Phytochemical Study of the Bioactive Fractions of *Chrysanthemum frutescens* L. Cultivated in Egypt

Ammar N M1*, El-Hawary S S2, El-Anssary A A1, El-Desoky A H1,5, Abdelaal T A3, Abdelrahman R F4, Hattori M5

1Pharmacognosy Department, Pharmaceutical Industries Research Division, National Research Centre, Giza, Egypt.
2Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt.
3Pharmacology Department, Medical Sciences Division, National Research Centre, Giza, Egypt.
4Chemistry of Natural and Microbial Department, Pharmaceutical Industries Research Division, National Research Centre, Giza, Egypt.
5Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan.

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ABSTRACT
Phytochemical study of the aerial flowering parts of *Chrysanthemum frutescens* L. Family Astraecae, cultivated in Egypt, revealed the presence of an appreciable percentage of volatile oil and phenolic compounds. The volatile oil was prepared (0.5%) and analyzed by GC/MS which revealed the identification of 20 compounds, the major is (Z)-1-farnesene (37.21%). The volatile oil was tested for antimicrobial activity and inhibited the growth of *Staphylococcus aureus*, *Bacillus subtilis*, *Esherichia coli*, *Pseudomonas aerugenosa* and *Candida albicans*. The non-polar extract and the volatile oil showed significant anti-inflammatory activity, using carrageenan-induced oedema in rats while the non-polar extract showed analgesic activity using acetic acid induced writhing with reduction of the writhing score (23.8%). The DPPH free radical scavenging activity of the polar aqueous methanolic extract of *C. frutescens* was evaluated. Bioassay guided fractionation led to the isolation of 6 compounds 4,5-di-o-cafeoyl quinic acid (IC50 = 16.18 µg/ml), methyl 4,5-di-o-cafeoyl quinic (IC50=13.25 µg/ml), 1,5-di-o-cafeoyl quinic acid (IC50=42.9µg/ml), methyl 1,5-di-o-cafeoyl quinate (IC50=77.0 µg /ml), apigenin 7-o-glucopyranoside (IC50=78.00mg/ml) and melilotoside (IC50>200 µg/ml).

Keywords: *Chrysanthemum frutescens* L., anti-inflammatory, analgesic, antimicrobial, volatile oil, dicafeoyl quinic acid.

INTRODUCTION
The genus *Chrysanthemum* also known as *Argyranthemum* (Astraeaceae) includes 25 species with numerous subspecies1. The natural distribution of the genus is restricted to the Canary Islands, Madeira, Desertas and Selvagens2. *C. frutescens*, also known as “Marguerite Daisy” is cultivated either as cut flowers or pot plants. It has been in cultivation for more than 200 years. The genus *Chrysanthemum* is a good source of secondary metabolites, such as polyacetylenes, sesiquiterpene lactones, aromatic esters3 and spirostanic ethers4. Generally, the genus *Chrysanthemum* is widely used in traditional medicine for the cure of many complaints. The inflorescence or bud of *Chrysanthemum indicum* has a long history of usage as a Chinese traditional medicine5. *Chrysanthemum indicum* possesses anti-bacterial, anti-viral, anti-oxidant, anti-inflammatory and immunomodulatory properties6. *Chrysanthemum cinerariefolium* has insecticidal activity7. *Chrysanthemum parthenium* treats fevers, reduces swelling and used for its analgesic properties, promotes menstrual flow as well as in rheumatoid and migraine8. *Chrysanthemum frutescens* is well reputed in Egyptian folk medicine for the treatment of oral inflammatory diseases and as analgesic.

General experimental procedures
During our continuous prospect for natural anti-inflammatory and analgesic preparations; some preparations from the aerial parts of *Chrysanthemum frutescens* showed significant hits. Different preparations of the aerial parts of *Chrysanthemum frutescens* were prepared for the study, including the volatile oil and successive extracts. Modified Likens and Nikerson apparatus was used for preparation of volatile constituents by hydrodistillation. GC/MS: Gas chromatograph coupled with a mass spectrometer GC/MS Finnigan Mat SSQ 7000, Digital DEC EL, 70 eV for GC/MS analysis of volatile compounds. UV-Visible Spectrophotometer: UV–VIS double beam UVD–3500 spectrophotometers, Labomed, Inc. was used for recording UV spectra and measuring the absorbance in UV and visible range. Preparative HPLC, was conducted on Tosoh HPLC instrument on a TSK ODS-80Ts C18 column (250 × 10 mm, 5 µm) and UV detector at λ, 280 nm. LC-MS were conducted using an Agilent LC-MS ion trap mass spectrometer (Bruker Daltonics, Bremen,
Preparation of volatile oil

