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Improvement of Pharmaceutically Active Materials from the Fruit of the Prickly Pear *(Opuntia ficus-indica)* Using Ultrasound (UAE) and Microwave (MAE) Assisted Extractions

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Abstract: Conventional extraction methods have many limitations and drawbacks, including prolonged extraction time, present safety concerns, and environmental risks with low-quality extracts. We report the use of ultrasonic (UAE) and microwave (MAE) assisted technologies as techniques for improving the extraction of pharmaceutically active materials from *Opuntia ficus-indica* (OFI), a species of prickly pear. The pulp and peel of the plant fruit were used, and the total phenolic and flavonoid contents were evaluated. Antioxidant assay (DPPH) was employed to prescreen different extracts. The Hepa1c1c7 model was used for testing the induction of chemopreventive marker protein NAD (P) H-quinone oxidoreductase 1 (NQO1). The *in vitro* anti-inflammatory activity was performed on RAW 264.7 macrophage model induced for nitric oxide (NO) release in the presence of lipopolysaccharide. *In vivo* study included testing the therapeutic potential of some extracts on carrageenan-induced paw edema in adult rats. Our data showed that fruit peels had the highest contents of phenolic and flavonoid compounds of OFI extracted using microwave-assisted extraction (MAE) at 800 W power for 5 min extraction time (EXM1) with a percentage increase of 74.1% and 115.3%, respectively when compared to conventional maceration. DPPH prescreen revealed the potency of the EXM1 among other tested extracts, recording EC50 148 µg/mL. Although *in vitro* chemopreventive as an anti-inflammatory model revealed no activity on (NQO1) induction of EXM1, the *in vivo* model gave positive results. The edema size reduction percentage of EXM1 was 104% after 4 hours. Anti-inflammatory markers indicated that EXM1 inhibited COX-2, IL-6, TNF-α, and TGF-β1 more significantly than indomethacin and conventional extraction methods. The data obtained confirmed the beneficial value of MAE and UAE technologies for the extraction of the active material of (OFI).

Keywords: *Opuntia ficus-indica* fruit; ultrasonic and microwave-assisted extraction techniques; total phenolic and flavonoid contents; antioxidant and anti-inflammatory activity.

1. Introduction

Opuntia ficus-indica (L.) (OFI) Mill. belongs to the Cactaceae family with the greatest economic relevance in the world [1]. It has been a domesticated plant in Latin America, Africa, Mediterranean countries, the Middle East, Egypt, India, and Australia [2, 3]. It is a medicinal herb [4] containing mixtures of bioactive compounds whose profiles change with species, cultivars, and climate conditions [5]. It has been used as a food source for animals and humans [6]. It is a relevant source of phytochemicals with proven biological activities and high-added value in the food/ nutraceutical industry [7]. There is an increasing usage of fresh fruit to produce several products such as juice, jelly, etc.[8].

It has been reported that (OFI) contains polyphenolic compounds such as flavonoids and phenolic acids. Quercetin, isorhamnetin, kaempferol glycosylated derivatives, gallic acid, catechin, and rutin were detected in different OFI parts [9, 10]. Polyphenols are an important group of compounds associated with (OFI) fruits which were found to possess antioxidant, anti-inflammatory activity [11] and antimicrobial properties [12]. Opuntia fruit/pulp extracts possess antidiabetic [13], cardioprotective, neuroprotective [14], anti-inflammatory [15], and hepatoprotective properties [16].

In the biological system, oxidative stress rises after increased contact with oxidants, reducing the system's antioxidant capability. It is frequently related to ROS generation and free radicals [17]. Cellular damage is caused by this oxidative stress and leads to many diseases like cardiovascular disease and cancer [18]. Antioxidants protect cells against ROS molecules and help organisms to deal with oxidative stress caused by free radicals such as a-enzymatic antioxidant defenses, including catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD), b-non-enzymatic antioxidants, including glutathione (GSH), flavonoids and carotenoids [19].

Natural products such as herbs have inroad powerful antioxidants and anti-inflammatory to inhibit the process of cellular transformation. Also, nitric oxide (NO) is presented as a critical mediator in the inflammation process which is produced by iNOS at the site of inflammation [20].

Agents such as TGF-ß and IL-10 are involved in the downregulation of inflammatory reactions [21]. Cytokines are categorized as pro-inflammatory IL-6, TNF- α, and the inflammatory-cytokines IL-10 and TGF-ß produced predominantly by activated macrophages, involved in the up-regulation of inflammatory reactions [22]. COX enzymes are the main enzymes responsible for prostaglandin biosynthesis. The modulation of the enzymes can assist in anti-inflammatory treatments owing to the key role of PG, especially PGE2, in the inflammatory response [23].

Nowadays, the extraction development of polyphenolic components from Opuntia spp. has great attention among researchers. This could be attributed to Opuntia spp. oxidant/antioxidant potential [24].

In the last years, efforts have been oriented to exploiting new extraction methods, such as ultrasonic-assisted extraction (UAE) [25,26], microwave-assisted extraction (MAE) [27],

pressurized liquid extraction (PLE) [28], and supercritical fluid extraction [29], which have emerged as energy-saving technologies.

In addition to its economic impact, the food and nutraceutical industry prefers green extraction to obtain high-quality products [30].

The extraction technique based on ultrasonic waves was utilized to reduce solvent consumption and extraction time and to obtain better yields [31]. The UAE mechanism is attributed to the mechanical and cavitation effects, which increase the mass transfer of targeted compounds due to cell wall breakage of plant material [32]; ultrasonic waves also ease the extraction of the solute from inside the plant matrix to the extraction solvent at a faster rate [33-35].

Another green extraction technique is microwave-assisted extraction (MAE). This technique involves irradiation of the samples soaked in a solvent. Furthermore, compared to conventional extraction methods (oil baths, sand baths, and heating mantles) that cause the thermal decomposition of substrates, microwaves can pass through the walls of the reaction container, thereby heating the reactants and the solvent [36] directly.

Microwave-assisted extraction is utilized on a large laboratory scale because it has numerous advantages reducing energy consumption and the amount of organic solvents, reducing waste, and making it possible to obtain better yield with respect to the conventional methods of extraction [37]. However, extraction efficiency is reliant on several factors, such as the nature of the compounds to be extracted, temperature, pH, nature, and volume of solvents.

In several published articles comparing microwave-assisted extraction with other innovative and conventional extraction methods, microwave-assisted extraction has been known as a potential and powerful alternative for the extraction of bioactive components from herbal material [38].

For the above reasons, it is essential to select the suitable method for extraction of bioactive compounds, one which aids in reserving their antioxidant properties. Therefore, the current study has examined the effect of two advanced green techniques on the chemical content of (OFI) different parts extracts and their influence on their biological activity.

2. Materials and Methods

2.1. Chemicals and reagents.

The chemicals and reagents used in the study, such as gallic acid (3, 4, 5-tri-hydro benzoic acid), rutin, methanol (MeOH), Folin-Ciocalteu phenol reagent, aluminum chloride ($AlCl_3$), and sodium bicarbonate (Na_2CO_3), were purchased from Merck (Darmstadt, Germany). The stable free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH), carrageenan, and indomethacin were purchased from Sigma-Aldrich (Darmstadt, Germany). Also, COX-2, IL-6, TNF-α, TGF-β1, NO, MDA, GSH, SOD concentrations were measured using ELISA kits purchased from (Wuhan Fine Biotech Co., China) and spectrometric kits purchased from Sigma-Aldrich (Darmstadt, Germany).

2.2. Cell culture.

The murine hepatoma cell line Hepa-1c1c7 was maintained as a monolayer culture in α- modified Minimum Essential Medium Eagle (α-MEME) supplemented with 10% (v/v) heat- and charcoal–inactivated fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin sulfate in a humidified incubator (Sartorius CMAT, Germany, 5%

CO_2/95% air). At about 80% confluence, cells were routinely subcultured with Trypsin EDTA solution.

2.3. Plant collection and identification.

Opuntia ficus-indica fruits were obtained from Abo-Hammad, Sharkiya, Egypt, during the harvesting season (August 2019), during which fruits had the optimum maturity, as a representative sample comprised of 500 fruits was used in the study. The taxonomic identity of the plant was confirmed with Prof. Dr. Ibrahim Ahmed El Garf, Botany and Microbiology Department, Faculty of Science, Cairo University, Egypt.

2.4. Plant preparation.

Opuntia ficus-indica fruits in the ripe stage of yellow to green color were washed with running water to remove glochids and impurities, air-dried, and carefully hand-peeled. The peels (with a thickness of about 3-4mm) were separated from fruit pulp, then chopped into small pieces and stored at -18 ºC in the deep freezer.

The fruit juice was extracted using a blender (Moulinex, LM30214A, France). The obtained juice was filtered using a sterile cheesecloth to separate the seeds from the pulp. Then, the juice and the peel were lyophilized separately in a freeze dry system and stored at −18 °C until analysis [39].

2.5. Plant extractions.

2.5.1. Conventional extraction methods.

2.5.1.1. Maceration.

The extraction was performed at room temperature by mixing 2.5 g of powdered (OFI) different parts (pulp and peel) with 25 mL 80% aqueous methanol in a sealed conical flask. Conventional maceration extraction was performed at different time intervals (15 and 30 min) [40].

2.5.2. Innovative extraction methods.

2.5.2.1. Ultrasonic assisted extraction (UAE) (20 kHz Probe).

A 20 kHz probe (Ultrasonic Processor UP400S (400 watts, 24 kHz, Hielscher, Germany) was employed for direct sonication extraction [41]. The horn tip was kept 1 cm below the solvent extraction surface inside the extraction vessel. Extractions of 2.5 g of OFI in different parts (pulp and peel) using 25 mL 80% aqueous methanol were performed at different time intervals, 15 and 30 mins.

2.5.2.2. Microwave assisted extraction (MAE).

This method was performed using opened system microwave apparatus (MARS 240v/ 50Hz); 2.5 g of OFI different parts (pulp and peel) were extracted using 25 mL 80% aqueous methanol at different time intervals (5, 15, and 30 mins). Extractions were performed at two different powers (400 and 800 W). The temperature of extraction using MAE was fixed at 65-70 °C considering the boiling point of methanol 64.7 °C [42].

