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

Amira A. El-anssary1, Gehan Fawzy Abdel Raoof1∗, Dalia Osama Saleh2, Hossam Mohammed El-Masry3

1Pharmacognosy Department, Pharmaceutical and Drug Research Industries Division, National Research Centre, Dokki, P.O. 12622, Giza, Egypt
2Pharmacology Department, Medical Division, National Research Centre, Dokki, P.O. 12622, Giza, Egypt
3Chemistry of Natural Microbial Products Department, National Research Centre, Dokki, P.O. 12622, Giza, Egypt

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-alpha (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 µg/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.

Keywords: Cucumis melo seeds, Anti-inflammation, Immunomodulatory agent, Antimicrobial agent, Physicochemical parameters, α-Tocopherol

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.

Please cite this paper as: El-anssary AA, Abdel Raoof GF, Saleh DO, El-Masry HM. 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. J Herbmed Pharmacol. 2021;10(4):476-485. doi: 10.34172/jhp.2021.55.

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...
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


cellular and solid human tumors were evaluated using the one-way analysis of variance (ANOVA).

Cells were treated with the extracts for 48 hours and then incubated for an additional 24 hours. Cell viability was determined using the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay. The percentage of viable cells was calculated as the ratio of the absorbance of the treated cells to that of the untreated controls.

Statistical analysis

All data are presented as mean ± SE. GraphPad Prism was used for the statistical analysis. Variance analyses were analyzed using the one-way analysis of variance (ANOVA) method followed by the Tukey-Kramer test for multiple comparisons.
Table 1. The physicochemical characterization 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 µg/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, constituting 8.76% and 6.24%, respectively. The results showed that the identified components consisted of 71.5% unoxygenated 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 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  | 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_{30}      |
| 2,4-bis(1,1-dimethylethyl) phenol      | 0.78| 1.03   | 191 | 206              | C_{17}H_{36}O     |
| Pygmaein                               | 0.80| 1.75   | 91  | 194              | C_{19}H_{38}O_{2} |
| Thujopsanol                            | 0.82| 1.71   | 91  | 222              | C_{20}H_{40}O     |
| 1-Hexadecanol                          | 0.84| 1.74   | 55  | 242              | C_{20}H_{42}O     |
| Hexadecane                             | 0.85| 6.24   | 57  | 226              | C_{16}H_{32}      |
| 1-butylheptyl benzene                  | 0.87| 1.37   | 91  | 232              | C_{13}H_{26}      |
| 1-propyloctyl benzene                  | 0.88| 4.78   | 91  | 232              | C_{13}H_{26}      |
| Benzene, (1-ethylnonyl)                | 0.90| 3.99   | 91  | 232              | C_{13}H_{26}      |
| Heptadecane                            | 0.92| 3.62   | 57  | 240              | C_{17}H_{36}      |
| 1-pentyloctyl benzene                  | 0.94| 4.95   | 91  | 246              | C_{12}H_{26}      |
| 1-butyldecyl benzene                   | 0.95| 4.60   | 91  | 246              | C_{12}H_{26}      |
| 1-propylnonyl benzene                  | 0.96| 3.60   | 91  | 246              | C_{12}H_{26}      |
| 1-ethyldecybenzene                     | 0.97| 3.57   | 91  | 246              | C_{12}H_{26}      |
| 1-Heptadecan                           | 0.98| 1.44   | 55  | 256              | C_{16}H_{32}      |
| 2-Methyl 1-Hexadecan                   | 0.99| 2.56   | 57  | 256              | C_{16}H_{32}      |
| (1-methyldecy)benzene                  | 1   | 8.76   | 105 | 260              | C_{15}H_{30}      |
| 1-butylbenzene                         | 1.02| 2.65   | 91  | 260              | C_{12}H_{26}      |
| 1-Propylbenzene                        | 1.03| 1.91   | 91  | 260              | C_{12}H_{26}      |
| 1-heptylbenzene                        | 1.05| 2.05   | 91  | 260              | C_{12}H_{26}      |
| 3-(Prop-2-enoyloxy)tridecane           | 1.06| 4.25   | 55  | 254              | C_{12}H_{26}O     |
| Dodecanoic acid, hex-3-enyl ester      | 1.17| 0.92   | 82  | 282              | C_{12}H_{26}O     |
| Nonadecane                             | 1.18| 2.46   | 57  | 268              | C_{19}H_{38}      |
| Octadecane                             | 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_{19}H_{38}O     |
| 1-Heneicosene                          | 1.25| 1.57   | 83  | 294              | C_{20}H_{42}      |
| Phytol                                 | 1.27| 1.54   | 71  | 296              | C_{20}H_{42}O     |
| Stigmasterol                           | 1.28| 2.60   | 55  | 412              | C_{20}H_{42}      |
| n-Tricosane                            | 1.29| 2.84   | 57  | 324              | C_{22}H_{46}      |
| n-Pentacosane                          | 1.31| 1.59   | 57  | 352              | C_{23}H_{48}      |
| 1-Hexacosene                           | 1.34| 1.99   | 97  | 364              | C_{24}H_{50}      |

