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Chemical Composition and Bioactivities of Egyptian *Opuntia ficus-indica* Seeds Oils Obtained by Conventional and Ultrasound-assisted Extraction Techniques

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Abstract.

The main objective of the current work was to assess the suitability of ultrasound-assisted extraction (UAE) for the oil recovery from *Opuntia ficus-indica* (L.) Mill. (*O. ficus indica*) seeds as compared to conventional extraction technique; Soxhlet extraction (SE). In the present study, the recovery oil yield, the saponifiable and unsaponifiable lipids of seeds oils obtained by different extraction methods, were compared. In addition, determination of the wound healing and analgesic activities of the plant seed oil, *in vitro* and *in vivo*, were evaluated.

UAE and SE methods recovered total oil yields of 2.15% and 2.01%; respectively. Study of fatty acids composition exposed that the predominant fatty acids were linoleic acid (75.2% and 84.9%), oleic acid (14.01% and 8.18%) and palmitic acid (8.91% and 5.71%) for UAE and SE, respectively. Moreover, the most abundant sterol was *β*-Sitosterol (73.41% and 72.12%) followed by campesterol (8.84% and 8.57%) for UAE and SE, respectively. UAE significantly improved bioactivities of the oil with a significant increase in wound healing rate (85.7%) and inhibition of abdominal writhing (71.0%) compared to those obtained for oil prepared by SE (73.3% and 28.1%, respectively). Conclusion: Compared to conventional extraction method SE, the application of the innovative technique UAE for oil extraction increased the obtained oil yield of *O. ficus indica* and enhanced the biological activities *in-vitro* as well as *in-vivo*.

Key Words: *Opuntia ficus-indica*, Soxhlet extraction, ultrasound-assisted extraction, GC / MS, analgesic activity, wound healing activity.

Introduction

Prickly pear (*O. ficus indica* (L.) Mill.), belongs to the Cactaceae family, is a very interesting herbal plant due to its active components abundance, precisely in the cladodes [1], roots [2], flowers [3], fruits [4] and seeds [5]. Approximately 80% of the world residents still use plant-based drugs including Opuntia species, conferring to the World Health Organization. [6]

O. ficus indica is a multiuse crop, not only to afford food and feed, but also a source of bioactive components that give the plant pharmacological properties, besides its medicinal applications. These molecules include a variety of secondary metabolites known for their antioxidant, anti-inflammatory [7], antimicrobial, antidiabetic [8], cardioprotective, neuroprotective [9] and hepatoprotective properties [10].

The cactus seeds comprise 10-15% of the cactus pear pulp, which are considered as a waste and discarded after the pulp is removed [11]. Prickly pear seed oil contains high contents of important nutrients that make the oil an admirable applicant in food and healthy products industry [12].

Prickly pear seeds extracted oil is primarily sold for cosmetics because of the high price associated with the labor-intensive and time-consuming production method [13]. This oil has several biological activities, including antioxidant [14], anti-inflammatory [15], anti-ulcer [16], antimicrobial [17], antitumor [18], antidiabetic [19], hypolipidemic [20] and UV radiation protection [21].

Extraction is the main step in seed oil procedure, where it evaluates both the quantity and the quality of the obtained oil. Seed oils are extracted by means of traditional methods such as maceration and Soxhlet. Ultrasound-assisted extraction (UAE), microwave and supercritical fluids are techniques that employ a physical effect on the sample [22]. Ultrasound has been used to extract bioactive components from various plant parts counting the seeds. UAE uses

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sound waves to produces cavitation micro-bubbles by using sound waves, which collapse violently in the sample and facilitate the extraction of numerous active molecules [23]. Few studies have stated the innovative methods use as ultrasound in *O. ficus indica* seed oil extraction [12].

The purpose of the present study was to investigate the cactus pear seed oil extraction by using ultrasound waves compared with conventional extraction method, based on wound healing and analgesic activities, in addition to oil extraction yields and their chemical composition.

Experimental

Plant material

O. ficus indica fruits were obtained from Abo-Hammad, Sharkiya, Egypt, during the harvesting season (August 2019), when the fruits had their optimum maturity. The plant taxonomic identity was verified by Prof. Dr. Mona Marzouk, Herbarium of National Research Centre, Giza, Egypt.