Fresh flowering aerial parts of *Chrysanthemum frutescens* L. (500 gm) were covered with water in a round bottom flask and subjected to hydro-distillation in a modified Likens and Nickerson apparatus, which allowed the distillation and simultaneous extraction of the volatile components in an organic solvent (n-pentane). The n-pentane layer was collected and cautiously evaporated, dehydrated over anhydrous sodium sulfate and stored in dark tightly closed container at 4°C to be analyzed by GC/MS.

**GC/MS analysis of volatile constituents**

GC/MS analysis of the volatile constituents was carried out on a gas chromatograph directly coupled to mass spectrophotometer (Finnigan SSQ 7000) using capillary column of fused silica, 30m length, 0.25mm ID and 0.25mm thickness. Stationary phase DB-5, carrier gas Helium at flow rate 1 mL/min and pressure 13 psi, temperature programming 50-260°C at a rate of 3°C/min., ion source temperature180°C and ionization voltage70 eV, and injection volume 1 µL.

**Investigation of bioactive non-polar fractions**

All successive extracts were subjected to TLC and paper chromatography. Petroleum ether (60-80°C), ether and chloroform extracts were combined together as total mixed non-polar extract. Methanol and 50% aqueous methanol were combined together as total mixed polar extract. Total mixed non-polar extract (20 g) was subjected to silica gel open column 50x10 cm with a stepwise gradient elution using hexane and increasing 10% folds of ethyl acetate till reaching 100% ethyl acetate to yield 8 fractions. Fraction 4 (200 mg) was then applied to silica gel column and eluted with hexane and ethyl acetate (9.5:0.5 v/v) to yield compound (1) (55 mg) as colorless needle crystals. Fraction 6 (59 mg) was subjected to preparative ODS-C18 HPLC (COSMOSIL 5C18-AR-II, Nacalai Tesque Inc., 20 x 250 mm; COSMOSIL 5C18-MS-II, Nacalai Tesque Inc., 20 x 250 mm), eluted with methanol as solvent (A) and 0.1% trifluoroacetic acid in water as solvent (B) with a flow rate of 5 ml/min and elution gradient (35% A) from 0 to 20 min., then to (50% A) from 20 to 40 min., then to (70% A) from 40 to 50 min. to yield compound (2) (4 mg).

**Investigation of bioactive polar fractions with antioxidant activity**

The total mixed polar extract was applied to Diaion HP20 column eluted with water, 25% MeOH in water, 50% MeOH in water, 75% MeOH in water and MeOH respectively to yield 5 fractions. The 50% methanol/water fraction showed the highest DPPH free radical scavenging activity (IC<sub>50</sub> =47.8 µg/mL). While the 25% MeOH in water, 75% MeOH in water and MeOH fractions had IC<sub>50</sub> 59.24 µg/mL, 48.89 µg/mL and more than 200 µg/mL, respectively. The most active fraction...
was applied to Sephadex LH20 (Pharmacia LKB) using 50% methanol/water for elution to yield 7 fractions. Fraction 7 (14 mg) was compound (3)\textsuperscript{12}. Upon standing the fifth fraction yielded compound (8)\textsuperscript{13} as colorless rosette crystals (40 mg). Fraction 5 was separated with 15% (A) 0-20 min., 35% (A) 20-35 min., 50% (A) 35-50 min., 70% (A) 50-60 min., 100% (A) 60-70 min. with a flow rate 2 ml/min. to yield compound (4) (5 mg)\textsuperscript{12} and compound (7) (3 mg)\textsuperscript{14}. Fraction 6 was separated with preparative-HPLC using the same conditions as fraction 5 to yield compound (5) (8 mg) and compound (6) (2 mg)\textsuperscript{12}. Isolated compounds were identified by comparing their $^1$HNMR and ESIMS spectra with literature.

