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Phytochemical Constituents of Natural Extract of Prickly Pads (*Opuntia Ficus-Indica*) from Northern and Middle Provinces of the Midle East Area using (ICP-EAS)/(GC-MS) techniques

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ABSTRACT

Prickly Pads species of Opuntia ficus-indica, which naturally grows in different areas of the Middle East like Jordan and Saudi Arabia. Samples were collected and chemically prepared investigated to their phytochemical constituents using Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-EAS) for determining twenty-two trace of heavy elements and analyzing the oils extracted for identifying thirty-two organic compounds by Gas Chromatography-Mass (GC-MS) Spectroscopy Detection (GC-MS). In this research study, results of mean concentrations for heavy metals were ranged from non-detectable to 277 ppm, and the extracted oil analysis was dominated by 2- furancarboxaldehyde, 2,5 -dihydroxy-3,6-dimethylhyroxy-1,4 propanal, formic acid, propionic acid, undecanoic acid, 1,2-bisphenyloxycarbonylhydrazine, 2,3-dihydroxy acetic acid, 2,2-dihydroxyacetic acid, 2-Ethoxyethyl acrylate, acetic acid, methyl acetoacetate, 2- (2 methoxyethoxymethyl) oxirane, ethane, 2-hydroxy-1,2-

diphenylethanone, 2-Hydroxyethyl propanoate, Hydrazine, 4H-Pyran-4-one, 2,4,5-trimethyl-1,3-dioxolane, 5-hydroxymethyl-2-furaldehyde, 4-methyl-4-hepten-3-one, 1H-Pyrazole 1,3,5-trimethyl-1-butene and 2-chloro-3-methyl-cyclohexene as major and minor components were ranged from very low percentage to 700 ± 0.20 %.

Key words: *Opuntia ficus-indica*, Phytochemical, (ICP-EAS), (GC-MS) techniques.

INTRODUCTION

Species of *Opuntia ficus-indica* of Prickly Pads of is a Mediterranean fleshy plant, it heights ranged between 2-4 m with a bifurcated branching system producing ovate to elliptic fleshy compressed delicious fruits as seen in Figure 1. It is commonly used in many Mediterranean countries as a fencing plant to protect private gardens, orchards and cultivated areas specially those within or close to villages [1].

Figure 1: Prickly pads of the species (*Opuntia ficus-indica*).

The use of the plant in this way has multiple functions. First it acts as formidable protection to the crops within the fence due to its ridged growth and more important scary spiny nature of the plant. Secondly the fruits of this of the plant have high demand in the market and can produce extra money to the farmer, and thirdly the soft cladodes can be used as a green feed to animals especially in the long dry summer season [2,3]. For centuries the medicinal plants like *Opuntia ficus-indica* species, fruits, stems and their fiber content in its maturity stage have been prescribed traditionally in folk medicine in several countries for several purposes [4,5]. Previous studies addressed that young and advanced maturity stages of prickly pads are rich in proteins vitamins, minerals, and other substances important for good health, but most current literature and several studies reported a high levels of

amino acids, especially proline, taurine and serine [6,7]. Almost no change in the form or the way they have been used and the strong belief in their ability to cure diseases [8]. Therefore, the clinical pharmacologic interest in terms of the efficacy and safety of the phytochemicals present in *Opuntia ficus-indica* has grown during recent years due to the fact that many people using this plant in self-medicate [9,10]. There are clinical and experimental evidences about the effects of phytochemical from *Opuntia ficus-indica* on health in order to give some ideas of the potential benefits of one commonly used plant around the world. So far, there is no report about the adverse/toxic effects on humans [11,12]. El-Moghazy et al. were isolated and identified N-methyl tyramine, tyramine and mescaline of *Opuntia ficus-indica* (L) Mill, also the flavonoids, Pendleton, kaempferol, luteolin, quercitrin and rutin were isolated too. The study showed 80-85% w/w of carbohydrates and acids like pentose, hexoses, hexuronic acids in addition to 0.094% of organic acids companied by ascorbic acids [13].

