

CHEMICAL COMPOSITION AND ANTI-INFLAMMATORY ACTIVITY OF *Myrtus communis* L. ESSENTIAL OIL

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Abstract.- *The aim of this study is to evaluate the chemical composition and the anti-inflammatory activity of the common myrtle essential oil (Myrtus communis L.). The essential oil composition of Myrtus communis L. extracted by steam distillation, was characterized by a high oxygenated monoterpene fraction (80.9%). The major components were myrtenyl acetate (38.7%), 1,8-cineole (12.7%), α -pinene (13.7%) and linalool (7.00%). For the anti-inflammatory essay, five different groups were established and the essential oil was administered orally in three different doses. The common myrtle essential oil (100 mg/kg) was able to significantly reduce the paw edema with a comparable effect to that observed with Diclofenac (positive control). This is the first report to demonstrate a significant anti-inflammatory activity of Algerian common myrtle essential oil*

Key words: *Myrtus communis L., essential oil, composition, anti-inflammatory activity*

COMPOSITION CHIMIQUE ET ACTIVITE ANTI-INFLAMMATOIRE DE L'HUILE ESSENTIELLE DE L'ESPECE *Myrtus communis* L.

Résumé.- *L'objectif de cette étude consiste à évaluer la composition chimique et l'activité anti-inflammatoire de l'huile essentielle de myrte commun (Myrtus communis L.). La composition de l'huile essentielle de Myrtus communis L. extraite par distillation à la vapeur, a été caractérisée par une fraction élevée de monoterpènes oxygénés (80,9%). Les principaux composants sont l'acétate de myrtényle (38,7%), 1,8-cinéole (12,7%), α -pinène (13,7%) et le linalool (7,00%). Pour le test anti-inflammatoire, cinq groupes différents ont été mis en place et l'huile essentielle a été administrée par voie orale en trois doses différentes. L'huile essentielle de myrte commun (100 mg / kg) a été en mesure de réduire significativement l'œdème de la patte avec un effet comparable à celle observée avec le Diclofénaç (contrôle positif). Ce rapport est le premier à démontrer une activité anti-inflammatoire significative d'huile essentielle de myrte commun algérien.*

Mots clés: *Myrtus communis L., huile essentielle, la composition, l'activité anti-inflammatoire*

Introduction

Commercially available anti-inflammatory drugs exert a wide range of side effects and are either too potent or too weak. Consequently, the search for new anti-inflammatory compounds has been a priority for the pharmaceutical industry. Medicinal plants continue to be an important source of new chemical substances with potential therapeutic effects [1].

Many natural products have been tested in various animal models for the development of new anti-inflammatory agents. Plant essential oils (EO) are used as folk medicines against different inflammatory diseases. Some of them have been scientifically shown to possess medicinal properties, including anti-inflammatory activities [2].

In Algeria, the use of medicinal plants as anti-inflammatory drugs is a common practice, although in most cases the active principles of the plants are unknown. Therefore,

the study of plant species should still be seen as a logical research strategy, in search for new anti-inflammatory drugs. The variety of climatic and geographic conditions in Algeria provides a rich source of vegetation, comprising many species of plants. Among these plants, the common myrtle, which belongs to the Myrtaceae family, it is a medicinal plant endemic to the Mediterranean area and it has been used by locals for its culinary and medicinal properties since antiquity [3]. The common myrtle essential oil (CMEO) is extracted from the fresh leaves and stems of this plant by steam distillation. This essence is composed of various chemical constituents such as cineole, α -pinene and other oxygenated monoterpenes [4, 5].

The common myrtle is one of the most fragrant species and its essential oil is used in the perfumery and cosmetics industry [6, 7]. It is also used as a flavoring agent and as a spice. Further, the essential oil of this plant is non-toxic, non-irritant, generally non-sensitizing, and it is not known to cause any other side effects [8]. The therapeutic properties of this essence include being an antiseptic and wound-healing [9, 10]. It may also be one of the best oils for several dermatological problems such as dry or congested skins, eczema, and dermatitis [11].