2.6. Determination of total phenolic acids or phenols (TPC)

Total phenolic content was determined by Folin–Ciocalteu according to the method described by [43] with some modification. Briefly, 20 µl of the sample was mixed with 100 µl of Folin–Ciocalteu reagent (diluted 1:10 with deionized water). After 15 min incubation, the mixture was neutralized with 80 µl of saturated Na_2CO_3. The absorbance of the mixture was measured at 760 nm after 2 h of incubation. Gallic acid was used as a standard for the calibration curve. The concentration of the total phenolics was determined from a standard calibration curve. The mean of three readings was used, and the total phenolics were expressed as micrograms of gallic acid (GAE) equivalent per 100 milligrams of the extract (µg /100 mg extract).

2.7. Determination of total flavonoids (TFC)

The total flavonoid content was determined according to the method adopted by [44, 45]. Briefly, 40 µl of the sample was mixed with 40 µl of $AlCl_3$ (2 % in methanol for HPLC grade), and the mixture was allowed to stand for 15 min. The absorbance was measured at 430 nm with a Zenyth 200rt microplate reader (Biochrom Ltd, Cambridge, UK) against an appropriate blank. Rutin was used as a standard, and the total flavonoids content was determined using the standard curve; the mean of three readings was recorded, and the total flavonoids were expressed as microgram of rutin (RU) equivalent per 100 milligrams of the extract (µg /100 mg extract).

2.8. Determination of in vitro free radical scavenging activity (DPPH assay).

Various concentrations of (OFI) methanolic extracts (1 mL) were mixed with 1 mL of a methanolic solution containing DPPH radicals (6.10-5 mol/L). The assay method used in the present study was based on a modified procedure [46], essentially based on previously published literature [47]. The mixture was shaken vigorously and left to stand for 30 min at room temperature in the dark. Absorbance was read using a microplate reader (tristar LP2, Berthol, Germany) at 520 nm. Rutin was used as the reference compound. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation:

$$\%RSA = [(A0 – A1)/A0] \times 100$$

where A0 is the absorbance of the control (blank, without extract), and A1 is the absorbance in the presence of the extract. The extract concentration providing 50% of radical scavenging activity (EC_{50}) was calculated from the graph of RSA percentage against extract concentration.

2.9. In vitro anti-inflammatory study.

RAW 264.7 Cells (500000 cell/mL) were seeded onto 96-well plates for 24 h. Cells were treated with either 0.1% v/v DMSO (negative control lipopolysaccharide {LPS}), 100ng/mL LPS+) or LPS in the presence of 6.26-100µg/mL of the extracts. Griess assay was performed to determine N in triplicates of culture supernatants following 24 h exposure time in all groups. Briefly, 100µL of culture supernatant from every well was mixed with an equal volume of Griess reagent mixed at room temperature; absorbance was measured at 540 nm on a Tristarlb 942® microplate reader (Berthold, Germany). Inhibition (%) was calculated relative to the LPS-only group (LPS+) [48].

2.10. In vitro chemopreventive study.

2.10.1. NQO1 assessment.

The induction of NQO1 in Hepa-1C1C7 cells was evaluated. Briefly, cells (3 × 105 cells/mL) were seeded onto 6-well plates and left for 24 h to adhere and form semi-confluent monolayers. Monolayers were treated with either vehicle (final concentration 0.1% v/v DMSO), peel MW 800 extracts (final concentrations of 100, 25, and 6.25 µg/mL) for an additional 24 h [48]. In parallel, 4-bromoflavone (4-BF) was used as a positive control for NQO1 induction. Monolayers were washed with ice-cold Dulbecco's PBS (1 mL/well). Cells were then scrapped in ice-cold homogenization buffer (25 mM Tris-HCl, pH 7.4, 250 mM sucrose, and 5 µM FAD). Cell suspensions were then sonicated on ice for 5 s (20% amplitude). Sonicates were then centrifuged (15,000 ×g for 10 min), and the supernatants (cytosolic fractions) were aliquoted and stored at a −80 °C freezer until tested for protein expression.

2.10.2. Western blot analysis of (NQO1).

Hepa-1C1C7 cells were cultured and treated as mentioned above. NQO1 protein expression was assessed in cell sonicates by Western blotting as previously described with some modifications [49]. At the end of exposure, samples including vehicle control, positive controls (4-BF), and peel (MAE) at 800 W sample increasing concentration (6.25, 25, 100 µg/mL total proteins/lane) were resolved under denaturing conditions by electrophoresis (SDS-PAGE) on 10% acrylamide/bisacrylamide gel (200 Volts for 1 h). Resolved proteins were then transferred to a nitrocellulose membrane at 100 V for 60 min. Membranes were blocked in 5% non-fat milk in Tris-buffered saline with 0.1% Tween 20 (TBST) for 1 h at 25°C and then probed overnight (4°C) with primary antibodies against NQO1 and ***β***-actin (Abcam, UK). After three washes in TBST (5 min each), membranes were probed with 1:10000 dilutions of appropriate secondary antibodies (Abcam, UK) for 1 h at 25°C, washed three times in TBST, and then developed using enzyme chemiluminescence (ECL, Pierce, USA, Invitrogen, USA), and bands were detected using CCD camera (UVP, UK).

2.11. Toxicity studies of different Opuntia ficus-indica extractions.

2.11.1. *In vivo* toxicological studies.

Swiss albino mice were housed in groups in stainless steel cages and kept under standard laboratory conditions. They were drinking water ad libitum and given pelleted food. The mice were acclimatized to the laboratory conditions for at least five days before the initiation of the experiments.

2.11.2. Toxicity and determination of median lethal dose (LD_{50}).

An acute oral toxicity test was performed per OECD guidelines [50]. Mice weighing 22-29 gm were randomly classified into four treated groups. LD_{50} of the promising extracts was assessed using the method described by [51]. All groups were orally administered 1, 2, 3, 4, 5, 6, and 7 g/kg body weight of four (OFI) extracts. The LD_{50} of each extract was calculated according to the formula:

$\mathbf{LD_{50}} = Dm\text{-}\Sigma\ (Zxd)/n$

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 in each group, and **Σ** is the sum of (**Zxd**).

The animals were observed continuously for the first 24 h and after 14 days for any signs of behavioral changes, mortality, and body weight.

2.12. Determination of anti-inflammatory activity (Experimental design).

The anti-inflammatory activities of the extracts were evaluated *in vivo* using the carrageenan-induced rat paw edema model [13, 52] with some modifications. Forty-two adult male Sprague–Dawley rats weighing 160–190 g will be classified into 7 Groups (*n*=6) as follows:

G1 served as the healthy negative control group (NCT) and received a saline solution.

G2 served as the positive control group (PCT) induced with carrageenan (CARR) only, 0.1 mL of 1% freshly prepared solution of carrageenan in saline solution [53] in the sub-plantar region of the left hind paw.

G3 was induced with carrageenan and received (indomethacin; 10 mg/kg, p.o) as a reference drug [54] half an hour after the carrageenan challenge.

G4 was induced with carrageenan and was orally dosed with 1/10 of LD_{50} dose of (OFI) peel extracted using the conventional method half an hour after the carrageenan challenge.

G5 was induced with carrageenan and orally dosed with 1/10 of LD50 dose of (OFI) peel extracted using the MAE technique half an hour after the carrageenan challenge.

G6 was induced with carrageenan and orally dosed with 1/10 of LD50 dose of (OFI) pulp extracted using the conventional method half an hour after the carrageenan challenge.

G7 was induced with carrageenan and orally dosed with 1/10 of LD50 dose of (OFI) pulp extracted using the MAE technique half an hour after the carrageenan challenge.

All animals were retained as per the approvals of the guiding principle in the care and use of laboratory animals permitted by the Medical Research Ethics Committee, National Research Centre, Cairo, Egypt, ethical approval number (20-048).

2.12.1. Calculation of rat paw edema inhibition %.

Rat paw edema was assessed by using Vernier Caliper (SMEC, Shanghai, China) after carrageenan injection at 4, 3, 2, 1, and zero h. The difference in paw volume, assessed after injection of carrageenan, indicated the severity of edema. The inflammation inhibition percentage was assessed for each animal compared to controls and calculated by the following formula:

$$\%I = 1 - (dt \backslash dc) \times 100$$

where '***dt***' is the difference in paw volume in the treated group, and "***dc***" is the difference in paw volume in the control group. "***I***" stands for inhibition.

2.12.2. Estimation of anti-inflammatory markers (COX-2, IL-6, TNF-***α***, and TGF-***β***1) in serum samples

This kit has relied on sandwich enzyme-linked immune-sorbent assay technology. Determination of IL-6 levels was performed using ELISA assay Kit. The color change is measured spectrophotometrically at a wavelength of 450 nm [55]. These kits were based on the Competitive-ELISA detection procedure. The provided microtiter plate has been pre-coated

with the target. Through the reaction, the target in the sample or standard competes with a fixed amount of target on the solid phase supporter for sites on the Biotinylated Detection Antibody specific to the target. Excess conjugate and unbound samples or standard were washed from the plate, and Streptavidin-Biotin Complex (SABC) was added to each microplate well and incubated. Then 3, 3′, 5, 5′-Tetramethylbenzidine (TMB) substrate solution is added to each well. The enzyme-substrate reaction is terminated, and the color change is measured spectrophotometrically at a wavelength of 450 nm. The target concentration in the samples is then determined by comparing the OD of the samples to the standard curve.

2.12.3. Estimation of antioxidant parameters in serum samples.

2.12.3.1. Nitric oxide measurement.

Nitric oxide (NO) was rapidly oxidized to nitrite and nitrate, which were utilized to quantitate NO production. NO colorimetric assay kit was used to measure the total nitrate/nitrite in a simple two-step process. Firstly, nitrate reductase was used to convert nitrate to nitrite. Then, the Griess reagent was used to convert nitrite to a deep purple azo complex. The amount of the azo chromophore accurately revealed nitric oxide quantity in the samples. Finally, optical density was measured at A540 nm by the microplate reader (BMG Labtech, Germany) [56].

2.12.3.2. Determination of malondialdehyde levels (MDA).

MDA levels, as a lipid peroxidation product, were assessed using ELISA assay Kit (Wuhan Fine Biotech Co., China). This method detected the thiobarbituric acid (TBA) reactive substances (TBARS) quantity as an MDA production index in serum samples based on nmol per mL of serum. The change in color is measured spectrophotometrically at a wavelength of 450 nm [57].