Previous studies and experimental analysis of Prickly pads of *Opuntia ficus-indica* showed that the main constituent is water (70-90%) followed by small amounts of carbohydrates (4-6%), fiber (1-3%), and protein (0.5-1%) [14]. Bioactive chemical constituents of the oils of *Opuntia ficus-indica* was identified and showed 21 according to GC-MS analysis techniques, including groups such as polymers, fatty acids, esters, aldehydes, phenolics acids and steroid which is illustrated in Figure 2. These organic mucilaginous components are only partly known and have not been quantitatively determined, but generally have pharmaceutical and industrial uses as seen in Figure 2 [15-17].

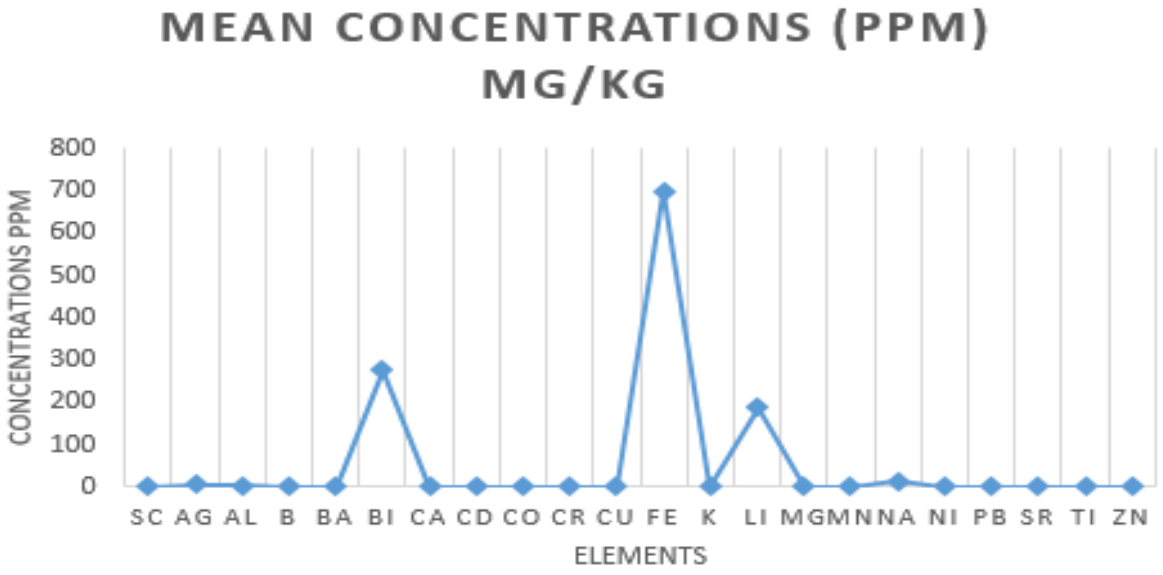


Figure 2: Phytochemical constituent of heavy metals of *Opuntia ficus-indica* in ppm.

The major objective of this research was to evaluate the *Opuntia ficus-indica* plant for its chemic The chemical active ingredients composition of medicinal properties and their pharmaceutical activities and uses in their ability to cure human diseases. So far, the chemical active ingredients and constituents of this plant species were identified, in particular organic (volatile, non-volatile) and inorganic component, using Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) and Gas Chromatography-Mass Spectroscopy Detection (GC-MS) [18].

MATERIALS AND METHODS

Method of powder samples

Representative samples of *Opuntia ficus-indica* were grown without chemical treatment, collected from several sampling areas of different maturity stages in summer 2015 (June, July to August) from the field located on a "Northern and Middle Provinces in Jordan and Saudi Arabia. About four kilograms samples were carefully washed with distilled water and disinfected using commercial 10% sodium hypochlorite solution; in order to remove dust particles and eliminate microorganisms. Thorns were removed manually and separated into two groups each one associated with specific analysis. Group 1 for heavy metals determination, small parts of *Opuntia ficus-indica* slices were dried in a vacuum oven system at temperature (45 ± 5°C) for 12 h at 10^{-2} Torr, in order to stop enzymatic activity and to avoid protein and carbohydrate damage. The dried sample was crushed using a mechanical grinding stainless steel mill equipped with 60-mesh sieve <180 μm to obtain a powder (flour) form before acid dissolution and stored. Ash for heavy metals content was prepared and analyzed by weighing accurately 2 g portion of a powder into a 50-ml crucible and placed in a muffle furnace at 550°C for 24 hr, to remove organic material; then cooled at room temperature in a desiccator in order to reach a constant weigh. Then the ashed sample was dissolved with 10 ml HCl 2N and boil for 10 minutes gently. Cool, filtrate using 10 ml of HCl 2N and deionized water in 100-ml volumetric flask, to prepare a mixture volume with deionized water to 100 ml [19].

