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Physicochemical and Biological Characterization of *Opuntia ficus-indica* (L.) Mill. Seed Oil Collected from The Bordj Bou Arreridj Region, Algerian Highlands

H. Guetarni[1,2], H. Baroun[1], A. Derabla[1], A. Ouradj[1]

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ABSTRACT

Background: The prickly pear is well adapted to arid and semi-arid areas. It occupies an important part in the human diet and it is also used as fodder for livestock. It is an interesting plant due to the environmental conditions in which it grows and its resistance to extreme climatic conditions. The objectives of our study were to determine the biological effects of oil extracted from prickly pear seeds (*Opuntia ficus-indica* L. Mill.).

Methods: The organoleptic characteristics and the physicochemical parameters of this oil were done. The antibacterial and antifungal effects of the oil were tested *in vitro* against certain numbers of pathogenic bacteria and fungi by the aromatogram method. The antioxydante activity and the GC-MS analysis were proceeded also in this study.

Result: The results show that the bacterial strains *Klebsiella pneumoniae* and *Enterococcus faecalis* are the most sensitive to the acid oil of *Opuntia ficus-indica* (L.) Mill. with an inhibition diameter varying between 23 mm and 25 mm, respectively. On the other hand, prickly pear seed oil exhibits an antifungal effect mainly against *Candida albicans* (25 mm). The oil extracted was strongest active (IC_{50} = 0.55 µg/ml). This indicates that our oil exhibited high reducing activity. 18 components were found in prickly pear seeds oil after analysis by GC-MS, which are presented mainly by phthalic acid (45.04%), monounsaturated fatty acid (18.56%), polyunsaturated fatty acid (16.69%) and other components (19.71%). The oil of *Opuntia ficus-indica* (L.) Mill. seeds showed good biological activity grace to its richness in components.

Key words: Antimicrobial activity, Antioxidant activity, GC-MS, *Opuntia ficus-indica* (L.) Mill., Prickly seed oils.

INTRODUCTION

Humans have used vegetable oils for centuries and still using it nowadays in food, medicine, cosmetics and as fuels. Plants generally accumulate oil in their seeds and fruits to provide energy for germination and the early stages of seedling development, which make seeds good sources of edible oils. Oilseed crops are primarily grown for edible oil. Recently, oilseeds attracted more attention due to an increasing demand for their healthy vegetable oils, livestock feeds, pharmaceuticals, biofuels and other oleochemical industrial uses. The increased interest resulted in an 82% expansion of oilseed crop cultivation areas and about a 240% increase in total world production over the last 30 years (Karaoui *et al*., 2020).

The prickly pear, called *Opuntia ficus-indica* (L.) Mill. is present in all parts of Algeria except the south. First widely used for the fight against drought and erosion, it is used for the consumption of its fruits or as animal feed. One of the objectives of the concept of sustainable integrated development set by the United Nations Development Program (UNDP) in collaboration with the FAO (Food and Agriculture Organization of the United Nations) aims to increase the agricultural potential of arid zones, in order to face the vagaries of the climate (Benkaddouri, 2011). The seeds of the fruit are pressed to extract the oil they contain. Virgin prickly pear seed oil is a very valuable oil extracted by cold pressing which is widely used in pharmaceuticals and cosmetics. It has multiple moisturizing, antioxidant and therefore anti-aging properties. Its exceptional richness in vitamin E and sterols gives it an exceptional ability to protect the skin against free radicals and boost cell renewal (Krifa, 2019). Phytotherapy is currently experiencing considerable growth as a result of the increasing discovery of effective plant extracts for the treatment of various diseases (Novais *et al*., 2004). Today, the World Health Organization (WHO) estimates that 80% of African populations are cured with natural remedies and 20% of the drugs produced and

[1]Department of Biology, University Bounaama Djilali of Khemis Miliana, 44225, Ain Defla, Algeria.
[2]Laboratory of Natural Substances Valorization, University Bounaama Djilali of Khemis Miliana, 44225, Ain Defla, Algeria.