2.12.3.3. Determination of superoxide dismutase (SOD).

Determination of SOD enzyme activity was performed using ELISA assay Kit (Wuhan Fine Biotech Co., China). In brief, after the reduction of 2-(4-iodophenol)-3-(4-nitrophenol)-5-phenyltetrazolium chloride by xanthine oxidase, a red formazan product was produced. This reduction was suppressed by SOD and produced a colored complex. Its absorbance was quantified at 450 nm [57].

2.12.3.4. Determination of reduced glutathione (GSH).

It was determined through the colorimetric method using 5, 5′-dithiobis-2-nitrobenzoic acid (DTNB) according to the protocol provided by the kit. Briefly, DTNB reacted with the reduced form of thiol (–SH) groups and made a complex. The absorbance was read at 450 nm to estimate the GSH levels [58].

2.13. Statistical analysis.

Statistical analysis was done using the SPSS (Statistical Package for the Social Sciences) version 20.0 statistics software (IBM Corp., Armonk, NY, USA, 2011). The mean (±standard deviation) values of bioactivity parameter analyses were evaluated using Microsoft

Excel spreadsheets. The t-test was applied at the confidence level of $p < 0.05$ for significance and $p < 0.01$ for high significance when comparing between groups.

3. Results and Discussion

3.1. Estimation of total phenolic and flavonoid contents of different (OFI) extracts.

The current study indicated that OFI peel extract showed the highest phenolic and flavonoid contents compared to pulp extracts. OFI peel extracted using MAE at W 800 for 5 min extraction (EXM1) showed the highest content of both phenolic and flavonoid contents among all extracts.

3.1.1. Effect of ultrasonic-assisted extraction.

A significant difference in the total amount of phenolic and flavonoid contents of peel and pulp belonging to (OFI) was observed. In general, (OFI) methanolic peel extract showed higher contents of both phenolic and flavonoid contents. A slight increase in TPC and TFC of (OFI) peel EXC1 [1880 µg/mL GAE 100mg extract and 480 µg/mL RE 100mg extract, respectively] and pulp EXC2 [433 µg/mL GAE 100mg extract and 57 µg/mL RE 100mg extract, respectively] was observed after increasing the conventional maceration extraction time from 15 to 30 mins.

We examined the effect of both ultrasonic waves and duration of extraction on the extractability of TPC and TFC from (OFI) peel and pulp. In the case of (OFI) pulp and peel, a significant increase in the extraction efficiency was observed on TPC and TFC using direct sonication (20 kHz) when compared to silence extraction, as shown in Figures 1 & 2.

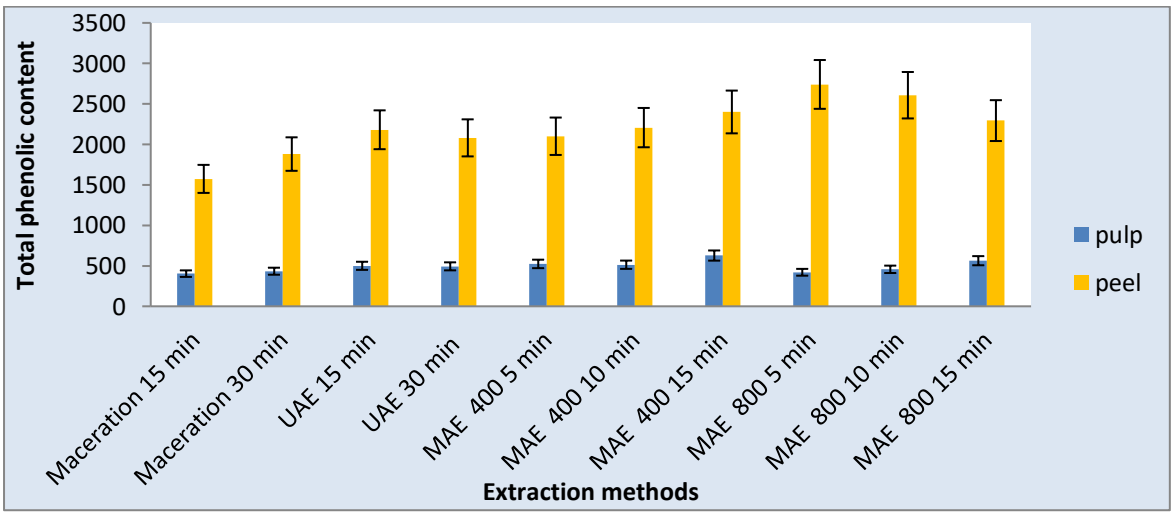


Figure 1. Total phenolic content for different (OFI) extracts.

The unique mechanism offered by ultrasonic waves, which depends mainly on the cavitation phenomena [59], increased the extractability of TPC and TFC from (OFI) different parts. The highest TPC and TFC from (OFI) peel and pulp were observed after 15 mins sonication EXU1 [2180 µg/mL GAE 100mg extract and 493 µg/mL RE 100mg extract, respectively] and EXU2 [500 µg/mL GAE 100mg extract and 66.67 µg/mL RE 100mg extract, respectively]. The extraction yield is elevated significantly due to the formation of several high-speed jets produced as a result of ultrasonic cavitation phenomena, which can strike the cell walls and increase the mass transfer from inside the cells of the vegetal material to the

surrounding extraction solvent. However, a slight decrease was observed at 30 mins extraction time using UAE as increasing the sonication time can cause degradation/breakdown of some compounds [60].

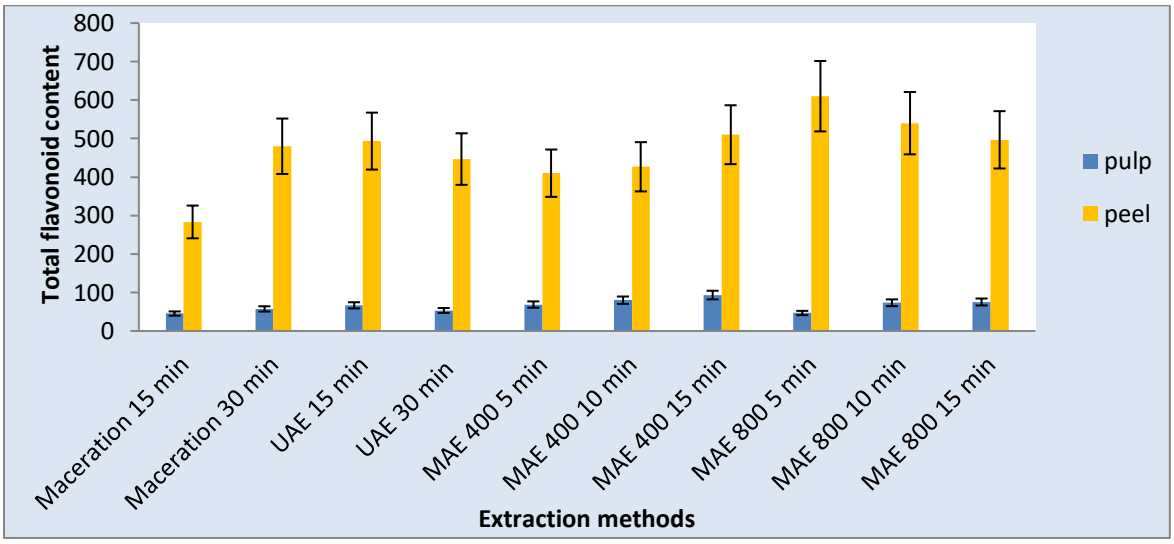


Figure 2. Total flavonoid content for different (OFI) extracts.

3.1.2. Effect of microwave-assisted extraction

The extractability of TPC and TFC from (OFI) peel and pulp using microwave-assisted extraction increased significantly when compared to conventional maceration. Moreover, MAE showed better extraction efficiency and reduced the extraction time of TPC and TFC from (OFI) peel and pulp compared to UAE. The highest TPC and TFC from (OFI) peel were observed after 5 mins microwave irradiation at 800 W (EXM1) [2740 µg/mL GAE 100mg extract and 610 µg/mL RE 100mg extract, respectively], whereas the highest TPC and TFC from (OFI) pulp were observed after 15 mins microwave irradiation at 400 W (EXM2) [626.66 µg/mL GAE 100mg extract and 93.33 µg/mL RE 100mg extract, respectively].

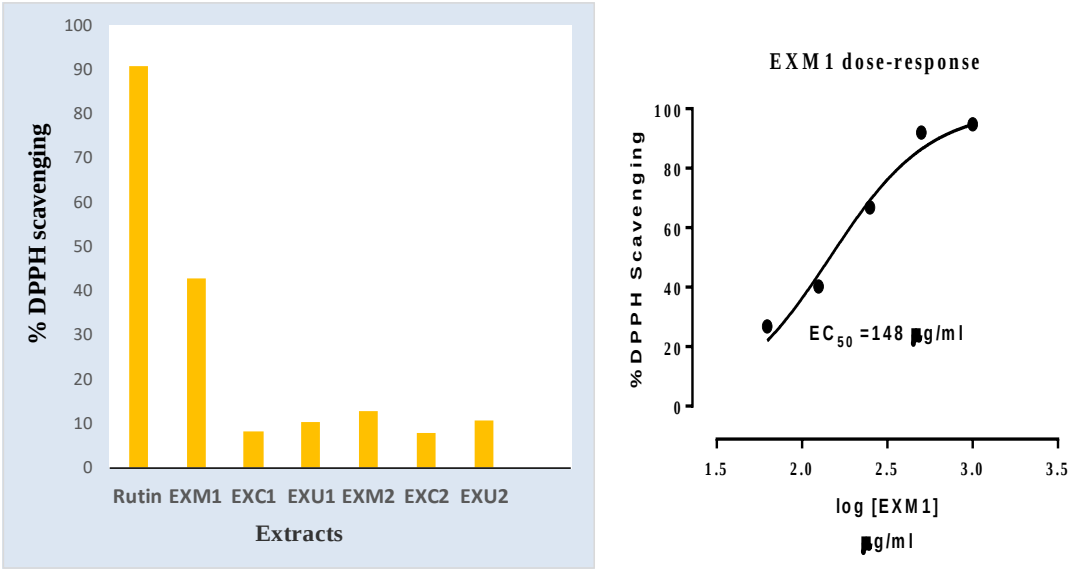
Microwave distinctive heating mechanism not only increases the solvent's penetration force to the cell wall of the plant matrix, but it also causes evaporation of the moisture content inside plant cells and generation of enormous pressure. The produced pressure pushes the cell wall from inside and ultimately ruptures it, facilitating the complete rupture of the plant cell and leaching out of the active constituents from the ruptured cells to the extraction solvent, thus improving the yield of phytoconstituents [27]. In addition, microwave increases the number of plant cell pores, which facilitates the release of the bioactive principles to the extraction solvent. Moreover, MAE increases the extraction selectivity of phenolic compounds when compared to conventional methods. Phenolic compound molecules are characterized by dipole moment. These compounds strongly absorb microwave energy. Consequently, it is considered one of the most successful MAE applications among all other classes of compounds [61, 62] reported an increase in the extractability of bioactive phenolic compounds about four times higher than other extraction methods. [63] and [64] reported a significant increase in the extraction efficiency of phenolic compounds using microwave energy from different herbal plants. The power used during MAE and the structure of the plant matrix under treatment greatly affect the extraction efficiency of bioactive compounds. In our study, extraction of (OFI) pulp using 400 W microwave irradiation showed better results when compared to 800 W at all time intervals. The physiological composition of the pulp is characterized by a softer structure (more

