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Psychotropic Activity and GC-MS Analysis of Cactus Seeds Extracts (*Opuntia ficus-indica* L.)

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ABSTRACT

Nopal a (prickly pear tree) is a kind of plant xerophytes belonging to the family of Cactaceae, the fruit is consumed exclusively in a fresh state. It is claimed to be an excellent source of natural oligoelements which may improve human health and nutrition, it also used in traditional medicine. The objective of this work is to extract and analyze the lipid fraction of prickly pear seeds (Opuntia ficus-indica L.) and to determine the psychotropic activity of prickly pear seed extracts (Opuntia ficus-indica) to achieve a primary pharmacological screening contained in these extracts (aqueous, hydro-ethanol). The oil of seeds is rich in fatty-acids poly-unsaturated, Main fatty acids identified were linoleic (C18:2), oleic (C18:1), palmitic (C16:0), and palmitoleic (C16:1) acids with respective contents 81.25%, 3.70% and 15.03%. These results are returned account that the food quality of prickly pear seeds is very important and its natural source of edible oil containing of the essential fatty-acids. The study of the pharmacological activity showed that the extracts à bases seeds with the tested amounts are endowed with sedative effects, myorelaxant and tranquillizing.

Keywords: *Opuntia ficus-indica*, Oil, Extracts, GC-MS analysis, Seeds, Psychotropic activity

INTRODUCTION

Cactus (*Opuntia ficus-indica*), commonly known as prickly pear, belongs to the family Cactaceae, is reported to contain about 130 genera and nearly 1500 species, which were originally native to the New World. Being so water-use efficient, they are highly useful in arid and semi-arid environments, particularly during prolonged dry spells or failure of the monsoon. The prickly cactus pear is widely distributed in Mexico, much of Latin America, South Africa and the Mediterranean area [1].

It produces sweet, nutritionally rich edible fruits; its tender cladodes are used as fresh green vegetable and salad. The fruit, as well as cactus stem are used to prepare value-added products, such as jam, squash, wine, pickle, body lotions, shampoo, creams, etc. It also has several medicinal and industrial uses. Its seeds constitute about 4 to 7% of the edible pulp, and can be used as flavouring agents [2].

Cactus pear "*Opuntia* sp." grows throughout Algeria and the fruits are consumed exclusively as fresh fruit, the extracted pigments from prickly pear fruits are used as additives in food, cosmetic and pharmaceutical preparations [3]. It is claimed to be an excellent source of natural oligoelements which may improve human health and nutrition, it also used in traditional medicine. The Cactus extracts have an important role in treating a number of diseases including hypoglycemic effects, anti-tumoral, and antioxidant activities [4-6].

The cactus has been largely ignored by scientists until the beginning of 1980; this renewed interest is partly attributed to the multifunctionality of prickly pear fruit. Recent studies have revealed their high levels of certain chemical compounds, which can make this fruit an added nutritional value, such as calcium, magnesium, phenolic compounds and betalains [7,8]. It has been found that fruit juice contains a rich variety of natural antioxidants, numerous phenolic compound, ascorbic acid, betalans, beneanines and a flavonoid fraction composed mainly of rutine and isorhamnetine [9,10].

All the authors have agreed that *Opuntia ficus-indica* seed oil was rich in polyunsaturated fatty acids and vitamins and may potentially be included in animal and human diets. Therefore, the present study was therefore to analyze the fatty acids of the seeds of the Algerian cactus and to evaluate the psychotropic activity in order to determine the possible beneficial effects on the economy and health.

MATERIALS AND METHODS

Collection and Identification of plant material

The plant material used in this study is composed of prickly pears were collected in August 2013 in the region of Ain el rahma a town about thirty kilometers from the Relizane province in north-west Algeria. The prickly pear fruits was hand peeled. The skin and seeds were separated from the juicy pulp, washed abundantly with distilled water then dried at room temperature. The dried plant materials were grounded into fine powder using the electric blender. The plant was identified in Laboratory of Ecology and Management of Natural Ecosystems of the University of Tlemcen (Algeria). A voucher specimen of the plant was deposited in the Laboratory.