Presently, there are no published scientific data to validate the popular claims of the anti-inflammatory effect of this plant. The purpose of the present study was to evaluate the anti-inflammatory activity of the CMEO. In addition, we identify of the various constituents of this essential oil analyzed by gas chromatography-mass spectrometry (GC-MS).

1.- Materials and methods

1.1.- Plant material and essential oil extraction

Aerial parts of common myrtle were collected in July 2013 during the flowering stage in Zaccar region (table I). The plant was identified as at the botanical laboratory of The National Institute of Agronomy (Algiers). The freshly-cut aerial parts were submitted to a steam distillation, using a *Clevenger* type apparatus during 3 hours. The essential oil obtained was dried over anhydrous sodium sulfate, filtered, and stored in dark glass bottles at +4°C.

Table I.- Geographical coordinates of the harvesting site

Area	Localisation	Altitude	Latitude	Longitude	Bioclimatic floor
Zaccar	Ain Defla	522 m	36°18' North	2°16' East	Semi-arid

1.2.- Gas Chromatography-Mass Spectrometry (GC/MS) analysis

The GC/MS analysis was performed on an HP 5972 mass spectrometer (Agilent technologies, California, USA) with electron impact ionization (70 eV). An HP-5MS capillary column (30 m, 0.25 mm coated with 5% phenyl methyl silicone, 95% dimethylpolysiloxane, 0.25 μ m film thickness) was used. Oven temperature was programmed to rise from 50 to 240°C at a rate of 5°C/min, transfer line temperature was 250°C. The carrier gas was Helium with a flow rate of 1.2 ml/min and a split ratio of 60:1. Scan time and mass range were 1 s and 40-300 m/z, respectively.

The identification of volatile components was assigned by comparison of their retention indices (RI) with those of literature or with those of authentic compounds available in the authors' laboratory. Further identification was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC/MS data system and other published mass spectra [12]. Determination of the percentage composition was based on peak area normalization without using correction factors.

1.3.- Experimental animals

Carrageenan-induced paw edema was carried out on male Swiss mice (25-30 g). Male animals were purchased from the laboratory of toxicology of Antibiotic Company (Medea, Algeria) and were housed in groups of six per standard cage, on a 12 h light/dark cycle with free access to food and water. They were acclimatized to laboratory conditions for at least 1 week before testing. The food was withdrawn on the day before the experiment, but free access to water was allowed. A minimum of six animals was used in each group.

1.4.- Determination of median lethal dose (LD₅₀)

LD₅₀ of the CMEO was estimated in mice by using the method of HILAN *et al.* (2009) [13]. In a preliminary test, animals in groups of three received 10, 100, or 1000 mg/kg of CMEO suspended in the vehicle (1% v/v Tween 80). Animals were observed for 24 h to register any signs of toxicity and number of deaths. The LD₅₀ was calculated as the geometric mean of the dose that resulted in 100% mortality and that which caused no deaths.

1.5.- Anti-inflammatory assay: Carrageenan-induced paw edema in mice

The anti-inflammatory activity was evaluated by the carrageenan-induced paw edema test [14]. Paw edema was induced by injecting 0.1 ml of the carrageenan 1% suspension in isotonic saline (w/v) into the sub-plantar region of the left hind paw of the mouse.

CMEO doses of 100, 200, or 400 mg/kg and vehicle (0.2% Tween 80 in 0.9% NaCl) were administered (0.5 ml per animal) orally (per os) 30 min before injection of the edematogenic agent to different groups of mice for each treatment ($n=6$ per group). Diclofenac sodium dissolved in 0.9% NaCl (50 mg/kg, oral) was used as a reference drug. Paw thickness was measured before the application of the inflammatory substance and every 30 min for 4 h after induction of inflammation. The difference in footpad thickness was measured by a gauge calliper (Facom, France).