Group 2 used for the isolation of oils (organic) extracts, small parts of Opuntia Ficus Indica, samples the extracts were filtered using Whitman filter paper (No.1). Extracts were stored in a freezer at 4°C for further tests. Sample of ~1 g of *Opuntia ficus-indica*, was ground and mixed with 5 ml methanol (absolute), then transferred into two separate 1-L glass flasks filled with methanol the mixtures were and then kept in the dark for 48 hrs, at 20-25°C. After magnetic stirring for 10 h, the extract was centrifuged at 500 rpm for 3 min and the supernatant was filtered through a GH Polypro 0.45 μm filter and then concentrated under reduced pressure at 40°C using a rotary evaporator. A small portion 1 μL was injected for the GC/MS analysis model Shimadzu 2010 with a gas chromatograph coupled to MSD quadrupole mass spectrometer. Built-in data-handling program for all

identification were carried out and provided by the manufacturer of the gas chromatograph. The compositions were reported and the identification of the extracted compounds was based on a comparison of their retention times, compared to published data and spectra of authentic compounds using their mass spectra compared to the Wiley version 7.1 library. All analyses were done in triplicate according to the methods as described in the Association of Official Analytical Chemists (AOAC) techniques [20].

EXPERIMENTAL PROCEDURE

Equipments

Inductively coupled plasma - Atomic Emission Spectrometer ICP- AES

ICP-AES spectrometer Perkin Elmer, Model Optima 3000, and AS-90 Serial no., N069-0158, carried out by the AOAC method for the analysis of trace metals in plants. Argon was used as the coolant and carrier gas for the determination of the mineral contents of elements like Scandium (Sc), silver (Ag), Aluminum (Al), Born (B), Barium (Ba), Bismuth (Bi), Calcium (Ca), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Iron (Fe) Potassium (k), Lithium (Li), Mg, Manganese (Mn), Sodium (Na), Nikel (Ni), Lead (Pb) Titanium (Ti), Zinc (Zn), Strontium (Sr). ICP- AES method provides a rapid and precise means of monitoring up to 50 elements simultaneously for minerals at trace- levels [21].

Gas Chromatography-Mass Spectrometry GC-MS

The identification and quantification of extracted oils components were also performed using GC-MS with limit of detection in ppb. Analyses were done in triplicate according to the methods as described in the Association of Official Analytical Chemists (AOAC) [21]. The system of Mass spectroscopy detection was equipped with capillary column and electron ionization mode used with a specific mass range. The helium carrier gas and mass selective detector, *hp* 5890 series II, Hp 0.5 cross link 5% pH Me Silicone, column 25 m × 0.2 mm × 0.33 m and Film thicken was used. The detector and injector initial temperatures were set at 250°C and 280°C, respectively. Temperature column program started at 35°C to 250 C^{o} at a rate degree of 5°C/minute, with the lower and upper temperatures being held for 1 and 5 minutes, respectively. The carrier gas (helium) rate flow was 1 ml/min. The solvent retention time was 5 min. The oven temperature also programmed initially at 60°C (held for 1 min.), then increased to 240°C at a rate of 3°C/min (held for 5 min). The injector temperature was 230°C and the split injection mode was (1:5). The ion source and the transfer line temperatures were 200° and 250°C, respectively. The MS instrument was operated in positive electron ionization mode (EI^{+}) with automatic gain control, with 70 eV of electron energy and 250 mA of emission current with in the full scan operated mode started from 35 to 300 m/z [18].

