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Research Paper

Chemical composition and anti-inflammatory activity of *Asteriscus graveolens* essential oils from south-western Algeria

BENAISSA KEDDAR Youcef[1]*, MEGHERBI Aicha[1], SAID Mohammed El-Amin[1], BENYAMINA Abdelfettah[1], TOUMI Fouzia[1] and BETTAYEB Zouaouia[1]
1Laboratory of Ecodevelopment of Spaces, Faculty of Natural and Life Sciences, University of Djillali-Liabès, DZ-22000 Sidi-Bel-Abbès, ALGERIA

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Abstract

The objective of this work is the study of the chemical compositions, the acute toxicity and the anti-inflammatory activity of the essential oils (EOs) of the leaves and flowers from *Asteriscus graveolens*, collected in the south-west of Algeria. The chemical composition of EOs was established by gas chromatography (GC) and gas chromatography / mass spectrometry (GC/MS). The acute toxicity was assessed using Lorke's approach in Animal Models. The anti-inflammatory test was performed using an animal model and determined by carrageenan induced paw oedema. Thirty-four compounds were identified, accounting for 98.7 and 99.25% of the total EOs of leaves and flowers respectively. The major chemical components characterizing the EOs were oxocyclonerolidol, *cis*-chrysanthenyl acetate and *cis*-acetoxychrysanthenyl acetate, but the percentage of each compound varied depending on the EOs. The results obtained from the anti-inflammatory test showed that the EOs have a very high anti-inflammatory power with a dose dependent effect.

Keywords: *Asteriscus graveolens*, essential oil, chemical composition, acute toxicity, anti-inflammatory.

Introduction

Asteriscus graveolens (*AG*), commonly known as "Taffs" is an endemic medicinal plant widely used by the local population in south-western Algeria[1]. Belonging to the Asteraceae family, it is a perennial plant with a strong odor as a shrub that can grow up to 60 cm[2]. It grows in arid and semi-arid climates, stretching from northern Africa to the Central Asian desert, but it is widely represented in southwest Algeria and southeast Morocco[4]**.** In folk medicine, *AG* is used for various diseases such as fever, gastrointestinal tract complaints, headache and bronchitis. Several studies have shown that the plant has an anti-inflammatory[3], an antibacterial[5,6] and an antifungal effect[2,7]. Furthermore, *AG* EO exhibits different biological activity such as antioxidant, antibacterial, antifungal, hemolytic, anti-cancerous activity and anticorrosion activities[2,5,12].

To our knowledge, eight articles deal with the chemical composition of the EOs of this plant in the literature. The first one described the flowering part of *AG* from Sinai (Egypt)[9]. Three articles deal with plants from southeast Morocco[2,3,8]. And four articles deal with aerial part of plant in Algeria[1,4,5,11]. In these articles, the EO of aerial part (flowers and leaves) of *AG* is characterized by a high content of sesquiterpene compounds such as oxocyclonerolidol, *cis*-chrysanthenyl acetate and *cis*-*acetoxychrysanthenyl* acetate which are described in other species of Asteriscus and can be considered from a chemo-taxonomic point of view as markers of the genus[26]**.** All previously cited articles dealing with biological activities and chemical composition didn't find any study dealing with *in-*

vivo tests especially anti-inflammatory activity of *AG* EOs. Thus, the aim of this study is the chemical characterization of leaves and flowers of *AG* EOs, their acute toxicity and anti-inflammatory activity.

Materials and Methods

Plant material

AG leaves and flowers were collected from the region of Bechar (latitude: 31° 54′ 59″ N; longitude: 2° 18′ 0″ W; altitude: 870 m) during the flowering stage. Specimens were then dried in the open air for 15 days and stored in a cool place before extraction. A voucher specimen is deposited at the Herbarium of the University of Sidi Bel Abbes (Algeria).

Essential Oil Extraction

Dried leaves and flowers were subjected to hydrodistillation for 3 h using a Clevenger apparatus. The EO yields were 0.17 and 0.45 (% w/w) for leaves and flowers EOs respectively. The EOs obtained were dried over anhydrous sodium sulfate and stored in a sealed vial in the dark at 4° before analysis.

