.7 ml/min, the injected volume was 20 µL. Detection: UV detector set at 273 nm and data integration by claritychrom@ software. This method was the modified of methods Goupy et al. [30] and Mattila et al. [31] for fractionate the polyphenols and flavonoids, respectively.

Antioxidant activity. DPPH free Radical Scavenging activity (RSA). The antioxidant activity of fruits and peels of *Opuntia ficus-indica* extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable DPPH method as modified by Park et al. [32] (2006). The reaction mixture containing 1 ml of the extract at different concentrations (40, 80, 120, 150 µg/ml) and 1ml of DPPH (0.2mM) was vigorously shaken and incubated in darkness at room temperature for 30 minutes. The absorbance was read at 517nm using UV-visible spectrophotometer. Radical scavenging activity was expressed as percent of inhibition and was calculated using the following formula:-

%DPPH = [Absorbance of Control – Absorbance of Sample / Absorbance of Control] x 100

Determination of reducing power. The ability of the tested extracts to reduce Fe^{3+} was assayed by the method of Chou et al. (2009). The absorbance was measured at 700 nm. The results were expressed as µg of gallic acid equivalent per 100 g DW.

Antibacterial activity. Agar disc diffusion method was used to evaluate the antibacterial activity of pulps and peels of *Opuntia ficus-indica* fruits as described by Bauer et al. [33]. The strains were grown on Mueller-Hinton agar slants at 37°C for 24 h and checked for purity. After the incubation, the cells were washed off the surface of agar and suspended in sterile physiological solution. The number of cells in 1 ml of suspension for inoculation measured by McFarland nefelometer was 5 × 107 CFU/ml. 1 ml of these suspensions was homogenized with 9 ml of melted (45°C) Mueller-Hinton agar and poured into Petri dishes. On the surface of the agar, 5 mm diameter paper discs (HiMedia®, Mumbai, India) were applied and impregnated with 15 µl of samples. The plates were incubated at the optimum temperature for each indicator strain (Table 1) and tested after 24, 48 and 72 h. Growth inhibition was scored positive in the presence of a detectable clear zone (ZI) around the disc and expressed in mm. Experiments were carried out in triplicates and the inhibition zone was recorded as the average of the replicates± SD.

In Vitro cytotoxicity assay. Liver cell line (HepG2), Colorectal adenocarcinoma (Caco-2) and Breast cell line (MCF-7) were purchased from CURP, faculty of Agriculture at Cairo University (Egypt). Cells were maintained in (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 100 µg/ml streptomycin and 100 units/ml penicillin g potassium, in a humidified 90% and 5% (V/V) CO_2 atmosphere at 37ºC. The cytotoxicity of ethanolic extracts was tested by the neutral red (NR) assay as previously described by Repetto et al. [35]. Exponentially growing cells were collected using 0.25% Trypsin-EDTA and seeded in 96- well plates at 20000 cells/well. After incubation (overnight), extracts were added in various concentrations (10, 50, 100, 200, 400, and 800 µg/ml); 4 wells for each concentration. After treatment with extracts for 24h., the media were removed and cells were exposed to neutral red solution for 4 hours at 37ºC. Destin solution was used to dissolve the NR stained cells and color intensity was measured at 540nm microplate reader (Biotek, ELX808).

Prickly pear pulp and peel extraction for GC/MS analysis. The fruits were cleaned, shade dried and pulverized to a powder in a mechanical grinder. Required quantity of powder was weighed and transferred to Stoppard flask and treated with methanol until the powder is fully immersed. The flask was shaken every hour for the first 6 hours and then it was kept aside and again shaken after 24 hours. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using a vacuum distillation unit. The final residue obtained was then subjected to GC-MS analysis.

GC-MS Analysis. GC-MS analysis of these extracts was performed using an Agilent 7000 Series Triple, Quad Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS/) equipped With a Elite-5MS (5% diphenyl/ 95% dimethyl poly siloxane) fused a capillary column (30 x 0.25 µm ID x 0.25µm df)). For GC-MS detection an electron Ionization system with ionizing energy of 70ev was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate 1ml/min and injection volume of 2µl was employed (split ratio of 10:1); injector temperature 250º C; ion-source temperature 200° C. The oven temperature programmed from 110º C (iso thermal for 2 min) With an increase of 10º c/min to 200º C, then 5º C/min to 280º C, ending with a 9 min iso thermal at 280º C, mass spectra were taken at 70ev: a scan interval of 0.5 second and fragments from 45 to 450Da, total GC Running time was 36 minutes. The relative % amount of each component was calculated by comparing its average Peak area to the total areas. Software adopted to handle mass spectra and chromatograms was Turbomass.

Interpretation of mass spectrum GC-Ms was conducted using the database of Nationl Institue of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the of the unknown components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained [36].

Statistical analysis. All results were expressed as mean values ± standard deviation. Comparisons were performed by analysis of variance (ANOVA). Statistical analyses were run using SAS software.

RESULTS AND DISCUSSION

Chemical composition of Prickly pear pulp and peel. The chemical composition of the pulp and peel of cactus showed that pulp has high content of proteins, moisture and lipid but low content of total fiber and ash as compared to the peel (Table 2). The peels of the cactus were considerably higher in fiber (5.83%) than the pulp. The beneficial effects of fiber in human health have been shown to act together with other bioactive compounds in the prevention of chronic diseases [37, 38].

Glucose and fructose was the predominant sugars in pulp samples and present in lesser amounts in peel samples of prickly pear fruit. On the other hand, sucrose was the predominant sugars in peel samples and present in lesser amounts in pulp samples. The principle carbohydrate constituents of cactus pear fruits are the free sugars composed mainly of glucose and fructose with a small amount of sucrose [39]. These results are in accordance with previous study who reported that sucrose was the predominant sugar in peel samples and present in lesser amounts in pulp and juice samples of prickly pear fruit [40]. The relative total content of glucose and fructose differed among species and within fruit tissues. Also, Abdel-Nabey [41] found that fruit pulp content was about 53% glucose, and 47% fructose and this amount of glucose was notable, as this sugar was the sole energetic metabolite for the brain and nerve cells and was presented in prickly pear as free sugar, directly absorbable by the body. Also fructose was easily absorbed, enhances flavor, and it was sweeter than either glucose or sucrose [42].

