



Bioactivities, physicochemical parameters and GC/MS profiling of the fixed oil of *Cucumis melo* L seeds: A focus on anti-inflammatory, immunomodulatory, and antimicrobial activities

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ABSTRACT

Introduction: Recently, the recovery of waste products from plants as a source of biologically active compounds has increased interest. Therefore, the current research aims to evaluate the anti-inflammatory, immunomodulatory and antimicrobial activities of the fixed oil of *Cucumis melo* L seeds, as well as to investigate its physicochemical parameters and chemical composition.

Methods: Anti-inflammatory activity was examined using carrageenan-induced rat paw edema assay. The antimicrobial activity was assayed against *Staphylococcus aureus*, *Micrococcus luteus*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Candida albicans* by well diffusion method. The chemical composition of the oil was determined by gas chromatography/mass spectrometry (GC/MS), α -tocopherol was estimated by high-performance liquid chromatography (HPLC).

Results: *Cucumis melo* oil had no toxicity and possessed a promising anti-inflammatory activity. Moreover, the oil exhibited a reasonable decrease in the pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α), and a significant increase in the anti-inflammatory cytokine (IL-10). The oil exhibited a reasonable antimicrobial activity against all tested organisms. The major identified compound in the unsaponifiable matter was (1-methyldodecyl) benzene (8.76%), while the major fatty acid was methyl linoleate (14.10%). The results of physicochemical characterization revealed the better quality of *Cucumis melo* oil. The amount of α -tocopherol in the oil was 23.5 μ g/mL, which is considered a reasonable amount.

Conclusion: These findings indicate that the fixed oil of *Cucumis melo* L seeds might be used as a safe natural anti-inflammatory, immunomodulatory and antimicrobial agent.

Implication for health policy/practice/research/medical education:

The current study suggests that *Cucumis melo* oil might be exploited in the food industry and used as a potent anti-inflammatory, immunomodulatory and antimicrobial agent.

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Introduction

Inflammation is a defensive response that intends to eradicate the source of cell injury and remove/ repair the damaged tissue. One of the most common signs of inflammation is the release of numerous mediators; serotonin, histamine, prostaglandin, leukotrienes, and

cytokines (1). These mediators are classified into two common types, which are pro-inflammatory and anti-inflammatory mediators. The most widely investigated inflammatory mediators are cytokines such as tumor necrosis factor- α (TNF- α) and interleukins (IL) (1). Pro-inflammatory cytokines, TNF- α and IL-6, are linked

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to immune and inflammatory disorders produced by different cells, resulting in several cellular changes (2). Conversely, IL-10 is an anti-inflammatory cytokine that plays a vital role in constraining the host's immune response to pathogens, protecting the tissue from damage, and maintaining normal tissue homeostasis (2).

Since the discovery of antibiotics, the medical community has believed that infectious diseases will be eliminated. However, infection diseases are resurfacing in emergent resistant forms to antibiotic treatments (3). These resistant microbes cause epidemics that are considered a common global problem affecting public health (3). So, discovering new antimicrobial agents from natural sources is necessary as an alternative to overcome the resistance to synthetic drugs (4).

Medicinal plants play an essential role in human and animal health care due to diverse biologically active compounds (5). Most people prefer plant-based medications over synthetic ones because they are more readily available, have low side effects, and are easier to administer (6).

Cucumis melo L., commonly known as cantaloupe, belongs to the Cucurbitaceae family. The fruits of *Cucumis melo* L. have been used as a food for decades, while its seeds have been regarded as a waste product (7). In recent years, seeds are a rich source of oils and biologically active compounds such as; carotenoids, phytosterols, and fatty acids (8). Cantaloupe seeds were discovered to possess high levels of carbohydrates, fibers, proteins, and essential amino acids such as leucine, isoleucine, and phenylalanine (7). *Cucumis melo* L. has been reported to exhibit antioxidant, cytotoxic (9,10), antimicrobial (5), anti-hyperlipidemic (11), diuretic, nephroprotective (12), anti-inflammatory, and anti-ulcer activities (13,14). *Cucumis melo* is rich in biologically active compounds that play a vital role in plant bioactivities (7,8).

The current research tends to evaluate the anti-inflammatory, immunomodulatory and antimicrobial properties of the fixed oil of *Cucumis melo* L. seeds, as well as its physicochemical parameters and chemical composition. This trial highlights the importance of adequately treating fruit by-products for use in the food and drug industries and properly disposing of waste to reduce environmental pollution.