Gas chromatography (GC) / mass spectrometry (GC/ MS) analysis

GC analyzes were performed on a 7890A GC (Agilent Technologies) system with a flame ionization detector (FID) equipped with an HP5 capillary column (30 x 0.25 mm, film thickness 0.25 µm). Experimental conditions were: oven temperature 2 min at 80°C, then 80°C to 200°C (5°C / min), then 200°C to 260°C (20°C /min), and held at final temperature for 5 min. Injector and detector temperatures were set at 250°C. Hydrogen was the carrier gas at a flow rate of 1.2 ml/min. One µL of diluted EO (0.05 g in 1.5 ml of CH_2Cl_2) was injected. Linear retention indices were calculated with reference to n-alkanes (C_8–C_{28}). The identification of the compounds was based on the comparison of their retention indices with those of authentic samples in literature[13].

GC/MS analysis of the EO was carried out on an Agilent Technologies GC instrument equipped with a GC 7890A gas chromatograph system, an MS 5975C VL MSD mass spectrometer detector, and an HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm). The data acquisition and processing were performed using the MSD Chemstation E.01.01.335 (Agilent) software. One µL of diluted EO (0.05 g in 1.5 ml of CH_2Cl_2) was injected. The experimental conditions were: solvent delay, 2 min, column temperature program, 2 min at 80°C, then 80 to 200°C (5°C/min), then 200 to 260°C (20°C/min), and held at the final temperature for 5 min, temperature injector (split ratio 60) and detector were 250°C, carrier gas was helium at a flow rate of 1.2 ml/min, ionization voltage 70 eV, electron multiplier, 1 kV. The identification of the components was based on the comparison of their mass spectra with those of the Wiley and NIST libraries.

Animals and products

The study of acute toxicity and anti-inflammatory activity was carried out on mice (BALB/C) from the breeding of Ecodevelopment of Spaces Laboratory (Djilali Liabes University of Sidi Bel Abbes). Mice are of sexes, aged 05 to 06 months and weigh 30 to 40 g, they are housed, fed and kept under normal conditions with a light/dark cycle (12 h/12 h). 14 hours before experiments, food was withheld, but animals had free access to drinking water. The EOs were suspended in a vehicle (Tween 80, 0.9% in saline). The intraperitoneal injection (IP) volume is 10 mL/Kg of the animal's body weight as recommended by the American Association for laboratory animal science[14,15].

Acute toxicity study

The toxicity of our oils was determined according to according to Lorke's approach[16]. In the first step of this method, it is necessary to determine the approximate extent of toxicity. This last was obtained by forming three batches of three mice with each batch receives IP doses of 10, 100, 1000 mg/Kg body weight respectively. The animals were observed during the two hours following the administration of the EOs and symptoms of toxicity were noted. The number of mortalities was recorded after 24H and this will be used to complete the next step of the test. The second step consists of administering specific doses chosen according to the number of deaths in the first phase as shown in Table 1.

Table 1: Doses required according to Lorke's approach in mice[16]

First step doses (mg/kg body weight)			Second step doses (mg/kg body weight)			
10	**100**	**1000**				
0/3[a]	0/3	0/3	/	1600	2900	5000
0/3	0/3	1/3	600	1000	1600	2900
0/3	0/3	2/3	200	400	800	1600
0/3	0/3	3/3	140	225	370	600
0/3	1/3	3/3	50	100	200	400
0/3	2/3	3/3	20	40	80	160
0/3	3/3	3/3	15	25	40	60
1/3	3/3	3/3	5	10	20	40
2/3	3/3	3/3	2	4	8	16
3/3	3/3	3/3	1	2	4	8

[a]: Number of animals died/number of animals used

In the second step, only three animals were used which was divided into three groups, one animal each and the mortalities are noted after 24H. The number of mortalities, toxic effects and/or changes in general behavior were noted after 24 hours. The test is completed by determining the lethal dose for 50% of the animals (LD_{50}) using the following formula:

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

D_0 = Highest dose that gave no mortality, D_{100} = Lowest dose that produced mortality.

The acute toxicity experiments were performed according to the ethical principles of the European Union Directive (2010/63/EU) on the protection of animals used for scientific experiments.