Corresponding Author: H. Guetarni, Department of Biology, University Bounaama Djilali of Khemis Miliana, 44225, Ain Defla, Algeria. Email: kmhg2009@yahoo.fr

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marketed worldwide come from medicinal plants (WHO, 2022). As part of the development of new antidiabetic agents of plant origin, ethnobotanical investigations have revealed the use of about a hundred medicinal plants for the treatment of diabetes. Among these species is *Opuntia ficus-indica* (L.) Mill. (Benkaddouri, 2011). Cactus plant (*Opuntia ficus-indica* L. Mill.) has long been used in traditional medicine for the treatment of several diseases. As a result, during the last two decades, the search for health-promoting compounds in *O. ficus-indica* (L.) Mill became increasingly popular. In fact, this Caryophyllal-belonging plant was revealed to be rich in a variety of bioactive secondary metabolites, namely flavonoids, betalain pigments and tocopherols, which are known to play an important role in human health protection and prevention from different pathologies (Benramdane *et al*., 2022).

The purpose of this work is to study the biological properties of the oil extracted from prickly pear seeds collected from Bordj Bou Arreridj region (antimicrobial and antioxidant activities) and to determine their chemical components by GC-MS.

MATERIALS AND METHODS

Workplace

The present study was carried out during 2022 within the University Bounaama Djilali of Khemis Miliana and at the Dr. Zibouche Abdellah medical analysis laboratory of Ain Defla.

Prickly pear seed oil extraction

It is extracted by cold pressing by Dr. Saifi Mounir (University Bounaama Djilali of Khemis Miliana) at his small company Aromabiol from the seeds of *Opuntia ficus-indica* (L.) Mill. harvested from Bordj Bou Arreridj region (36° 4′ 0″ North, 4° 46′ 0″ East) (Fig 1) (Google Map, 2022).

This method is used mainly to produce extra-virgin edible oils or by small capacity units and allows extraction by cold pressing by passing the seeds through a screw oil press which causes an increasing pressure at around 60 °C. The oil obtained is then filtered and checked that no contamination has occurred during the process. The pure prickly pear oil thus obtained is packaged in glass bottles. The recovered oil was decanted, weighed and then stored at -20 °C. This technique allows the preservation of the content of essential fatty acids and natural antioxidants and therefore avoids an alteration of the properties of the oil (Nitiema *et al*., 2012). Oil extraction by cold screw pressing is an alternative method and has been found to be a substitute to extraction of solvent. This process has the advantage of being less oil producing than others but is safer, simpler, less expensive, hygienic, no chemical residue and ecologically friendly. Cold-pressed oils improve the quality of oil and are rich with bioactive components such as essential fatty acids, sterols, tocopherol and phenolics (Alqurashi *et al*., 2022).

Microbial strains

Nine microorganisms were used for testing the antimicrobial activity of *O. ficus-indica* oil (Table 1). These microbial strains were maintained by subculturing on nutrient agar (CONDA603012) favourable to the growth of bacteria at 37 °C for 24 h and at 30 °C for 48 h for yeasts.



Fig 1: Prickly pear seed harvest site (Google Map, 2022).

Table 1: Strains used in antibacterial and antifungal activity.

Microorganisms	Gram⁺	Gram⁻	Sources
Bacteria	*Enterococcus faecalis*	/	Laboratory of medical analyzes directed by Dr. N.Houti, Khemis Miliana.
	Bacillus spizizenii ATCC 6633 *Staphylococcus aureus* ATCC 6538	*Klebsiella pneumoniae* *Escherichia coli* ATCC 25922 *Pseudomonas aeruginosa* ATCC 7756	Laboratory of medical analyzes
Yeasts	*Candida albicans* *Microsperme canie* 1893 *Microsperme canie* 1815		Central laboratory of Hadjout hospital

Organoleptic properties of *Opuntia ficus-indica* seed oil

The organoleptic examination is one of the criteria for evaluating the quality of the oils. It concerns odour, color and appearance at 20 °C.

Analysis of physicochemical parameters

The physicochemical parameters of prickly pear seed oil (PPSO) studied are: yield (AFNOR, 1996), pH (Actu-Environnement, 2022); refractive index (AURA, 2022); the acid index and acidity (Jacques *et al.*, 2012), the saponification index (AFNOR, 1984) and the ester index (Novidzro *et al.*, 2019).