The minerals content of cactus pulp and peel is summarized in Table (2). Cactus pulp extract showed to be rich source of calcium, potassium and magnesium than peel extract, but contained low amount of sodium. These results are in harmony with those of Bakari et al. (2017) [43] who found that cactus fruit contain magnesium, calcium, iron, cupper, manganese and zinc. Cactus pulp has high levels of calcium and magnesium, which are used for energy and sports drinks to uphold the mineral pool during periods of physical exhaustion [44] and the low level of sodium has an advantage for people with renal and blood pressure problems [45].

Calcium is an important element in pulp than peel and known to help ease insomnia, regulate the passage of nutrients through cell walls and stimulate muscle [46]. Also, calcium was represented three-quarters of minerals of the body and were found fundamentally in bones, which serve as an important reservoir [41]. The second important element found in pulp is magnesium in the pulp than peel provides its usefulness for the reactions involved in converting vitamin D to its active form and therefore, leading to the formation of Adenosine triphosphate (ATP), as constituent, to release parathyroid hormone and to relax the muscles [47]. Zinc and Cupper are distributed widely in plant and animal tissues and occurs in all living cells. It functions as a cofactor and is a constituent of many enzymes. Zn dependent enzymes are involved in macronutrient metabolism and cell replication [48]. Intakes of Mn and Fe from cactus can similarly be highlighted. Both trace elements, have commonly been associated with protection against oxidative damage [49]. The mineral pattern depends on the fruit origin and factors on the site of cultivation [50].

TABLE 2
Chemical composition *of Opuntia ficus-indica* fruit and peels samples (% w/w, DW)

Constituents	Pulp	Peel
Moisture	90.66± 2.32	88.92±1.98
Protein	1.62±0.26	1.53±0.18
Lipids	0.56±0.04	0.32±0.02
Total Fibers	4.65±0.37	5.83±0.42
Ash	2.6±0.29	3.4±0.31
Carbohydrate composition (g/100g)		
Fructose	20.17	1.24
Glucose	30.8	12.16
Sucrose	0.34	1.86
Mineral composition (mg/100 g DW)		
Ca	49.04	26.48
P	2.82	4.76
Na	0.70	1.80
K	410.7	549
Mg	18.6	12.7
Fe	3.2	4.2
Mn	1.08	1.17
Zn	0.78	1.18
Cu	1.53	1.70

TABLE 3
Quantitative phytochemical analysis of Prickly pear fruit and peel extracts

Constituents	Pulp extracts		Peel extracts	
	Ethanolic	Ethyl acetate	Ethanolic	Ethyl acetate
Total phenolic (mg Gallic acid /g DW)	15.74±0.66	8.69±0.24	121.26±0.38	57.373±0.54
Total flavonoid (mg Quercetin /g DW)	13.99±0.37	3.89±0.39	36.69±0.84	25.12±0.44
Total tannin (mg Tannic acid /g DW)	3.60±0.27	1.98±0.12	25.98±0.19	18.34±0.15
Total alkaloid (g/100g DW)	2.44±0.11	1.36±0.06	2.50±0.08	1.54±0.09
Total anthocyanin (mg cy-3-glu /100g FW)	471.41±2.62	245.45±1.83	56.73±0.24	36.48±0.35

Values are mean ± SD of three replicate analyses

Phytochemical compounds of Prickly pear pulp and peel. Phytochemical compounds of the two extracts of cactus pulp and peel are presented in table (3). The ethanolic and ethyl acetate extracts of peel have higher concentrations of total phenols, flavonoids, tannins and alkaloids than the pulp extracts. The ethanolic extract in both pulp and peel gave higher concentrations of phytochemical compounds than the ethyl acetate extract. On the other hand, the ethanolic and ethyl acetate extracts of pulp contain high concentrations of total anthocyanin than peel extracts. These results are in accordance with Abou-Elella and Ali (2014) [51] who found that the higher amount of phenol compounds was obtained by solvent which has high polarity. These findings may be due to the fact that phenolics are often extracted in higher amounts in more polar solvents such as aqueous methanol/ethanol as compared with absolute methanol/ ethanol [52].

Phytochemical screening of bioactive plant extracts has revealed the presence of alkaloids, tannins and flavonoids in Cactus (*Opuntia ficus-indica*), flavonoids and tannins have been linked to antibacterial activity and antidiarrheal activity [53, 54]. Different phytochemicals display various mechanisms of action such as increasing colonic water and electrolyte re absorption and inhibiting intestinal motility, while some components have been shown to inhibit specific pathogens [55]. Phytochemicals such as flavonoids, tannins and alkaloids have anti-inflammatory effects [56]. Tannins were present in Cactus (*Opuntia ficus-indica*) [57] and may be responsible for the good antibacterial activity. Previous studies have shown that tannins have been found to form irreversible complexes with proline-rich proteins, resulting in the inhibition of the cell protein synthesis, they bind proteins and adhesions, inhibit enzymes and complex with cell wall [58]. Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions, have been assigned to tannins [59]. Alkaloids have been reported to be responsible for the antibacterial activity in some plants [60]. Studies have demonstrated that alkaloids have pharmacological effects and could be associated with inhibition of nucleic acid, protein, and membrane phospholipids biosynthesis [61]. The mechanism of action of highly aromatic planar quaternary alkaloids such as berberine an important representative of the alkaloid group and humane is attributed to their ability to intercalate with DNA [62]. This probably explains the reason as to why the plants containing these basic alkaloids and alkaloid salts showed good antibacterial activity. Previous studies on other plants have reported that flavonoids being phenolic compounds are water soluble antioxidants and free radical scavengers which are capable of preventing oxidative cell damage and have strong anticancer activity [49]. The potent antioxidant activity of flavonoids, their ability to scavenge hydroxyl radicals, superoxide anions and lipid peroxide radicals may be the most important function of flavonoids [63].