Materials and Methods

Plant material

Cucumis melo fruits were acquired at the commercial ripening stage from a local market. The seeds were separated from the fruits and rinsed well under running water, air-dried for three days, and ground into a fine powder.

Preparation of the lipoidal matter

Using the maceration process, the dried seeds of *Cucumis melo* (200 g) were thoroughly extracted with

light petroleum (60–80°C). The petroleum ether residue was obtained by evaporating the extract under reduced pressure.

Investigation of the lipoidal matter

Saponification of the petroleum ether extract

The petroleum ether extract (3.4 g) was saponified by refluxing with 10% alcoholic potassium hydroxide. The solvent was evaporated, and the extract was diluted with water; the unsaponifiable matter was extracted with ether. The ether extract was evaporated, weighed, and kept for further investigation (15).

Preparation of fatty acid methyl esters

The methylation of free fatty acids was performed according to the method described by Liu (16). Briefly, the aqueous mother liquor was acidified with 10% HCl, and the liberated fatty acids were extracted with ether. After evaporation of the solvent, the residue was weighed and kept for studying the total fatty acids. The methylation of free fatty acids was performed by refluxing for 2 hours with absolute methanol (50 mL) and sulphuric acid (2 mL). The methylated fatty acids were extracted with diethyl ether.

GC/MS analysis

Gas chromatography/mass spectrometry (GC/MS) analysis was applied on an Agilent 6890 gas chromatograph conjugated with an Agilent mass spectrometric detector to determine the contents of both unsaponifiable and saponifiable matters. The identification of the compounds was based on comparing their mass spectral fragmentation patterns with those reported in database libraries, NIST (National Institute of Standards and Technology, Colorado, USA), Wiley (Wiley International, Colorado, USA) and/or published data (17), while the quantitative determination was based on the integration of peak area.

The physicochemical characteristics of *Cucumis melo* oil

The physicochemical qualities determine the suitability of oils. The physicochemical parameters of the oil, including the color, odor, as well as acid, saponification, iodine, and peroxide values, were examined as previously described (18), while the ester value was considered by subtracting the acid value from the saponification value.

Estimation of α -tocopherol, a fat-soluble vitamin, in *Cucumis melo* oil

Alpha-tocopherol in the oil of *Cucumis melo* was quantitatively estimated using high-performance liquid chromatography-ultraviolet (HPLC-UV) technique that applied on Agilent 1100 chromatographic system equipped with a fluorescence detector (emission 325 nm, excitation 292 nm) as reported by (19). The identification and estimation of α -tocopherol were based on comparing the retention time and peak area with standard α -tocopherol.

Biological studies

Animals

Adult male Wistar rats (weight 200–250 g), aged from 10 to 16 weeks as well as six weeks old mice of either sex (weight 20–25 g), were selected from the animal house of National Research Center (NRC), Giza, Egypt and maintained under standard laboratory conditions at NRC. Animals were fed with basal diet pellets, supplied with water ad libitum, and kept in a temperature-controlled environment (20–22°C).

Acute toxicity study

The acute toxicity of the extract was assayed using the up and down procedure as recommended by the Organization for Economic Cooperation and Development (OECD), recommendation no. 423, 2001 (20). Mice of either sex (five females and five males, weight 20–25 g) were given a dose of 2 g/kg extract orally. For the first four hours after treatment, the animals were observed for toxic signs. Any abnormal variations regarding eyes, salivation, lacrimation, abdominal cramps, discoloration, skin ulcers, diarrhea, hair loss, neurological behavior, lethargy, hyper/hypoactivity and/or deaths were recorded (21). Finally, after 24 hours, the number of survivors was recorded and maintained for daily observations for another 13 days.

Acute anti-inflammatory and immunomodulatory study

Eighteen male Wistar rats were divided into three groups: Group I received saline; Group II received indomethacin (oral dose of 10 mg/kg body weight; p.o.); Group III received orally 200 mg/kg of oil of *Cucumis melo* seeds. The anti-inflammatory activity was examined using carrageenan-induced rat paw edema assay (22,23). Edema was induced in all rats by sub-plantar injection of 100 µL of 1% freshly prepared solution of carrageenan in distilled water into the right hind paws of each rat. Paw thickness was measured using vernier caliper before and after 1, 2, 3, 4, and 24 hours after carrageenan injection and compared to its baseline. Blood samples were collected after light anesthesia using diethyl ether. Blood samples were then centrifuged for 15 minutes. Sera were then harvested and stored at –20°C for further biochemical assessment. The immunomodulatory activity was evaluated by determination of inflammatory cytokines levels (IL-6, IL-10, and TNF- α) using ELISA kits (IBL America, Minneapolis, MN; DRG International Inc., NJ; KOMA BIOTECH Inc., Korea; respectively) in picogram per milliliter (pg/mL) using ELISA Reader (LisaPlus, Germany).