juice/water) than the peel, which requires less drastic extraction conditions to prevent the degradation of the extracted compounds. Contrastingly, extraction of (OFI) peel needed more powerful extraction conditions, using 800 W microwave irradiation power for 5 mins, due to the physiological composition of the peel, which is characterized by higher fiber content [1]. However, a slight decrease in the total phenolic content of (OFI) peel was observed after prolonged extraction under drastic conditions due to the possible degradation of the target compounds. Similar behavior was observed by [65].

3.2. Estimation of in vitro antioxidant activity for different (OFI) extracts by using DPPH assay (Radical scavenging activity).

The DPPH is a stable free radical used for the study of structural characteristics to the radical scavenging activity of compounds. As shown in Figure 3a The preliminary screening of OFI peel and pulp extracts (EXM1, EXC1, EXU1, EXM2, EXC2, EXU2) showed that EXM1 had the highest radical scavenging potency among the tested extracts compared to rutin reference radical scavengers by 90.8%. Concentration-response experiment showed a gradual increase in the DPPH radical activity with increasing concentration. The effective concentration that scavenges 50% of DPPH (EC_{50}) in the vehicle control was calculated on GraphPad prism software to be 148 µg/mL (Figure 3b). EXM1 had the highest DPPH activity recorded in the present study among other extracts.

The DPPH is a stable free radical. EXM1 had the highest DPPH activity recorded in the present study, among other extracts. The obtained activity correlates well with this extract's total phenolic and flavonoid content, indicating the influence of phenolic constituents on antioxidant capacity. Consistent with our findings, previous studies reported DPPH activity [66-70].



(a) **(b)**

Figure 3. Preliminary screening of (OFI) different parts extracts obtained using conv., MAE, and UAE methods compared to rutin (0.36 µg/mL) as the reference radical scavengers: **(a)** Prescreen ; **(b)** EXM1 EC_{50}.

3.3. Estimation of in vitro anti-inflammatory activity for EXM1.

The NO inhibition assay was employed to assess the inhibition of LPS-induced NO from culture RAW macrophage. EXM1 showed very weak inhibition of NO release (Figure 4).

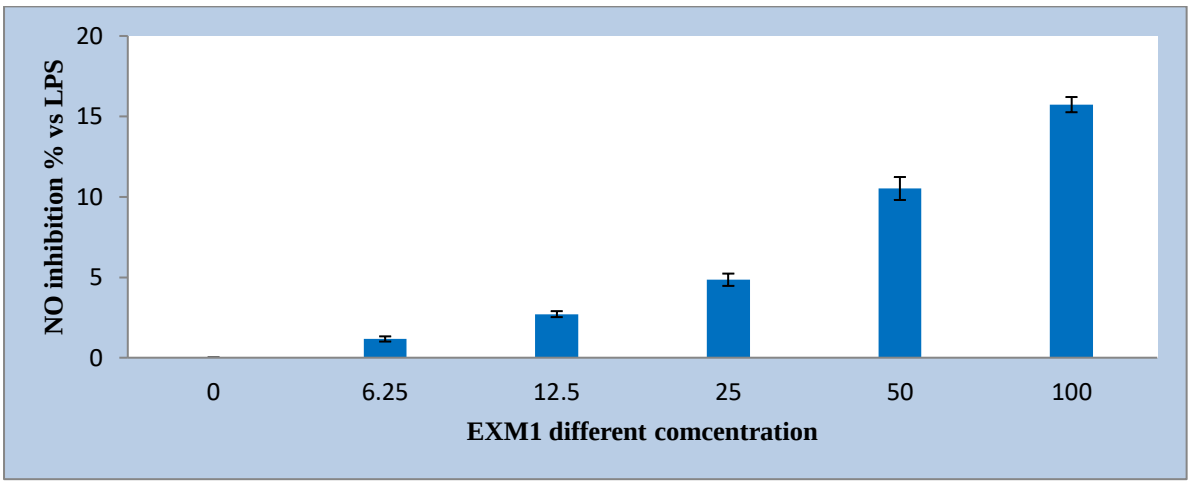


Figure 4. Determination of the (NO) inhibition % for EXM1 at different concentrations.

3.4. Estimation of in vitro chemopreventive activity for EXM1 at different concentrations.

Assessment of the cancer chemopreventive potential by Western blot analysis of the NQO1 protein expression for EXM1 showed no effect within the concentration range tested (6.25, 25, and 100 µg/mL), as shown in Figure 5.

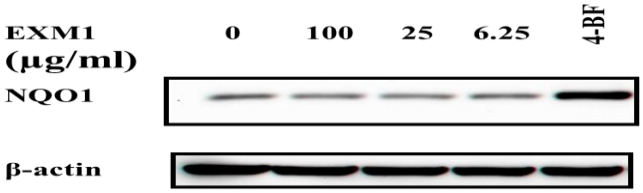


Figure 5. Assessment of the cancer chemopreventive potential by Western blot analysis of NQO1 protein expression for EXM1 at different concentrations *in vitro*.

3.5. Toxicity in vivo studies of different (OFI) extracts.

After 24 hours of oral administration of four extracts (EXM1, EXC1, EXM2, and EXC2) at different doses (7, 6, 5, 4, 3, 2, and 1g/kg body weight), the behavior pattern of mice was tracked. All tested animals were dead after oral administration of EXC1, EXM2, and EXC2 extracts at 5, 6, and 7 g/kg body weight doses. The death rate decreased with decreasing the given dose to 4, 3, 2, and 1g/kg body weight. $\boldsymbol{LD_{50}}$ of EXC1, EXM2, and EXC2 extracts were 1.2gm/kg, 0.8g/kg, and 1.2g/kg, respectively (Table S2, S3, and S4). Six, 3, and 2 animals were dead after oral administration of EXM1 at 5, 4, and 3 g/kg body weight doses, respectively, with LD_{50} 4g/kg body weight (Table S1). Living animals did not show any negative changes in their behavioral patterns as a decrease in food intake or weight loss.

3.6. Biological in vivo anti-inflammatory and antioxidant studies for different (OFI) extracts.

3.6.1. Estimation of rat paw edema curve.

The inhibition rate of edema size slightly increased with the time of extract injection. After 3 h and 4 h of carrageenan induction, EXM1 (G5) showed percentage inhibition of edema size (89.2% and 100%), respectively, while EXM2 (G7) showed edema percentage inhibition (69.6% and 94.6%) respectively. A reduction in inhibition percentage of edema size percentage was observed by extracts obtained using conventional extraction methods after 4 h of carrageenan induction. EXC1 (G4) and EXC2 (G6) showed edema size percentage inhibition (42% and 58%), respectively. Treatment with reference standard indomethacin drug (G3)

showed inhibition of edema size percentage (97.5%) after 4 h of carrageenan induction (Figure 6).

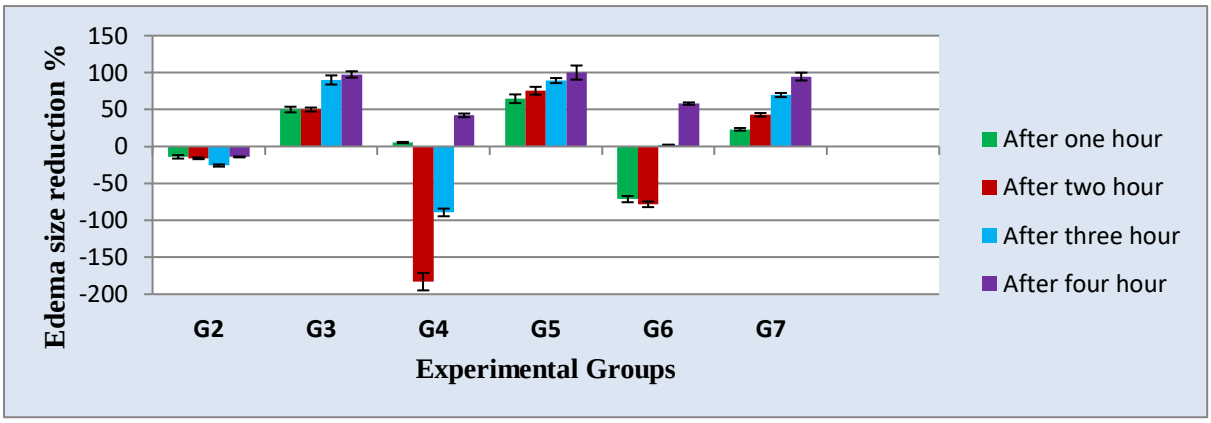


Figure 6. Rat paw edema curve of different treated groups where G2: treated with carrageenan only (PCT), G3: treated with indomethacin (NCT), G4: treated with EXC1 extract, G5: treated with EXM1 extract, G6: treated with EXC2 extract and G7: treated with EXM2.

3.6.2. Estimation of antioxidant enzymes (MDA, NO, SOD and GSH) and anti-inflammatory markers (COX-2, IL-6, TNF-α, and TGF-β1) in serum samples.