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                                              | RR  | Area %  | BP  | Molecular weight | Molecular formula |
|-------------------------------------------------------|-----|---------|-----|------------------|-------------------|
| Dimethyl 3-methyloctanedioate                         | 0.45| 11.26   | 55  | 216              | C_{6}H_{14}O      |
| 9-Tetradecenoic acid methyl ester (Methyl myristoleate)| 0.80| 7.86    | 55  | 240              | C_{11}H_{24}O     |
| 9,15-Octadecadienoic acid methyl ester (Methyl 9,15-linoleate) | 0.85| 12.38   | 41  | 294              | C_{19}H_{38}O     |
| 9,12-Octadecadienoic acid methyl ester (Methyl linoleate) | 1  | 14.10   | 67  | 294              | C_{18}H_{36}O     |
| 9-Octadecenoic acid methyl ester (Methyl oleate)      | 1.21| 4.86    | 55  | 296              | C_{18}H_{38}O     |
| 11-Nonadecenoic acid methyl ester                     | 1.23| 5.89    | 55  | 310              | C_{19}H_{40}O     |
| 5-Eicosenoic acid methyl ester                        | 1.37| 6.03    | 55  | 338              | C_{20}H_{42}O     |
| 11-Docosenoic acid methyl ester                       | 1.74| 5.65    | 55  | 352              | C_{19}H_{38}O     |
| 15-Tetracosenoic acid methyl ester                    | 1.84| 5.26    | 55  | 380              | C_{22}H_{46}O     |
| 16-Pentacosenoic acid methyl ester                    | 1.94| 3.27    | 55  | 394              | C_{21}H_{44}O     |
| 19-Octacosenoic acid methyl ester                     | 1.95| 4.54    | 55  | 436              | C_{22}H_{46}O     |
| 9-Methyl 2-phenyl 1,3-dioxolan-4-yl-octadecenoate     | 1.97| 5.48    | 43  | 444              | C_{22}H_{46}O     |
| 2-Tetradecyloxy ethyl palmitate                       | 2.34| 3.49    | 43  | 496              | C_{24}H_{50}O     |

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.
Bioactivities and GC/MS profiling of *Cucumis melo* oil

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

| Groups | 1st hour | Inhibition% | 2nd hour | Inhibition% | 3rd hour | Inhibition% | 4th hour | Inhibition% | 24 hours | Inhibition% |
|--------|----------|-------------|----------|-------------|----------|-------------|----------|-------------|----------|-------------|
| Control | 94.00±10.06 | -- | 136.75±18.2 | -- | 154.41±9.26 | -- | 157.02±7.07 | -- | 110.32±5.15 | -- |
| IND (10 mg/kg) | 70.12±5.23 | 35.61 | 66.40 | 57.14 | 61.99±4.73 | 3.92 | 131.38±5.05 | 16.28 | 61.99±4.73 | 43.80 |
| OCM (200 mg/kg) | 96.69±5.9 | 88.04±10.48 | 100 | 66.16±5.33 | 69.21±10.44 | 55.92 | 37.06±2.61 | 37.06±2.61 | 66.40 |

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

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

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.

http://www.herbmedpharmacol.com
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 µg/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 unoxygenated 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, alpha-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 auroarius*.
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.

**Acknowledgments**

The authors express their gratitude to the National Research Centre (NRC), Dokki, Giza, Egypt, for providing the facilities and funds.

**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).

**Funding/Support**

This study is a part of an internal project funded by the National Research Centre (NRC) Dokki, Giza, Egypt. Special appreciation is extended to the NRC offering the facilities for this study.