Fatty acid and sterols compounds of Prickly pear pulp and peel. The ethanolic extract of cactus pulp and peel contain 11 fatty acids, 7 saturated fatty acids and 4 unsaturated fatty acids (Table 4). The main constituent is C18:2 Linoleic acid followed by C16:0 Palmitic acid, C18:1 Oleic acid and C18:3 Linolenic acid. Peel extract contains high amount of saturated fatty acids (24.65%) than pulp extract which contain 22.36%. On the other hand, pulp extract contains high amount of unsaturated fatty acids (77.64) than peel extract which contain (75.35 %). These results are in accordance with Bakari et al. [43] which reported that cactus extract contain palmitic acid (20.9%), oleic acid (11.0%) and linoleic acid (7.0%). These compounds were known for their beneficial health effects such as unsaturated fatty acids (oleic and linoleic acid) which can prevent cardiovascular diseases [64,65]. Several studies have indicated that cactus particularly; fruits, pulp, seed and prickly pear peel were rich in linolenic, oleic and palmitic acids [66]. High level of omega-6 linoleic acid was reported in cactus pulp and peel. 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 [67]. Omega-3 linolenic acid is known to be beneficial for health, cardiovascular diseases, inflammatory conditions, autoimmune disorder and diabetes [68].

Data in Table (4) showed that the ethanolic extract of pulp and peel of cactus contains 6 compounds of sterols separated by HPLC. The main constituent is β-Sitosterol followed by Campesterol. Ergosterol found only in peel extract. These results are in accordance with others [69] documented β-sitosterol as the major sterol extracted from different parts of the fruit oils: pulp, skin and seeds.

Water soluble vitamins. Fruits are important sources of vitamins for local people at the natural growth sites of the plant. The results in Table 5 reported that the Pulp of cactus contains vitamin C (35.6 mg/100g DW), vitamin B3 (0.48 mg/100g DW), vitamin B6 (0.32 mg/100g DW) and vitamin B9 (0.25 mg/100g DW). While the peel contains vitamin C (27.3 mg/100g DW), vitamin B3 (0.26 mg/100g DW), vitamin B6 (0.19 mg/100g DW) and vitamin B9 (0.11 mg/100g DW). Vitamin B12 (cobalophilin) is not found in both pulp and peel of cactus. These results are similar to previous studies who found that Cactus pear ranged from 1 to 41 mg/100g of vitamin C, which is higher than that found in other common fruits like apple, banana or grape [70, 71]. While other vitamins, such as carotenoids, thiamin, riboflavin, pyridoxine, Folic acid and niacin are in trace amounts [45]. It is known that the fruit contains vitamin C higher than peel [68]. It's reported that, vitamin c plays an important role in human nutrition, including growth and maintenance of tissues, the production of neurotransmitters, hormones and immune system responses. It's an important antioxidant and reduces the adverse effects of reactive oxygen species which caused damage to macromolecules such as lipids, DNA and proteins, which are related to cardiovascular disease, cancer and neurodegenerative diseases [72].

Phenolic and flavonoids compounds of Prickly pear pulp and peel. The growing interest in polyphenols results from their antioxidant potential, which is involved in health benefits such as the prevention of inflammation, cardiovascular dysregulation and neurodegenerative diseases. Polyphenols have also proven anticancer activity [73].

TABLE 4
Fatty acids and sterols compositions percent of prickly pear oils

Lipid Composition	Constituents	pulp	Peel
Fatty acids	C12:0 Lauric acid*	0.42	0.95
	C14:0 Myristic acid*	0.21	1.22
	C16:0 Palmitic acid*	14.82	15.76
	C16:1Palmitolic acid	0.16	0.40
	C18:0 Stearic acid *	4.09	4.15
	C18:1 Oleic acid	12.80	12.55
	C18:2 Linoleic acid	52.38	50.31
	C18:3 Linolenic acid	12.30	12.24
	C20:0 Arachidic acid*	0.77	0.71
	C22:0 Behenic acid*	1.34	0.93
	C24:0 Lignoceric acid*	0.71	0.95
	*SFA	22.36	24.65
	USFA	77.64	75.35
	USFA/SFA	3.47	3.06
Sterols	Ergosterol	-	0.75
	Campesterol	39.44	29.18
	Stigmasterol	4.86	2.54
	Lanosterol	3.36	2.18
	β-Sitosterol	47.2	61.4
	Δ^5-Avenasterol	5.14	3.95

*Total saturated fatty acids

TABLE 5
Water soluble vitamins contents of Prickly pear fruit and peel extracts

Vitamin contents	Values (mg/100g DW)	
	Pulp	**Peel**
Vitamin C (Ascorbic acid)	35.6	27.3
Vitamin B3 (Niacin)	0.48	0.26
Vitamin B6 (Pyridoxine)	0.32	0.19
Vitamin B9 (Folic acid)	0.25	0.11
Vitamin B_{12} (cobalophilin)	-	-

Values are mean ± SD of three replicate analyses

TABLE 6
HPLC analysis of phenolic and flavonoid compounds of Prickly pear pulp and peel extracts