Anti-inflammatory activity

The test used is based on the induction of carrageenan-induced of rats or mice paw oedema. It is a simple and common animal model. It is widely used for the evaluation of this activity without injury or damage to the inflamed paw[17,22]. It is used to test new anti-inflammatory drugs and the mechanisms involved in inflammation. Approximately 400 articles in the literature derived from anti-inflammatory activities use this model[23].

The animals were randomized into groups of five. Group I (control) was treated with saline, Group II with diclofenac sodium (10 mg/kg), the remainder of Groups III, IV and V were treated with EO from the flowers and leaves of the plant at 50, 100 and 150 mg/kg respectively. After 30 minutes of intraperitoneal administration of the treatments, 50 µl of carrageenan (1% in 0.9% NaCl) was injected subcutaneously into the sub-plant tissue of the right hind leg of each mouse. The injection site was near the centre of the plantar region, an important point compared to mice, which did not have a well-defined padding region on the underside of the hind leg. The diameters of the legs were measured prior to the injection of carrageenan and again at 1, 2, 3, 4 and 5 hours after injection. Leg thickness was measured in the dorsal-plantar axis at the metatarsal level using a caliper. The measurement point was marked on the top of the foot with an indelible pen to serve as a reference point for subsequent measurements.

The mean leg swelling in treated animals was compared to that of the control and the percentage inhibition (anti-inflammatory activity) of edema was determined using the following formula[24].

$$\% \text{ inhibition} = [(A-B)/A] \times 100$$

Of which **A** represented the volume of edema in the control group and **B** the volume of leg edema in the treated groups.

Statistical analysis

One-way ANOVA analysis followed by Tukey's multiple comparisons procedure was used to determine significant differences between both samples of *AG* and standards analyzed. All determinations were performed in triplicate, in three separate experiments and the results are expressed as mean ± SD. All data were performed using the statistical software Minitab 18.

Results and Discussion

Chemical composition of essential oils

The chemical composition of *AG* EOs (leaves and flowers) is represented in table 2, the analyses carried out using GC-MS identified 34 compounds, 98.7% in the leaves and 99.25% in the flowers of to the total oil composition. The oils obtained from the leaves were characterized by three major compounds: oxocyclonerolidol (68.16%) followed by 6-hydroxycyclonerolidol (5.96%) and *cis*-chrysanthenyl acetate (5.83%). On the other hand, flower's EOs has a high content of *cis*-chrysanthenyl acetate (37.33%) followed by *cis*-acetoxychrysanthenyl acetate (31.52%), then oxocyclonerolidol (18.48%).

Table 2: Chemical composition of *AG* EO (leaves and flowers) from south-western Algeria

Compound	RI[a]	Leaves	Flowers	Compound	RI[a]	Leaves	Flowers
α-Pinene	925	0.66	4.7	β-Caryophyllene	1432	0.14	0.14
Camphene	945	0.27	0.1	*α*- Caryophyllene	1467	1.77	0.2
Sabinene	974	0.25	0.12	Alloaromadendrene	1474	0.25	0.1
6-Methyl-5-hepten-2-one	981	0.16	0.26	Germacrene D	1491	0.27	0.11
Myrcene	985	0.16	0.27	γ- Cadinene	1527	0.23	0.16
α-Phellandrene	1013	0.15	0.13	δ- Cadinene	1534	0.53	0.2
p-Cymene	1026	0.68	0.11	Oxocyclonerolidol	1578	68.16	18.48
Limonene	1034	0.52	0.11	*cis*-*Acetoxychrysanthenyl* acetate	1599	2.89	31.52
γ-Terpinene	1058	0.28	Tr	Caryophyllene oxide	1605	0.31	0.27
α-Terpinolene	1091	0.11	Tr	Humulene epoxide II	1623	0.42	0.11
α-Thujone	1107	1.93	0.18	6-Hydroxycyclonerolidol	1650	5.96	0.1
β-Thujone	1115	0.14	0.19	Ʈ-Cadinol	1654	2.15	2.93
α-Campholene aldehyde	1140	0.36	Tr	β-Eudesmol	1665	1.72	Tr
Camphor	1150	0.21	0.34	α-Cadinol	1667	0.34	0.21
Terpinen-4-ol	1183	0.10	0.12	Intermedeol	1675	0.32	0.31
Myrtenol	1212	Tr	0.24	Bisabolene oxide	1727	0.31	0.1
cis-Chrysanthenyl acetate	1272	5.83	37.33	**Total identified**		98.7	99.25
Bornyl acetate	1293	0.12	0.11				

[a]RI: Retention indices on HP-5 capillary column. Tr: Trace.