Oil extraction

Powder of ground seeds (67 g) was extracted at 80°C with cyclohexane (800 ml) for 6 h in a soxhlet extractor. Then the solvent was removed at 40°C under reduced pressure using a rotary evaporator. The residual oil was stored at -20°C until use.

Preparation of the extracts

The vegetable matter (seeds) dried beforehand and crushed until the constant weight, was reduced out of fine powder, and then macerated under agitation at room temperature during 2 h, a weight of 2 g in 25 ml of water distilled for the aqueous extract which corresponds the first extract (Ext Aq) and of 2 g in 25 ml of a mixture of distilled water and ethanol mixture in equal volumes for hydro-alcoholic extract or extract ethanol 50% (Ext EtOH 50%), after filtration, the obtained two filtrates were evaporated under pressure by rotavapor. Finally the extracts were kept frozen at -20°C for further analysis [11].

Phytochemical prospecting

The phytochemical tests to detect the presence of flavonoids, tannins, sterols, and alkaloids were used by simple qualitative methods. The tests were based on the visual observation of color change or formation of precipitate after the addition of specific reagents.

Esterification of fatty acids

The fatty-acids are cross esterified out of methyl esters (FAMEd) by heating in boron trifluoride (BF₃) (solution at 12% in methanol), as a catalyst, the mixture of fatty acids was first saponified with solution (2%) the sodium hydroxide in methanol (MeOH). A typical procedure was performed as follows: oil (1 g) was hydrolyzed on reflux for 10 min with 25 ml the solution of complex (BF₃-MeOH), water (25 ml) was added to the cold solution and the mixture was extracted thrice with hexane (20 ml), and the combined organic extracts were dried over anhydrous sodium sulfate (Na₂SO₄) and the solvent was removed *in vacuo*. The resulting esters mixture needed no further purification and was stored at -20°C until analysis [12].

FTIR analysis

Infrared spectra were acquired using a Mattson Genesis II FTIR spectrometer using neat samples on NaCl cells. Absorption bands were identified according to their wave numbers (cm⁻¹). FTIR analysis of FAMES was used to check the efficiency of the protocol for esterification. As a rule, GC and/or GC-MS analysis of fatty acids is usually performed after their conversion into methyl esters. Therefore, previous to GC/MS analysis, one should give evidence for the full conversion of fatty acids into their methyl esters and not perform analysis with mixture of carboxylic acids and esters. That is why FTIR analysis was used to check the full disappearance of carboxylic absorption bands in the region 3200-2500 cm⁻¹ and the presence of esters. All samples showed spectral features namely characteristic absorption bands for carbonyl C=O at 1744.21 cm⁻¹ and C-O stretching vibration at 1170.19 cm⁻¹, 1195.56 cm⁻¹ and 1242.03 cm⁻¹. The absorption at 723 cm⁻¹ was due to C-H bond from alkane long chains [13].

Gas chromatography (GC-MS) analysis

The GC-MS was performed by using GC-TOF/MS Master. DN Wax capillary column was used. Dimension 30 Mts, ID: 0.25 mm, Film: 0.25 µm. Carrier gas: He, Flow: 0.5 ml/min. Temp Prog: Oven temp 70°C raised to 240°C at 2°C/min. Injection volume 1 µl.

Identification of compounds

Interpretation mass spectrum of GC-MS was conducted using the database of National Institute Standard and Techniques (NIST) which consist of more than 62,000 patterns. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the known component inherent in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Pharmacological study

The products tested are aqueous extract (Ext Aq), and the hydro-ethanolic extract (Ext EtOH 50%) and the reference products are Chlorpromazine (LARGACTIL[®]) 25 mg (C), and Thiopental (NESDONAL[®]) (T) injectable aqueous solution with 10%.