**Biological study**

**Materials for bioactivity studies**

**Plant extracts and fractions**

Both total non-polar and polar extracts as well as volatile oil were prepared from the aerial parts of *Crysanthemum frutescens* L.

**Animals**

Adult rats of both sexes weighing 150-200 g and adult mice weighing 20-25 g were used in the experiments. Animals were housed under standardized conditions of light and temperature and received standard rat chow and tap water \textit{ad libitum}. Animals were randomly assigned to different experimental groups, each kept in separate cage. All animal procedures were performed after approval from the Ethics Committee of the National Research Centre and in accordance with the recommendations for the proper care and use of laboratory animals\textsuperscript{15}.

**Drugs and Chemicals**

Aspirin (acetyl salicylic acid) used as positive control in acetic acid induced writhing test (Bayer, Germany), Indomethacin capsules, used as positive control in carrageenan- induced paw oedema assay (Kahira Pharmaceutical and Chemical Company, Cairo, Egypt), Carrageenan for the induction of footpad oedema in carrageenan- induced paw oedema assay (Sigma Aldrich chemical company, USA), Acetic acid 1% for the...
Table 1: Anti-inflammatory activity of C. frutescens preparations against carrageenan induced paw oedema in rats (n=6). Values are means ± SE (n=6). *P<0.05 in comparison to control group (Dunnett's test).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound</th>
<th>Mol. formula</th>
<th>M.W</th>
<th>B.P.</th>
<th>R_t (min)</th>
<th>*R_t</th>
<th>Relative area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α-pinene</td>
<td>C_{10}H_{16}</td>
<td>136</td>
<td>57</td>
<td>10.00</td>
<td>0.17</td>
<td>2.16</td>
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<tr>
<td>2</td>
<td>myrcene</td>
<td>C_{10}H_{16}</td>
<td>136</td>
<td>57</td>
<td>12.00</td>
<td>0.34</td>
<td>9.78</td>
</tr>
<tr>
<td>3</td>
<td>L-limonene</td>
<td>C_{10}H_{16}</td>
<td>136</td>
<td>59</td>
<td>13.33</td>
<td>0.41</td>
<td>1.27</td>
</tr>
<tr>
<td>4</td>
<td>cis-ocimene</td>
<td>C_{10}H_{22}</td>
<td>142</td>
<td>71</td>
<td>13.56</td>
<td>0.45</td>
<td>8.46</td>
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<td>5</td>
<td>unknown</td>
<td>C_{20}H_{36}O_{6}</td>
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<td></td>
<td>16.28</td>
<td>0.46</td>
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<td>6</td>
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<td></td>
<td></td>
<td>22.22</td>
<td>0.47</td>
<td>1.27</td>
</tr>
<tr>
<td>7</td>
<td>trans-caryophyllene</td>
<td>C_{13}H_{24}</td>
<td>204</td>
<td>58</td>
<td>22.82</td>
<td>0.53</td>
<td>1.05</td>
</tr>
<tr>
<td>8</td>
<td>b-cubebene</td>
<td>C_{15}H_{24}</td>
<td>204</td>
<td>121</td>
<td>23.59</td>
<td>0.54</td>
<td>6.27</td>
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<td>9</td>
<td>α-cadinene</td>
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<td>204</td>
<td>161</td>
<td>23.86</td>
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<td>24.71</td>
<td>0.57</td>
<td>0.54</td>
</tr>
<tr>
<td>11</td>
<td>(z)-b-farnesene</td>
<td>C_{15}H_{24}</td>
<td>204</td>
<td>57</td>
<td>25.58</td>
<td>0.58</td>
<td>37.21</td>
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<tr>
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<td>(-)-calarene</td>
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<td>161</td>
<td>26.36</td>
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<td>3.83</td>
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<tr>
<td>13</td>
<td>unknown</td>
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<td></td>
<td></td>
<td>26.70</td>
<td>0.61</td>
<td>0.78</td>
</tr>
<tr>
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<td>viridiflorol</td>
<td>C_{13}H_{26}O</td>
<td>222</td>
<td>57</td>
<td>27.01</td>
<td>0.63</td>
<td>2.70</td>
</tr>
<tr>
<td>15</td>
<td>d-cadinene</td>
<td>C_{15}H_{24}</td>
<td>204</td>
<td>57</td>
<td>27.25</td>
<td>0.67</td>
<td>1.04</td>
</tr>
<tr>
<td>16</td>
<td>Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1α,4α,8α) α-cedrene</td>
<td>C_{16}H_{26}</td>
<td>240</td>
<td>71</td>
<td>27.79</td>
<td>0.68</td>
<td>1.40</td>
</tr>
<tr>
<td>17</td>
<td>α-cedrene</td>
<td>C_{15}H_{12}O</td>
<td>240</td>
<td>57</td>
<td>28.13</td>
<td>0.69</td>
<td>1.65</td>
</tr>
<tr>
<td>18</td>
<td>3α-methoxy-11-oxa-5-pregnane</td>
<td>C_{25}H_{36}O_{2}</td>
<td>320</td>
<td>57</td>
<td>28.77</td>
<td>0.70</td>
<td>0.68</td>
</tr>
</tbody>
</table>
induction of writhing in acetic acid induced writhing test,
DPPH free radical (Sigma Aldrich Chemical Company,
USA) were all purchased.