**References**

1. Abdulkhaleq LA, Assi MA, Abdullah R, Zamrias-Saad M, Taufiq-Yap YH, Hezmeen MNM. The crucial roles of inflammatory mediators in inflammation: a review. Vet World. 2018;11(5):627-35. doi: 10.14202/vetworld.2018.627-635.
2. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol. 2014;6(10):a016295. doi: 10.1101/cshperspect.a016295.
3. Serwecińska L. Antimicrobials and antibiotic-resistant bacteria: a risk to the environment and to public health. Water. 2020;12(12):3313. doi: 10.3390/w12123313.
4. Sibanda T, Okoh AI. The challenges of overcoming antibiotic resistance: plant extracts as potential sources of antimicrobial and resistance modifying agents. Afr J Biotechnol. 2007;6(25):2886-96. doi: 10.5897/ajb2007.000-2458.
5. Thakur HA. Antimicrobial and antifungal activity of Cucumis melo L. (Cucurbitaceae) and Pergularia daemia Forsk. (Asclepiadaceae) as ethnomedicinal plants. Int J Bioassays. 2015;4(1):3661-5.
6. Mirzaeian R, Sadoughi F, Tahmasebian S, Mojahedi M. The role of herbal medicines in health care quality and the related challenges. J Herbbmed Pharmacol. 2021;10(10):156-65. doi: 10.34172/jhph.2021.17.
7. Mallek-Ayadi S, Bahloul N, Kechaou N. Cucumis melo L. seeds as a promising source of oil naturally rich in biologically active substances: compositional characteristics, phenolic compounds and thermal properties. Grasas Aceites. 2019;70(1):e284. doi: 10.3989/gya.0215181.
8. Rabadán A, Nunes MA, Bessada SME, Pardeo JR, Oliveira M, Álvarez-Ortí M. From by-product to the food chain: melon (Cucumis melo L.) seeds as potential source for oils. Foods. 2020;9(1):1341. doi: 10.3390/foods9101341.
9. Rolim PM, Fidelis GP, Padilha CEA, Santos ES, Rocha HAO, Macedo GR. Phenolic profile and antioxidant activity from peels and seeds of melon (Cucumis melo L. var. reticulatus) and their antiproliferative effect in cancer cells. Braz J Med Biol Res. 2018;51(4):e6069. doi: 10.1590/1414-431x20176069.
10. Zhang X, Bai Y, Wang Y, Wang C, Fu J, Gao L, et al. Anticancer properties of different solvent extracts of Cucumis melo L. seeds and whole fruit and their metabolite profiling using HPLC and GC-MS, Biomed Res Int. 2020;2020:5282949. doi: 10.1155/2020/5282949.
11. Bidkar JS, Ghanwat DD, Bhujbal MD, Dama GY. Anti-hyperlipidemic activity of Cucumis melo fruit peel extracts

http://www.herbmedpharmacol.com
in high cholesterol diet induced hyperlipidemia in rats. J Complement Integr Med. 2012;9(1):Article 22. doi: 10.1515/1533-3840.1580.

12. Saleem M, Javed F, Asif M, Baig MK, Arif M. HPLC analysis and in vivo renoprotective evaluation of hydroalcoholic extract of Cucumis melo seeds in gentamicin-induced renal damage. Medicina (Kaunas). 2019;55(4). doi: 10.3390/medicina5504107.

13. Moustafa SF, Gabr NM, Zaki JT, El Awdan SA, Mina SA. The anti-inflammatory, anti-ulcer activities and phytochemical investigation of Cucumis melo L. cv. Ismailawi fruits. Nat Prod Res. 2020;2020:1-5. doi: 10.1080/14786419.2020.1803314.

14. Singh S, Devi B. Anti-inflammatory activity of Cucumis melo L. subsp. agrestis (Naudin) Pangalo. Int J Pharm Sci Res. 2020;11(8):3819-23. doi: 10.13040/ijpsr.0975-8232.11(8).3819-23.

15. Tsuda K, Sakai K, Tanabe K, Kishida Y. Isolation of 22-dehydrocholesterol from Hypnea japonica. Chem Pharm Bull. 1959;7(6):747. doi: 10.1248/cpb.7.747.

16. Liu KS. Preparation of fatty acid methyl esters for gas-chromatographic analysis of lipids in biological materials. J Am Oil Chem Soc. 1994;71(11):1179-87. doi: 10.1007/bf02540534.

17. Adams RP. Identification of Essential Oils by Ion Trap Mass Spectroscopy. New York: Academic Press; 1995. doi: 10.1002/ffj.2730050215.

18. Mir MA, Farooq R, Jassal MM, Mir BA, Kaur S, Mishra D. Studies on the physicochemical parameters of the fixed oils of Cinnamomum zeylanicum. Biomed J Sci Tech Res. 2017;1(1):81-4. doi: 10.26717/bjstsr.2017.01.000118.