Statistical analysis

All data are presented as mean \pm SE. GraphPad Prism was used for the statistical analysis. Group variances were analyzed using the one-way analysis of variance (ANOVA) method followed by the Tukey-Kramer test for multiple comparisons.

Methods for antimicrobial activity

Sample preparation

A stock solution was prepared by dissolving the plant extract in DMSO to give a 150.0 mg/mL concentration.

Tested organisms

The microorganisms used in the *in vitro* antimicrobial study were as follows:

1. Gram-positive bacteria: *Micrococcus luteus* (ATCC 10240), *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 29212), and *Staphylococcus epidermidis* (ATCC 12228).
2. Gram-negative bacteria: *Pseudomonas aeruginosa* (ATCC 27853).
3. Pathogenic yeast: *Candida albicans* (ATCC 10231).

Antimicrobial assay

Qualitative evaluations were executed in nutrient agar plates according to a previous study (24). The inoculation of all microorganisms was prepared from fresh overnight broth cultures using Brain Heart infusion Broth medium incubated at 37°C (24). The inoculum size of these pathogenic strains was prepared and adjusted to approximately 0.5 McFarland standard (1.5×10^8 CFU/mL); 25.0 µL of both bacterial and yeast inocula were inoculated into each plate containing 20.0 mL of the sterile nutrient agar medium (NA). After the media cooled and solidified, 100.0 µL of the prepared samples were separately applied on the 0.9 cm well of that inoculated agar plates prepared previously by using 1.0 cm cork borer applying Well Diffusion Method. These inoculated plates were placed in the refrigerator for one hour, followed by incubation at 37°C for 24 hours, and zones of inhibition (ZI) were measured in mm (24). The sample was passed to another test for determination of minimum inhibitory and bactericidal concentrations values (MIC, MBC, respectively), using Tryptic Soy Broth medium (24).

Results

The oil yield of *Cucumis melo* seeds

The extraction of *Cucumis melo* seeds with petroleum ether presented a yield of 60 g, representing 30% of the dried part. Moreover, the results revealed that the unsaponifiable matter and the total fatty acid constituted 61.54% and 38.01%, respectively. As a result, the oil's profile of physicochemical characteristics should be studied.

The physicochemical analysis of *Cucumis melo* oil

Table 1 displays the physicochemical characterizations of *Cucumis melo* oil. The appearance of *Cucumis melo* oil was yellow to golden yellow with a pleasant odor. The extraction of *Cucumis melo* seeds with petroleum ether presented a yield of 60 g, representing 30% of the dried part. The results of physicochemical characterization revealed that acid, peroxide, saponification, iodine, and

Table 1. The physicochemical characterizations of *Cucumis melo* oil

Test	Unit	Results
Oil yield	%	60
Color	-	Yellow to golden yellow
Odor	-	Pleasant
Acid value	mg/g	4.59
Peroxide value	mEq/kg	5.58
Iodine value	g/100 g	63.39
Saponification value	mg/g	39.47
Ester value	mg/g	34.88

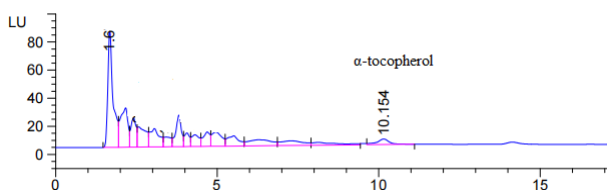
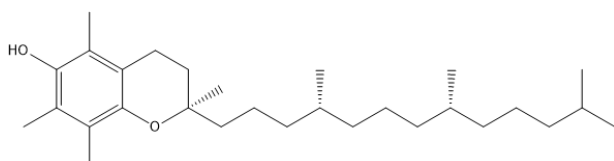
ester values were 4.59 mg/g, 5.58 mEq/kg, 39.47 mg/g, 63.39 g/100 g, and 34.88 mg/g, respectively. These results showed better quality of *Cucumis melo* oil.

α -Tocopherol determination

The estimation of α -tocopherol in the oil of *Cucumis melo* was determined quantitatively using the HPLC-UV technique by a comparison of retention time and area with that of standard α -tocopherol. The amount of α -tocopherol in the oil of *Cucumis melo* was 23.5 μ g/mL, which should be considered a reasonable amount (Figure 1). The structure of α -tocopherol is illustrated in Figure 2.