Antibacterial and antifungal activity

We adopted the aromatogram method on agar medium using sterile discs (Somaie *et al.*, 2013). In each inoculated Petri dish, 6 blotting paper discs are applied (Andrews, 2001): (A) Negative control, (B) Pure oil, (C) Oil diluted 1:2 (v/v), (D) Oil diluted 1:4 (v/v), (E) Oil diluted 1:8 (v/v), (F) Oil diluted to 1:12 (v/v). In this experiment, the oil was dilute in dimethyl sulfoxide (DMSO) which is inert on antibacterial activity. The prepared Petri dishes are incubated at 37 °C for 24 h for bacteria and at 30 °C for 48 h for fungi. The reading of the results was made by measuring the diameters of disc inhibition halos using aslide gauge. The results can be symbolized by signs according to the sensitivity of the strains to oil (Hombach *et al.*, 2013).

Antioxidant activity

The ability of prickly pear seed oil to scavenge the free radical DiPhenylPicrylHydrazyl (DPPH) is evaluated using the method described by Jaamali *et al.* (2013). The stock solution of oil in ethanol is prepared at a concentration of 100 µg/ml. A series of dilutions were carried out to obtain concentrations ranging from 25 µg/ml to 100 µg/ml. The reaction mixture is stirred vigorously and incubated for 30 min in the dark and at room temperature. Absorbances are measured at 517 nm. The control samples are composed of 50 µl of methanol to which 2 ml of the ethanoic solution of DPPH has been added. Ascorbic acid is used as a positive control. The survey is carried out using a visible UV spectrophotometer. Antioxidant activity is expressed as per cent inhibition calculated using the following equation:

$$\text{Antioxidant activity (\%)} = 100 \times \frac{\text{A control - A sample}}{\text{A control}}$$

Inhibitory concentration (IC50) is the concentration of the test sample required to reduce 50% of the DPPH radical. IC50 is calculated from the graph of the antioxidant activity (in %) according to different concentrations of oil and vitamin C tested. A low IC50 value indicates a high capacity of the oil to act as a DPPH scavenger (Molyneux, 2004). The reducing power of a compound is associated with its antioxidant power, an increase in absorbance corresponds to an increase in reducing power (Chikhi, 2013).

Oil analysis by GC-MS

The chromatographic analysis of the *O. ficus-indica* oil was carried out with a gas phase chromatograph type TQ 8030 coupled to a mass spectrometer.

The fragmentation is carried out by electron impact at 70 eV. The operating conditions were: column temperature 40 to 250 °C, helium flow rate as carrier gas 3 mL/min, split pressure 49.5 KPa, volume of oil injected 0.5 µl, computerized database (Q3 Scan).

RESULTS AND DISCUSSION

Organoleptic examination

In the present study from a seeds of *O. ficus-indica* collected from Bordj Bou Arreridj region, the oil was extracted by cold pressing method. This oil was examined and its organoleptic characteristics were determined before studying it's biological effects. *Opuntia ficus-indica* seed oil is found to have a clear appearance, light yellow color and very mild odor. Oils are usually liquid at room temperature and volatile. Our oil are more or less colored. It is important to appreciate the organoleptic characteristics of the oil of OFI [*Opuntia ficus-indica* (L.) Mill.] in which its colour, flavor and smell are the parameters that give a general idea of quality (AFNOR, 2000).

Physicochemical analysis

The oil extracted from PPSO had a pH equal to 4.50. It gave a yield equal to 4%. The refractive index of *Opuntia ficus-indica* oil at 20 °C is 1.469. From the results obtained, we found that the value of the acid index is equal to 2.80 mg of KOH/g. Others physicochemical parameters are determined such as acidity, acidification index and ester, whose recorded values are: 1.40%, 182.32 mg of KOH/g and 179.52 mg of KOH/g, respectively. The pH of PPSO oil is 4.5. Our oil is acidic. For our oil extracted by cold pressing, its saponification index is equal to 186.63 mg of KOH. Physicochemical parameters are influenced by soil and climatic conditions as well as plant growing conditions (Gildo, 2006), geographical separation, harvest season, cultural practices and extraction technique (Akrout *et al.*, 2010). Indeed, this oil requires 1 ton PPSO and 25 kg of the seeds to extract 1 litre of oil (Krifa, 2019).