Materials for antimicrobial study
Tested microorganisms
Tested microorganisms including, gram positive bacterial
strains (Bacillus subtilis and Staphylococcus aureus),
gram negative bacteria strains (Escherichia coli and
Pseudomonas aeruginosa), fungi (Aspergillus niger,
Penicillium digitatum, Macrophomina phaseoli and
Fusarium oxysporium) and yeasts (Candida albicans and
Saccharomyces cerevisiae) were kindly provided by the
Chemistry of Natural and Microbial Products Laboratory,
Chemistry of Natural and Microbial Department, NRC,
Cairo, Egypt.

Media
All the chemicals used in the preparation of the media
were of the analytical grade. Distilled water was used.
Routine sterilization was done by autoclaving for 20
minutes at 15 psi. (121°C).

Potato- dextrose agar growth medium (PDA), yeast
extract peptone dextrose medium (YPED medium) and
Lauria – Bertani medium (LB medium) were used.

Biological methods
Antimicrobial activity
Nutrient agar media for the bacteria, fungi and yeast were
prepared and sterilized then distributed in sterile petri
dishes each of 12 cm diameter. Each suspension of the
test organism was separately inoculated onto a number of
petri dishes. Each antibiotic assay disc (6mm diameter)
was loaded with 100 μg/disc of the tested volatile oil of
C. frutescens. The discs after air-dried were firmly
applied to the surface of the inoculated agar plates.
Ampicillin as antibacterial (200 μg/disc) and Canesten
(100 μg/disc) as antifungal were used as reference drugs.
This assay was replicated three times. All these steps
were done under aseptic conditions. Bacterial plates were
incubated at 37°C for 24 hours, while those containing
yeast and fungi were incubated at 28°C for 48 – 72 hours.
The diameter of the inhibition zone was recorded for each
replicate and the average diameters are calculated.

Acute inflammation test (carrageenan- induced paw
eodema assay)
Thirty adult male albino rats, divided into five groups,
each of six animals, were orally treated with polar extract
(500 mg/kg), non-polar extract (160 mg/kg), volatile oil
(160 mg/kg), Indomethacin (20mg/kg) as positive control
and saline as negative control. One hour after oral
administration, all animals were given a sub-plantar
injection of 100 μL of 1 % carrageenan solution in saline
100 mL in the right hind paw. The contralateral hind paw
received the same volume of saline and served as normal
control. Hind foot-pad thickness was measured with a
micrometer caliper16,17 before, and at 1, 2, 3 and 4 hrs
after carrageenan injection, as carrageenan caused visible
redness and pronounced swelling that was well
-developed by 4 hrs and persisted for more than 48 hrs.18.

Peripheral analgesic test (Acetic acid –induced writhing
test)
Three separate groups, (6 mice each), were administered
the vehicle and/or 50 mg/dl of the tested extracts. After
60 min. interval, an I.P. injection of 0.6% acetic acid was
administered19,20. Each mouse was then placed in an
individual clear plastic observational chamber, and the
total number of writhes made by each mouse was counted
for 20 min.