Seeds collection and preparation

Fruits were hand-peeled after being air-dried and cleaned under running water to get rid of glochids and other contaminants. Fruit pulp and peels were separated. The peeled fruits were mixed in a blender (Moulinex, LM30214A, France). The collected juice was filtered to remove the pulp from the seeds using a sterile cheesecloth. The seeds were then cleaned three times with tap water, allowed to dry for 24 hours at room temperature, and weighed. Finally, a blender type A11 basic was used to grind the seeds into a fine powder (IKA, Germany). The seeds powder (740g) was kept at -18ºC until additional analysis.

Extraction

Soxhlet extraction (SE)

SE of *O. ficus indica* seeds was performed according to [24, 18]. Seeds powder (100g), hexane (1000 mL) and a universal fat extraction system (Büchi Labortechnik AG, Flawil, SG, Switzerland) were used. SE was performed for one hour. The extraction process was repeated in triplicate and yields obtained were recorded.

Ultrasound Assisted Extraction (UAE)

For direct sonication extraction, a 24 kHz probe (Ultrasonic Processor UP400S (400 watts, 24 kHz, Hielscher, Germany) was used; inside the extraction vessel, the horn tip position of the probe was one cm below the extraction solvent level [25]. The temperature inside the extraction mixture was measured by a thermocouple, and an ice bath was used to maintain a constant temperature of (25°C ±5°C) around the extraction vessel. Similar to SE conditions, the same amount of milled seeds, extraction solvent (hexane) and extraction time were employed to assess the obtained results. This process was repeated in triplicate and the yields obtained were recorded.

Saponification of the lipophilic fraction and formation of fatty acid methyl esters

Three grams of the obtained oils were saponified according to the method described by [26], by refluxing with 100 mL alcoholic potassium hydroxide (10%) for four hours to give the unsaponifiable matter (0.82 g) and fatty acids fraction (0.74 g). The fatty acids fraction was subjected to methylation according to the method adopted by [27], by refluxing with absolute methanol (30 mL) and sulphuric acid (3 mL) for two hours, to give fatty acids methyl esters.

Analysis of unsaponified matter and fatty acid methyl esters by gas chromatography–mass spectrometry (GC/MS)

A mass spectrometer detector (5977A) and Agilent gas chromatograph (7890B) were used to analyze the composition of fatty acids and unsaponified materials. DB-WAX column, which has an internal diameter of 30 m x 250 µm internal diameter and a film thickness of 0.25 µm, was installed in the GC. Hydrogen was used as the carrier gas for the fatty acid analysis with a flow rate of 1.9 mL/min at a split ratio of 50:1, an injection volume of 1 µl was used.

The following temperature program was used during the analysis: 50°C as the initial temperature for one minute, increasing the temperature by 25°C/minute to 200°C and kept for five minutes, then increasing the temperature by 3°C/minute to 220°C and kept for ten minutes, and finally increasing the temperature again by 5°C/minute to 240°C and kept for eight minutes. The injector final temperature was held at 250°C, while the detector final temperature was kept at 290°C. By utilising electron ionisation (EI) at 70 eV, a spectral range of m/z 50–550, and a solvent delay of one minute, mass spectra were produced. A percent relative peak area was used to study the identification of various ingredients. On the basis of comparing the relative retention times of the compounds and comparing the fragmentation patterns of the spectra with those found in the NIST, WILLY library data of the GC/MS system, a preliminary identification of the compounds was performed.

Analysis of unsaponified matter was performed using hydrogen as the carrier gas at a flow rate of 1.0 Ml /min at a split ratio of 10:1, injection volume of 1 µl, and the following temperature program was used during the analysis: 240°C as the initial temperature; increasing at a rate of 10 °C/min to 265°C and holding for 1 minute, then reaching 300°C at a rate of 15°C/min and holding at 300°C for 25 minutes. The injector final temperature was held at 280°C, while the detector final temperature was kept at 290°C. By employing electron ionisation (EI) at 70 eV, a spectral

range of m/z 50–550, and a solvent delay of three minutes, mass spectra were produced. By correlating the spectrum fragmentation pattern with those found in the Wiley and NIST Mass Spectral Library data, a preliminary identification of the compounds was performed [28].

