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Research Article

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

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ABSTRACT
Phytochemical study of the aerial flowering parts of *Chrysanthemum fructesens* L. Family Astraceae, 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, Escherichia coli, Pseudomonus aerugenos*a 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*-caffeoyl quinic acid (IC_{50} = 16.18 µg/ml), methyl 4,5-di-*o*-caffeoyl quinic (IC_{50}=13.25 µg/ml), 1,5-di-*o*-caffeoyl quinic acid (IC_{50}=42.9µg/ml), methyl 1,5-di-*o*-caffeoyl quinate (IC_{50}=77.0 µg /ml), apigenin 7-*o*-glucopyranoside (IC_{50}=78.00mg/ml) and mellilotoside (IC_{50}>200 µg/ml).

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

INTRODUCTION

The genus *Chrysanthemum* also known as *Argyranthemum* (Asteraceae) includes 25 species with numerous subspecies[1]. The natural distribution of the genus is restricted to the Canary Islands, Madeira, Desertas and Selvagens[2]. *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, sesquiterpene lactones, aromatic esters[3] and spirostanic ethers[4]. Generally, the genus *Chrysanthemum* is widely used in traditional medicine for the cure of many complains. The inflorescence or bud of *Chrysanthemum indicum* has a long history of usage as a Chinese traditional medicine[5]. *Chrysanthemum indicum* possesses anti-bacterial, anti-viral, anti-oxidant, anti-inflammatory and immunomodulatory properties[6]. *Chrysanthemum cinerariefolium* has insecticidal activity[7]. *Chrysanthemum parthenium* treats fevers, reduces swelling and used for its analgesic properties, promotes menstrual flow as well as in rheumatoid and migraine[8]. *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 prospection 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-MSD ion trap mass spectrometer (Bruker Daltonics, Bremen,

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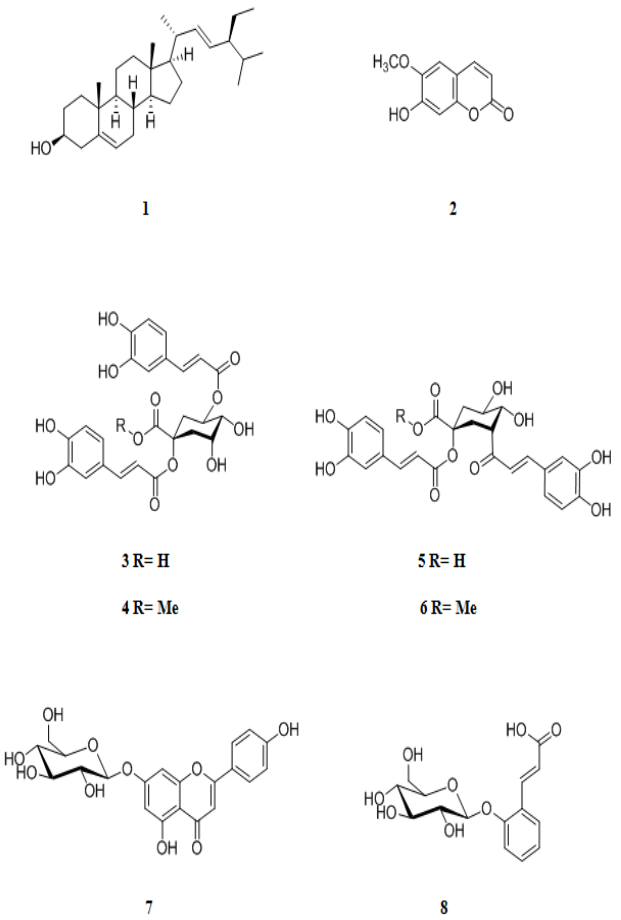


Figure 1: Structures of 1-8

Germany) equipped with 1100 series HPLC and a Cosmosil waters $5C_{18}$ (150 mm × 4.6 mm, 5 µm) column. The UV detection was set at 280 nm. NMR spectra were recorded on a Varian Inova –500, Varian Gemni unity plus 300 NMR and JEOL delta 400 spectrometer apparatus using DMSO-d_6, $CDCl_3$, CD_3OD as solvent and TMS as internal standard. The data are expressed in δ-values in ppm and *J* values in Hz.