The chemical composition of our EOs is in agreement with those previously reported by the bibliography where the major compounds of our oils are oxocyclonerolidol, *cis*-chrysanthenyl acetate and *cis*-acetoxychrysanthenyl acetate according to the results reported by Saïd *et al.* and Cristofari *et al.*[3,4]. On the other hand, the results obtained by Cheriti *et al.* are very different from our results despite the fact that the samples are of the same geographical origin[1].

The results of the chemical characterization of the EOs of the flowers of our plant confirm the bibliographical data brought by Aouissi *et al*. which consists that the majority compounds of the EOs of the flowers of *AG* are *cis*-chrysanthenyl acetate and *cis*-8-acetoxychrysanthenyl acetate[11]. On the other hand, our results of chemical analysis of the EOs of the flowers and leaves of the plant are totally different from the results obtained by Fahmy and Faiza Chaib *et al.* with the exception of *cis*-chrysanthenyl acetate which is a common majority compound of our oils and those of Faiza Chaib *et al.* [5,9]**.** Oxygenated sesquiterpenes can be considered as a marker of the genus. This is the case of the results obtained by the chemical analysis of our oils and those of Znini *et al.* and Cristofari *et al.* [2,3,8].

Acute Toxicity

In vivo, acute toxicity test for *AG* EOs were estimated in mice using Lorke's test. The results of the first step which determines the approximate toxicity after the administration of the 10mg/Kg, 100mg/Kg, 1000mg/Kg doses are presented in table 3.

Table 3: Results of the first step of the toxicity test of *AG* EOs.

Step 1			
Dose (mg/kg)	10	100	1000
Number of mortalities after 24 h			
Leaves EO	0/3	0/3	1/3
Flowers EO	0/3	0/3	1/3

Intraperitoneal administration of *AG* EOs at doses of 10 and 100 mg/Kg had no apparent effect on animals behaviour (no animal mortality was reported for leaves and flowers after 24H), which means that these doses are not toxic for mice. On the other hand, the administration of the 1000mg/Kg dose caused a change in behavior and the appearance of some signs of toxicity after about 15min of injection. Initially, hyperactivity, abdominal contortions and irritation were noted and then sedation was noticed, the mobility of the animals gradually decreased to the point of an inability to move. Other symptoms were observed after almost 18H of injection, malaise, anorexia (loss or decrease of appetite) and tachycardia (increased heart rate). One animal out of three had a lowered heart rate (bradycardia) and finally died after about 24 hours. The survivors resumed their normal behavior and all symptoms disappeared after 48 hours.

From the first phase of the test we were able to determine the doses to be administered in the second phase according to Lorke's protocol (table 1 materials and methods). The results of the second stage are presented in (table 4).

Table 4: Results of the second step of the toxicity test

Step 2					LD_{50} (mg/kg)
Dose (mg/kg)	**600**	**1000**	**1600**	**2900**	
Number of mortalities after 24 h					
Leaves EO	0/1	1/1	1/1	1/1	774.60
Flowers EO	0/1	0/1	1/1	1/1	1264.91

The LD_{50} (lethal dose 50 or median lethal dose) is a quantitative indicator of toxicity. The second step of the Lorke's test allowed us to determine the LD_{50} of the leaves which is equal to 774.60 by the calculation of the geometric mean between the 600 and 1000 mg/Kg dose, and the LD_{50} of flowers which is equal to 1264.91 resulting from the calculation of the geometric mean between the 1000 and 2900 mg/Kg dose.