Compounds	Conc. µg/mg DW	
	pulp	peel
Pyrogallol	25.20	36.05
Quinol	9.67	61.62
Gallic acid	4.70	8.39
Catechol	53.91	32.25
p-Hydroxy benzoic acid	214.30	500.34
Caffeine	-	76.24
Chlorgenic acid	21.80	169.80
Vanillic acid	39.22	330.23
Caffeic acid	1.08	-
Syringic acid	1.71	43.91
Vanillin	26.45	15.83
p-Coumaric acid	6.18	16.56
Ferulic acid	11.34	256.92
Benzoic acid	462.72	1597.62
Rutin	32.20	45.98
Ellagic acid	179.77	1481.23
o- Coumaric acid	2.43	1.095
Salicylic acid	191.24	11.12
Cinnamic acid	1.31	17.20
Flavonoids compounds		
Myricetin	141.39	405.81
Quercitin	-	92.95
Rosmarinic acid	-	56.44
Neringenin	207.42	102.54
Kaempferol	29.02	48.90

Data in Table (6) showed that the ethanolic extract of pulp and peel cactus contains 20 phenolic compounds. The main constituent in pulp extract is Benzoic acid followed by *p*-Hydroxy benzoic acid, Salicylic acid and Ellagic acid. In addition, the main constituent in peel extract is Benzoic acid followed by Ellagic acid, *p*-Hydroxy benzoic acid, Vanillic acid and Ferulic acid. In addition, the ethanolic extract of pulp and peel cactus contains a number of flavonoids compounds such as myricetin, Quercitin, Rosmarinic acid, Neringenin and kaempferol. Quercitin and Rosmarinic acid found only in peel extract. Quercetin possesses antiproliferative, anticarcinogenic and antioxidant activities [74]. The peel extract contains high concentrations of Myricetin. While the pulp extract contains higher concentrations of Neringenin.

Cactus extract contains the main constituent being ferulic acid (34.8%), caffeic acid (2.6%) and vanillic acid occurring in small quantities (0.1%) [43]. These compounds vanillic acid and *p*-coumeric acid are used as an antimicrobial agent [75]. Ferulic acid can inhibit the photo-peroxidation of linoleic acid at high concentrations and caffeic acid was found to possess higher antioxidant activity [76, 77].

Antioxidant activity of Prickly pear pulp and peel. In DPPH scavenging assay, the antioxidant activity was measured by the decrease in absorbance as the DPPH radical received an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule [78]. The capability of DPPH reduction was determined by the decrease in its absorbance at 517 nm, which is increased by antioxidants. Scavenging of superoxide radical is important because it is one of the precursors of the singlet oxygen and hydroxyl radicals. During oxidation reactions at a cellular level, superoxide radicals are normally formed first, and their effects can be magnified because they produce other kinds of cell-damaging free radicals and oxidizing agents [79]. The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells. This radical has the capacity to join nucleotides in DNA and can cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity [80].

The ethanolic extract of both pulp and peel cactus gave high antioxidant activity as compared with ethyl acetate extract and the peel extract gave the hight antioxidant activity than pulp extract. The scavenging of DPPH radicals increased with increasing extract concentration from 40, 80, 120 and 150 µg /mL (Table 7). The IC_{50} value of ethanolic and ethyl acetate extracts of peel cactus was lower than pulp extract. IC_{50} values indicate the concentration

of the test sample required to inhibit 50% of the free radicals. The IC_{50} value is a parameter widely used to measure the free radical scavenging activity; a smaller IC_{50} value corresponds to a higher antioxidant activity [81].

The antioxidant effects are due to the major flavonoids encountered in cactus fruits. Flavonoids are more efficient antioxidants than vitamins, since phenolic compounds are able to delay pro-oxidative effects in proteins, DNA, and lipids by the generation of stable radicals [82]. Polyphenolic compounds in *O. ficus indica* have been shown to induce a hyperpolarization of the plasma membrane and to raise the intracellular pool of calcium in human Jurkat T-cell lines [83]. Also, health beneficial effects of cactus polyphenols might be conditioned by their antioxidant and radical scavenging activities. For instance, gallic acid, largely found in cactus flowers, exhibits high antioxidant activity responsible for its ability to reduce DNA damage and to buffer free radicals [84, 85]. In addition, the ethanol extract of the stem of *Opuntia ficus indica* was found to be potential in protecting plasmid DNA against the strand breakage induced by hydroxyl radicals. The ethanol extract was determined as containing a high amount of phenolic compounds responsible for antioxidant activity of the extract [86]. As the concentration of phenolic compounds or the degree of hydroxylation of the phenolic compounds increases DPPH radical scavenging activity increases, and with it antioxidant activity [87]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [88]. Phenolic compounds in plants play a major role in their antioxidant activities. The major secondary metabolites from plants are polyphenols, which are reported to possess antioxidant and free radical scavenging activity. Several studies have shown that polyphenols act as antioxidants by inhibiting free radicals [89].

The reducing power capacity reflects the presence of an antioxidant for the reduction of ferricyanide ions $[Fe(CN)6]^{3-}$ to ferrocyanide ions $[Fe(CN)6]^{4-}$. This reducing property is generally associated with the presence of a reducer exercising an antioxidant action by breaking the free radical chains; yielding and hydrogen atom [89]. The ethanolic extract of both pulp and peel cactus gave high reducing power activity than ethyl acetate extract and the peel extract gave high reducing power activity than pulp extract (Table 7). The ethanolic extracts of prickly pear peels may act as electron donors and could react with free radicals to convert them into more stable products and then terminate the free radical chain reactions. The peel extract contains high phenolic compounds than pulp extract (Table 3). Therefore, the antioxidant activity is well correlated with the amount of phenolics constituent found in the extract. Therefore, phenolic compounds of prickly pear peels are good electron donors and could terminate the radical chain reaction by converting free radical to more stable products [51].

TABLE 7
Antioxidant activity of Prickly pear pulp and peel extracts against DPPH method.

Conc. (µg/ml)	pulp		peel	
	DPPH % in ethanolic extract	DPPH % in ethyl acetate extract	DPPH % in ethanolic extract	DPPH % in ethyl acetate extract
40	55.05	50.29	59.79	55.91
80	63.40	51.20	66.8	58.58
120	68.69	56.63	70.44	60.49
150	74.77	61.11	76.44	61.59
IC_{50} (µg/ml)	25.75	39.77	20.45	35.77
Reducing power activity (µg Gallic acid /100g DW)	12.58	8.42	20.53	15.19

TABLE 8
Antimicrobial activities of Prickly pear pulp and peel extracts against selected bacterial strains and fungus.