The current study was performed to investigate the anti-inflammatory activity of (OFI) different extracts against carrageenan-induced rat paw edema. The activities of these extracts were directly correlated to their constituents as antioxidant agents (phenolic and flavonoid contents) and enhanced using advanced green technology (Figure 7).

In the current study, it was indicated from the results that GSH levels were significantly decreased ($p<0.05$) in PCT (G2) compared to NCT (G1). GSH levels were significantly increased in G3, G5, and G7 compared to PCT (G2). In addition, SOD levels were significantly decreased ($p<0.05$) in PCT (G2) compared to NCT (G1). SOD levels were significantly increased ($p<0.05$) in G5 and G7 compared to PCT (G2) (Figure 7a).

Furthermore, NO and MDA levels were significantly increased ($p<0.05$) in PCT (G2) compared to NCT (G1). Moreover, NO and MDA levels were significantly decreased ($p<0.05$) in G5, and G7 ($p<0.01$) compared to PCT (G2) (Figure 7b). Our findings also indicated that cytokines; IL-6, TNF-α, and TGF-β1 levels were significantly increased ($p<0.05$) in PCT (G2) compared to NCT (G1) and significantly decreased ($p<0.05$) in G5 and G7 compared to PCT (G2), while G4 and G6 showed a significant increase ($p<0.05$) in IL-6, TNF-α, and TGF-β1 levels compared to PCT (G2) (Figure 7c). Finally, COX-2 levels were significantly increased ($p<0.05$) in PCT (G2) compared to NCT (G1). Also, G3, G4, and G6 showed a significantly increased in COX-2 levels compared to PCT (G2), especially G4 and G6, which showed a high significance increase ($p<0.01$), while G5 and G7 showed a significantly decreased in COX-2 levels ($p<0.05$) compared to PCT (G2) (Figure 7d).

In light of the strong interconnection between inflammations and redox-modulating properties of phytochemicals plants, phenolic and flavonoid content play an important role either as antioxidant or anti-inflammatory agents with different mechanisms. For example, the biological system depends on numerous endogenous defense mechanisms to protect against cell damage induced by free radicals through antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH), which offer the cellular first-line defense against toxic free radicals. The major role of GSH is to detoxify the radicals such as NO and MDA through scavenging them or by acting as a co-substrate in the glutathione peroxidase

(GPx)-catalyzed reduction of lipid peroxides and hydrogen peroxide. SOD activity protects cells and the extracellular matrix from the harmful effects of superoxide anion and its derivatives, such as hydroxyl radical [71].

In acute inflammation, as the carrageenan-induced paw edema model, oxidative stress which responsible for inflammation associated with reactive oxygen species (ROS), exceeding the capacity of the endogenous antioxidant system [72]. From the obtained results, we suggested that bioactive components in (OFI) MAE extracts protect cells from toxins such as free radicals by maintaining and increasing GSH and SOD levels. Furthermore, in addition to the decreased MDA, the main final gent of lipid peroxidation, which gathers at the site of inflammation [73] and nitric oxide (NO) levels, also proposed that these extracts might mediate the ROS-relevant lipid peroxidation that leads to a significant decrease of lipids damage in cell membranes. In agreement with our results, the extract of (OFI) flower has been reported to reduce the lipid peroxidation products (MDA) of inflammation processes which are related to NO production [74].

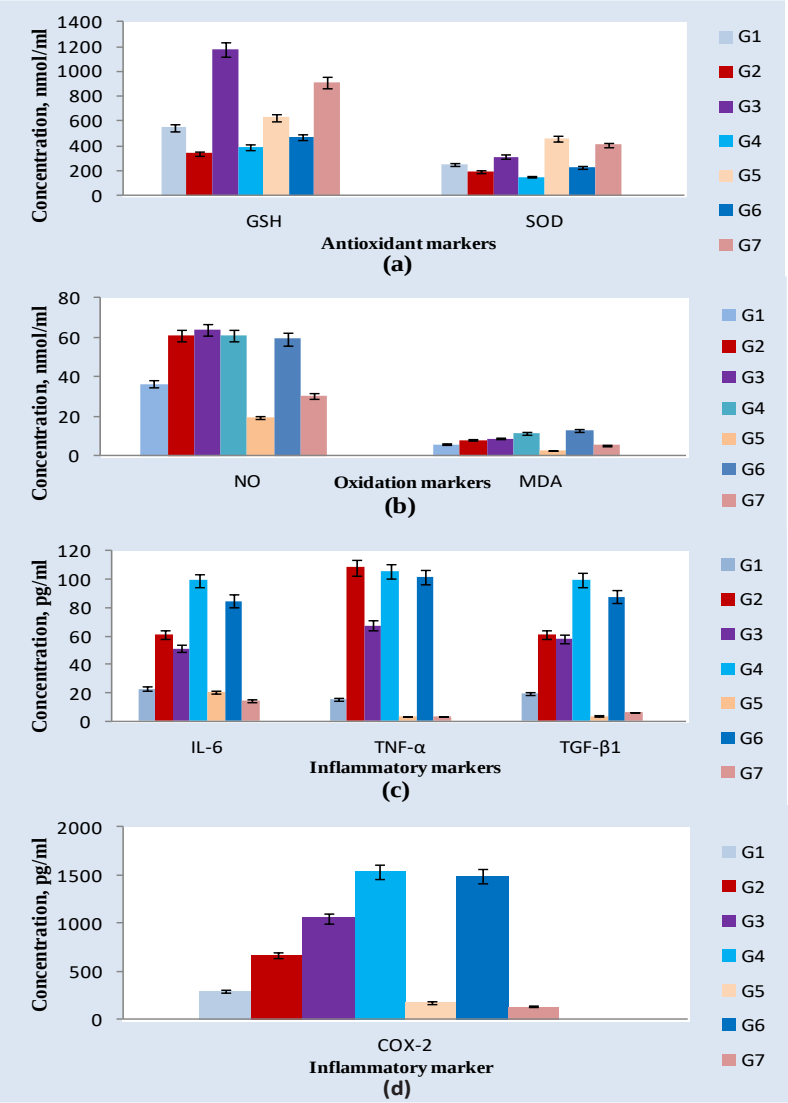


Figure 7. Determination of antioxidant enzymes (MDA, NO, SOD, and GSH) and anti-inflammatory markers (COX-2, IL-6, TNF-*α*, and TGF-*β*1) in serum samples **(a)** Determination of GSH, SOD; **(b)** NO, MDA ; **(c)** IL-6, TNF-*α*, and TGF-*β*1 ; **(d)** COX-2 in serum samples of different treated groups where G1: negative control (NCT) , G2: treated with carrageenan only (PCT), G3: treated with indomethacin after carrageenan challenge, G4: treated with EXC1 extract, G5: treated with EXM1 extract, G6: treated with EXC2 extract and G7: treated with EXM2.

Consistent with our findings, some studies have demonstrated that the ability of some flavonoids to inhibit cytokine production may contribute to their anti-inflammatory properties [75,76]. In addition, ameliorate inflammation by HO-1 mRNA expression upregulation with a simultaneous reduction in the cytokine, TNF-α release [54]. Pro-inflammatory cytokines activate NF-κB, which regulates several genes important in immunity and inflammation. It is well known that endothelial adhesion molecule expression is regulated by several redox-regulated transcription factors, including the master minder NF-κB [77]. A study reported that the anti-inflammatory activity of OFI, both *in vitro* and *in vivo*, inhibited the adhesion molecule overexpression and NF-κB transcriptional activity [78]. Moreover, IL-6 induces inflammatory responses in the cells via activation of the transcription factor STAT3 [79]. Other transcription factors, such as NF-κB and TGF-β1, seem to be involved in the transcription regulation of IL-6. Previous studies suggested several mechanisms for the anti-inflammatory effects of plant extracts containing phenolics [80].

The edema size reduction is a good indicator to determine the anti-inflammatory activity of different extracts. Pro-inflammatory markers and cytokines were evaluated in rat serum samples to confirm the results from the edema size inhibition. COX-2 is an inducible enzyme and is only expressed after an inflammatory stimulus [81]. The role of COX-2 is to synthesize prostaglandins to induce inflammation [82]. COX-2 is an important marker associated with anti-inflammatory mechanisms and inhibition of edema. This edema model includes the release and the synthesis of inflammatory mediators at the site of inflammation, such as prostaglandins for the second phase of inflammation (3–4 h after CARR injection) [83]. Previous studies suggested that some flavones and flavonols may act as preferential suppressors of COX-2 [84, 85] while working on anti-inflammatory activity and the inhibition of arachidonic acid metabolism by flavonoids revealed that flavonoids inhibit the arachidonic acid metabolism through the enzyme pathway which responsible for anti-inflammatory properties of these compounds [86, 87]. Based on the obtained results in the present study, further studies should be conducted for the possible industrialization or scale-up of MAE and UAE of pharmaceutically active materials from the fruit of OFI.