GC/MS analysis of *Cucumis melo* oil

GC/MS analysis of the unsaponifiable matter of *Cucumis melo* oil revealed the identification of thirty-four compounds representing 92.64% of the total composition. 1-Methyldodecyl benzene and hexadecane were presented as the major compounds, constituted 8.76% and 6.24%, respectively. The results showed that the identified components consisted of 71.5% unoxxygenated compounds and 21.14% oxygenated compounds (Table 2). On the other hand, thirteen compounds were identified from the saponifiable matter representing 90.07% of the total composition (Table 3). The unsaturated fatty acids

**Figure 1.** HPLC analysis of α -tocopherol in the oil of *Cucumis melo* seeds.**Figure 2.** α -Tocopherol structure.

constituted the major percentage (75.32%), while the saturated fatty acids represented 14.75%. The major fatty acids were 9,12-octadecadienoic acid methyl ester (methyl linoleate) (14.10%), 9,15-octadecadienoic acid methyl ester (12.38%), and dimethyl 3-methyloctanedioate (11.26%).

Acute toxicity study

The acute toxicity of the oil of *Cucumis melo* seeds was studied, and the results revealed that *Cucumis melo* oil was nontoxic up to the dose of 2 g/kg, as there were no general behavior changes, toxicity, or mortality between the tested animals indicating the safety of the oil under investigation.

Acute anti-inflammatory study

Following carrageenan injection, an inflammatory response was observed in the rat hind paw. The oil of *Cucumis melo* seeds inhibited the edema by 16.28% after 4 hours, while indomethacin, the standard anti-inflammatory drug, reduced the edema by 55.92% (Table 4).

Immunomodulatory study

The anti-inflammatory response was confirmed by immunomodulatory study via assessing the pro-inflammatory cytokines (TNF- α and IL-6) and the anti-inflammatory cytokine (IL-10). The oil of *Cucumis melo* seeds showed a reasonable decrease in the pro-inflammatory cytokines; TNF- α and IL-6 (Figures 3 & 4, respectively) and a reasonable increase in the anti-inflammatory cytokine; IL-10 (Figure 5) compared to group I (control group). These results specify that *Cucumis melo* oil can be considered an active anti-inflammatory and immunomodulatory agent.

Antimicrobial activity

Petroleum ether extract of *Cucumis melo* seeds was screened for its *in vitro* antimicrobial activity against *Staphylococcus aureus*, *Micrococcus luteus*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Candida albicans* compared with levofloxacin, erythromycin, and gentamicin as standard antibiotics and Miconaz cream 20 g as a fungicidal and bactericidal drug. The *Cucumis melo* oil exhibited a reasonable antimicrobial activity against all tested organisms. The extract showed the highest susceptibility against *Micrococcus luteus* with a maximum inhibition zone of 22 mm, while the least susceptibility was shown against *Staphylococcus aureus* with a zone of 11 mm. Moreover, the extract exhibited antimicrobial activity with similar inhibition zone (16 mm) against *Enterococcus faecalis*, *Staphylococcus epidermidis*, and *Candida albicans*, while it showed a reasonable antimicrobial activity with an inhibition zone of 13 mm against *Pseudomonas aeruginosa* (Figure 6).

Figure 7 illustrates the minimum inhibitory

Table 2. The identified compounds in unsaponifiable matter of *Cucumis melo* oil by GC/MS