Cytotoxicity of *O. ficus indica* seeds oils

Cell culture

The following operations were all carried out in a biosafety class II level laminar flow cabinet in a sterile environment (Baker, SG403INT, and Sanford, ME, USA). Plant extracts were tested against a human normal immortalized fibroblast cell line (BJ.1). The BJ.1 cell line was maintained at 37°C in 5% CO2 and 95% humidity while after being cultured in DMEM-F12 media with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (2 mg/mL). Previously obtained from the American Type Culture Collection, the cell line was graciously provided by Professor Stig Linder, Oncology and Pathology department, Karolinska Institute, Stockholm, Sweden (ATCC).

Cytotoxicity bioassay on BJ.1 cell line

Cells were seeded in 96-well plate at a concentration of (10×10^3) cells/well for BJ.1 cell line.

24 hours after seeding, media was aspirated and replaced with serum-free culture media containing the extracts at different concentrations (100 µg/mL – 7.8 µg/mL) for 48 hours in triplicates. Doxorubicin served as positive control and DMSO (0.5 %) was served as negative control. The cytotoxicity was evaluated using the MTT assay and according to the following equation:

% cytotoxicity = [1- (AVx / AVNC)] x 100,

Where AVx: average absorbance of sample wells and AVNC: average absorbance of negative control wells measured at 595 nm with reference at 690 nm [29].

Wound healing activity of different seeds oils *via* wound scratch assay

The scratch assay method was used to measure the migration rates of BJ.1 cells [30]. In a 24-well plate, cell density of "2×10^5 cells" was seeded into each well, before it was incubated at 37°C and 5% CO_2 with complete medium. The monolayer confluent cells were horizontally scrapped with a sterile P200 pipette tip after 24 hours of incubation. PBS was used to wash the debris. The cells were treated with samples with concentration 100 µg/mL (concentration less than of IC_{50} for all extracts) as follows:

The cells without treatment used as negative control G1, while G2 and G3 served as treated groups, in which the application of *O. ficus indica* oil extracts of SE and UAE, respectively were tested on wound area. Prior to incubation with the samples, the scratch-induced wound was imaged at 0 time using phase contrast microscopy at a magnification of x40. The second batch of photos was captured to assess the rate of migration following a 24-hour of incubation period. Using the software "Image J," the images were analysed, and the percentage of the closed area was measured and compared to the value obtained at 0 time. Cell migration was indicated by an increase in the closed area's percentage. The data were recorded and statistically evaluated using SPSS, and experiments were carried out in triplicate.

$$\text{Wound closure (\%)} = \frac{(\text{Measurement at 0 time} - \text{Measurement at 24 hours})}{\text{Measurement at 0 time.}} \times 100$$

***In vivo* studies**

Animals

For the evaluation of toxicity and analgesic efficacy, adult female albino mice weighing 25–30 g were used, obtained from The National Research Centre's Animal-Breeding Unit, Giza, Egypt. Animals were kept under controlled conditions, temperature (23 ± 2°C), light cycle (12 hours of light/dark), and relative humidity (55 ± 5%) in ventilated cages with ad libitum access to tap water and a standard pelleted diet. The National Research Centre's ethical committee's guidelines for the use of laboratory animals were followed in all animal procedures (Number: 20048).

Acute toxicity

Acute oral toxicity test was performed according to OECD guidelines [31]. To determine the acute toxicity, the protocol reported by Wilbrandt [32] was followed with slight modifications. After one week of acclimatization, animals were divided randomly into different groups, each group of 6 mice (n=6). *O. ficus indica* oil obtained by SE and UAE were administered by oral gavage at different doses (1000, 2000, 3000, 4000, 5000, 6000 and 7000 mg/kg body weight) to a total of 6 healthy mice for each concentration. One group (n=6) was given orally the respective amount of DMSO and left as control. Animals were observed continuously for the initial four hours followed by further monitoring for 20 hours.