Mean values of treated groups were compared with those of control group (vehicle) and analyzed statistically. The data obtained for the various groups are reported as means \pm standard deviation (SD) and expressed in mm. The percentage inhibition of the inflammatory reaction was determined for each animal by comparison to the controls and calculated by the formula:

$$I(\%) = \left[1 - \frac{\Delta(PV)t}{\Delta(PV)c} \right] \times 100$$

Where I (%) = percentage inhibition of edema, $(\Delta PV)_t$ = the change in paw volume in the treated mice, and $(\Delta PV)_c$ = the change in paw volume in the control mice.

1.6.- Statistical analysis

Results of the paw edema of the mice are reported as mean \pm SD. Comparison between groups was made by one-way analysis of variance (ANOVA). Differences with $P < 0.05$ were considered statistically significant. Statistical data analysis was determined using XLStats 2013 statistical software (Addinsoft, France).

2.- Results and discussion

2.1.- Chemical composition of the common myrtle essential oil

The EO was obtained by steam distillation from fresh aerial part of the common myrtle. The CMEO obtained is a yellowish-green liquid. It has a strong camphred odor.

The EO was obtained with a yield of 0.195% (v/w). It seems to be less than that reported by AYDIN and OZCAN (2007) [15]. It didn't vary significantly with seasons as reported by JAMOSSI *et al.* (2005) [16].

Essential oils are odorous and volatile compounds found only in 10% of the plant kingdom [17]. Essential oils and their components can be very promising biological agents, because of their relative safety, wide acceptance by consumers and exploitation for potential multipurpose use [18]. They are stored in plants in special brittle secretory structures, such as glands, secretory hairs, secretory ducts, secretory cavities or resin ducts [19-25].

The total essential oil content of plants is generally very low and rarely exceeds 1% by mass [26]. For example, the essential oil yields in leaf, stem and flower of *M. communis* were respectively 0.61%, 0.08% and 0.30% (w/w) [27]. Essential oils are hydrophobic and thus only slightly soluble in water. They are soluble in alcohol, non-polar or weakly polar solvents, waxes and oils. Most essential oils are colorless or pale yellow, liquid and have lower density than water [28, 29]. Essential oils are complex mixtures comprising many various compounds.

The data presented in table 2 demonstrated that CMEO is characterized by a high monoterpen percentage (80.9%) as the most important components. However, our results diverge from those published by other studies [30]. The main compounds identified in this essential oil were myrtenyl acetate (38.7%), 1,8-cineole (12.7%), α -pinene (13.7%), and linalool (7.00%).

The chemical profile of this sample has to be classified in the chemotype group of Myrtenyl acetate/ α -pinene proposed by BRADESI *et al.* (1997) [5]. However, the chemical composition of the CMEO differs from those reported in previous studies [31, 32, 33, 27, 34], it could be due to a number of factors, including the geo-climatic locations and growing conditions, including concentration of nutrients, temperature, humidity, soil type, day length, climate, altitude and the amount of available water. The chemical composition also depends on season or vegetative period of plant, i.e. before or after flowering [35-37].

Table II.- Chemical profile of the CMEO extracted by steam distillation
 [RI* (retention indices) were calculated on the HP-5 MS
 column relative to C7-C28 n-alkanes.]

	Compounds	R*	Content %
1	Isobutyl isobutyrate	898	3.00
2	α -thujene	936	0.30
3	α -Pinene	941	13.7
4	<i>B-pinene</i>	975	0.10
5	δ -3-carene	988	0.10
6	Myrcene	990	0.30
7	δ -terpinene	1050	0.10
8	Sabinene	970	0.50
9	Limonene	1025	Tr
10	E-ocimene	1038	0.10
11	A-terpinolene	1083	0.20
12	1,8-cineole	1027	12.7
13	Linalool	1098	7.00
14	Tras-pinocarveol	1131	0.20
15	Terpinen-4-ol	1171	0.20
16	A-terpineole	1186	1.80
17	Myrtenol	1191	3.50
18	Linalyl acetate	1258	2.50
19	Trans-pinocarveyl acetate	1297	0.70
20	Myrtenyl acetate	1322	38.7
21	P-menth-1-en-8-ol acetate	1347	0.39
22	Neryl acetate	1366	2.00
23	Geranyl acetate	1384	0.40
24	Trans-cryophyllene	1412	0.20
25	A-humulene	1447	0.31
26	Caryophyllene oxide	1576	0.50
27	Humulene epoxide	1588	1.30
<i>Monoterpene</i>			
Oxygenated monoterpene			70.1%
Monoterpene hydrocarbons			10.8%
<i>Sesquiterpene</i>			
Oxygenated sesquiterpene			4.49%
Sesquiterpene hydrocarbons			5.41%
<i>Total identified</i>			90.8%
<i>Oil yield</i>			0.195%