4. Conclusions

This work demonstrates the possibility of exploiting microwave (MAE) and ultrasound (UAE) techniques to obtain *Opuntia ficus-indica* extracts remarkably enriched in valuable antioxidants and anti-inflammatory forms. The optimum condition was found as an extraction time of 5 min, 800 W, and 15 min, 400 W for peel and pulp extracted using microwave-assisted extraction (MAE), respectively. A significant increase in total phenolic and flavonoid values was found in peel and pulp extracted using microwave-assisted extraction by 74.1%, 115.3%, and 55.4%, 105.8%, respectively, compared to conventional maceration. A high correlation between total phenolic and flavonoid contents for peel and pulp extracted using microwave-assisted extraction and their *in vivo* biological activities were observed. The peel extracted using microwave-assisted extraction with the optimum condition at 5 min, and 800 W reported the best antioxidant and anti-inflammatory activity. Overall, considering the results obtained, these advanced laboratory extraction methods could advantageously be scaled to a commercial plant within the framework of Green Chemistry. It is essential to provide new studies to isolate and identify the main constituent and its mechanism of action responsible for the anti-inflammatory activity.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. De Wit, M.; Du Toit, A.; Osthoff, G.; Hugo, A. Antioxidant content, capacity and retention in fresh and processed cactus pear (*Opuntia ficus-indica* and *O. robusta*) fruit peels from different fruit-colored cultivars. *Front. Sustain. Food Syst.* **2020**, *133*, https://doi.org/10.3389/fsufs.2020.00133.
2. Kaur, M.; Kaur, A.; Sharma, R. Pharmacological actions of *Opuntia ficus indica*: A Review. *J. Appl. Pharm. Sci.* **2012**, *2*, 15-18, https://doi.org/10.7324/JAPS.2012.2703.
3. Aragona, M.; Lauriano, E. R.; Pergolizzi, S.; Faggio, C.J. *Opuntia ficus-indica* (L.) Miller as a source of bioactivity compounds for health and nutrition. *Nat. Prod. Res*. **2018**, *32*, 2037-2049, https://doi.org/10.1080/14786419.2017.1365073.
4. Nharingo, T.; Moyo, M. Application of *Opuntia ficus-indica* in bioremediation of wastewaters, A critical review. *J. Environ. Manage*. **2016**, *166*, 55-72. https://doi.org/10.1016/j.jenvman.2015.10.005.
5. Paiva, P. M.; de Souza, I. F.; Costa, M. C.; Santos, A. D.; Coelho, L. C. *Opuntia* sp. Cactus: biological characteristics, cultivation and applications. *Adv. Res.* **2016**, *7*, 26125, https://doi.org/10.9734/AIR/2016/26125.
6. Osuna-Martínez, U.; Reyes-Esparza, J.; Rodríguez-Fragoso, L. Cactus (*Opuntia ficus-indica*): A Review on its Antioxidants Properties and Potential Pharmacological Use in Chronic Diseases. *Nat. Prod. Chem. Res.* **2014**, *2*, https://doi.org/10.4172/2329-6836.1000153.
7. Mena, P.; Tassotti, M.; Andreu, L.; Nuncio-Jáuregui, N.; Legua, P.; Del Rio, D.; Hernández, F. Phytochemical characterization of different prickly pear (*Opuntia ficus-indica* (L.) Mill.) cultivars and botanical parts: UHPLC-ESI-MSn metabolomics profiles and their chemometric analysis. *Int. Food Res. J.* **2018**, *108*, 301-8, https://doi.org/10.1016/j.foodres.2018.03.062.
8. Cardador-Martínez, A.; Jiménez-Martínez, C.; Sandoval, G. Revalorization of cactus pear (*Opuntia* spp.) wastes as a source of antioxidants. *Food Sci. Technol.*, **2011**, *31*, 782-8, https://doi.org/10.1590/S0101-20612011000300036.
9. Abdel-Hameed, E.S.S.; Nagaty, M.A.; Salman, M.S.; Bazaid, S.A. Phytochemicals, nutritionals and antioxidant properties of two prickly pear cactus cultivars (*Opuntia ficus indica* Mill.) growing in Taif, KSA. *Food Chem*. **2014**, *160*, 31–38, https://doi.org/10.1016/j.foodchem.2014.03.060.
10. De Wit, M.; Hugo, A.; Shongwe, N. South African Cactus Pear Seed Oil: A Comprehensive Study on 42 Spineless Burbank *Opuntia ficus-indica* and *Opuntia robusta* Cultivars. Eur. *J. Lipid Sci. Technol.* **2018**, *120*, 1700343, https://doi.org/10.1002/ejlt.201700343.
11. Antunes-Ricardo, M.; Gutiérrez-Uribe, J. A.; Martínez-Vitela, C.; Serna-Saldíva,r S. O. Topical anti-inflammatory effects of isorhamnetin glycosides isolated from *Opuntia ficus-indica.* Biomed. *Res. Int.* **2015**, http://dx.doi.org/10.1155/2015/847320.
12. Khatabi, O.; Hanine, H.; Elothmani, D. Hasib A. Extraction and determination of polyphenols and betalain pigments in the Moroccan Prickly pear fruits (*Opuntia ficus indica*). Arab. *J. Chem*. **2016**, *9*, S278-81, https://doi.org/10.1016/j.arabjc.2011.04.001.
13. Hassan, F.; El-Razek, A.; Hassan, A. A. Nutritional value and hypoglycemic effect of prickly cactus pear (*Opuntia ficus-indica*) fruit juice in alloxan-induced diabetic rats. Aust. *J. Basic Appl. Sci*. **2011**, *5*, 356-77.
14. Gambino, G.; Allegra, M..; Sardo, P.; Attanzio, A.; Tesoriere, L.; Livrea, M. A.; Ferraro, G.; Carletti, F. Brain distribution and modulation of neuronal excitability by indicaxanthin from *Opuntia ficus indica* administered at nutritionally-relevant amounts. *Front. Aging Neurosci.* **2018**, *10*, https://doi.org/10.3389/fnagi.2018.00133
15. Gómez-Maqueo, A.; García-Cayuela, T.; Welti-Chanes, J.; Cano, M. P. Enhancement of anti-inflammatory and antioxidant activities of prickly pear fruits by high hydrostatic pressure: A chemical and microstructural approach. Innov. *Food Sci. Emerg. Technol.* **2019**, *54*, 132-142, https://doi.org/10.1016/j.ifset.2019.04.002

16. Serra, A. T.; Poejo, J.; Matias, A. A.; Bronze, M. R.; Duarte, C. M. Evaluation of *Opuntia* spp. derived products as antiproliferative agents in human colon cancer cell line (HT29). *Int. Food Res. J*. **2013**, *54*, 892-901, https://doi.org/10.1016/j.foodres.2013.08.043.
17. Zejnullahu, V. A.;Kosumi, E. The role of oxidative stress in patients with recurrent pregnancy loss: A review. *Reprod. Health*. **2021**, *18*, 1, 1-12, https://doi.org/10.1186/s12978-021-01257-x.
18. Panche, A.; Chandra, S.; Diwan, A. D.; Harke, S. Alzheimer's and current therapeutics: A review. Asian J. *Pharm. Clin. Res.* **2015**, *8*, 14-9, t: https://www.researchgate.net/publication/333775193.
19. Irato, P.; Santovito, G. Enzymatic and non-enzymatic molecules with antioxidant function. *Antioxidants*, **2021**, *10*, 579, https://doi.org/10.3390/antiox10040579.
20. Lee, C. K..; Lee, E. Y.; Kim, Y. G.; Mun, S. H.; Moon, H. B.; Yoo B. Alpha-lipoic acid inhibits TNF-*α* induced NF-κB activation through blocking of MEKK1–MKK4–IKK signaling cascades. *Int. Immunopharmacol*., **2008**, *8*, 362-70, https://doi.org/10.1016/j.intimp.2007.10.020.
21. Zhao, H.; Wu, L.; Yan, G.; Chen, Y.; Zhou, M.; Wu, Y.; Li, Y. Inflammation and tumor progression: Signaling pathways and targeted intervention. Signal Transduct. *Target. Ther*. **2021**, *6*, 1-46, https://doi.org/10.1038/s41392-021-00658-5.
22. Liu, C.; Chu, D.; Kalantar-Zadeh, K..; George, J.; Young, H.A.; Liu, G. Cytokines: from clinical significance to quantification. *Adv. Sci.* **2021**, *8*, 15, 2004433, https://doi.org/10.1002/advs.202004433.
23. Jiang, X.; Renkema, H.; Pennings, B.; Pecheritsyna, S.; Schoeman, J.C.; Hankemeier, T.; Smeitink, J.; Beyrath, J. Mechanism of action and potential applications of selective inhibition of microsomal prostaglandin E synthase-1-mediated PGE2 biosynthesis by sonlicromanol's metabolite KH176m. *Sci. Rep*. **2021**, *11*, 1-14, https://doi.org/10.1038/s41598-020-79466-w.
24. El-Mostafa, K.; El Kharrassi, Y.; Badreddine, A.; Andreoletti, P.; Vamecq, J.; El Kebbaj, M. H.; Latruffe, N.; Lizard, G.; Nasser, B.; Cherkaoui-Malki, M. Nopal cactus (*Opuntia ficus-indica*) as a source of bioactive compounds for nutrition, health and disease. *Molecules.* **2014**, *19*, 14879-901, https://doi.org/10.3390/molecules190914879.
25. Jerman, T.; Trebše, P.; Vodopivec, B. M. Ultrasound-assisted solid liquid extraction (USLE) of olive fruit (*Olea europaea*) phenolic compounds. *Food Chem*. **2010**;*123*;175-82, https://doi.org/10.1016/j.foodchem.2010.04.006.
26. Adetunji, L. R.; Adekunle, A.; Orsat, V.; Raghavan, V. Advances in the pectin production process using novel extraction techniques: A review. *Food Hydrocoll.* **2017**, *62*, 239-50, https://doi.org/10.1016/j.foodchem.2010.04.006.
27. Dahmoune, F.; Nayak, B.; Moussi, K.; Remini, H. Madani K. Optimization of microwave-assisted extraction of polyphenols from *Myrtus communis* L. leaves. *Food Chem*. **2015**, *166*, 585-95 https://doi.org/10.1016/j.foodchem.2014.06.066.
28. Luthria, DL. Influence of experimental conditions on the extraction of phenolic compounds from parsley (*Petroselinum crispum*) flakes using a pressurized liquid extractor. *Food Chem*. **2008**, *107*, 745-52, https://doi.org/10.1016/j.foodchem.2007.08.074.
29. Camel, V. Recent extraction techniques for solid matrices—supercritical fluid extraction, pressurized fluid extraction and microwave-assisted extraction: their potential and pitfalls. *Analyst.* **2001**;*126*;1182-93, https://doi.org/10.1039/b008243k.
30. Chemat, F.; Rombaut, N.; Sicaire, A. G.; Meullemiestre, A.; Fabiano-Tixier, A. S.; Abert-Vian, M. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrason. Sonochem.* **2017**, *34*, 540-60, https://doi.org/10.1016/j.ultsonch.2016.06.035.
31. Senrayan, J.; Venkatachalam, S. Optimization of ultrasound-assisted solvent extraction (UASE) based on oil yield, antioxidant activity and evaluation of fatty acid composition and thermal stability of *Coriandrum sativum* L. seed oil. *Food Sci. Biotechnol.* **2019**, *28*, 377-86, https://doi.org/10.1007/s10068-018-0467-1.
32. Hossain, M. B.; Brunton, N. P.; Patras, A.; Tiwari, B.; O'donnell, C. P.; Martin-Diana, A. B.; Barry-Ryan, C. Optimization of ultrasound assisted extraction of antioxidant compounds from marjoram (*Origanum majorana* L.) using response surface methodology. *Ultrason. Sonochem.* **2012**, *19*, 582-90, https://doi.org/10.1016/j.ultsonch.2011.11.001. .
33. Manzoor, M.; Anwar, F.; Bhatti, I. A.; Jamil, A. Variation of phenolics and antioxidant activity between peel and pulp parts of pear (*Pyrus communis* L.). fruit. Pak. *J. Bot.* **2013**, *45*, 1521-5, https://doi.org/10.3390/molecules17066491.