The acute toxicity study by the IP route shows an LD_{50} of 774.60 mg/Kg and 1264.91 mg/Kg for the leaves and flowers EOs of *AG* respectively, which are relatively high values, showing a low toxicity of these EOs. According to the toxicity scale established by Hodge and Sterner, the LD_{50} of our oils are classified at level four, which corresponds to slightly toxic substances[25]. To our knowledge, there is no study of this plant toxicity. On the other hand, the aerial part (flowers and leaves) is rich in EOs and characterized especially by a high content of oxygenated terpene compounds such as chrysanthenyl acetate and oxocyclonerolidol. These are described in other species of *Asteriscus* and can be considered taxonomically as markers of the genus[4,26], thus assuming that the toxicity may be due to the major compounds or has a synergistic effect between several compounds of the EO.

Anti-inflammatory activity

The difference in diameter between the healthy leg (before carrageenan injection) and the inflamed leg (1, 2, 3, 4 and 5 hours after carrageenan injection) was calculated and classified in Table 5, which represents the volumes of oedema.

Table 5: Effects of leaves and flowers EOs of AG on the evolution of the diameter of plantar oedema in mice

	Dose (mg/kg)	1 hour	2 hours	3 hours	4 hours	5 hours
Witness	**/**	0.910±0.050	0.893±0.038	0.915±0.029	0.923±0.025	0.953±0.061
Diclofénac	**10**	0.524±0.048**	0.362±0.048**	0.346±0.079**	0.456±0.033**	0.490±0.024**
Leaves	**50**	0.500±0.047**	0.460±0.060**	0.350±0.030**	0.290±0.072**	0.260±0.075**
	100	0.492±0.040**	0.350±0.077**	0.300±0.057**	0.240±0.068**	0.200±0.080**
	150	0.380±0.072**	0.310±0.060**	0.210±0.036**	0.140±0.052**	0.140±0.057**

	Dose (mg/kg)	1 hour	2 hours	3 hours	4 hours	5 hours
Flowers	**50**	0.380±0.097**	0.261±0.047**	0.259±0.062**	0.260±0.098**	0.302±0.075**
	100	0.230±0.045**	0.220±0.075**	0.230±0.097**	0.200±0.063**	0.300±0.072**
	150	0.150±0.062**	0.090±0.062**	0.080±0.071**	0.120±0.055**	0.160±0.040**

Asterisks indicate statistically significant differences from the control. *P< 0.05, **P< 0.001.

The injection of carrageenan into mice feet of the control batch resulted in oedema, which reached a maximum of 0.953 mm in the fifth hour. IP administration of diclofenac and EOs from both parts of the plant reduced the oedema very significantly during the five hours of the test. The reduction of oedema was recorded for the leaves EO in a dose-dependent and chronological manner with a maximum effect in the fourth and fifth hour (0.140mm). The same think are observed in the flowers EO with a maximum oedema reduction in third hour (0.080mm). These results reveal that the EOs have an anti-inflammatory effect. This effect is confirmed with the percentage of oedema inhibition which was calculated and illustrated in figure 1.