Determination of median lethal dose (LD_{50})

On healthy mice, the LD50 of *O. ficus indica* oil samples extracted using various techniques was calculated to establish the threshold toxic dose. Thus, the LD50 of each extract was calculated using Wilbrandt's method [32]. Each extract's LD50 was determined using the following formula:

$LD_{50} = Dm - \Sigma (Z \times d)/ n$

Where, Dm is the minimum dose that kills all animals in the group;

Z is the mean of dead animals in two successive groups;

d is the constant factor between two successive groups;

n is the number of animals of each group;

Σ is the sum of (Z x d).

Determination of analgesic activity *via* writhing test

The peripheral analgesic profile of different seed oils was carried out by performing the writhing test [33]. Adult female Swiss albino mice were classified into 5 groups (n=6) as follows:

G1 served as the control group and received the vehicle (saline solution). G2 and G3 were injected intraperitoneally (i.p.) with diclofenac sodium (12.5 and 25 mg/kg, respectively) as a reference drug, whereas G4 and G5 were orally administrated 1/10 LD_{50} of different seed oils (SE 6.75 gm/kg and UAE 3.8 gm/kg, respectively). After 30 minutes of receiving an intraperitoneally (i.p.) injection of the vehicle, reference medication, or *O. ficus indica* oils, animals received a fresh preparation of acetic acid [2% (w/v) in saline, 10 mL/kg body weight]. For a 30 minute interval, the mice were separated for individual observation. Throughout the observation period, each mouse's numbers of writhes were counted. Abdominal writhing activity is defined as contraction of the abdominal muscles, rotation of the pelvis, and extension of the hind limbs [34].

The inhibition percentage of abdominal writhing was calculated using the following equation:

% Inhibition of abdominal writhing = $[(Nc - Nt) / Nc] \times 100$

Where, *Nc* is the number of writhes of the control group and *Nt* is the number of writhes of the treated group.

Statistical analysis

For each experimental group of six mice, the data are presented as mean ± standard error of mean. The mean values of the quantitative variables were compared between the groups using the Students' test and one-way analysis of variance, and then Dunnett's multiple t-test for unpaired data was used to evaluate whether there was a significant difference between the groups at $P < 0.05$.

Results

Extraction yield:

Total mass yield (g/100g) of *O. ficus indica* seeds oils obtained by using UAE technique and SE are illustrated in table (1).

Table (1): Total mass yield (g/100g) of *O. ficus indica* seeds oils

Method	Solvent	Yield (g/100g)
SE	*n*-hexane	2.01 ±0.52
UAE	*n*-hexane	2.15 ±0.61

According to the obtained data, there was a slight improvement (0.14%) in the oil total yield obtained using UAE as compared to SE at the same time period.

GC/MS analysis

GC/MS analysis of the unsaponified and fatty acids methyl esters fractions of seed oils revealed the presence of 13 and six phytochemical compounds, respectively. Tables (2) and (3) represent relative retention time and peak area in percentage of the active principles. The probabilities of the detected 13 compounds of the unsaponifiable matter are listed in table (2). In addition, six fatty acids were identified, and their probabilities are listed in table (3).

Beneficial substances found in prickly pear seed oil, like tocopherols and sterols, have been scientifically proven to decrease or treat human ailments like cancer, atherosclerosis and diabetes [35]. The results obtained from table (2) indicated that *β*-sitosterol and campesterol were found to be the main phytosterols compounds in the two samples with no significant concentration difference. Other phytosterols including cholesterol and stigmasterol, were found to be the minor sterols. Squalene and communic acid were only detected in oil prepared using UAE.

Table (2): Unsaponifiable matter of *O. ficus indica* oils

Constituent	Relative amount (%)*		Relative retention time (RR_t)*
	SE	**UAE**	
Saturated fatty alcohol			
1-Hexacosanol	0.11	0.12	0.002
1-Heptatriacotanol	2.7	3.35	0.04
Hydrocarbons			
Squalene	-	0.17	0.002
Heptacosane	0.16	0.25	0.003
Steroids			
Brassicasterol acetate	0.13	-	0.002
Cycloeucalenyl acetate	2.48	-	0.01
γ- Sitosterol	1.33	1.07	0.01
Stigmastanol	7.72	7.01	0.09
Campesterol	8.57	8.84	0.12
Stigmasterol	3.41	4.04	0.55
β - Sitosterol	72.12	73.41	1.00
Phenolic constituents			
γ- Tocopherol	1.27	0.72	0.01
Terpenes			
Communic acid	-	1.34	0.02

*RRt, relative to *β*-Sitosterol retention time = 7.11 min.