Phytochemical study

Plant material

Samples of the aerial parts of *Chrysanthemum frutescens* L. (Astraceae) were collected from Ornamental gardens, 6th of October city, Egypt, and were kindly authenticated by Dr. Abdelhaleem Abdelmotagaly, Department of Flora, the Agricultural Museum, Dokki, Giza, Egypt. Fresh samples were used for preparing the volatile oil. Samples for solvent extraction were air-dried, powdered and reduced to mesh no. 36 and kept in tightly-closed containers.

Phytochemical methods

Preparation of successive extracts with selective organic solvents

500 grams of air-dried powdered aerial parts of *Chrysanthemum frutescens* L. were separately extracted in continuous extraction apparatus (Soxhlet) successively and exhaustively using solvents of increasing polarity in the following order: petroleum ether (60-80°C), ether, chloroform, methanol and 50% aqueous methanol. For each solvent the extraction was continued till exhaustion. In each case, the solvent was stripped off by distillation under reduced pressure at a temperature not exceeding 40°C and dried at a constant weight in a vacuum desiccator over anhydrous calcium chloride.

Investigation of volatile components

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[9]. 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, 0.32mm 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[10]. 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% trifluroacetic 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)[11].

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_{50} =47.8 µg/mL). While the 25% MeOH in water, 75% MeOH in water and MeOH fractions had IC_{50} 59.24 µg/mL, 48.89 µg/mL and more than 200 µg/mL, respectively. The most active fraction

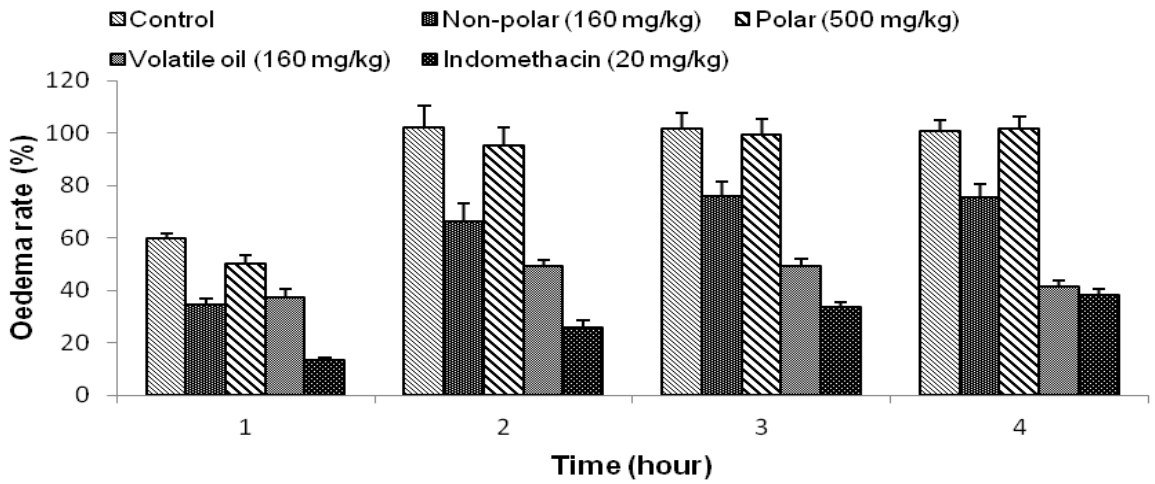


Figure 2: Oedema rate of control (untreated), non-polar extract (160 mg/kg), polar extract (200 mg/kg), volatile oil (160 mg/kg) and Indomethacin (20 mg/kg) after 1, 2, 3 and 4hours after carrageenan injection.