A low refractive index of oil indicates its low refraction of light which could promote its use in cosmetic products (Chouitah, 2011). Acidity value varies according to the stage of maturity of the plant (Charef *et al.*, 2008). A high value means poor conservation of the fruits before oil extraction or a harvest made from unripe fruit (Djerrou, 2014). Acidity is the percentage of fatty acid expressed as oleic acid for most fatty substances. A low acidity level contributes to the stability of the oil against oxidation by air. It is recommended for an edible oil to have a low acidity level (less than 3.3%, standard imposed by Codex Alimentarius, to withstand long storage without deterioration (Onyeike *et al.*, 2002). A high saponification index indicates that the oils have a high triglyceride content and are therefore very useful in cosmetology (Gharbi *et al.*, 2015).

Antibacterial activity

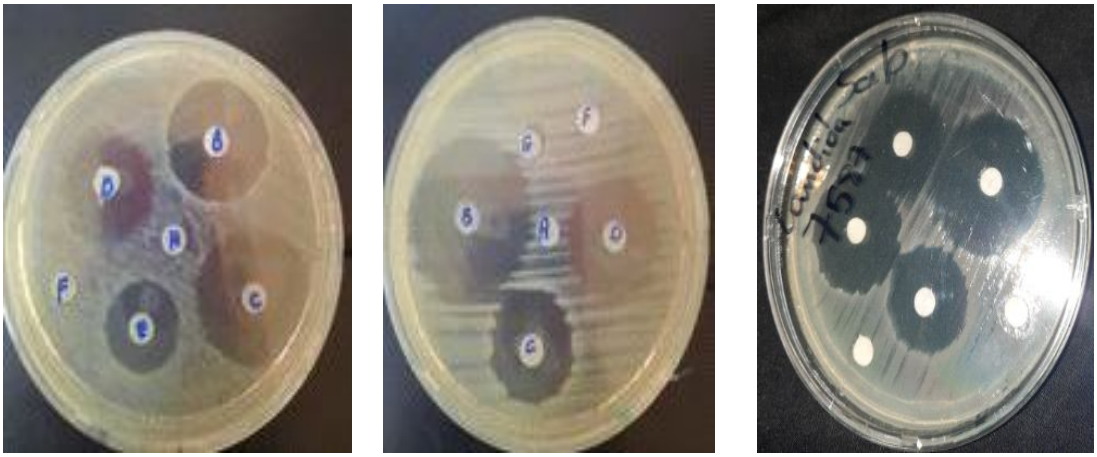
According to the results obtained, Gram-positive bacteria are the most sensitive to the antibacterial activity of *Opuntia*

ficus-indica. seed oil (Table 2, Fig 2). The oil inhibited the growth of *Enterococcus faecalis* whose diameters of the zones of inhibition are 25 mm, 22 mm, 20 mm, 16 mm and 10 mm obtained with B, C, D, E and F, respectively. We also recorded the presence of *Klebsiella pneumoniae* inhibition zones whose diameters vary from 18 mm to 23 mm obtained with the same dilutions. The results show that *O. ficus-indica* (L.) Mill. seed oil proved inactive against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 7756, *Bacillus spizizenii* ATCC 6633 and *Staphylococcus aureus* ATCC 6538. Gram-positive bacteria are increasingly susceptible compared to Gram-negative bacteria. The difference in the outer layers of these bacteria, which Gram-positive bacteria apart from the cell membrane, have an additional layer which consists of phospholipids, proteins and lipopolysaccharides. The outer membrane of Gram-positive bacteria is highly charged. It acts as a barrier to vegetable oils (Chouitah, 2011). For Gram-negative bacteria, proteins that are in the outer membrane are deactivated before they reach the cytoplasmic membrane and cytoplasm (Fujisawa *et al.*, 2009). This membrane is impermeable to most molecules, which reflects the resistance of the other strains tested (Turkmen *et al.*, 2007). *Escherichia coli* ATCC 25922, a Gram-negative bacterium, also develops resistance to a number of oils (Delamare *et al.*, 2007). It has been described that this sterol can inhibit the growth of certain microorganisms such as *Pseudomonas aeruginosa* ATCC and *Bacillus spizizenii* (Ogbe *et al.*, 2015). The antibacterial activity of this oil can be attributed mainly to its major constituents, such as terpene components, which are particularly active against microbial cells because they are soluble in aqueous media and cause damage to the cell walls of microorganisms (Fillippi *et al.*, 2006). In terms of antibacterial activity, linalool was shown to be the most effective and inhibited 17 bacteria such as *Enterococcus faecalis*, *Escherichia coli*, *Bacillus spizizenii* and *Staphylococcus aureus* (Peana *et al.*, 1999).