Free radical scavenging assay
Radical scavenging activity of the tested polar extract and
compounds were measured by slightly modified
method21. The assay is based on the incubation of
reaction medium of the tested samples of C. frutescens
for 30 min at 37 ºC in an ethanolic solution of 150 μM.

*Rrt = retention time relative to 9,12-Octadecadien-1-ol = 1
Table 2: Components of the volatile oil of C. frutescens as detected by GC/MS analysis.

<table>
<thead>
<tr>
<th>Group</th>
<th>1 hour</th>
<th>2 hour</th>
<th>3 hour</th>
<th>4 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>59.83±2.04</td>
<td>102.18±8.07</td>
<td>101.41±6.09</td>
<td>100.58±4.22</td>
</tr>
<tr>
<td>Non–polar (160 mg/kg)</td>
<td>34.28±2.70a</td>
<td>66.38±8.83a</td>
<td>75.65±5.99a</td>
<td>75.58±4.91a</td>
</tr>
<tr>
<td>Polar (500 mg/kg)</td>
<td>50.03±3.27</td>
<td>95.09±7.07</td>
<td>99.27±5.95</td>
<td>101.59±4.76</td>
</tr>
<tr>
<td>Volatile oil (160 mg/kg)</td>
<td>37.1±3.22</td>
<td>49.0±2.42</td>
<td>49.2±2.62</td>
<td>41.4±2.33a</td>
</tr>
<tr>
<td>Indomethacin (20 mg/kg)</td>
<td>13.07±1.34a</td>
<td>25.71±2.97a</td>
<td>33.45±1.87a</td>
<td>38.19±2.47</td>
</tr>
</tbody>
</table>

Inhibition rate (%)

<table>
<thead>
<tr>
<th>Group</th>
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<th>4 hour</th>
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</tr>
<tr>
<td>Non–polar (160 mg/kg)</td>
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<td>34.28±2.70a</td>
<td>50.03±3.27</td>
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<tr>
<td>Polar (500 mg/kg)</td>
<td>99.27±5.95</td>
<td>101.59±4.76</td>
<td>50.03±3.27</td>
<td>500 mg/kg</td>
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<tr>
<td>Volatile oil (160 mg/kg)</td>
<td>49.2±2.62</td>
<td>41.4±2.33a</td>
<td>37.1±3.22</td>
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<tr>
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<td>38.19±2.47</td>
<td>13.07±1.34a</td>
<td>25.71±2.97a</td>
</tr>
</tbody>
</table>

Inhibition rate (%)

P<0.05: Statistically significant from control (Dunnett’s test).

DDPH and the absorbance is measured afterwards at 515 nm. The antioxidant activity was expressed as IC50, which is defined as the extract concentration required to scavenge 50 % DPPH free radicals.

Statistical Analysis

Results are expressed as mean ± S.E. Differences between vehicle control and treatment groups were tested using one-way ANOVA followed by the least significant difference (L.S.D). Methods of statistical analysis were done according to22.

RESULTS

Antimicrobial activity

The prepared essential oil of the flowering aerial parts of C. frutescens (100 µg/disc) was tested for antimicrobial activity and inhibited the growth of Staphylococcus aureus (inhibition zone 12 mm), as well as, Bacillus subtilis (inhibition zone 10 mm), Escherichia coli (inhibition zone 9 mm), Pseudomonas aeruginosa (inhibition zone 7 mm) and Candida albicans.

Determination of acute anti-inflammatory effect (carrageenan-induced paw oedema assay)