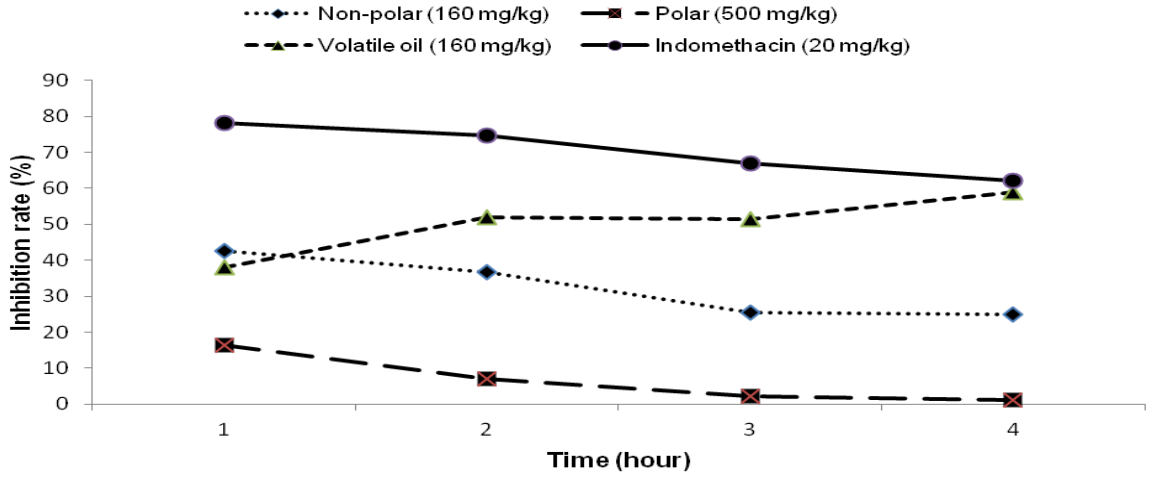


Figure 3: Inhibition rate, as percent of control, of non-polar extract (160 mg/kg), polar extract (200 mg/kg), volatile oil (160 mg/kg) and Indomethacin (20 mg/kg) after 1, 2, 3 and 4hours after carrageenan injection, showing the course of activity of each preparation.

was applied to Sephadex LH20 (Pharmacia LKB) using 50% methanol/water for elution to yield 7 fractions. Fraction 7 (14mg) was compound (3)[12]. Upon standing the fifth fraction yielded compound (8)[13] as colorless rosette crystals (40mg). 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)[12] and compound (7) (3 mg)[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)[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 fructescens* L.

Animals

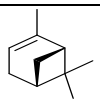
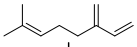
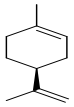
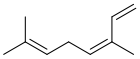
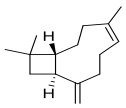
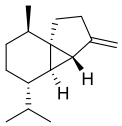
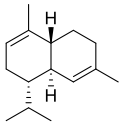
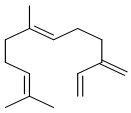
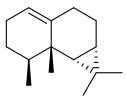
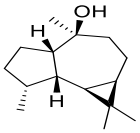
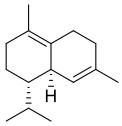
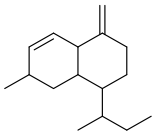
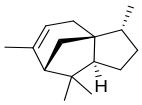
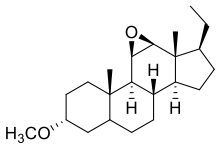
Adult rats of both sexes weighing 150-200 g and adult mice weighing 20-25 g were used in the experiments. preparative-HPLC using a Tosoh HPLC instrument on a TSK ODS-80Ts C18 column (250 × 10 mm, 5 μm) with a gradient elution system of two solvents: (A) 0.01% trifluroacetic acid in water and (B) methanol; as follows,

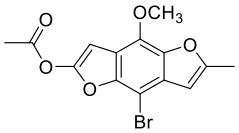
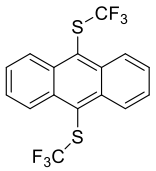
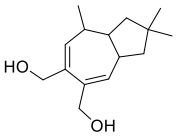
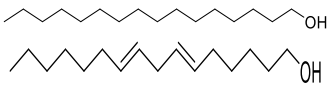
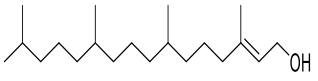
Animals were housed under standardized conditions of light and temperature and received standard rat chow and tap water *ad libitum*. Animals were randomly assigned to different experimental groups, each kept in separate cage. All animal procedures were performed after approval from the Ethics Committee of the National Research Centre and in accordance with the recommendations for the proper care and use of laboratory animals[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). [a], P<0.05 in comparison to control group (Dunnett's test).