Samples	Inhibition zone (mm)*					
	Gram positive bacteria		Gram negative bacteria		Fungus	
	S. aureus	*B. cereus*	*E. coli*	*S. typhimureum*	*A. niger*	*C. albicans*
Pulp ethanolic extract	17.26±0.19	10.13±0.11	12.20±0.16	12.14±0.19	5.48±0.13	6.45±0.10
Pulp ethyl acetate extract	13.06±0.27	9.25±0.18	10.32±0.10	9.28±0.08	3.12±0.06	4.45±0.08
Peel ethanolic extract	19.35±0.34	13.46±0.22	14.35±0.41	13.82±0.33	6.18±0.14	8.20±0.13
Peel ethyl acetate extract	15.14±0.41	9.68±0.24	11.17±0.18	11.32±0.14	4.53±0.11	5.70±0.17

Values are mean ± SD of three replicate analyses

TABLE 9
Anticancer activities of Prickly pear fruit and peel ethanolic extracts

Concentrations (μg/ml)	Liver cell line (HepG2) Viability %		Colorectal adenocarcinoma (Caco-2) cell line Viability %		Breast cell line (MCF-7) Viability %	
	pulp	Peel	pulp	Peel	pulp	Peel
500	91.5	100	98.8	98	100	100
1000	79.7	90.5	97.6	92.8	88.6	98
1500	57	81	82.6	88.4	82.8	95
2000	50.6	74	74	78	75	81.5
3000	43	66	69	72	70.4	79
IC_{50} (μg/ml)	18.3	50.3	120	170	400	790

Antimicrobial activity of Prickly pear pulp and peel. The antimicrobial activity of Prickly pear pulp and peel extracts varied according to the species of bacteria and fungi tested and the solvents used (Table 8). The data indicate that the ethanolic extract of pulp and peel of cactus exhibited the highest activity against the investigated food pathogens as compared with the ethyl acetate extract. In addition, peel extract produced potent antimicrobial activity as compared with pulp extract. All extracts of pulp and peel cactus showed high antibacterial activity (The diameter of zone of inhibition increased) against *Staphylococcus aureus and Escherichia coli* than *Bacillus cereus* and *Salmonella typhimureum*. Also, ethanolic and ethyl acetate extract gave high antifungal activity against *Candida albicans* than *Aspergillus niger*. These results are in accordance with [90] who reported that the antibacterial activity of methanol, ethanol, chloroform extracts of cladodes and skin fruit extracts of *Opuntia ficus indica* have demonstrated greater antibacterial activity against both gram positive and gram negative bacteria. The antibacterial activity might be due the presence of various bioactive constituents in the extracts.

The antibacterial potency of peel extract may be due to the presence of many potent compounds such as sterols, flavonoids, tannins, phenolics and alkaloids as shown in Table (3). Phytochemicals are as antimicrobial compounds, have made great contribution for quick and effective management of plant disease and microbial contamination in several agricultural conditions [91]. Alkaloids were present in in Cactus (*Opuntia ficus-indica*) and may be responsible for the good antibacterial activity. The antibacterial activity that they exhibited against the three bacterial species; *S. Marcescens*, *E. coli* and *S. thermophiles.* These active compounds in these plants could find place in treatment of various bacterial infections in poultry where they can be used as alternatives to conventional antibacterial drugs [57]. Also, the antimicrobial activity of flavonoids may be due to their ability to complex with extracellular, protein and bacterial cell wall while that of tannins was explained by their ability to inactivate microbial adhesions, enzymes and cell envelope. In addition, phenolic compounds have been shown to inhibit enzymes by reacting with the sulfhydryl groups of amino acids [92]. Flavonoids and tannins form complexes with the nucleophilic amino acids of proteins which leads to their inactivation. Flavonoids lacking hydroxyl groups on there β-rings are more active against microorganisms than are those with the two OH groups; this finding supports the idea that their microbial target is the membrane. It has been shown that phenolic alcohols (thymol, carvacrol, eugenol) are the strongest inhibitors of enzymatic processes. This is attributed to its lipophilic characteristic and its free OH groups [95]. Some fungi secrete hydrolytic enzymes that diffuse into host cells prior to the advance of microorganisms, which can be inhibited by free radicals of oxidized phenols that function as nonspecific inhibitors; such as tannins, cyanidin, delphinidin and malvidin anthocyanindins [95]. Sterols have been reported to account for the exertion of antimicrobial activity by plants containing them. The presence of these sterols may contribute to the good antibacterial activity. Also, the mode of antimicrobial action of tannins may be related to their ability to inactivate microbial adhesions, enzymes, and cell envelope transport proteins, they also complex with polysaccharide [95].

The Gram positive bacteria isolates were found to be more susceptible to the inhibitory action of the pulp and peel extracts than the Gram negative bacteria isolates. This was due to the presence of an extra outer membrane in Gram negative bacteria, which contains lipo-polysaccharide that make the cell wall of the bacteria impermeable to extract. Similar inhibitory activity of extracts against Gram positive bacteria was reported by Durgesh and Tumane [96].