Antifungal activity

The results of antifungal activity of *Opuntia ficus-indica* (L.) Mill. oil are presented in Table 3 and Fig 2. The antifungal activity of the oils can occur through two different mechanisms: some constituents cause the leakage of electrolytes and the depletion of amino acids and sugars and the other can be inserted into membrane lipids, therefore there are loss membrane functions (Suppakul *et al.*, 2003).

The antifungal action of *Opuntia ficus-indica* (L.) Mill. oil against *Candida albicans* is due to an increase in the permeability of the plasma membrane followed by a rupture of the latter leading to leakage of the cytoplasmic content and therefore death yeast (Cox *et al.*, 2000).



1): *Enterococcus faecalis*. 2): *Klebsiella pneumoniae*. 3): *Candida albicans*.

Fig 2: The antimicrobial potency of the OFI seeds oil against microbial strains.

Table 2: Diameter of the zones of inhibition of the bacterial strains tested in mm by *Opuntia ficus-indica* oil.

Bacterial strains	Inhibition zone diameters (mm)					
	A	B	C	D	E	F
Enterococcus faecalis	-	25	22	20	16	10
Bacillus spizizenil ATCC 6633	-	-	-	-	-	-
Staphylococcus aureus ATCC 6538	-	-	-	-	-	-
Klebsiella pneumoniae	-	23	20	18	-	-
Escherichia coli ATCC 25922	-	-	-	-	-	-
Pseudomonas aeruginosae ATCC 7756	-	-	-	-	-	-

A: Negative control, B: Pure oil, C: Oil diluted 1:2 (v/v), D: Oil diluted 1:4 (v/v), E: Oil diluted 1:8 (v/v), F: Oil diluted to 1:12 (v/v).

Table 3: Measurement of inhibition zone diameter of the fungal strains tested by *Opuntia ficus-indica* seed oil.

Yeasts	Inhibition zone diameters (mm)					
	A	B	C	D	E	F
Candida albicans	-	25	23	15	12	5
Microsperm canie 1893	-	-	-	-	-	-
Microsperm canie 1815	-	-	-	-	-	-

A: Negative control, B: Pure oil, C: Oil diluted 1:2 (v/v), D: Oil diluted 1:4 (v/v), E: Oil diluted 1:8 (v/v), F: Oil diluted to 1:12 (v/v).

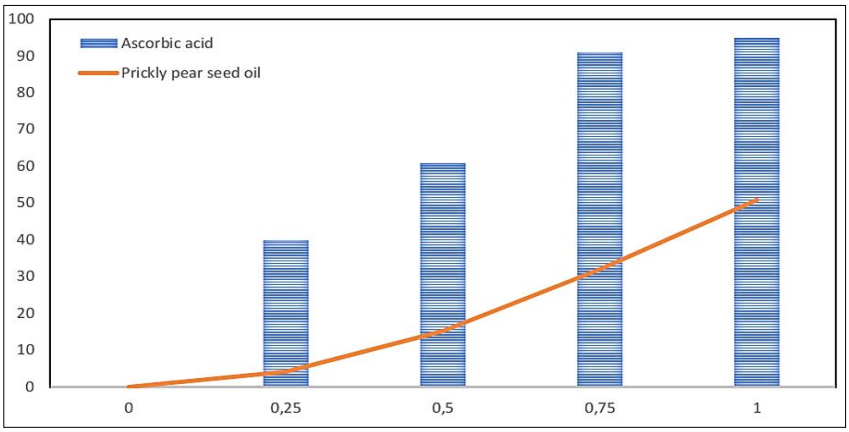


Fig 3: DPPH inhibition rate as a function of *Opuntia ficus-indica* seed oil concentrations.