The fatty acid compositions of the oil extracted by different methods are listed in table (3).

Linoleic acid and oleic acid were the major fatty acids of the oil content extracted using SE and UAE representing (84.99% and 75.2%) and (8.18% and 14.01%); respectively. In addition, saturated fatty acids (palmitic acid and stearic acid) were detected in both oil samples. However, fatty acids (caproic acid and palmitoleic acid) were only detected in oil sample extracted using UAE.

Table (3): Fatty acids of *O. ficus indica* oil

Constituent	Relative amount (%)*		Relative retention time (RR_t)*
	SE	UAE	
Saturated fatty acids			
Stearic acid	1.12	1.62	0.01
Caproic acid	-	0.02	0.02
Palmitic acid	5.71	8.91	0.07
Unsaturated fatty acids			
Oleic acid	8.18	14.0	0.01
Palmitoleic acid	-	0.25	0.25
Linoleic acid	84.99	75.2	1

RRt, relative to linoleic acid retention time= 9.64 min.

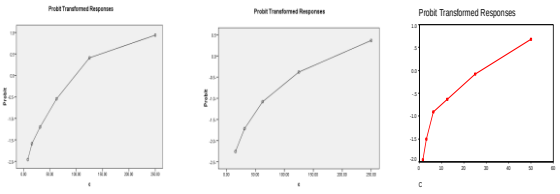
Determination of cytotoxicity on normal skin fibroblast cell line (BJ.1)

Cytotoxicity effect of *O. ficus indica* oils extracted by SE and UAE on normal skin fibroblast cell line (BJ.1)

O. ficus indica oil extracted using SE and UAE were screened on normal skin fibroblast cell line (BJ.1) at different concentrations (500 to 7.8µg/mL) for 48 hours. The results indicated that the IC_{50} were 132.4 and 194.2 μg/ml for SE and UAE after one hour extraction, respectively (Table 4 & Figure 1).

Table (4): IC_{50} and IC_{90} of *O. ficus indica* oil extracted using SE and UAE

Tested samples		IC_{50} (μg/mL)	IC_{90} (μg/mL)
(a)	SE	132.4	240.6
(b)	UAE	194.2	318.8
(c)	Doxorubicin	31.5	54.9



(a) **(b)** **(c)**

Figure 1: IC_{50} probet analysis of *O. ficus indica* different oil samples; **a)** SE, **b)** UAE and **c)** Doxorubicin as positive control**.**

Determination of wound healing activity *via* wound scratch assay (*in vitro*)

To assess the wound healing activity of different seed oils, *in vitro* normal skin fibroblast cell line (BJ.1) scratch assay technique was used. The pictures are displayed in Figure (2).

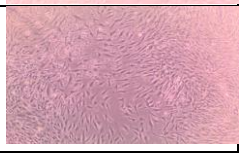
Groups	At zero time.	*After 24 hours.*
G1		
G2		
G3		

Figure 2: Images from *in vitro* scratch assay experiment at different time (0 and 24 hours) of BJ.1 dermal fibroblast cells treated with *O. ficus indica* different oil samples, where G2; SE., G3; UAE and G1 as a negative control. Scale bar = 500 µm, (× 40 magnification)

The assumed values were calculated during 24 hours based on the rate of scratch coverage as shown in Figure (3). All oil samples showed wound healing activity remarkably G3, which showed highly significant wound healing activity (85%), while G2 showed wound healing activity (73%), compared to G1 as a negative control group.