S. No.	Compound	Mol. formula	M W	B.P.	R_t (min)	*Rr_t	Relative area	Structure
1	*a*-pinene	$C_{10}H_{16}$	136	57	10.20	0.17	2.16	
2	myrcene	$C_{10}H_{16}$	136	57	12.00	0.34	9.78	
3	L-limonene	$C_{10}H_{16}$	136	59	13.33	0.41	1.27	
4	*cis*-ocimene	$C_{10}H_{22}$	142	71	13.56	0.45	8.46	
5	unknown	$C_{20}H_{34}O_6$	370		16.28	0.46	0.5	
6	unknown				22.22	0.47	1.27	
7	*trans*-caryophyllene	$C_{15}H_{24}$	204	58	22.82	0.53	1.05	
8	*b*-cubebene	$C_{15}H_{24}$	204	121	23.59	0.54	6.27	
9	*a*-cadinene	$C_{15}H_{24}$	204	161	23.86	0.56	1.84	
10	unknown				24.71	0.57	0.54	
11	(*z*)-*b*-farnesene	$C_{15}H_{24}$	204	57	25.58	0.58	37.21	
12	(-)-calarene	$C_{15}H_{24}$	204	161	26.36	0.59	3.83	
13	unknown				26.70	0.61	0.78	
14	viridiflorol	$C_{15}H_{26}O$	222	57	27.01	0.63	2.70	
15	*d*-cadinene	$C_{15}H_{24}$	204	57	27.25	0.67	1.04	
16	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1à,4aà,8aà)	$C_{16}H_{26}$	240	71	27.79	0.68	1.40	
17	*a*-cedrene	$C_{15}H_{32}O$	240	57	28.13	0.69	1.65	
18	3*α*-methoxy-11-oxa-5-preganae	$C_{22}H_{36}O2$	320	57	28.77	0.70	0.68	

19	unknown				29.49	0.73	0.96	
20	2-Acetyl-8-bromo-5-methoxy-6-methylbenzo(1,2-B:5,4-B')difuran	$C_{14}H_{11}BrO_5$	322	58	29.83	0.75	0.87	
21	9,10-bis(trifluoromethylthio) anthracene	$C_{16}H_8F_6S_2$	378	149	30.61	0.76	2.06	
22	vellerdiol	$C_{15}H_{24}O_2$	236	57	31.27	0.77	2.22	
23	Unknown				34.38	0.78	0.73	
24	1-Hexadecanol	$C_{16}H_{34}O$	242	71	35.29	0.80	7.79	
25	9,12-Octadecadien-1-ol	$C_{18}H_{34}O$	266	69	38.62	1	1.54	
26	3,7,11,15-tetramethyl-Hexadec-2-ene-1-ol,	$C_{20}H_{40}O$	296	74	39.63	1.2	1.50	

*Rrt = retention time relative to 9,12-Octadecadien-1-ol = 1

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 subitilis* and *Staphylococcus aureus), gram negative* bacteria strains *(Escherichia coli* and *Pseudomonas aeruginosa),* fungi **(***Asperigillus niger*, *Penicillium digilatum*, *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. (121ºC).

Potato- dextrose agar growth medium (PDA), yeast extract peptone dextrose medium (YEPD 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 37ºC for 24 hours, while those containing yeast and fungi were incubated at 28ºC 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 oedema 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 caliber[16,17] 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 administered[19,20]. 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 method[21]. 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

Table 2: Components of the volatile oil of C. frutescens as detected by GC/MS analysis.

Edema rate (%)				
Group	1 hour	2 hour	3 hour	4 hour
Control	59.83±2.04	102.18±8.07	101.41±6.09	100.58±4.22
Non –polar (160 mg/ kg)	34.28±2.70[a]	66.38±6.83[a]	75.65±5.99[a]	75.58±4.91[a]
Polar (500 mg /kg)	50.03±3.27	95.09±7.07	99.27±5.95	101.59±4.76
Volatile oil (160 mg/kg)	37.1±3.22	49.0±2.42	49.2±2.62	41.4±2.33[a]
Indomethacin (20 mg/kg)	13.07±1.34[a]	25.71±2.97[a]	33.45±1.87[a]	38.19±2.47
Inhibition rate (%)				
Group	1 hour	2 hour	3 hour	4 hour
Non-polar (160 mg/kg)	42.71	36.64	25.42	24.85
Polar (500 mg/kg)	16.39	6.94	2.11	1.00
Volatile oil (160 mg/kg)	38.00	52.05	51.48	58.84
Indomethacin (20 mg/kg)	78.15	74.84	67.02	62.03

[a] P<0.05: Statistically significant from control (Dunnett's test).