Compounds	RR _t	Area%	BP	Molecular weight	Molecular formula
Dodecane	0.39	1.57	57	170	C ₁₂ H ₂₆
Tridecane	0.49	2.52	57	184	C ₁₃ H ₂₈
3-Tetradecene	0.67	1.09	55	196	C ₁₄ H ₂₈
2,4-bis(1,1-dimethylethyl) phenol	0.78	1.03	191	206	C ₁₄ H ₂₂ O
Pygmaein	0.80	1.75	91	194	C ₁₁ H ₁₄ O ₃
Thujopsanol	0.82	1.71	91	222	C ₁₅ H ₂₆ O
1-Hexadecanol	0.84	1.74	55	242	C ₁₆ H ₃₄ O
Hexadecane	0.85	6.24	57	226	C₁₆H₃₄
1-butylheptyl benzene	0.87	1.37	91	232	C ₁₇ H ₂₈
1-propyloctyl benzene	0.88	4.78	91	232	C ₁₇ H ₂₈
Benzene, (1-ethylnonyl)	0.90	3.99	91	232	C ₁₇ H ₂₈
Heptadecane	0.92	3.62	57	240	C ₁₇ H ₃₆
1-pentylheptyl benzene	0.94	4.95	91	246	C ₁₈ H ₃₀
1-butylloctyl benzene	0.95	4.60	91	246	C ₁₈ H ₃₀
1-propylnonyl benzene	0.96	3.60	91	246	C ₁₈ H ₃₀
1-ethyldecyl benzene	0.97	3.57	91	246	C ₁₈ H ₃₀
1-Heptadecanol	0.98	1.44	55	256	C ₁₇ H ₃₆ O
2-Methyl 1-Hexadecanol	0.99	2.56	57	256	C ₁₇ H ₃₆ O
(1-methyldodecyl) benzene	1	8.76	105	260	C₁₉H₃₂
1-butynonylbenzene	1.02	2.65	91	260	C ₁₉ H ₃₂
1-Propyldecylbenzene	1.03	1.91	91	260	C ₁₉ H ₃₂
1-hexylhepty benzene	1.05	2.05	91	260	C ₁₉ H ₃₂
3-(Prop-2-enoyloxy)tridecane	1.06	4.25	55	254	C ₁₆ H ₃₀ O ₂
Dodecanoic acid, hex-3-enyl ester	1.17	0.92	82	282	C ₁₈ H ₃₄ O ₂
Nonadecane	1.18	2.46	57	268	C ₁₉ H ₄₀
Octadecene	1.20	1.82	55	252	C ₁₈ H ₃₆
1-Nonadecene	1.22	1.96	43	266	C ₁₉ H ₃₈
Isophytol	1.24	1.60	71	296	C ₂₀ H ₄₀ O
1-Heneicosene	1.25	1.57	83	294	C ₂₁ H ₄₂
Phytol	1.27	1.54	71	296	C ₂₀ H ₄₀ O
Stigmasterol	1.28	2.60	55	412	C ₂₉ H ₄₈ O
n-Tricosane	1.29	2.84	57	324	C ₂₃ H ₄₈
n-Pentacosane	1.31	1.59	57	352	C ₂₅ H ₅₂
1-Hexacosene	1.34	1.99	97	364	C ₂₆ H ₅₂
Total identified compounds		92.64%			

RRt: Relative Retention time in minute, BP: Base peak.

Table 3. The identified compounds in the saponifiable matter of *Cucumis melo* oil by GC/MS

Compound	RRT	Area %	BP	Molecular weight	Molecular formula
Dimethyl 3-methyloctanedioate	0.45	11.26	55	216	C ₁₁ H ₂₀ O ₄
9-Tetradecenoic acid methyl ester (Methyl myristoleate)	0.80	7.86	55	240	C ₁₅ H ₂₈ O ₂
9,15-octadecadienoic acid methyl ester (Methyl 9, 15-linoleate)	0.85	12.38	41	294	C₁₉H₃₄O₂
9,12-octadecadienoic acid methyl ester (Methyl linoleate)	1	14.10	67	294	C₁₉H₃₄O₂
9-Octadecenoic acid methyl ester (Methyl oleate)	1.21	4.86	55	296	C ₁₉ H ₃₆ O ₂
11-Nonadecenoic acid methyl ester	1.23	5.89	55	310	C ₂₀ H ₃₈ O ₂
5-Eicosenoic acid methyl ester	1.37	6.03	55	338	C ₂₂ H ₄₂ O ₂
11-Docosenoic acid methyl ester	1.74	5.65	55	352	C ₂₃ H ₄₄ O ₂
15-Tetracosenoic acid methyl ester	1.84	5.26	55	380	C ₂₅ H ₄₈ O ₂
16-Pentacosenoic acid methyl ester	1.94	3.27	55	394	C ₂₆ H ₅₀ O ₂
19-Octacosenoic acid methyl ester	1.95	4.54	55	436	C ₂₉ H ₅₆ O ₂
9-Methyl 2-phenyl 1,3-dioxolan-4-yl-octadecenoate	1.97	5.48	43	444	C ₂₈ H ₄₄ O ₄
2-(Tetradecyloxy) ethyl palmitate	2.34	3.49	43	496	C ₃₂ H ₆₄ O ₃

Saturated fatty acids, 14.75%; Unsaturated fatty acids, 75.32%; Total identified fatty acids, 90.07%.

RRt: Relative Retention time in minute, BP: Base peak.