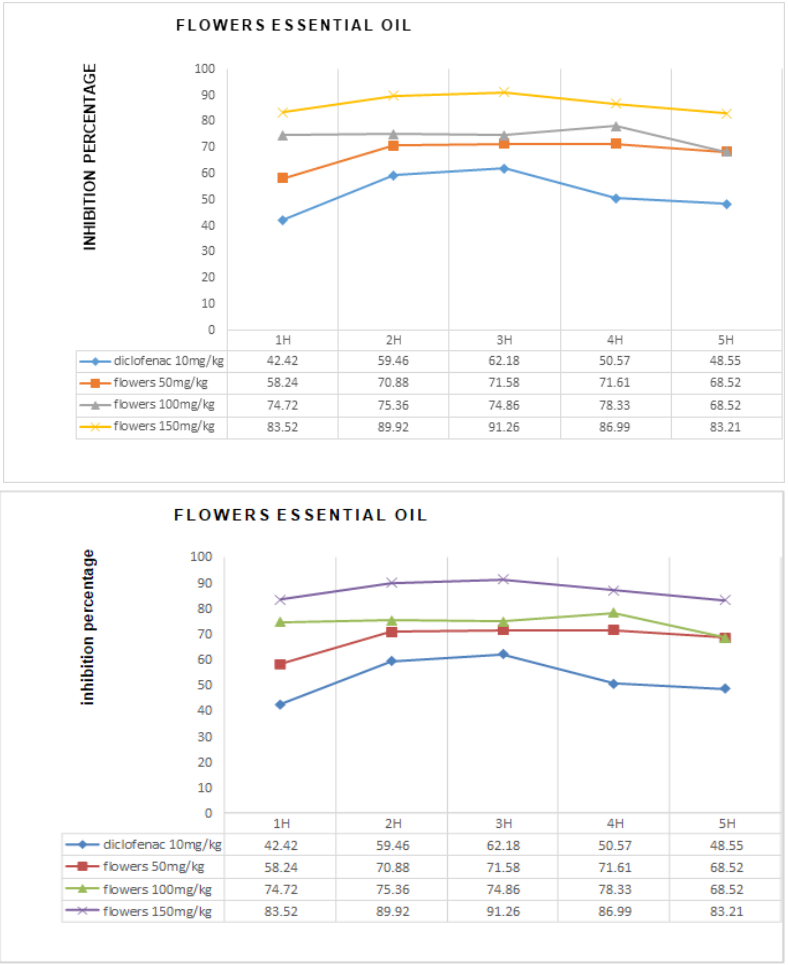

Figure 1: Percentage of edema inhibition by EOs of the leaves and flowers of *AG*

The results of the percentage of inhibition of oedema are very significantly (P<0.001) for both organs. For the leaves EO, the inhibition is dose dependent and higher than the standard even at the lowest dose from the third hour. The maximum inhibitory effect is provided by the dose of 150 mg/kg at the fifth hour (85.3%). The flowers EO also show a dose dependent effect and a higher inhibition than the standard for the three doses but it reaches its maximum at the third hour at a dose of 150 mg/kg (91.26%).

During the inflammation process several symptoms appear (redness and warmth which are due to increased blood flow and capillary dilation, swelling or oedema is caused by an accumulation of fluid, pain is due to the release of chemicals that simulate nerve endings). Inflammation is subdivided into three phases: the initial phase around the first hour involves the release of histamine, serotonin and

bradykinin, which promote vasodilation, plasma transudation and oedema[27]. The second phase uses kinins as mediators to increase vascular permeability and the third phase is believed to be mediated by prostaglandin[28] and it is sensitive to steroidal and non-steroidal anti-inflammatory agents[29,30]**.**

In our tests, Diclofenac Injection (a non-steroidal anti-inflammatory drug derived from phenylacetic acid) is used as the reference substance. It is administered IP in injectable form. The results obtained from anti-inflammatory tests show that the leaves and flowers EOs of *AG* have an effect on the three phases of inflammation and very significantly reduce the oedema induced by carrageenan with a good dose-effect relationship (P<0.001). The better inhibition is obtained with the maximum dose which is 150 mg/Kg at the third hour for the flowers and the fifth hour for the leaves. The action kinetics of our oils is superior to that of the reference treatment.

The effects of our oils manifest with the same anti-inflammatory mechanism due to diclofenac from the first hour of experimentation. This means that the flowers and leaves EOs of our plant contains anti-inflammatory substances whose action mechanism is probably related to the inhibition of prostaglandin synthesis by blocking COX enzymes[31] and also the important efficacy could be related to the chemical profile of the oils[32]. The results obtained from the strong anti-inflammatory power confirm the traditional use of this plant to the inflammatory diseases.

Conclusion

The results obtained by chromatographic techniques allowed us to identify a total of 34 compounds from the leaves and flowers of *AG*. *cis*-chrrsanthenyl acetate, oxocyclonerolidol and *cis*-acetoxychrysanthenyl acetate are the majority compounds in the essential oil of this plant. The study of the acute toxicity shows that the essential oils of two aerial parts are not toxic for the doses used in the experiment without excluding symptoms that disappear after a certain time. Essential oils from both organs of the plant confer a strong anti-inflammatory power with a dose-dependent and superior effect to the reference treatment. These results justify the traditional use of *AG*. However, pharmacodynamic and phytochemical studies must be carried out to establish the oil actions mechanism of this plant and identify the active ingredients responsible for the anti-inflammatory effect.

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