DPPH and the absorbance is measured afterwards at 515 nm. The antioxidant activity was expressed as IC_{50}, 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 tesFted using one-way ANOVA followed by the least significant difference (L.S.D). Methods of statistical analysis were done according to[22].

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* (inhibion zone 10 mm), *Escherichia coli* (inhibition zone 9 mm), *Pseudomonus 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 hrs, (101.41 ± 6.09%) 3 hrs, (100.58 ± 4.22%) 4hrs 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%, 36.64%, 25.42% and 24.85% at 1, 2, 3 and 4 hrs (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 hrs (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 IC_{50} value 40 µg/mL, while catechin (as standard) showed IC_{50} 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 data[23]. 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 unoxygenated, including sesquiterpene hydrocabons (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

compounds were (Z)-l-farnesene (37.21%), l-myrecene (9.78%), (Z)-β-ocimene (8.46%), 1-hexadecanol (7.79%), cubebene (6.27%), viridiflorol (2.7%).

Phytochemical 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 campesterol, stigmasterol and □-sitosterol inhibits carrageenan paw oedema in mice[24]. Meanwhile, scopoletin is reported to inhibit eicosanoid-release from ionophore-stimulated mouse peritoneal macrophages[25] and inhibits the production of inflammatory cytokines through inhibition of the IκB/NF-κB signal cascade in the human mast cell line HMC-1[26]. Therefore, stigmasterol and scopoletin are believed to be the major anti-inflammatory constituents of the non-polar extract of *Chrysanthemum frutescens* L.

Phytochemical investigation of the bioactive polar extract

The aqueous methanolic polar extract of the aerial parts of *C. frutescens* scavenged DPPH free radicals in a dose dependant manner with IC_{50} value of 40 µg/ml. The radical scavenging activity of the methanolic extract of *C. frutescens* can be attributed to its content of phenolic compounds. The aqueous methanolic extract of *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 -di-*o*-caffeoylquinic acid (IC_{50}=16.18 µg/ml), methyl 4,5-di-*o*-caffeoyl quinate (IC_{50}=13.25 µg/ml), 1,5-dicaffeoyl quinic acid (IC_{50}=42.9 µg/ml), methyl 1,5-dicaffeoyl quinate (IC_{50}=77.00 µg/ml) and apigenin7-o-glucopyranoside (IC_{50}=78.00 µg/ml), while the last compound mellilotoside had no antioxidant activity (IC_{50}>200 µg/ml).

DISCUSSION

Biological investigation of the aerial parts of *C. frutescens* was conducted to investigate its anti-inflammatory and analgesic activities using the carrageenan induced hind rat paw oedema and acetic acid induced writhing models, respectively. The results revealed remarkable and significant anti-inflammatory activity of the non-polar extract of *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[24-26]. Moreover, the polar extract of *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 *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 -di-*o*-caffeoyl quinic acid (IC_{50}=16.18 µg/ml), methyl 4,5-di-*o*-caffeoyl quinate (IC_{50}=13.25 µg/ml), 1,5-di-*o*-caffeoyl quinic acid (IC_{50}=42.9 µg/ml), methyl 1,5-di-*o*-caffeoyl quinate (IC_{50}=77.00 µg/ml) and apigenin7-*o*-glucopyranoside (IC_{50}=78.00 µg/ml) (12-14). The volatile oil was also tested for its antimicrobial activity and the results revealed that it inhibited the growth of *Staphylococcus aureus* (inhibition zone 12 mm) as well as, *Bacillus subtilis* (inhibion zone 10 mm), *Escherichia coli* (inhibition zone 9 mm), *Pseudomonas aeruginosa* (inhibition zone 7 mm) and *Candida albicans.* GC/MS analysis of the volatile oil revealed the presence of (Z)-l-farnesene (37.21%), l-myrecene (9.78%), and (Z)-β-ocimene (8.46%) (23). Reviewing the available current literature, nothing was previously reported concerning the volatile content of *Chrysanthemum frutescens* L. Major compounds of the prepared volatile oil possess antimicrobial activity as previously reported in the literature[27]. All the former results promote the advantage of the use of *Chrysanthemum frutescens* as a whole fresh herb without fractionation, if it is advised to be used, as a treatment of inflammatory conditions.

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