Chemical reagents and calibration

Argon and helium (high purity, 99%), methanol, ethanol (absolute) grade AR., Nitric acid (69-72%) grade AR., Hydrochloric acid (36%) grade AR., Sulfuric acid (98%) grade AR., perchloric acid conc. grade AR., commercial 10% sodium hypochlorite solution Deionized water (laboratory) used in preparation all reagents. A Perkin Elmer Optima 3100 XL axial viewing atomic emission spectrometer with inductively coupled plasma (ICP-AES) was used according to the operating conditions like RF generator/power: 40 MHz free-running/1300 W, viewing mode: Axial, Torch alumina injector, id: 2.0 mm, Argon flow rates: Auxiliary 0.5 l min^{-1} 21/nebulizer 0.85 l min^{-1} 21/plasma 15 l min^{-1}, Air flow rate (shear): 18 l min^{-1}, Water flow rate: 1 l min^{-1} (310–550 kPa), Spray chamber: Scott double-pass, Nebulizer: Gem tip cross flow, Pump Peristaltic: three-channel, Sample flow rate: 1.0 ml min^{-1}, Uptake tube: 0.5 mm id polyethylene, Polychromatic: Echelle, Resolution: 0.006 nm at 200 nm and Detector Segmented array charge coupled: (SCD). Standard solutions and internal standard solution were prepared by dissolving the, atomic spectroscopy standard 21 (QC), PE # N930-0281, Reference multi: Lot # 2-89BDREF, 1-70 CR, 9-138AS, 9-27 AS for calibrating the ICP-AES for elements. Linear regression of intensities was used for constructing a calibration curves in which were obtained by multi-element standards with equal mass concentration for all analytes. Then the analytical wavelengths were set for all elements accordingly and a 3-point background signal correction was used through the operating software of the instrument. For GC-MS standard solutions and internal standard solution were prepared by dissolving the pure organic substances in absolute methanol, for the calibration procedures. Blank calibration sample was prepared to ensure a very low loaded sample with the same matrix as the real sample.

STATISTICAL ANALYSIS

Results were expressed in terms of mean and standard error of three separate determinations. Tukey's range (Honestly Significant Difference) test were applied on raw data in conjunction with an ANOVA at significant level $p<0.05$ to test the means for significance differences, using GraphPad PRISM® version IV software, San Diego, CA, USA.

RESULTS AND DISCUSSION

For our refrence, the experimental analysis of Prickly pads of *Opuntia ficus-indica* were also determined according to standards methods of AOAC [20], the data results showed that the main constituent is about 90 ± 3.21% water followed by small amounts of 5 ± 0.30% carbohydrates, 4 ± 0.69% fiber 1 ± 0.21% protein and about 0.49 ± 0.081% ash. But the identification and quantification of the analysis of heavy metals showed the mineral contents like Scandium (Sc), silver (Ag), Aluminum (Al), Born

(B), Barium (Ba), Bismuth (Bi), Calcium (Ca), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Iron (Fe) Potassium (k), Lithium (Li), Mg, Manganese (Mn), Sodium (Na), Nikel (Ni), Lead (Pb) Titanium (Ti), Zinc (Zn), Strontium (Sr). The concentrations were ranged from non-detectable to 277 ppm (Table 1).

For instance, barium, calcium, cobalt, manganese and titanium were non-detectable, while zinc, nickel, magnesium, copper, chromium, cadmium, barium and scandium were showed concentrations below 1ppm. Elements like potassium, strontium and lead showed a concentration ranged between 1.20 to 1.50 ppm, also sodium and sliver of concentration 14.50 and 5.70 ppm respectively. The highest values were seen for lithium 187, bismuth 277 and iron 700 ppm (Figure 3).

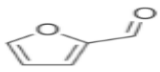
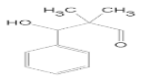
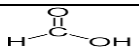
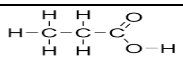
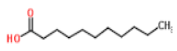
Table 1: *Opuntia ficus-indica* and its phytochemical constituent in ppm (mg/Kg)[1].