Table 4. Effect of *Cucumis melo* oil on the paw thickness in carrageenan-induced rat paw edema

Groups	Edema volume (% change from baseline)									
	1 st hour	Inhibition%	2 nd hour	Inhibition%	3 rd hour	Inhibition%	4 th hour	Inhibition%	24 hours	Inhibition%
Control	94.00±10.06	--	136.75±8.2	--	154.41±9.26	--	157.02±7.07	--	110.32±5.15	--
IND (10 mg/kg)	70.12±5.23*	25.40	88.04±10.48*	35.61	66.16±5.33*	57.14	69.21±10.44*	55.92	37.06±2.61*	66.40
OCM (200 mg/kg)	96.69±5.9	-2.86	131.38±5.05*	3.92	150.16±7.53	2.74	131.45±8.18*	16.28	61.99±4.73*	43.80

OCM: Oil of *Cucumis melo* seed; IND: Indomethacin.

Data are presented as mean ± SEM. * Significantly different from normal control group.

concentration (MIC) and minimal bactericidal concentration (MBC) values of *Cucumis melo* oil. The maximum MIC value was against *Pseudomonas aeruginosa* (150 mg/mL), while the extract revealed the least MIC value against *Micrococcus luteus* (90 mg/mL). Moreover, *Cucumis melo* oil showed similar MIC value (100 mg/mL) against *Staphylococcus epidermidis*, *Enterococcus faecalis*, and *Candida albicans*, while it showed MIC value of 120 mm against *Staphylococcus aureus*. On the other hand, the extract showed the most negligible MBC value against *Micrococcus luteus* (120 mg/mL), while the oil showed a similar MBC value (150 mg/mL) against the other tested organisms.

Discussion

The extraction of *Cucumis melo* seeds with petroleum ether presented a yield of 60 g, representing 30% of the dried part. This significant amount of oil may be considered economically significant, making *Cucumis melo* seeds suitable for application in different fields of the oil industry.

The acid value, peroxide value, ester value, saponification value, and iodine value are the quality parameters used to characterize edible oils. The acid value is used as an indicator for the edibility of an oil. The acidity of oil is dependent on the amount of free fatty acids. The level of free fatty acid should be low in the oils recommended for human dietary purpose. As an edible oil, palm oil has been shown to have an acid value of 19.3 mg/g (25). The oil of *Cucumis melo* was found to have an acid value of 4.59 mg/g, indicating its edibility. Peroxide value is an indicator of the deterioration of oils (26). The peroxide value of *Cucumis melo* oil was determined to be 5.58 milliequivalent/kg, and this reduced value implies that the oil can be stored without becoming rancid for an extended amount of time. The edible oils and fat were reported to have peroxide values ranging from 0.9 to 15.9 mEq/kg (26). The current results showed low acidity and peroxide values, indicating better quality of *Cucumis melo* oil. Saponification value plays a significant role in soap production, and it indicates the average molecular weight or chain length of all the fatty acids present (27). The saponification value was determined to be 39.47

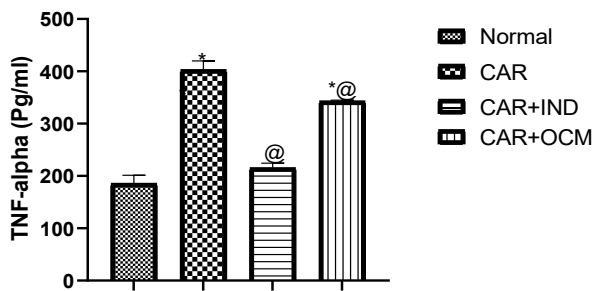


Figure 3. Effect of the oil of *Cucumis melo* L. seeds (OCM) on the serum level tumor necrosis factor alpha in carrageenan-induced inflammation. Data are presented as mean ± SEM. *Significantly different from normal control group, @Significantly different from inflamed group at P<0.05; IND: Indomethacin; CAR: carrageenan; OCM: Oil of *Cucumis melo* seed.

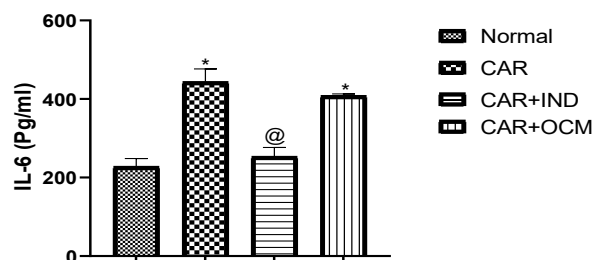


Figure 4. Effect of the oil of *Cucumis melo* L. seeds (OCM) on the serum level of interleukin -6 in carrageenan-induced inflammation. Data are presented as mean ± SEM. *Significantly different from normal control group, @Significantly different from inflamed group at P<0.05; IND: Indomethacin; CAR: carrageenan; OCM: Oil of *Cucumis melo* seed.