Anticancer activity of Prickly pear pulp and peel. Data in Table (9) showed that the cytotoxic activity of Prickly pear pulp and peel as an anticancer agent. The percentage of Liver cell line (HepG2), Colorectal adenocarcinoma (Caco-2) and Breast cell line (MCF-7) viability was decreased with increasing the concentrations of the ethanolic extract of pulp and peel cactus (500, 1000, 1500, 2000, 3000 μg/ml). The most pronounced reduction in the viability of cancer cells was detected after treatment with pulp extract than peel extract. The high concentrations of ethanolic extract of pulp and peel cactus caused the high reduction in the viability of cancer cells especially in Liver cell line (HepG2). Cancer is often associated with increased risk of death and the

toxic side effects caused by the modern medicine. These results are in accordance with others who concluded that prickly pear cactus effectively inhibited cell growth in several different immortalized and cancer cell cultures *in vitro* and suppressed tumor growth. The pear extracts significantly suppressed tumor growth in nude mice [97]. The anticancer effect of ethanolic extract may be due to presence of polyphenols that play an important role in antioxidant activity [98] and have evidently shown antiproliferative activity or cytotoxicity in human oral cancer cells, melanoma cells [99, 100] and lung metastasis induced by B16F10 melanoma cells [101].

Also, the ethanolic extract contains gallic acid also exerts a cytotoxic activity against tumoral cells from leukemia, lung and prostate cancer origins. In addition, sterols were found to inhibit tumor promotion in two-stage carcinogenesis in mice [102]. Cactus pear does alter the expression of certain genes related to cell growth and apoptosis. Quercetin is one of the components of cactus pear extracts [97]. Herzog et al., [103] suggested that quercetin might be one of the active compounds responsible for the anti-carcinogenetic and apoptosis-induction effects of cactus pear extracts.

GC- mass analysis of Prickly pear pulp and peel. 31 compounds were identified in the ethanolic extract of Prickly pear pulp by GC-MS analysis. The active principles with their retention time (RT) molecular formula, peak area and % of peak area are present in Table (10) and Fig. (1). The prevailing compounds were Cyclodeca[b]furan-2(3H)-one, 9-(acetyloxy)-3a,4,5,8,9,11a-hexahydro-4-hydroxy-6, 3-Amino-5-methylthio-1H-1,2,4-triazole, Ethyl 1-fluoro-2-methylenecyclopropane-1-carboxylate, Limonene, α-Terpinolene, 4-Methyl-4-hexen-1-ol, Naphthalene, Ropivacaine, Ethyl 4-oxo-8-methyldecanoate, H-Indole-2-methanamine, 5-methyl-1, 2,4-Di-tert-butylphenol, α-methyl-p-isopropyl hydrocinnamaldehyde, 4,4,5,5-Tetramethyl-2-phenyl-1,3,2-dioxaborolane, 1-Morpholino-1-cyclopentene, (2-Decyl)benzene, Dicinnamamide, α- Methyl-4-(2-methylpropyl) benzeneacetaldehyde, Benzamide, 4-ethyl-N-allyl, 4-Ethylbenzylamine, N,N-diheptyl-, 2- Phenylundecane, 1,2,4-Tributylbenzene, Disiloxane, 1,3-bis(1,1-dimethylethyl)-1,1,3,3-tetramethyl-, Dodecylbenzene, Tetraethylbenzene, Indolin-2-one, 1-methyl-3-t-butyl-, 1-(2'-Iodophenyl)-2-propanone, Ambrosiol (8,9-dihydroxy-6,9a-dimethyl-3-methylidene-decahydro-azuleno[4,5-b]furan-2(3h)-one), Calcitriol, Squalene and Gamma.-Sitosterol. These bioactive components which may possess several pharmacological properties. Squaleneis one among the compounds of the present study. Squalene possesses chemo-preventive activity against the colon carcinogenesis [104]. In addition, Squalene is the main component of skin surface. Polyunsaturated lipids show some advantages for the skin as an emollient and antioxidant and for hydration and its antitumor activities [105].

In addition, 27 compounds were identified in the ethanolic extract of the Prickly pear peel by GC-MS analysis. The active principles with their retention time (RT) molecular formula, peak area and % of peak area are present in Table (11) and Fig. (2). The prevailing compounds were 7-Methyl-7-(1-methylethenyl)-2-phenylbicyclo[4.2.0]oct-1-ene, γ-Terpinene, 1-Chloro-3-methyl-1-pentyn-3-ol, 4-amino-3,5-bis(dimethoxymethyl)-4H-1,2,4-triazole, 1-methyl-8-isopropyl-Tricyclo undeca-4,9-diene-3,6-dione, 2-Cyclohexylpropan-1-ol, 1,4-Cyclohexanedione, N-Acetyl-L-proline, 4-Methylhex-4-en-1-ol, Naphthalene, 2,2-Dimethylcyclohexanone, 3-Isopropenylthiophene, 1; 3'-Azido-3'-deoxythymidine, Ethyl 4-oxo-8-methyldecanoate, 3-Phenyl-1-pentene-3-yne, 1-Methyl 5-Methyl-imidazolecarboxylate, Trans-beta-Methylstyrene, 3,4-Bis(hydroxymethyl)furan, [Ethyl(methyl)seleno]-sulfenate, (1'-propenyl)thiophene, 2-Pentyl-3-methyl-2-cyclopenten-1-one, 4-Vinyl-1-methyl-3-oxabicyclo hexane, 2,4,4-trimethyl-3-vinylcyclopentanone, 1-Morpholino-1-cyclopentene, 2,4-DI-Tert-butylphenol, Thymol and cis-Chrysanthenyl acetate.

Thymol detected in peel extract and not found in pulp extract. Similar results are reported by Moosazadeh et al. [106] analysed the chemical composition and antifungal activity of *Opuntia stricta* fruit essential oil. Nineteen compounds were identified in the oil by Gas Chromatography-Mass Spectrometry (GC-MS), with thymol (42.7%) as the dominant component with antifungal activity against *Candida albicans* at low (2.5 to 40 mg/mL) concentrations, which was attributed to the high content of thymol. Also, terpenes are detected by GC- mass in the ethanolic extract of peel. These results are similar to Ammar et al. [107] found that the hexane extracts from *Opuntia ficusindica* and *O. stricta* inhibited both fungi *Aspergillus niger* and *Candida lipolytica*. Chemical composition of hexane extracts analysed by GC-MS, revealed the presence of secondary metabolites belonging to carboxylic acids (28–97%), terpenes (0.2–57%), esters (0.2–27%) and alcohols.