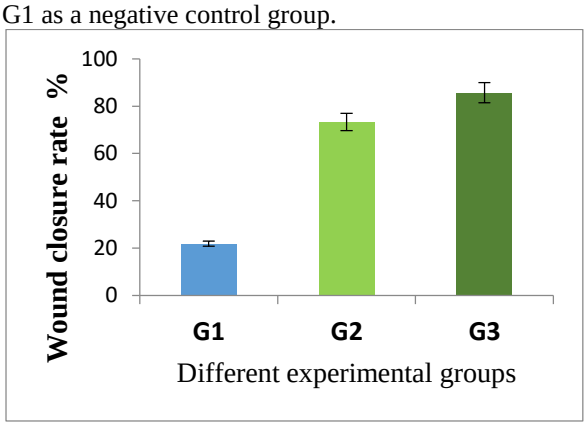


Figure 3: Wound closure rate % of *O. ficus indica* different oil samples, where G2; SE, G3; UAE and G1; negative control.

Acute toxicity studies, median lethal dose LD_{50} estimation.

This study was designed to evaluate the acute oral toxicity produced when the test sample, bioactive oil, was administered by oral gavage to mice. The tested oils were administered by oral gavage at different dose levels (7 to 1 gm/kg body weight) to a total of 6 healthy adult male mice according to the method described by

[33]. Animal management and experimental procedures were accepted by the NRC Animal Ethics Committee.

Table (5): Lethal dose LD_{50} of *O. ficus indica* oil extracted using SE

Dose (mg/kg	No. of	Dead mice	Z	d	(Z)x(d)
7	6	1	1	1	1
6	6	0	0	1	0.5
5	6	0	0	1	0
4	6	0	0	1	0
3	6	0	0	1	0
2	6	0	0	1	0
1	6	0	0	1	0

Neither death nor any abnormal behaviors were observed during our test period except only one mouse was dead at 7gm/kg. The 50% lethal dose (LD_{50}) of *O. ficus indica* oil extracted using SE was 6.75gm/kg b.wt (Table 5).

Table (6): Lethal dose LD_{50} of *O. ficus indica* oil extracted using UAE.

Dose (mg / kg b.wt.)	No. of mice	Dead mice	Z	d	(Z)x(d)
7	6	6	6	1	6
6	6	5	5.5	1	5.5
5	6	3	4	1	4
4	6	3	3	1	3
3	6	1	0.5	1	0.5
2	6	0	0	1	0
1	6	0	0	1	0

All mice were dead at dose 7gm/kg and the death rate was dose dependent as decreased to 3gm/kg b.wt. There is no any abnormal behaviors were observed during our test period. The 50% lethal dose (LD_{50}) of *O. ficus indica* oil extracted using UAE was 3.8gm/kg b.wt (Table 6).

For two oils the results indicated that these LD_{50} could be classified as slightly toxic according to Hodge and Sterner scale [36].

Determination of analgesic activity *via* writhing test (*in vivo*)

In the current study acetic acid induced writhing response was performed in mice as visceral model to evaluate the anti-nociceptive (peripheral analgesic activity) of *O. ficus indica* oil extracted using SE and UAE. In all treated groups, the writhing numbers reduced with maximum values observed in G5 ($P < 0.05$). The extent of writhing reduction in the treated groups with oil extracted by different methods was different when compared with the reference drug groups (G2 & G3), i.e., a significant decrease in G5 ($P < 0.05$), while non-significant decrease was observed in G4 as shown in Figure (4).

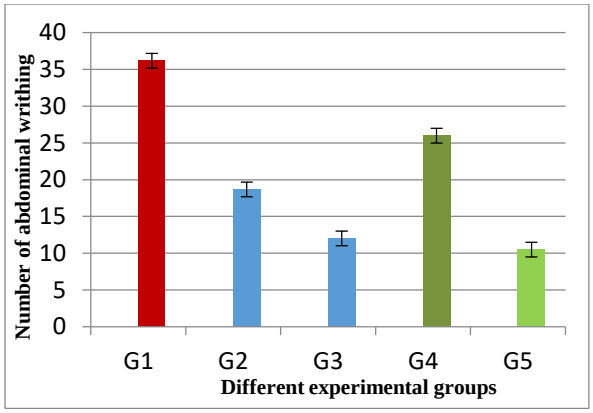


Figure 4: Total number of abdominal writhing of different experimental groups where; G1: negative control, G2: treated with diclofenac sodium (12.5 mg/kg), G3: treated with diclofenac sodium (25 mg/kg), G4: treated with SE oil and G5: treated with UAE oil.