Animals

The experiment is carried out on adult mice Swiss of the race Balb/c, coming from the breeding of the Laboratory of Biology in the Faculty of sciences of Rabat (Morocco). The weight of the mice ranges between 25 and 35 g. They were maintained under the same conditions of temperature (25 ± 2°C); the relative humidity was located between 50 ± 5% and the access to water and food at will. The use of the animals was made in conformity [14].

Psychotropic activity

The description of the sedative effect, tranquillizing and myorelaxant was carried out thanks to the following behavioural tests [15].

Test of traction

It consists in suspending mice by the forefeet to a horizontally tended wire, and one counts the time put by the mouse to bring at least one of the legs posterior to touching the wire. A normal Mouse carries out a re-establishment in less than 5 s (positive reaction), the contrary case means that the mouse is subjected to a sedative action (negative reaction).

Test of the chimney

It consists in placing a mouse in a tube of 30 cm length glass, laid out vertically. The answer is positive if the mouse reassembles the tube in less than 30 s.

Test of the board with holes

It makes it possible to explore the curiosity and the desire of escape of the animal. With this intention, one uses a 1.8 cm thickness board of 40 × 40 cm and, bored of 16 holes 3 cm in diameter, regularly spaced. The mouse is deposited in the center of the board and one count the number of times where the mouse plunges the head in one of the holes at the end of 1, 2, 3, 4 and 5 min and one calculates has average holes explored during 5 min.

Study of the medicamentous interactions

For each test, three batches of six animals are used and the administration is done exclusively by way intrapéritonéale:

- Batch pilot untreated.
- Batches of references receiving: Chlorpromazine with the following doses: 3 mg/kg, 5 mg/kg, 10 mg/kg. And it Thiopental whose amounts are: 20 mg/kg, 30 mg/kg.
- Batches treated by drugs (extracts) whose amount is 500 mg/kg, this amount was given on the basis of result of the study of the acute toxicity of these products.

RESULTS AND DISCUSSION

GC-MS chromatogram of *Opuntia ficus-indica* seed oil (Figure 1) clearly shows 3 peaks indicating the presence of 3 fatty-acids. The identification of FAMES was based on the peak area, retention time and molecular formula. The Table 1 shows the compound name with its molecular weight, molecular formula, and run time and % area.