34. Šic Žlabur, J.; Radman, S.; Opačić, N.; Rašić, A.; Dujmović, M.; Brnčić, M.; Barba, F. J.; Castagnini, J. M.; Voća, S. Application of Ultrasound as Clean Technology for Extraction of Specialized Metabolites From Stinging Nettle (*Urtica dioica* L.). *Front. nutr* **2022**, *684*, https://doi.org/10.3389/fnut.2022.870923.
35. Soni, A.; Samuelsson, L. M.; Loveday, S. M.; Gupta, T. B. Applications of novel processing technologies to enhance the safety and bioactivity of milk. Compr. Rev. *Food Sci. Food Saf.* **2021**, *20*, 4652-77, https://doi.org/10.1111/1541-4337.12819.
36. Shah, J. J.; Mohanraj, K. Comparison of conventional and microwave-assisted synthesis of benzotriazole derivatives. *Indian J. Pharm. Sci*. **2014**, *76*, 1, 46-53, https://pubmed.ncbi.nlm.nih.gov/24799738/.
37. Grigonis, D.; Venskutonis, P.R.; Sivik, B.; Sandahl, M.; Eskilsson, C.S. Comparison of different extraction techniques for isolation of antioxidants from sweet grass (*Hierochloe odorata*). *J. Supercrit. Fluids.* **2005**, *33*, 223-33, https://doi.org/10.1016/j.supflu.2004.08.006. .
38. Veggi, P. C.; Martinez, J.; Meireles, M. A. Fundamentals of microwave extraction. In: Chemat F., Cravotto G, editors. Microwave-assisted extraction for bioactive compounds, Boston: *Springer* **2013**, 15-52, https://link.springer.com/chapter/10.1007/978-1-4614-4830-3_2.
39. Moussa-Ayoub, T.E., Abd El-Hady, E.S.A., Omran, H.T., El-Samahy, S.K., Kroh, L.W. and Rohn, S., 2014. Influence of cultivar and origin on the flavonol profile of fruits and cladodes from cactus *Opuntia ficus-indica*. *Food Res. Int. 64*, 864-872, https://doi.org/10.1016/j.foodres.2014.08.021.
40. Saleh, I. A.; Vinatoru, M.; Mason, T. J.; Abdel-Azim, N. S.; Aboutabl, E. A.; Hammouda, F. M. A possible general mechanism for ultrasound-assisted extraction (UAE) suggested from the results of UAE of chlorogenic acid from *Cynara scolymus* L. (artichoke) leaves. *Ultrason. Sonochem*. **2016**, *31*, 330-336, https://doi.org/10.1016/j.ultsonch.2016.01.002.
41. Vinatoru, M. An overview of the ultrasonically assisted extraction of bioactive principles from herbs. *Ultrason. Sonochem*. **2001**, *8*, 303-13, https://doi.org/10.1016/S1350-4177(01)00071-2.
42. Li, H.; Deng, Z.; Wu, T.; Liu, R.; Loewen, S.; Tsao, R. Microwave-assisted extraction of phenolics with maximal antioxidant activities in tomatoes. *Food Chem.* **2012**, *130*, 928-36, https://doi.org/10.1016/j.foodchem.2011.08.019.
43. Singleton, V. L.; Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965,** *16*, 144-58, https://www.ajevonline.org/content/16/3/144.
44. Arvouet-Grand, A.; Vennat, B.; Pourrat, A.; Legret, P. Standardization of propolis extract and identification of principal constituents. *J. Pharm. Belg*. **1994**, *49*, 462-468, https://pubmed.ncbi.nlm.nih.gov/7884635/.
45. Meda, A.; Lamien, C.E.; Romito, M.; Millogo, J.; Nacoulma, O.G. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chem*. **2005**, *91*, 571-7, https://doi.org/10.1016/j.foodchem.2004.10.006.
46. Hamed, A. R.; Soltan, M.; Fry, J.; Hammouda, F.; Zaki, A..Antioxidant and Cytoprotective Properties of Three Egyptian Cyperus Species Using Cell-free and Cell-based Assays. *Pharmaceutical Crops*, **2012**, *3*, 88-93. https://doi.org/10.2174/2210290601203010088.
47. Nara, K.; Miyoshi, T.; Honma, T.; Koga, H. Antioxidative activity of bound-form phenolics in potato peel. Biosci. *Biotechnol. Biochem.* **2006**, *70*, 1489-91, https://doi.org/10.1271/bbb.50552.
48. Hamed, A. R.; El-Hawary, S.S.; Ibrahim, R.M.; Abdelmohsen, U.R.; El-Halawany, A. M. Identification of chemopreventive components from halophytes belonging to Aizoaceae and Cactaceae through LC/MS—Bioassay guided approach. *J. Chromatogr. Sci.* **2021**, *59*, 618-26, https://doi.org/10.1093/chromsci/bmaa112.
49. Hamed, A. R.; Hegazy, M. E.; Higgins, M.; Mohamed, T.A.; Abdel-Azim, N. S.; Pare, P. W.; Dinkova-Kostova, A. T. Potency of extracts from selected Egyptian plants as inducers of the Nrf2-dependent chemopreventive enzyme NQO1. *J. Nat. Med.* **2016**, *70* , 683-8, https://doi.org/10.1007/s11418-016-0994-0.
50. Wang, L. L.; Ding, J. J.; Pan ,L.; Fu, L.; Tian, J. H.; Cao, D. S.; Jiang, H.; Ding, X.Q. Quantitative structure-toxicity relationship model for acute toxicity of organophosphates via multiple administration routes in rats and mice. *J. Hazard. Mater.* **2021**, *401*, 123724, https://doi.org/10.1016/j.jhazmat.2020.123724. .
51. Wilbrandt, W. Behrens methods for calculation of LD_{50}. *Arzneimittelforschung*. **1952**, *2*, 501-3, https://pubmed.ncbi.nlm.nih.gov/13031942/.
52. Winter, C. A.; Risley, E. A.; Nuss, G. W. Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med*. **1962**, *111*,544-7, https://doi.org/10.3181/00379727-111-27849,
53. Gupta, M.; Mazumder, U. K.; Kumar, R. S.; Gomathi, P.; Rajeshwar, Y.;, Kakoti, B. B.; Selven, V. T. Anti-inflammatory, analgesic and antipyretic effects of methanol extract from *Bauhinia racemosa* stem bark in animal models. *J. Ethnopharmacol*. **2005**, *98*, 267-73, https://doi.org/10.1016/j.jep.2005.01.018.