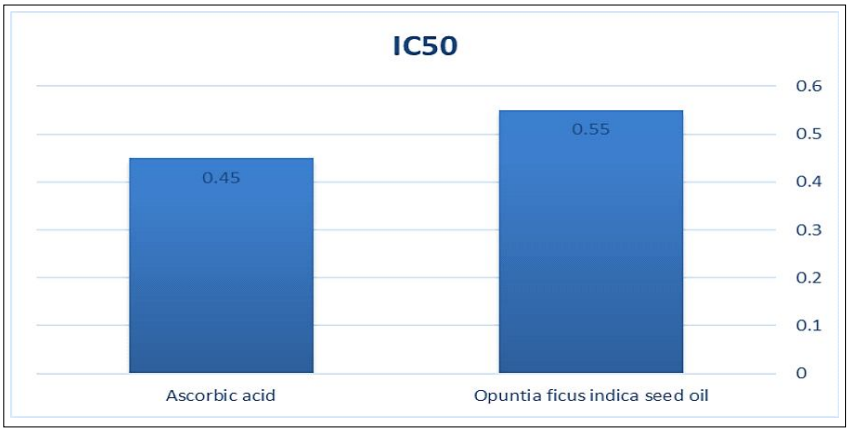


Fig 4: Representative histogram of IC50 inhibitory concentration value of *Opuntia ficus-indica* seed oil.

Antioxidant activity by DPPH

We were able to graphically determine the corresponding 50% inhibition concentration (IC50), which constitutes the antioxidant activity of the oil (Fig 3). According to the recorded results, the IC50 of the oil is equal to 0.55 μg/ml. It is relatively low for ascorbic acid, the value of which is around 0.45 μg/ml. The IC50 is proportional to the antioxidant activity of the oil (Fig 4). Concerning extracts from the seeds of three different species of *Opuntia ficus-indica* (L.) Mill. in which the percentages of root inhibition of DPPH are higher in the polyphenol and the fractions rich in flavonoids more precisely than in the other variety. The ability to reduce DPPH, this antioxidant activity due to phenolic compounds (Prior *et al.*, 2005). Our results are in agreement with those obtained by Chougui *et al.* (2013).

This appears to be consistent with other results in the literature, where the mechanism of interaction between antioxidants and the DPPH radical depends on the structural conformation of the antioxidant and the latter interacts with the DPPH radical by reducing an equal number of hydroxide groups carried by the radical DPPH (Molyneux, 2004). Ascorbic acid, tocopherols, flavonoids and tannins are antioxidant molecules that reduce and decolorize DPPH due to their ability to yield hydrogen (Pooter *et al.*, 1986). The synergistic interactions between the different constituents of vegetable oil is at the origin of much greater antioxidant power (Vardar *et al.*, 2003). Antioxidants are considered as reducers and inactivators of oxidants (Siddhuraju, 2007). The reducing power of a compound can serve as a significant indicator of its potential antioxidant activity (Bougandoura, 2013).

Table 4: Chemical constituents of prickly pear seed oil.

Chemical components	Rate %
2-, Heptenal, (E)-	0.74
2-Isopropyl-5-Methylcyclohexanol#	2.16
3-trifluoromethylbenzoic acid	0.33
3,3,6,6,11,11-Hexa Chlorotetra Cyclo[8.1.0.0(2,4).0(5,7)]Undec-8-Ene	0.62
2,4-Decadenal, (E,E)-	0.93
Deca-2(E), 4(E)-dienal	1.52
Cycloheptasiloxane, tetradecamethyl-	1.65
Cyclo Heptasiloxane, hexadecamethyl-	1.63
Cyclopentasiloxane, Ethyl Tetrade	1.25
Palmitic acid	2.80
Heptasiloxane, hexadecamethyl-	1.14
9,12-Octadecadienoic acid (Z,Z)-	16.69
Octadec-9-enoic acid	18.56
Octadecanoic acid	1.70
Spiro [7H-Cyclohepta[B]Furan-7,2'(5'H)-Furan]-2,5'(3H)-Dione,	
Octshydro-8-Hydroxy-6,8-Dimethyl-3-Methylene-, [3AS-(3A.Alpha.,6.B	0.54
1-Oxaspiro [4.5] Decan-7-One, 2,10,10-Trimethyl-6-Methylene-, (R*,S*)-(.+-.)-	1.01
Cyclopentasiloxane, Ethyl Tetrade	1.69
1 2-benzenedicarboxylic acid mono(2-ethylhexyl) ester	45.04
Total	100