The anti-inflammatory effects of total polar extract, total non-polar extract and volatile oil were evaluated adopting the carrageenan induced rat hind paw oedema. In control group, the paw thickness increased by (59.83 ± 2.04%) 1 h, (102.18 ± 8.07%) 2 h, (101.41 ± 6.09%) 3 h, (100.58 ± 4.22%) 4 h after carrageenan injection as compared with pre-carrageenan control values. The non-polar extract (160 mg/kg) showed significant inhibition of the oedema formation by 42.71%, 38.00%, 52.05%, 51.48% and 58.84% at 1, 2, 3 and 4 h (carrageenan injection); respectively as compared with saline-treated control group, showing its maximum inhibition at the first hour then starts to decline gradually and reaches its minimum at the fourth hour. The volatile oil (160 mg/kg) showed significant inhibition of the oedema formation by 38.00%, 52.05%, 51.48% and 58.84% at 1, 2, 3 and 4 h (carrageenan injection); respectively as compared with saline-treated control group, showing its maximum inhibition at the fourth hour, which has been increasing gradually since the first hour of carrageenan injection in an opposite manner to the effect of the non-polar extract. The polar extract (500 mg/kg) showed no significant inhibition of the oedema formation. The positive control Indomethacin (20 mg/kg) showed significant inhibition of the oedema formation by 78.15%, 74.84%, 67.02% and 62.03% at 1, 2, 3 and 4 h (carrageenan injection); respectively as compared with saline-treated control group. (Table 1 and Figure 1)

Analgesic activity: (acetic acid induced writhing test)

Acetic acid induced writhing was significantly reduced in mice receiving tested extract of C. frutescens at in a dose of 500 mg/kg. The antinociceptive activity of the non-polar extract inhibited the writhing score by 23.8% indicating peripheral analgesic effect, while the response of the positive control aspirin was 46%.

Antioxidant activity

The polar extract of the aerial parts of C. frutescens scavenged DPPH free radicals in a dose dependant manner with IC50 value 40 µg/mL, while catechin (as standard) showed IC50 value 9.2 µg/mL.

Phytochemical investigation volatile oil of C. frutescens by GC/MS analysis

GC/MS analysis was carried out on a gas chromatograph directly coupled to mass spectrophotometer using the conditions described previously. Identification of components was performed by comparing the relative retention times and mass spectra with those of the available database libraries [Wiley (Wiley Int.) USA and NIST (Nat. Inst. St. Technol., USA)] and or published data23. Quantitative determination was carried out based on peak area integration. The results of GC/MS analysis of the volatile oil of the aerial parts of C. frutescens are compiled in Table (2). Furthermore, the chemical classes of these volatiles are presented in Table (2). The volatile oil obtained by hydro-distillation was light yellow in color with pleasant fragrant odor. GC/MS analysis of the volatile oil of the aerial parts of Chrysanthemum frutescens L. revealed the identification of 20 compounds out of total 26 compounds (representing about 95.32% of the oil), 11 compounds (representing 75.96%) were unxygenated, including sesquiterpene hydrocarbons (54.29%) and monoterpene hydrocarbons (21.67%), 9 compounds (19.36%) are oxygenated, including sesquiterpene alcohols (4.92%), ketones (0.87%), aromatic compounds (2.06%), aliphatic alcohols (10.83%), epoxy compounds (0.68%). The major
investigation of the bioactive non polar extract
Since the total non-polar extract showed significant inhibition of carrageenan induced swelling of the hind rat paw (P < 0.05), it was necessary to investigate the chemical composition of this extract to stand for the constituents which may be responsible for the biological activity. Stigmasterol (1) and scopoletin (2) were isolated from the non-polar extract adopting the method previously described. Sterols are reported to have anti-inflammatory activity as the sterol fraction rich in camposterol, stigmasterol and \( \alpha \)-sitosterol inhibits carrageenan paw oedema in mice\(^4\). Meanwhile, scopoletin is reported to inhibit eicosanoid-release from ionophore-stimulated mouse peritoneal macrophages\(^5\) and inhibits the production of inflammatory cytokines through inhibition of the \( \text{IkB} \)NF-\( \kappa \text{B} \) signal cascade in the human mast cell line HMC-1\(^6\). Therefore, stigmasterol and scopoletin are believed to be the major anti-inflammatory constituents of the non-polar extract of \textit{Chrysanthemum frutescens} L.