Our data demonstrated that G5 showed better analgesic activity than reference drug in G2 (48%) and G3 (66%), with a significant inhibition of the abdominal writhing (71%), when compared to reference drug in G2. However, G4 showed weak abdominal writhing inhibition percentage value of 28% when compared to reference drug groups as shown in Figure (5).

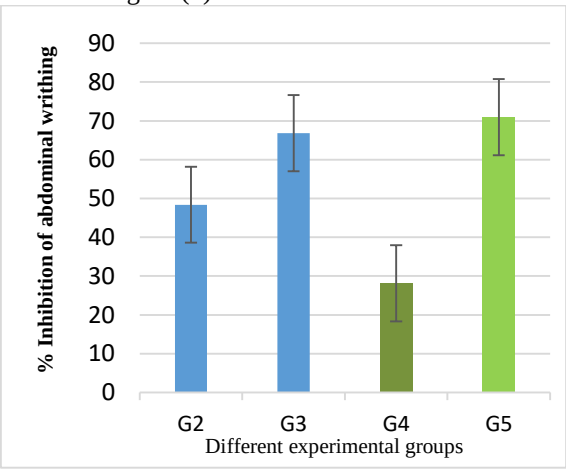


Figure 5: Analgesic activity of different experimental groups where; G2: treated with diclofenac sodium (12.5 mg/kg), G3: treated with diclofenac sodium (25 mg/kg), G4: treated with SE and G5: treated with UAE.

Discussion

The total mass yield (g/100g) of *O. ficus indica* seed oils obtained by UAE technique and SE indicated that the use of the UAE provides a slight increase in the yield of oil obtained at the same time interval (one hour). Contradictorily, prickley pear seeds preferred SE with a yield of 9.3–9.5%, while UAE acquired 5.4–5.6% as previously reported [37]. This could be attributed to the longer extraction time of SE, typically lasts several hours (6–24 h), under drastic conditions.

Several studies have been conducted on seed oil extracted by using UAE. Almost studies clearly prove that UAE is able to increase the oil quality and yield using a considerably milder conditions, lower energy consumption and shorter extraction time than traditional oil extraction methods [38, 39, 40 and 41]. In this context, our results indicated that the application of UAE for one hour affected and improved the detected unsaponifiable matter (*β*-sitosterol, campesterol and stigma sterol) as well as the evaluated fatty acids (stearic, oleic, palmitic and palmitoleic acids) compared to SE. This could be attributed to UAE process, which depends on the "*cavitation phenomena*". When a liquid is ultrasonically irradiated, acoustic cavitation bubbles occur, leading to localized hot spots (~5000 K and ~2000 atm) [42]. The bubbles collapse is symmetric in a homogenous liquid and asymmetric when the collapse occurs near a surface or when the liquid is loaded with suspended particles (as in case of extraction). UAE mechanism can increase the extraction yield and shorten the extraction time, thanks to the high shear forces together with shock waves generated by symmetric collapsing bubbles, which enhance the rinsing out of cell contents into the surrounding extraction solvent. Asymmetric collapsing bubbles also generate shear forces and shock waves, but in addition they create high speed jets directed towards the herb surface [43]. The latter aspect of asymmetric collapse plays a major role in ultrasonic assisted extraction because it helps to break the outer layer or surface of the plant material. This process releases the plant material content into the surrounding solvent and in addition, the jet formed during asymmetric bubbles collapse enhances the solvent penetration into the plant particles. The jets could also contribute to cell pore enlargement acting like a micro-pump which force the solvent into the cell, where it dissolves the required compounds and transports them back into the bulk solvent. The produced energy can also result in herbal particle size reduction, which produces a higher plant surface accessible to solvent [44, 23].

O. ficus indica oil is usually known as an oil rich in polyunsaturated fatty acids, followed by monounsaturated fatty acids and saturated fatty acids Table (3). These results are in accordance with Juhaimi *et al*., 2019, who demonstrated that the major fatty acid present in *O. ficus indica* seed oil extraction was linoleic acid ranged between 49.4 and 74.0% then oleic acid (8.8 to 23.5%), palmitic acid (7.2 to 17.8%), and stearic acid (1.5 to 5.8%) while palmitoleic and linolenic acid were less than 1% [45]. *O. ficus indica* oil content of linoleic acid is higher than other commonly consumed oils such as corn, soybean and cotton seed and close to that of sunflower oil [46].