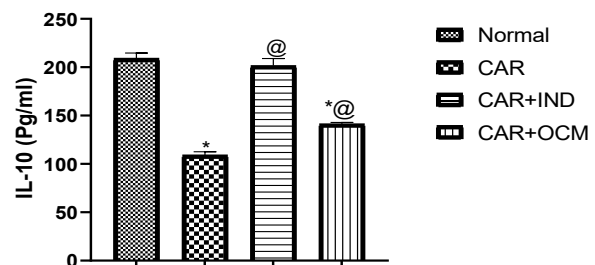


Figure 5. Effect of the oil of *Cucumis melo* L. seeds (OCM) on the serum level of interleukin-10 in carrageenan-induced inflammation. Data are presented as mean ± SEM. *Significantly different from the normal control group, @Significantly different from inflamed group at P<0.05; IND: Indomethacin; CAR: carrageenan; OCM: Oil of *Cucumis melo* seed.

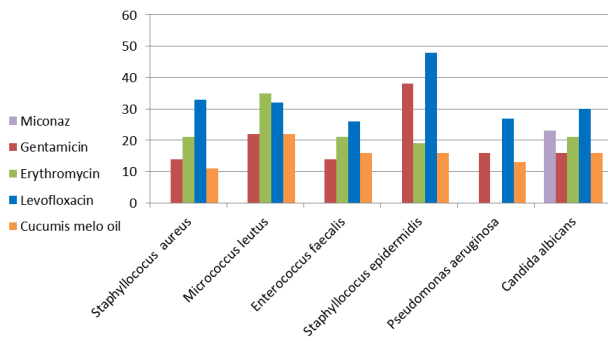


Figure 6. The antimicrobial activity of *Cucumis melo* oil, the antibiotic standards, and commercial antifungal (Miconaz cream) applying well diffusion method by inhibition zone diameter determination (millimeter).

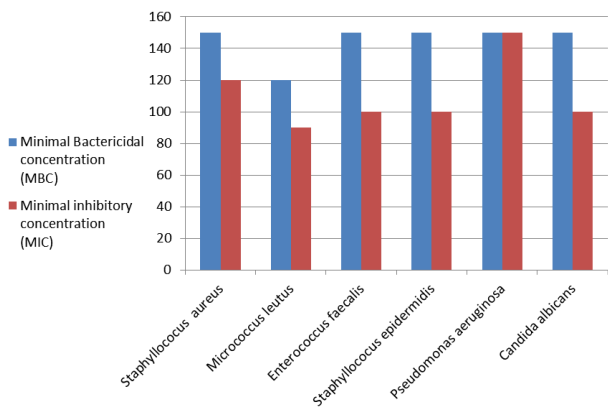


Figure 7. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of *Cucumis melo* oil (mg/mL).

mg/g. This high value indicates that *Cucumis melo* oil has a soap-making potential. Ester value is obtained as the difference between the saponification value and the acid value. The ester value of *Cucumis melo* oil was 34.88 mg/g; this low value indicates high durability (26). The iodine value determined the degree of unsaturation of oils and fats. The oil of *Cucumis melo* was found to have a high iodine value (63.39 g/100 g) due to its high content of unsaturated fatty acids, indicating good edible and drying qualities of *Cucumis melo* oil (28).

Vitamin E is a group of tocotrienols and tocopherols, among which α -tocopherol is believed to be the most biologically active form of vitamin E (29). α -Tocopherol is a fat-soluble vitamin that exhibited a potent antioxidant activity (30). Vitamin E has been suggested to inhibit microbial adhesion on implant surfaces by influencing microbial adhesion and modifying the surface of the substratum (30). The concentration of tocopherols in the seed oil is significantly affected by the method of extraction (31). Thus, the amounts of α -tocopherol (23.5 μ g/mL) were significantly higher than that was reported by Azhari et al (2.70 mg/100 g oil) (32), while it was in agreement with what was reported by da Silva and Jorge

(21.97 mg/kg) (33). Moreover, it was lower than what was reported by Rabadán et al (37.42 mg/kg) for the oil of *Cucumis melo* seeds (8).