19. Hussein AMS, Fouda K, Mehaya FM, Mohamed DA, Mohammad AA, Abdelgayed SS, et al. Fortified vegetarian milk for prevention of metabolic syndrome in rats: impact on hepatic and vascular complications. Helionyx. 2020;6(8):e04593. doi: 10.1016/j.helionyx.2020.e04593.

20. OECD (Organization for Economic Cooperation and Development). Guidelines for Testing of Chemicals, no. 423. Acute Oral Toxic. 2001. doi: 10.1787/20745788.

21. Botham PA. Acute systemic toxicity--propects for tiered testing strategies. Toxicol In Vitro. 2004;18(2):227-30. doi: 10.1016/s0887-2333(03)00143-7.

22. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Proc Soc Exp Biol Med. 1962;111:544-7. doi: 10.3181/00379727-111-27849.

23. Hisamuddin N, Shaik Mosaad QM, Sulaiman MR, Abas F, Leong SW, Kamarudin N, et al. Anti-edematogenic and anti-granuloma activity of a synthetic curcuminoid analog, [5-(3,4-Dihydroxyphenyl)-3-hydroxy-1-(2-hydroxyphenyl) penta-2,4-dien-1-one], in mouse models of inflammation. Molecules. 2019;24(14). doi: 10.3390/molecules24142614.

24. Mostafa FA, Abd El Aty AA, Hamed ER, Eied BM, Ibrahim NA. Enzymatic, kinetic and anti-microbial studies on Aspergillus terreus culture filtrate and Allium cepa seeds extract and their potent applications. Biocatal Agric Biotechnol. 2016;5:116-22. doi: 10.1016/j.bcab.2016.01.005.

25. Gui MM, Lee K, Bhatia S. Feasibility of edible oil vs. non-edible oil vs. waste edible oil as biodiesel feedstock. Energy. 2008;33(11):1646-53. doi: 10.1016/j.energy.2008.06.002.

26. Tavakoli HR, Naderi M, Jafari SM, Naeli MH. Postmarketing surveillance of the oxidative stability for cooking oils, frying oils, and vanaspati supplied in the retail market. Food Sci Nutr. 2019;7(4):1455-65. doi: 10.1002/fsn3.982.

27. Tavakoli J, Emadi T, Hashemi SMB, Moussavi Khanehagh A, Munekata PES, Lorenzo JM, et al. Chemical properties and oxidative stability of Arjan (Amygdalus reuteri) kernel oil as emerging edible oil. Food Res Int. 2018;107:378-84. doi: 10.1016/j.foodres.2018.02.002.

28. Nkafamiya II, Maina HM, Osimeahon SA, Modibbo UU. Percentage oil yield and physiochemical properties of different groundnut species (Arachis hypogaea). Afr J Food Sci. 2010;4(4):418-21. doi: 10.5897/ajfs.9000217.

29. Brigelius-Flohé R, Traber MG. Vitamin E: function and metabolism. FASEB J. 1999;13(10):1145-55. doi: 10.1096/fasebj.13.10.1145.

30. Campoccia D, Visai L, Renò F, Cangini I, Rizzi M, Poggi A, et al. Bacterial adhesion to poly-(D,L)-lactic acid blended with vitamin E: toward gentle anti-infective biomaterials. J Biomed Mater Res A. 2015;103(4):1447-58. doi: 10.1002/jbm.a.35284.

31. Górná S, Siger A, Juheviči K, Lács G, Šné E, Seglín D. Cold-pressed Japanese quince (Cyaenomeles japonica (Thunb.) Lindl. ex Spach) seed oil as a rich source of α-tocopherol, carotenoids and phenolics: a comparison of the composition and antioxidative activity with nine other plant oils. Eur J Lipid Sci Technol. 2014;116(5):563-70. doi: 10.1002/ejl.201300425.

32. Azhari S, Xu YS, Jiang QX, Xia WS. Physicochemical properties and chemical composition of Seinat (Cucumis melo var. tibish) seed oil and its antioxidative activity. Grasas Aceites. 2014;65(1):e008. doi: 10.3899/ga.074913.

33. da Silva AC, Jorge N. Bioactive compounds of oils extracted from fruits seeds obtained from agroindustrial waste. Eur J Lipid Sci Technol. 2017;119(4):1600024. doi: 10.1002/ejl.201600024.