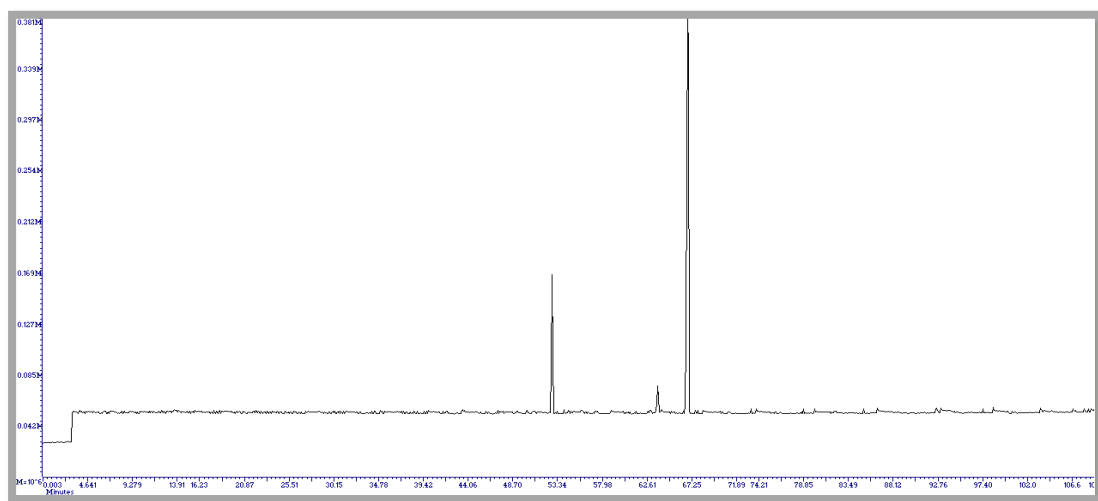


Figure 1: GC-MS chromatogram of cactus (*Opuntia ficus-indica*) seeds oil

GC-MS analysis of FAMES showed our sample contained one saturated fatty acid ester namely methyl palmitate, along with two unsaturated fatty acid esters, i.e., methyl linoleate, methyl oleate. The following fatty acids were found and they are mentioned with their percentages and retention times (rt): Linoleic (C18:2) was the main fatty acid that accounted for 81.25% (rt 66.84) of the mixture, followed by palmitic acid (C16:0), 15.036% (rt 52.77) whereas minor components were oleic acid (C18:1) 3.707% (rt 63.72). The base peak in all mass spectra of saturated FAMES was observed at m/z 74 corresponding to $\text{CH}_3\text{OC}(=\text{OH}^+)\text{CH}_2$ fragment which was a result of the well-known McLafferty rearrangement process followed by an alpha fragmentation [16].

Table 1: Fatty acids composition from oil of *Opuntia ficus -indica*

Compound name	Run time	Area %	Molecular formula	Molecular weight
Hexadecanoic acid, methyl ester (C16:0)	52.77	15.03	$\text{C}_{17}\text{H}_{34}\text{O}_2$	270
13-Octadecenoic acid, methyl ester (C18:1)	63.72	3.70	$\text{C}_{19}\text{H}_{36}\text{O}_2$	264
9,15-Octadecadienoic acid, methyl ester. (C18:2)	66.84	81.25	$\text{C}_{19}\text{H}_{34}\text{O}_2$	294

Pharmacological study

For the psychotropic Activities the results are expressed compared to the pilot batch and that of the reference:

Test of traction

The time of re-establishment of the mice treated by the products tested is clearly lengthened compared to the batch of the pilot mice. In more we recorded falls in the batch of the mice treated by hydro-ethanolic extract.

Test of the chimney

We noted a loss of initiative and curiosity in the batch of the mice treated by the extract ethanolic 50%.

Test of the board with holes

The products tested especially hydro-ethanolic extract decrease the cumulative number of holes explored (in connection with curiosity) and the number of spaces traversed between two holes (in connection with the motor activity). This activity psychotropic due to the chemical composition of secondary metabolites present in the extracts such as polyphenols, flavonoids.

Drug interaction

The batch treated by the chlorpromazine with the three doses used prolongs the sedative effect compared to the batch treated by the thiopental. The whole of the results seems indicated that the hydro-ethanolic extract with the amount 500 mg/kg exert a sedative effect on the central nervous system compared to the aqueous extract (Ext Aq) with the same amount observes on the psychomotor behaviour of the mice. Opposite the chlorpromazine, the hydro-ethanolic extract with a sedative action less important than that of the thiopental Table 2.

Table 2: Sedative action of the Opuntia seeds extracts

Lots	500 mg/Kg					
		Witness	T	C	Ext Aq	Ext Etoh 50%
Traction	Number of de mice	6	6	6	6	6
	Number of falls	0	3	6	0	2
	Reponse (+) ou (-)	+	+	-	+	+
Cheminy	Number of de mice	6	6	6	6	4
	Reponse (+) ou (-)	+	+	-	+	+
Hole Board	Holes explored After 5 min	48	42	6	34	21

CONCLUSION

In this paper, we investigate the composition of oil from seeds of cactus (*Opuntia ficus-indica* L.) as well as the effect of the extracts, the latter tested seem to exert a sedative effect, tranquilizing and myorelaxant important on the central nervous system, with regard to the psychomotor behaviour of the mice, whose the hydro-ethanolic extract (Ext Etoh 50%) presents a higher activity compared to the aqueous extract (Et Aq). One basing itself on these results one can conclude that the prickly pear fruit represents a very important source which can be exploited in various industrial sectors (nutritional, cosmetic, pharmaceutical).

Recently the researchers are interested more and more in the therapeutic effect of prickly pear.

Statistical analysis

All data were presented as mean \pm S.D. or relative percentage. Statistical comparisons between groups were performed using Student's t test. $P < 0.05$ was considered statistically significant.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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