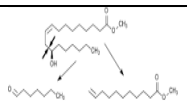
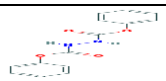
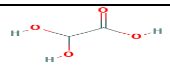
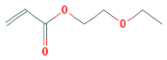
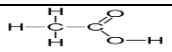
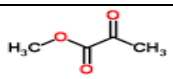
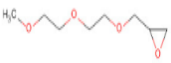
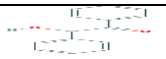
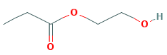
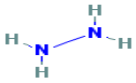
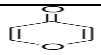
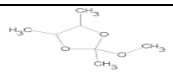
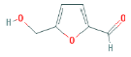
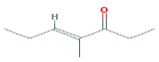
Elements	Mean concentrations (ppm) mg/Kg[1]	*RSD	R^2
Sc	0.35 ± 0.03	3.02%	0.99925
Ag	5.70 ± 0.29	2.77%	0.99985
Al	2.45 ± 0.34	7.89%	0.99924
B	0.85 ± 0.09	2.15%	0.99947
Ba	n.d	-	0.99978
Bi	277 ± 0.56	1.44%	0.99936
Ca	12.80 ± 0.23	8.12%	0.99994
Cd	0.25 ± 0.01	1.18%	0.99925
Co	nd	-	0.99937
Cr	0.20 ± 0.02	6.60%	0.99942
Cu	0.90 ± 0.06	1.89%	0.99999
Fe	700 ± 0.20	1.12%	0.99977
k	1.50 ± 0.08	0.04%	0.99995
Li	187 ± 0.12	5.68%	0.99924
Mg	0.40 ± 0.02	3.59%	0.99321
Mn	nd	-	0.99996
Na	14.50 ± 0.02	6.30%	0.99899
Ni	0.55 ± 0.03	3.27%	0.99944
Pb	1.45 ± 0.08	6.94%	0.99956
Sr	1.20 ± 0.40	2.11%	0.99967
Ti	nd	-	0.99991
Zn	0.70 ± 0.04	5.66%	0.99990

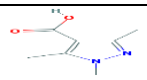
Note: [1]Values are mean and ± standard error of three separate determinations (n = 3), [2]RSD: Relative Standard Deviation.
*Significant level at $p < 0.05$

The whole observation of oil of *Opuntia ficus-indica* in terms of their chemical structures was identified and showed 21 components according to GC-MS analysis techniques, including groups such as polymers, fatty acids, esters, aldehydes, phenolics acids and steroid which is illustrated in Table 2 and Figure 3 [19]. These organic mucilaginous components are only partly known and have been qualitatively determined by their indices and mass spectra compared to those stored in the GC/MS built in libraries, Experimentally, some of the oil content have pharmaceutical and industrial uses sumerized in Table 2 [20,21]. Figure 4 represented a typical chromatogram of oil profile for chemical constituents detected of *Opuntia ficus-indica*.

Table 2: Organic compounds and their pharmaceutical and industrial uses.

S. No	Pharmaceutical and industrial uses	Name of organic compounds	Chemical structure	Chemical structure
1	Flavouring agent food additives and extraction solvent. Solvents (which become part of product formulation or mixture).	2-Furancarboxaldehyde	$C_5H_4O_2$; C_4H_3OCHO	
2	Reduction of cholesterol values and triglyceride levels.	2,5-Dihydroxy-3,6-dimethylhyroxy-1,4 propanal *(Mevalonic acid)*	$C_6H1_2O_4$	
3	In Pharmaceutical and pesticide industry. As local stimulation drugs, foaming paste and astringent and so on, camphor, vitamin B1, cocoa alkali borneol, caffeine, analgin, metronidazole, carbamazepine, aminopyrin, aminophylline, toluene imidazole drugs such as an important raw material, in a new process of synthesis of insulin.	Formic acid	CH_2O_2	
4	Formulation of steroidal and non-steroidal anti-inflammatory (NSAIDS) medications. In manufacturers as a preservative (at low doses) and as an antimicrobial agent in foods produced for human consumption. The carboxyl acid also makes the compound's use in foods created for livestock and poultry advantageous. May also be synthetically created using ethylene or natural gas. Pharmaceutical companies commonly include it as an ingredient in NSAIDS. Prescription anti-inflammatory preparations commonly contain propionic acid as an ester and medication's active ingredients and in the formulation for breast cancer and hormone replacement therapy.. Fluticasone inhalers used for respiratory conditions. The compound frequently accompanies steroid medications, including testosterone.	propionic acid	$C_3H_6O_2$	
5	An active ingredient in medications for skin infections, and to relieve itching, burning, and irritation associated with skin problems and against fungal skin infections, such as athlete's foot, ringworm, tinea cruris, or other generalized infections by Candida albicans.	Undecanoic acid	$C_{11}H_{22}O_2$	