34. Pedersen BK, Steensberg A, Schjerling P. Muscle-derived interleukin-6: possible biological effects. J Physiol. 2001;536(Pt 2):329-37. doi: 10.1111/j.1469-7793.2001.0329c.xd.

35. Amdekar S, Roy P, Singh V, Kumar A, Singh R, Sharma P. Anti-inflammatory activity of lactobacillus on carrageenan-induced paw edema in male wistar rats. Int J Inflam. 2012;2012:752015. doi: 10.1155/2012/752015.

36. Morris CJ. Carrageenan-induced paw edema in the rat and mouse. Methods Mol Biol. 2003;225:115-21. doi: 10.1385/1-59259-374-7:115.

37. Ammar NM, Abou El-Kassem LT, Ayoub NA, El-Ahmady SH, Moharam ME, AbouZeid EM. Anti-inflammatory and antimicrobial activities of the successive extracts of the aerial parts of Rumex pictus Forsk. growing in Egypt. J Herbmmed Pharmacol. 2021;10(1):116-22. doi: 10.34172/jhp.2021.12.

38. Ezzat SM, Raslan M, Salama MM, Menze ET, El Hawary SS. In vivo anti-inflammatory activity and UPLC-MS/MS profiling of the peels and pulps of Cucumis melo var. cantalupensis and Cucumis melo var. reticulatus. J Ethnopharmacol. 2019;237:245-54. doi: 10.1016/j.jep.2019.03.015.

39. Vouldoukis I, Lacan D, Kamate C, Coste P, Calenda A, Mazier D, et al. Antioxidant and anti-inflammatory properties of a Cucumis melo LC. extract rich in superoxide dismutase activity. J Ethnopharmacol. 2004;94(1):67-75. doi: 10.1016/j.jep.2004.04.023.

40. Wal P, Wal A, Sharma G, Rai AK. Biological activities

http://www.herbmedpharmacol.com
of lupeol. Syst Rev Pharm. 2011;2(2):96-103. doi: 10.4103/0975-8453.86298.

41. Yoon BK, Jackman JA, Valle-González ER, Cho NJ. Antibacterial free fatty acids and monoglycerides: biological activities, experimental testing, and therapeutic applications. Int J Mol Sci. 2018;19(4). doi: 10.3390/ijms19041114.

42. Murru E, Carta G, Manca C, Sogos V, Pistis M, Melis M, et al. Conjugated linoleic acid and brain metabolism: a possible anti-neuroinflammatory role mediated by PPARα activation. Front Pharmacol. 2020;11:587140. doi: 10.3389/fphar.2020.587140.

43. Wallert M, Schmölz L, Galli F, Birringer M, Lorkowski S. Regulatory metabolites of vitamin E and their putative relevance for atherogenesis. Redox Biol. 2014;2:495-503. doi: 10.1016/j.redox.2014.02.002.

44. Das S. Natural therapeutics for urinary tract infections-a review. Futur J Pharm Sci. 2020;6(1):64. doi: 10.1186/s43094-020-00086-2.

45. Wahdan O, Bassuony N, Abd El-Ghany Z, El-Chaghaby G. Antioxidant activity, antibacterial screening, proximate composition and GC-mass spectrometry analysis of cantaloupe seeds. J Agric Chem Biotechnol. 2016;7(12):291-5. doi: 10.21608/jacb.2016.41152.

46. Kumar P, Lee JH, Beyenal H, Lee J. Fatty acids as antibiofilm and antivirulence agents. Trends Microbiol. 2020;28(9):753-68. doi: 10.1016/j.tim.2020.03.014.

47. Casillas-Vargas G, Ocasio-Malavé C, Medina S, Morales-Guzmán C, Del Valle RG, Carballéa NM, et al. Antibacterial fatty acids: an update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents. Prog Lipid Res. 2021;82:101093. doi: 10.1016/j.plipres.2021.101093.

48. Kumar V, Bhatnagar AK, Srivastava JN. Antibacterial activity of crude extracts of Spirulina platensis and its structural elucidation of bioactive compound. J Med Plants Res. 2011;5(32):7043-8. doi: 10.5897/jmpr11.1175.

49. Bidossi A, Bortolin M, Toscano M, De Vecchi E, Romanò CL, Mattina R, et al. In vitro comparison between α-tocopheryl acetate and α-tocopheryl phosphate against bacteria responsible of prosthetic and joint infections. PLoS One. 2017;12(7):e0182323. doi: 10.1371/journal.pone.0182323.