CONCLUSION

This research is following a trend to effectively identify various compounds found in the ethanolic extract of pulp and peel cactus and find its prophylactic role in designing and developing pharmacological drugs with less side effects. *In vitro* investigations in the present study provide substantial evidence that cactus peel; an inedible waste product is a potent source of antioxidant, antimicrobial agent and anticancer activity thereby indicating its use as a value-added component for functional.

TABLE 10
Compounds present in the Prickly pear pulpt using GC-MS analysis

No.	Peak name	Formula	Retention time	Peak area	% Peak area
1	Cyclodeca[b]furan-2(3H)-one, 9-(acetyloxy)-3a,4,5,8,9,11a-hexahydro-4-hydroxy-6 MW: 306.358	$C_{17}H_{22}O_5$	3.673	167134.3	0.71
2	3-Amino-5-methylthio-1H-1,2,4-triazole MW: 130.17	$C_3H_6N_4S$	4.398	770234.75	3.88
3	Ethyl 1-fluoro-2-methylenecyclopropane-1-carboxylate MW:144.144	$C_7H_9FO_2$	5.565	61206.88	0.2
4	Limonene MW: 136.238	$C_{10}H_{16}$	5.831	99463.99	0.33
5	α-TERPINOLENE MW: 136.23	C_6H_{12}	6.475	140495.77	0.47
6	1,4-Cyclohexanedione MW:112.13	$C_6H_8O_2$	8.158	152331.62	0.5
7	4-Methyl-4-hexen-1-ol MW: 114.186	$C_7H_{14}O$	8.4	170527.57	0.56
8	Naphthalene MW: 128.174	$C_{10}H_8$	8.674	188832.28	0.63
9	Ropivacaine MW: 274.4054	$C_{17}H_{26}N_2O$	8.955	38288.47	0.13
10	Ethyl 4-oxo-8-methyldecanoate MW: 228.332	$C_{13}H_{24}O_3$	10.051	341518.62	1.13
11	1H-Indole-2-methanamine, 5-methyl- MW: 174.24	$C_{11}H_{14}N_2$	10.341	228042.57	0.76
12	2,4-Di-tert-butylphenol Mw: 206.32	$C_{14}H_{22}O$	15.1	103419.73	2.13
13	α-methyl-p-isopropyl hydrocinnamaldehyde MW: 190.281	$C_{13}H_{18}O$	15.543	438959.59	1.45
14	4,4,5,5-Tetramethyl-2-phenyl-1,3,2-dioxaborolane MW: 104.073	$C_{12}H_{17}BO_2$	15.768	453023.61	1.5
15	1-Morpholino-1-cyclopentene MW: 153.22	$C_9H_{15}NO$	16.05	348351.12	1.51
16	(2-Decyl)benzene MW: 218.384	$C_{16}H_{22}$	16.203	1007758.8	3.34
17	Dicinnamamide MW: 174.24	$C_{11}H_{14}N_2$	16.445	100062.47	0.33
18	α- Methyl-4-(2-methylpropyl) benzeneacetaldehyde MW: 190.286	$C_{13}H_{18}O$	16.557	731101.47	2.42
19	Benzamide, 4-ethyl-N-allyl MW: 189.258	$C_{12}H_{15}NO$	16.718	1247208.27	4.13
20	4-Ethylbenzylamine, N,N-diheptyl- MW: 331.588	$C_{23}H_{21}N$	16.96	1330485.85	4.41
21	2- Phenylundecane MW: 232.411	$C_{17}H_{28}$	17.379	2354613.91	18.39
22	1,2,4-Tributylbenzene MW: 246.438	$C_{18}H_{30}$	17.661	1611935.21	5.34
23	Disiloxane, 1,3-bis(1,1-dimethylethyl)-1,1,3,3-tetramethyl- MW: 246.5370	$C_{12}H_{30}OSi_2$	17.717	1496295.44	4.96
24	Dodecylbenzene MW: 246.43	$C_{18}H_{30}$	18.095	1292006	11.35
25	Tetraethylbenzene MW: 190.324	$C_{14}H_{22}$	18.748	1598155.42	5.29
26	Indolin-2-one, 1-methyl-3-t-butyl- MW: 203.280	$C_{13}H_{17}$	18.828	1031238.25	3.42
27	1-(2'-Iodophenyl)-2-propanone MW: 260.074	C_9H_9IO	18.973	758398.95	2.51
28	Ambrosiol (8,9-dihydroxy-6,9a-dimethyl-3-methylidene-decahydro-azuleno[4,5-b]furan-2(3h)-one) MW: 266.337	$C_{15}H_{22}O_4$	22.275	305929.76	1.01
29	Calcitriol MW: 416.646	$C_{27}H_{44}O_3$	25.665	49114.01	0.54
30	Squalene MW: 410.7180	$C_{30}H_{50}$	32.333	3084411.66	10.22
31	Gamma.-Sitosterol MW: 414.718	$C_{19}H_{50}O$	40.354	1770579.18	6.45