				
6	--	1,2- bisphenyl-oxycarbonylhydrazine	$C_{14}H_{12}N_2O_4$	
7	Used in topical cosmetic preparations for the treatment of oily hair and seborrheic skin	2,3-Dihydroxy acetic acid	$C_2H_4O_4$	
8	-	Ethoxy- ethyl ester *2-Ethoxyethyl acrylate* |	$C_7H_{12}O_3$	
9	As a generic medication, at low concentrations as vinegar which used as a medication to treat a number of conditions. As an eardrop it is used to treat external infections of the ear canal and an ear wick. As a liquid it is used to flush the bladder in those who have a urinary catheter in an attempt to prevent infection or blockage and as a gel it may be used to adjust the pH of the vagina. It may also be applied to the cervix to help detect cervical cancer during screening; also, It works against both bacterial and fungal causes of external ear infections.	acetic acid	CH_3COOH	
10	-	*Methyl acetoacetate* |	$C_5H_8O_3$	
11	-	*2-(2-Methoxyethoxymethyl) oxirane*	$C_6H_{12}O_3$	
12	-	Ethane	C_2H_6	$CH_3{-}CH_3$
13	-	*2-Hydroxy-1,2-diphenylethanone*	$C_{28}H_{24}O_4$	
14	-	*2-Hydroxyethyl propanoate*	$C_5H_{10}O_3$	
15	As Hydrazine sulfate for treating colon and rectal (colorectal) cancer, lung cancer, brain cancer (neuroblastoma). Hodgkin's disease (lymph cancer). Also may use to reduce the size of tumors cancer called glioblastoma and nerve cancer (neuroblastoma).	Hydrazine	N_2H_4	
16	-	4H-Pyran-4-one	$C_5H_4O_2$	
17	-	2,4,5-trimethyl-1,3-dioxolane	$C_6H_{12}O_2$	
18	Used in trials studying the treatment and prevention of Hypoxia, Anemia, Sickle Cell, and Sickle Cell Disease	*5-(Hydroxymethyl)-2-furaldehyde*	$C_6H_6O_3$	
19	-	*4-Methyl-4-hepten-3-one*	$C_8H_{14}O$	

20	-	1H-Pyrazole,1,3,5-trimethyl-1-butene	-	
21	-	2-chloro-3-methyl-cyclohexene	$C_7H_{11}Cl$	