Healing of wound occurs through an inflammatory phase, proliferative phase, and remodeling phase [47]. Studying skin tissue repair of the wounded area in the present work, fibroblast migration impacted by *O. ficus indica* oils resulted in re-epithelialization of the wounded tissue enhancing wound closure rate [48]. This could be due to fatty acid profile of *O. ficus indica* oils found to be rich in two essential fatty acids: linoleic acid (omega-6 fatty acid) and oleic acid. Bonferoni *et al*., 2014, reported that linoleic acid biphasic effects on the inflammatory phase of tissue repair improved wound healing process and maintained hydration [45].

Similarly, Silva *et al*., 2018, demonstrated that linoleic acid in pumpkin and lucuma nut oils is responsible for its wound healing activity [49]. Linoleic acid has beneficial properties for skin and it could be utilized by industrial cosmetics products [50, 48].

UAE oil showed better wound healing activity more than SE oil which could be attributed to higher content of *β*-sitosterol and oleic acid. *β*-sitosterol is considered as an angiogenic factor which is responsible for neovascularize stimulation and the motility of human umbilical vein endothelial cells in wound migration assay (*in vitro*), in addition to the role of oleic acid in increasing fibroblast migration and producing differential wound repair activity [51, 50]. Moreover, palmitoleic acid was only detected in UAE oil which possesses antifungal, antibacterial and antioxidant activities [52], which led to improvement of tissue repair process.

Due to steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs) side effect profiles, natural compounds, such as herbal remedies and dietary supplement which have been used to reduce pain and inflammation, is attracting growing interest. *Opuntia* species have been used as analgesic and anti-inflammatory agents and reported to promote wound healing and improve platelet function [53].

Our data indicated that UAE oil showed better analgesic activity than diclofenac sodium (reference drug), due to the presence of potentially active nutrients as campesterol, *β*-sitosterol, lupeol, saponins, phenolic compound or alkaloids and their multipurpose functions [54] which make *Opuntia* fruits ideal candidates of phytopharmaceutical components production with analgesic and anti-inflammatory activities [55, 56]. The possible mechanism by which *O. ficus indica* oils produced peripheral analgesia might be associated with suppression of peripheral nociceptors sensitivity in the

peritoneal free nerve endings and inhibition of various endogenous inflammatory mediators' synthesis [57].
In addition, treatment with UAE oil provoked better antinociceptive effect than SE oil, which could be attributed to the presence of squalene and communic acid in oil extracted by UAE. Squalene and communic acid had antioxidant, anti-inflammatory, and anti-atherosclerotic properties *in vivo* and *in vitro* [58] as well as their potential as antibacterial and anticholinesterase agents [59]. In addition to a higher content palmitic acid detected in UAE oil, which is considered as anti-nociceptive-bearing bioactive compounds [57].

Conclusion:

Based on these results, *O. ficus indica* seed oil is considered as a potential source of edible oil, due to the valuable fatty acids profile and can be used in the treatment of skin wounds due to its ability to modulate both immune responses and inflammation in skin injuries.
Ultrasound-assisted extraction application for oil extraction as an innovative technique, restored favorable active components, and increased the yield and the quality of *O. ficus indica* seeds oil; in addition UAE improved its analgesic and wound healing activities.

Abbreviations:

Declarations:

- **Competing interests**

The authors declare that they have no competing interests.

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- **Ethics approval**

The study was approved for the use of animals and human cell lines by the Medical Research Ethics Committee (MREC), National Research Centre, Dokki, Egypt

- **Availability of data and materials:**

All data are available as figures and supporting figures.

- **Consent for publication:**

All authors consent to submission of this work for publication.

Authors' contributions:

A.F., I.S., K.S. and N.A.; designed the plan of work as well as methodology; I.S., E.B., K.M. and A.A. conducted research and performed the experiments; E.B., A.F., M.A. and A.A. contributed to data collection, investigation and interpretation of the results; I.S. contributed to writing and drafting the article; A.F., provided critical revision of the article; All authors discussed the results and contributed to the final manuscript.

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