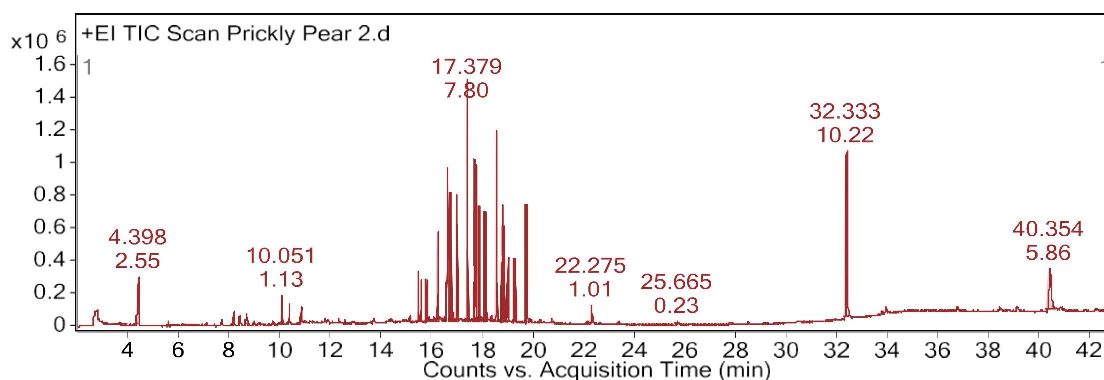

FIGURE 1
GC-MS profiles of Prickly pear pulp

TABLE 11
Compounds present in the Prickly pear peel using GC-MS analysis

No.	Peak name	Formula	Retention time	Peak area	% Peak area
1	7-Methyl-7-(1-methylethenyl)-2-phenylbicyclo[4.2.0]oct-1-ene MW: 238.374	$C_{18}H_{22}$	2.578	267652.55	10.55
2	γ-Terpinene MW: 136.2340	$C_{10}H_{16}$	3.633	18161.96	0.72
3	1-Chloro-3-methyl-1-pentyn-3-ol MW: 132.588	C_6H_9ClO	3.931	7653.95	0.71
4	4-amino-3,5-bis(dimethoxymethyl)-4H-1,2,4-triazole MW: 232.237	$C_8H_{16}N_4O_4$	4.301	401388.9	15.82
5	1-methyl-8-isopropyl-Tricyclo undeca-4,9-diene-3,6-dione MW: 229.196	$C_{15}H_{20}O_2$	7.087	27265.99	1.31
6	2-Cyclohexylpropan-1-ol MW: 142.242	$C_9H_{18}O$	7.595	42669.38	1.68
7	1,4-Cyclohexanedione MW: 112.128	$C_6H_8O_2$	7.675	31798.07	1.25
8	N-Acetyl-L-proline MW: 157.17	$C_7H_{11}NO_3$	8.134	80597.37	3.18
9	4-Methylhex-4-en-1-ol MW: 114.188	$C_7H_{14}O$	8.335	120628.71	4.75
10	Naphthalene MW: 128.174	$C_{10}H_8$	8.625	126462.29	6.55
11	2,2-Dimethylcyclohexanone MW: 126.199	$C_6H_{14}O$	8.915	64592.91	2.55
12	3-Isopropenylthiophene MW: 124.203	C_7H_8S	9.173	9354.28	0.37
13	1; 3'-Azido-3'-deoxythymidine MW: 267.245	$C_{10}H_{13}N_5O_4$	9.672	20169.76	0.79
14	Ethyl 4-oxo-8-methyldecanoate MW: 228.332	C13H24O3	10.018	129008.79	5.08
15	3-Phenyl-1-pentene-3-yne MW: 142.201	$C_{11}H_{10}$	10.332	42629.14	1.68
16	1-Methyl 5-Methyl-imidazolecarboxylate MW: 141.11	$C_6H_9N_2O_2$	10.654	7513.51	0.3
17	Trans-beta-Methylstyrene MW: 118.179	C_9H_{10}	11.089	15434.68	0.61
18	3,4-Bis(hydroxymethyl)furan MW: 118.179	$C_6H_8O_3$	11.17	28588.72	1.13
19	[Ethyl(methyl)seleno]-sulfenate MW: 154.96	C_3H_7SeS	11.25	84956.87	4.21
20	(1'-propenyl)thiophene MW: 124.201	C_7H_8S	11.717	29120.59	1.15
21	2-Pentyl-3-methyl-2-cyclopenten-1-one MW: 166.26	$C_{11}H_{18}O$	12.023	15534.82	0.61
22	4-Vinyl-1-methyl-3-oxabicyclo hexane MW: 121.238	C_9H_{13}	12.168	36071.48	2.35
23	2,4,4-trimethyl-3-vinylcyclopentanone MW: 153.245	$C_{10}H_{17}O$	12.539	153717.38	6.06
24	1-Morpholino-1-cyclopentene MW: 153.22	$C_9H_{15}NO$	13.505	11813.41	12.17
25	2,4-DI-Tert-butylphenol MW: 206.329	$C_{14}H_{22}O$	15.1	25791.65	1.02
26	Thymol MW: 150.221	$C_{10}H_{14}O$	16.243	260939.62	10.28
27	MW: 194.274	$C_{12}H_{18}O$	16.718	79747.83	3.12

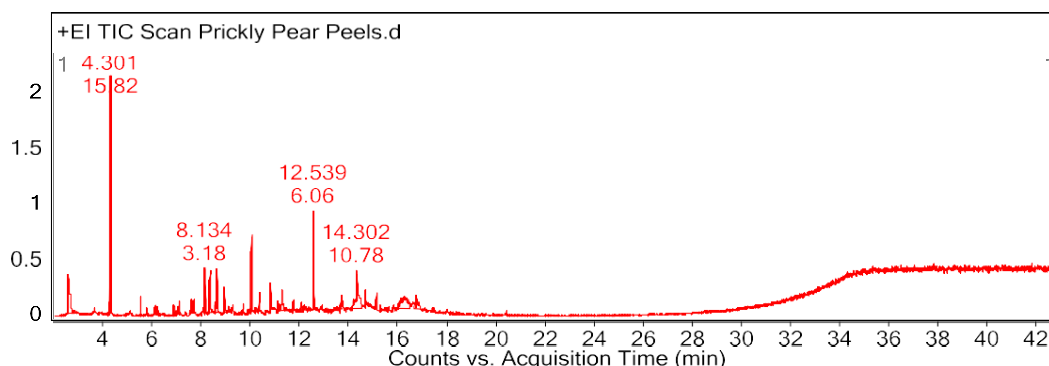

FIGURE 2
GC-MS profiles of Prickly pear peel

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Received: **04.11.2018**
Accepted: **22.01.2019**

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