**Phytochemical investigation of the bioactive polar extract**
The aqueous methanolic polar extract of the aerial parts of \textit{C. frutescens} scavenged DPPH free radicals in a dose dependant manner with IC\(_{50}\) value of 40 \( \mu \text{g/mL} \). The radical scavenging activity of the methanolic extract of \textit{C. frutescens} can be attributed to its content of phenolic compounds. The aqueous methanolic extract of \textit{Chrysanthemum frutescens} was subjected to bioassay guided fractionation on Diaion HP20, Sephadex LH20 and preparative HPLC to isolate biologically active compounds that were identified using spectral analysis (12-14). Six compounds were isolated and the free radical scavenging activity of each was measured using DPPH assay. These compounds are 4,5-\( \text{di-o-cafeoylquinic acid (IC}_{50}=16.18 \ \mu\text{g/mL}) \), methyl 4,5-\( \text{di-o-cafeoyl quinate (IC}_{50}=13.25 \ \mu\text{g/mL}) \), 1,5-\( \text{di-cafeoyl quinic acid (IC}_{50}=42.9 \ \mu\text{g/mL}) \), methyl 1,5-\( \text{di-cafeoyl quinate (IC}_{50}=77.00 \ \mu\text{g/mL}) \) and apigenin7-\( \text{o-glucopyranoside (IC}_{50}=78.00 \ \mu\text{g/mL}) \), while the last compound mellilotoside had no antioxidant activity (IC\(_{50}>200 \ \mu\text{g/mL})

**DISCUSSION**
Biological investigation of the aerial parts of \textit{C. frutescens} was conducted to investigate its anti-inflammatory and analgesic activities using the carrageenan induced hind paw oedema and acetic acid induced writhing models, respectively. The results revealed remarkable and significant anti-inflammatory activity of the non-polar extract of \textit{Chrysanthemum frutescens} (P<0.05) which reached its maximum value at the first hour from carrageenan injection; where the inhibition rate reached (42.71\%) then it started to decrease gradually. Meanwhile the prepared volatile oil of the same plant exhibited significant anti-inflammatory activity that increased gradually to reach its maximum inhibition rate (58.84\%) after 4 hours of carrageenan injection. This variation in time can refer to the advantage of using either the plant as a whole or using a mixture of the non-polar extract and the volatile oil to cover a wider time range for activity. Phytochemical investigation of the non-polar extract resulted in the isolation of stigmasterol and scopoletin. These two compounds are also reported to have anti-inflammatory activities\(^4\)^\(^-\)^\(^6\). Moreover, the polar extract of \textit{Chrysanthemum frutescens} revealed free radical scavenging activity using DPPH assay, and it is well known that oxidative stress participates strongly in inflammatory conditions and the release of such oxidative stress will participate in lowering the burden of inflammation. The polar extract of \textit{Chrysanthemum frutescens} was subjected to bioassay guided fractionation using DPPH free radical scavenging assay to isolate antioxidant compounds. Five compounds with free radical scavenging activity were isolated and identified as 4,5-\( \text{-d-o-cafeoyl quinic acid (IC}_{50}=16.18 \ \mu\text{g/mL}) \), methyl 4,5-\( \text{-d-o-cafeoyl quinate (IC}_{50}=13.25 \ \mu\text{g/mL}) \), 1,5-\( \text{-d-o-cafeoyl quinic acid (IC}_{50}=42.9 \ \mu\text{g/mL}) \), methyl 1,5-\( \text{-d-o-cafeoyl quinate (IC}_{50}=77.00 \ \mu\text{g/mL}) \) and apigenin7-\( \text{o-glucopyranoside (IC}_{50}=78.00 \ \mu\text{g/mL}) \) (12-14). The volatile oil was also tested for its antimicrobial activity and the results revealed that it inhibited the growth of \textit{Staphylococcus aureus} (inhibition zone 12 mm) as well as, \textit{Bacillus subtilis} (inhibition zone 10 mm), \textit{Escherichia coli} (inhibition zone 9 mm), \textit{Pseudomonas aeruginosa} (inhibition zone 7 mm) and \textit{Candida albicans}. GC/MS analysis of the volatile oil revealed the presence of (Z)-1-farnesene (37.21\%), \( 1\)-myrcene (9.78\%), and (Z)-\( \beta \)-ocimene (8.46\%) (23). Reviewing the available current literature, nothing was previously reported concerning the volatile content of \textit{Chrysanthemum frutescens} L. Major compounds of the prepared volatile oil possess antimicrobial activity as previously reported in the literature\(^7\). All the former results promote the advantage of the use of \textit{Chrysanthemum frutescens} as a whole fresh herb without fractionation, if it is advised to be used, as a treatment of inflammatory conditions.

**REFERENCES**
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