Free radicals can cause inflammation by inhibiting anti-inflammatory cytokine, IL-10, and stimulating the production of pro-inflammatory cytokines, including TNF- α and IL-6 (34). The carrageenan-induced rat paw edema assay has been widely used to evaluate the anti-inflammatory effect of drugs. Carrageenan can cause releasing of TNF- α , bradykinin, histamine, leukotrienes, prostaglandins, and other pro-inflammatory and inflammatory mediators (35).

An inflammatory reaction was observed after the injection of carrageenan into the rat hind paw, which has a biphasic action, regulating the action of numerous mediators and promoting edema (36). The initial inflammatory response to carrageenan (0-1 h) is caused by the release of bradykinin, serotonin, and histamine (36, 37). A second accelerated period of swelling (2-4 h) is associated with elevated prostaglandin activity (37).

The results revealed that the medicinal importance of *Cucumis melo* oil as an anti-inflammatory agent is mainly due to reduction of pro-inflammatory cytokines (TNF- α , IL-6) and production and stimulate of anti-inflammatory cytokine, IL-10.

Cucumis melo has been used traditionally for anti-inflammatory purposes (38). Vouldoukis et al have indicated the SOD scavenging activity of the *Cucumis melo* extract as an antioxidant thus promotes anti-inflammatory properties (39). A recent study demonstrated that methanol and petroleum ether extracts of *Cucumis melo* fruit exhibited edema inhibition of about 54.97% and 63.13%, respectively, after 4 hours (13). Another study revealed that the ethanol extract of *Cucumis melo* fruits had a potent anti-inflammatory activity higher than that of the leaves (14). The current study, in addition to previous findings, further supported the traditional use of *Cucumis melo* as an anti-inflammatory drug. This activity could be due to the presence of a wide range of phytoconstituents in petroleum ether extract of *Cucumis melo* seeds such as terpenoids, sesquiterpenes, oxygenated and unoxxygenated hydrocarbons, as well as unsaturated and saturated fatty acids which have been reported to exhibit anti-inflammatory activity (40). In addition, linoleic acid, the major fatty acid in the oil of *Cucumis melo* seeds, is reported to possess potent anti-inflammatory and antimicrobial activities (41,42). Moreover, α -tocopherol has been shown a potent anti-inflammatory activity (43).

The results indicate that *Cucumis melo* oil has a broad-spectrum antimicrobial activity toward bacteria and fungi. This result was in agreement with a previous study, which showed that the aqueous seeds extract of *Cucumis melo* had potent antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus*

faecalis (44). In contrast, in another study aqueous and ethanolic extracts of *Cucumis melo* seeds did not exhibit any activity against *Staphylococcus aureus* (45). Another research stated that the aqueous extract of *Cucumis melo* fruits showed minimum antimicrobial activity against the *Candida albicans* (5), which is not in agreement with our results.

In the current study, GC/MS analysis of *Cucumis melo* oil revealed a diversity of compounds that could play a vital role as anti-microbial agents. Several saturated and unsaturated fatty acids have been reported to exhibit a reasonable antibacterial activity against Gram-positive and Gram-negative bacteria (46). Recently, a study documented the possible antibacterial mechanisms of fatty acids as inhibition of metabolic routes, disruption of the cytoplasmic membrane, inhibition of protein synthesis, cell wall, and DNA/RNA replication (47). Moreover, hexadecane, one of the major compounds in an unsaponifiable matter of *Cucumis melo* oil, was reported to display a potent antibacterial activity (48). Furthermore, alpha-tocopherol enhances the antimicrobial activity (49). As a result, *Cucumis melo* oil can be classified as a natural broad-spectrum antimicrobial agent that can replace synthetic antibiotics drugs after applying the clinical studies.

Conclusion

Based on the preceding findings, it can be concluded that the oil of *Cucumis melo* seeds possesses potential and promising anti-inflammatory, immunomodulatory and antimicrobial activities. Moreover, the results disclosed the better quality and safety of *Cucumis melo* oil. Thus, it could be exploited in the food industry and employed as a potent anti-inflammatory, immunomodulatory, and antimicrobial agent after applying further clinical studies.

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Authors' contributions

AAE and GFA suggested the point, designed the study, and performed all the phytochemical parts, GFA wrote the manuscript with the interpretation of the results, DOS performed and analyzed the acute toxicity, anti-inflammatory, and immunomodulatory studies, HME performed and analyzed the antimicrobial assay. All authors read and approved the final manuscript.

Conflict of interests

The authors have no conflict of interests to declare.

Ethical considerations

The animal experiments were conducted after approval

from the Ethics Committee of the National Research Centre (19-278) and following the ethical guidelines for investigations in laboratory animals and comply with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

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