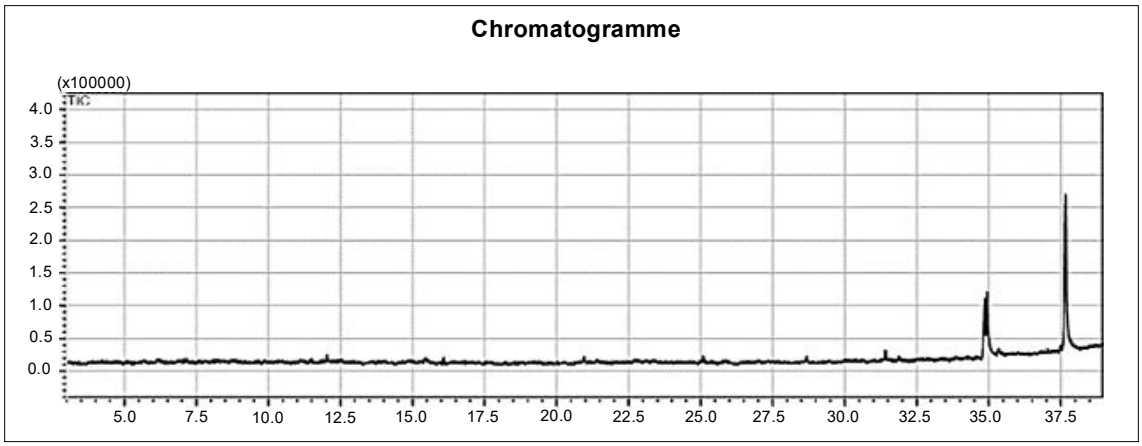


Fig 5: Chromatographic profile of prickly pear seed oil after analysis by GC-MS.

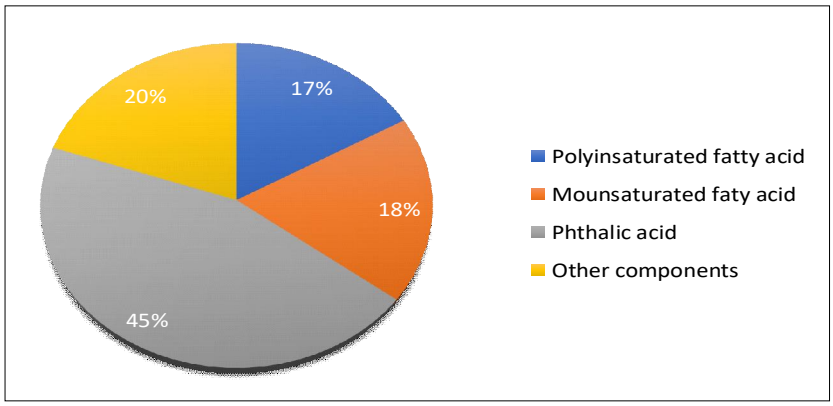


Fig 6: Level of chemical components present in prickly pear oil.

In the work of El-Faydy *et al.* (2017), showed that the series of the alkylthiomethyl-8-hydroxyquinoline present a significant antibacterial activity when compared to the Nitroxoline. The synthesized compounds demonstrated moderate antioxidant activities by DPPH in comparison to our oil which is a good antioxidant.

GC-MS analysis of *Opuntia ficus-indica* (L.) Mill. oil

Using this analysis technique, we were able to qualitatively and quantitatively study the chemical compounds of our oil (Fig 5, Table 4). Determination of oil chemical compound levels was based on peak area normalization without using correction factors. The constituents present in our oil are mainly presented by: phthalic acid (1,2-benzenedicarboxylic acid mono (2-ethylhexyl) ester), monounsaturated fatty acid (Octadec-9-enoic acid), polyunsaturated fatty acid (Acid 9, 12-octadecadienoic (Z,Z)-) and whose values were obtained: 45.04%, 18.56%, 16.69% and 19.71%, respectively (Fig 6).

This analysis made it possible to identify 18 bioactive compounds which have interesting inhibitory actions against pathogenic microorganisms. The antimicrobial activity is due to either one or more prickly pear seed oil constituents.

The influence of climatic conditions and soil conditions on vegetable oil composition has been established in other medicinal plants such as St. John's wort and thyme (Antonio, 2011). *Opuntia ficus-indica* (L.) Mill. seeds are a valuable source of essential unsaturated fatty acids. Polyunsaturated fatty acids are well known for their importance in the field of nutrition and use in the pharmaceutical industry (Chougui *et al.*, 2013).

CONCLUSION

The results of our work allowed us to assert that *Opuntia ficus-indica* (L.) Mill. seed oil could be a potential source of natural bioactive compounds which had a good inhibitory effect on pathogenic microorganisms.

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Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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