54. Heeba, G. H.; Mahmoud, M. E.; Hanafy, A. A. Anti-inflammatory potential of curcumin and quercetin in rats: role of oxidative stress, heme oxygenase-1 and TNF-*α*. *Toxicol. Ind. Health*. **2014**, *30*, 551-60, https://doi.org/10.1177/0748233712462444.
55. Wang, Z.; Li, S.; Wang, Y.; Zhang, X.; Chen, L.; Sun, D. GDNF enhances the anti-inflammatory effect of human adipose-derived mesenchymal stem cell-based therapy in renal interstitial fibrosis. *Stem Cell Res.* **2019**, *41*, 101605, https://doi.org/10.1016/j.scr.2019.101605.
56. Jackson, M. I.; Waldy, C.; Jewell, D. E. Dietary resistant starch preserved through mild extrusion of grain alters fecal microbiome metabolism of dietary macronutrients while increasing immunoglobulin A in the cat. *PloS one*. **2020**,*15*, e0241037, https://doi.org/10.1371/journal.pone.0241037.
57. Zhang, C.; Zhao, J.; Famous, E.; Pan, S.; Peng, X.; Tian, J. Antioxidant, hepatoprotective and antifungal activities of black pepper (*Piper nigrum* L.) essential oil. *Food Chem.* **2021**, *346*, 128845, https://doi.org/10.1016/j.foodchem.2020.128845.
58. Esmaeilzadeh-Gharehdaghi, E.; Razmara, E.; Bitaraf, A.; Jamshidi, A.; Mahmoudi, M.; Garshasbi, M. Functional analysis of *RELN S2486G* mutation and its contribution to pathogenesis of Ankylosing Spondylitis. *Arch. Iran. Med.* **2020**, *23*, 688-96, https://doi.org/10.34172/aim.2020.87.
59. Xia, E. Q.; Ai, X. X.; Zang, S. Y.; Guan, T.; Xu, X. R.; Li, H. B. Ultrasound-assisted extraction of phillyrin from *Forsythia suspensa*. *Ultrason. Sonochem.* **2011**, *18*, 549-52, https://doi.org/10.1016/j.ultsonch.2010.09.015.
60. Melgar, B.; Dias, M. I.; Barros, L.; Ferreira, I. C.; Rodriguez-Lopez, A. D.; Garcia-Castello, E. M. Ultrasound and microwave assisted extraction of *Opuntia* fruit peels biocompounds: Optimization and comparison using RSM-CCD. *Molecules*. **2019**, *24*, 19, 3618, https://doi.org/10.3390/molecules24193618.
61. Yahya, N. A.; Attan, N.; Wahab, R. A. An overview of cosmeceutically relevant plant extracts and strategies for extraction of plant-based bioactive compounds. *Food Bioprod. Process.* **2018**, *112*, 69-85, https://doi.org/10.1016/j.fbp.2018.09.002.
62. Gallo, M.; Ferracane, R.; Graziani, G.; Ritieni, A.; Fogliano, V. Microwave assisted extraction of phenolic compounds from four different spices. *Molecules.* **2010**, *15*, 6365-74, https://doi.org/10.3390/molecules15096365.
63. Xie, J. -H.; Dong, C. -j.; Nie, S. -P.; Li, F.; Wang, Z. -J.; Shen, M. -Y.; Xie, M. -Y. Extraction, chemical composition and antioxidant activity of flavonoids from *Cyclocarya paliurus* (Batal.) Iljinskaja leaves. *Food Chem*. **2015**, *186*, 97–105, https://doi.org/10.1016/j.foodchem.2014.06.106.
64. Wu, T.; Yan, J,.; Liu, R.; Marcone, M. F.; Aisa, H. A.; Tsao, R. Optimization of microwave-assisted extraction of phenolics from potato and its downstream waste using orthogonal array design. *Food Chem*. **2012**, *133*, 1292-8, https://doi.org/10.1016/j.foodchem.2011.08.002.
65. Alara, O. R.; Abdurahman, N. H.; Olalere, O. A. Optimization of microwave-assisted extraction of flavonoids and antioxidants from *Vernonia amygdalina* leaf using response surface methodology. *Food Bioprod. Process*. **2018**, *107*, 36-48, https://doi.org/10.1016/j.fbp.2017.10.007.
66. Lee, J. C.; Kim, H. R.; Kim, J.; Jang, Y. S. Antioxidant property of an ethanol extract of the stem of *Opuntia ficus-indica* var. saboten. J. Agri. *Food chem.* **2002**, *50*, 6490-6, https://doi.org/10.1021/jf020388c.
67. Alimi, H.; Hfaiedh, N.; Bouoni, Z.; Sakly, M.; Rhouma, K. B. Evaluation of antioxidant and antiulcerogenic activities of *Opuntia ficus indica f. inermis* flowers extract in rats. Environ. *Toxicol. Pharmacol.* **2011**, *32*, 406-16, https://doi.org/10.1016/j.etap.2011.08.007.
68. Chougui, N.; Tamendjari, A.; Hamidj, W.; Hallal, S.; Barras, A.; Richard, T.; Larbat, R. Oil composition and characterisation of phenolic compounds of *Opuntia ficus-indica* seeds. *Food Chem.* **2013**, *139*, 796-803, https://doi.org/10.1016/j.foodchem.2013.01.054.
69. Ivanov, I.; Vrancheva, R.; Marchev, A.; Petkova, N.; Aneva, I.; Denev, P.; Georgiev, V.; Pavlov, A. Antioxidant activities and phenolic compounds in Bulgarian Fumaria species. Int. J. Curr. Microbiol. *App. Sci*. **2014**, *3*, 296-306, http://www.ijcmas.com.
70. Farag, M. A.; Sallam, I. E.; Fekry, M. I.; Zaghloul, S. S.; El-Dine, R. S. Metabolite profiling of three *Opuntia ficus-indica* fruit cultivars using UPLC-QTOF-MS in relation to their antioxidant potential. *Food Biosci*. **2020**, *36*, 100673, https://doi.org/10.1016/j.fbio.2020.100673.
71. Fujii, J.; Homma, T.; Osaki, T. Superoxide Radicals in the Execution of Cell Death. *Antioxidants* **2022**, *11*, 501, https://doi.org/10.3390/antiox11030501.
72. Nathan, C. Neutrophils and immunity: challenges and opportunities. *Nat. Rev.Immunol.* **2006**, *6*, 173-182, https://www.nature.com/articles/nri1785. .

73. Paul, S.; Shin, H. S.; Kang, S. C. Inhibition of inflammations and macrophage activation by ginsenoside-Re isolated from Korean ginseng (*Panax ginseng* CA Meyer). *Food Chem. Toxicol*. **2012**, *50*, 1354-61, https://doi.org/10.1016/j.fct.2012.02.035.
74. Benayad, Z.; Martinez-Villaluenga, C.; Frias, J.; Gomez-Cordoves, C.; Es-Safi, N. E. Phenolic composition, antioxidant and anti-inflammatory activities of extracts from Moroccan *Opuntia ficus-indica* flowers obtained by different extraction methods. Ind. *Crop. Prod.* **2014**, *62*, 412-20, https://doi.org/10.1016/j.indcrop.2014.08.046.
75. Nair, M. P.; Mahajan, S.; Reynolds, J. L.; Aalinkeel, R.; Nair, H.; Schwartz, S. A.; Kandaswami, C. The flavonoid quercetin inhibits pro-inflammatory cytokine (tumor necrosis factor alpha) gene expression in normal peripheral blood mononuclear cells via modulation of the NF-κ*β* system. Clin. *Vaccine Immunol.* **2006**, *13*, 319-28, https://doi.org/10.1128/CVI.13.3.319-328.2006.
76. Ferraz, C. R.; Carvalho, T.T.; Manchope, M. F.; Artero, N. A.; Rasquel-Oliveira, F.S.; Fattori, V.; Casagrande, R.; Verri, Jr, W .A. Therapeutic potential of flavonoids in pain and inflammation: mechanisms of action, pre-clinical and clinical data, and pharmaceutical development. *Molecules*. **2020**, *25*, 762, https://doi.org/10.3390/molecules25030762.
77. Allegra, M.; d'Acquisto, F.; Tesoriere, L.; Livrea, M. A.; Perretti, M. Cross-talk between minimally primed HL-60 cells and resting HUVEC reveals a crucial role for adhesion over extracellularly released oxidants, Biochem. *Pharmacol*. **2011**, *81*, 396-401, https://doi.org/10.1016/j.bcp.2010.10.018.
78. Attanzio, A.; Diana, P.; Barraja, P.; Carbone, A.; Spanò, V.; Parrino, B.; Cascioferro, S. M.; Allegra, M.; Cirrincione, G.; Tesoriere, L.; Montalbano, A. Quality, functional and sensory evaluation of pasta fortified with extracts from *Opuntia ficus-indica* cladodes. *J. Sci. Food Agric.* **2019**, *99*, 4242-7, https://doi.org/10.1002/jsfa.9655.
79. Alonzi, T.; Maritano, D.; Gorgoni, B.; Rizzuto, G.; Libert, C.; Poli, V. Essential role of STAT3 in the control of the acute-phase response as revealed by inducible gene activation in the liver. *Mol. Cell. Biol.* **2001**, *21*, 1621-32, https://doi.org/10.1128/MCB.21.5.1621-1632.2001.
80. Gonzalez-Gallego, J.; Sánchez-Campos, S.; Tunon, M. J. Anti-inflammatory properties of dietary flavonoids. *Nutr. Hosp*. **2007**, *22*, 287-93, https://pubmed.ncbi.nlm.nih.gov/17612370/.
81. Faki, Y.; Er, A. Different Chemical Structures and Physiological/Pathological Roles of Cyclooxygenases. *Rambam Maimonides Med. J*. **2021**, *12*, e0003, https://doi.org/10.5041/RMMJ.10426.
82. Ju, Z.; Li, M.; Xu, J.; Howell, D. C.; Li, Z.; Chen, F. E. Recent development on COX-2 inhibitors as promising anti-inflammatory agents: The past 10 years. *Acta Pharm. Sin. B* . **2022**, *12*, 2790-2807, https://doi.org/10.1016/j.apsb.2022.01.002.
83. Orhan, D. D.; Hartevioğlu, A.; Küpeli, E.; Yesilada, E. *In vivo* anti-inflammatory and antinociceptive activity of the crude extract and fractions from *Rosa canina* L. fruits. *J. Ethnopharmacol*. **2007**, *112*, 394-400, https://doi.org/10.1016/j.jep.2007.03.029.
84. D'Mello, P.; Gadhwal, M. K.; Joshi, U.; Shetgiri, P. Modeling of COX-2 inhibitory activity of flavonoids. *Int. J. Pharm.Pharm. Sci*. **2011**, *3*, 33-40.
85. Alcaraz, M. J.; Ferrandiz, M. L. Modification of arachidonic metabolism by flavonoids. *J. Ethnopharmacol.* **1987**, *21*, 209-29, https://doi.org/10.1016/0378-8741(87)90101-2.
86. Slika, H.; Mansour, H.; Wehbe, N.; Nasser, S. A.; Iratni, R.; Nasrallah, G.; Shaito, A.; Ghaddar, T.; Kobeissy, F.; Eid, A.H. *Therapeutic potential of flavonoids in cancer: ROS-mediated mechanisms. Biomed. Pharmacother*. **2022**, *146*, 112442, https://doi.org/10.1016/j.biopha.2021.112442.
87. Al-Khayri, J. M.; Sahana, G. R.; Nagella, P.; Joseph, B. V.; Alessa, F. M.; Al-Mssallem, M. Q. Flavonoids as Potential Anti-inflammatory Molecules: A Review. *Molecules.* **2022**, *27*, 9, 2901, https://doi.org/10.3390/molecules27092901.

Supplementary materials

Table S1. Acute toxicity of EXM1 extract.

Dose (g / kg b.wt.)	Mice number	Dead mice number	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	1	2	1	2
3	6	0	0.5	1	0.5
2	6	0	0	1	0
1	6	0	0	1	0

LD_{50} of EXM1= 4g/kg b. wt.

Table S2. Acute toxicity of EXC1 extract.

Dose (g / kg b.wt.)	Mice number	Dead mice number	Z	d	(Z)x(d)
7	6	6	6	1	6
6	6	6	6	1	6
5	6	6	6	1	6
4	6	3	4.5	1	4.5
3	6	1	2	1	2
2	6	0	0.5	1	0.5
1	6	0	0	1	0

LD_{50} of EXC1= 0.8g/kg b. wt.

Table S3. Acute toxicity of EXM2 extract.

Dose (g / kg b.wt.)	Mice number	Dead mice number	Z	D	(Z)x(d)
7	6	6	6	1	6
6	6	6	6	1	6
5	6	6	6	1	6
4	6	2	4	1	4
3	6	0	1	1	1
2	6	0	0	1	0
1	6	0	0	1	0

LD_{50} of EXM2= 120g/kg b. wt.

Table S4. Acute toxicity of EXC2 extract.

Dose (g / kg b.wt.)	Mice number	Dead mice number	Z	D	(Z)x(d)
7	6	6	6	1	6
6	6	6	6	1	6
5	6	6	6	1	6
4	6	2	4	1	4
3	6	0	1	1	1
2	6	0	0	1	0
1	6	0	0	1	0

LD_{50} of EXC2= 120g/kg b. wt.