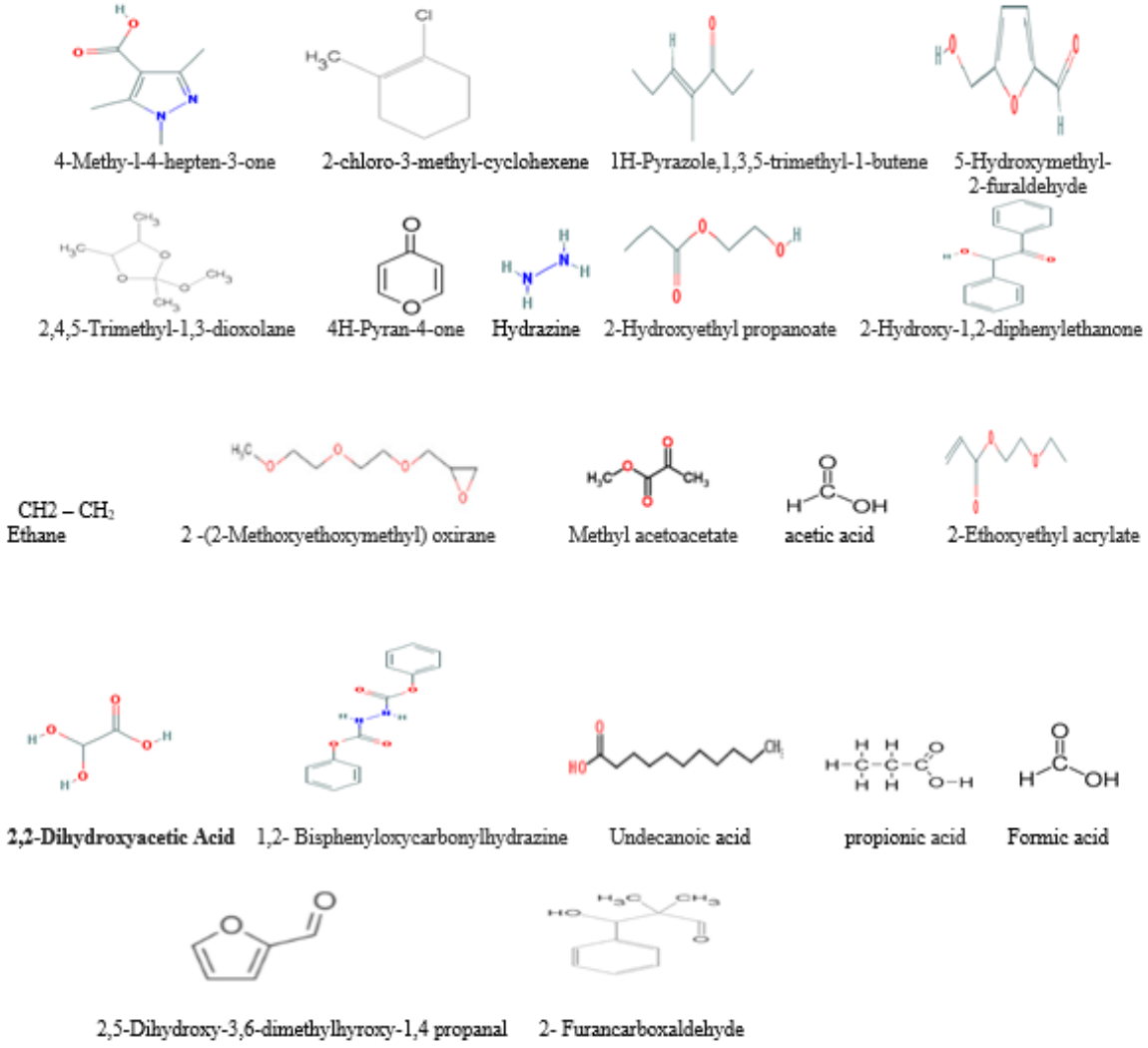


Figure 3: Chemical structure of oils constituents extracted from *Opuntia ficus-indica*.

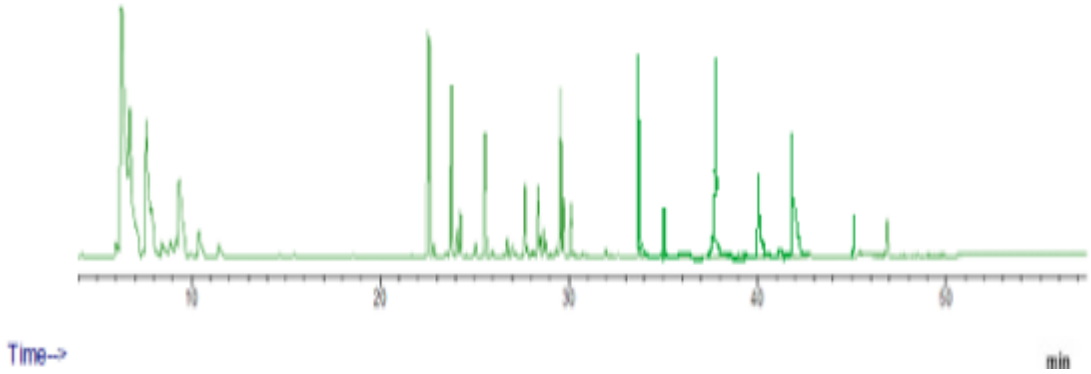


Figure 4: A typical chromatogram of chemical constituents detected of *Opuntia ficus-indica* of oil profile.

CONCLUSION

These results indicate that the chemical constituents of the species of Prickly Pads of *Opuntia ficus-indica* living in these harsh conditions of the area study are an important source of fibre, essential oil, vitamins and minerals. Also, the oil extracted profile indicate a unique ingredient set of the pharmaceuticals, nutrient treatment and their industry uses. The methods applied for identification like ICP-AES indicated the highest values of concentration (ppm) of three elements lithium, bismuth and iron while others have very low concentration to non-detectable. Whereas the GC-MS techniques approved that the constituents of the extracted oils contain volatile and non-volatile organic compounds associated with a mineral can be used for such type of medicinal treatment and industrial activities. The results obtained in this study are worth investigating for future studies on the bioactive compounds and their functional properties. Manufacturing of a natural medicine from this common neglected plant recorded for the first time under the studying area as far as we know on a scientific base.

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