According to these factors, plant biosynthetic pathways can change the relative proportion of the primary oil components. These variations in chemical composition led to the notion of chemotypes, which are generally defined as a distinct population within the same species that produces different chemical profiles for a particular class of secondary metabolites [17].

There are different essential oil chemotypes which can distinguish myrtle oil of different origins, as well as seasonal variations throughout the vegetative cycle of plants [5, 38, 39, 30]. Thus, the same species of plant can produce a similar essential oil, but with different composition and therapeutic activities. Essential oil composition also depends on

the plant parts used for oil preparation.

2.2.- Determination of median lethal dose (LD₅₀)

The CMEO did not cause any mortality in the mice in doses up to 1000 mg/kg. Therefore, we suggest that oral LD₅₀ of the tested volatile oil is higher than 1000 mg/kg. Thus, this oil can be considered as highly safe. Myrtle oils were granted GRAS status (Generally Recognized As Safe) and approved by the US Food and Drug Administration (FDA) for food use [40].

2.3. Anti-inflammatory activity of the common myrtle essential oil

The anti-inflammatory effect of EO was evaluated in carrageenan-induced paw edema test in mice, an animal model widely employed to assess the anti-edematogenic effect of natural products. Carrageenan is commonly used as a phlogistic (inflammation-inducing) agent. The resulting signs and symptoms of inflammation can be measured as an increase in paw thickness due to the edema.

The anti-inflammatory effect of the CMEO was evaluated in the paw edema model ($n=6$ per group). As shown in Table 3, the oral administration of EO at doses of 100, 200 and 400 mg/kg resulted in approximately 30.71, 38.44 and 72.90% reduction in paw edema respectively. Furthermore, the inhibition of paw edema resulting from a 100 mg/kg EO dose was not significantly different from that of positive control (50 mg/kg) (72.90% vs 80.70% respectively). This is the first demonstration that oral administration of common myrtle essential oil produces significant anti-inflammatory effects.

Table III.- Effect of the CMEO on carrageenan-induced paw edema test in mice

Treatment	Dose (mg/kg)	Thickness of the left hind paw (mm), mean±SD	Inhibition of paw edema (%)
Negative control	20	3.3±0.21	–
Common myrtle essential oil	400	3.0±0.17	30.71
	200	2.88±0.14	38.44
	100	2.81±0.12	72.90
Positive control (Diclofenac)	50	2.76±0.10	80.70

Groups of mice were pre-treated with vehicle (control group, 20 mg/kg, $n = 6$), Diclofenac (50 mg/kg) or common myrtle essential oil at doses of 100, 200 and 400 mg/kg $n = 6$ /group, 30 min before carrageenan-induced paw edema.

Attempts have been made to identify the component(s) responsible for such bioactivities [41]. Some plant constituents, particularly alcohol terpenoids, have been reported to be useful in the management of inflammatory processes [41, 42]. Our results are in agreement with those reported in the literature for other EOs rich in monoterpenic alcohol and showing a very strong anti-inflammatory effect. Further, the major constituents of the oil, namely α pinene, myrtenyl acetate and 1,8 cineole, were previously shown to possess anti-inflammatory activities [43, 44].

Conclusion

The common myrtle essential oil is used as an antimicrobial agent in folk medicine as well as a food preservative. The results of our present study give strong impulsion to consider the CMEO as a potentially useful anti-inflammatory substance to prevent inflammatory skin diseases. In addition, the study of the chemical constituents of CMEO might accelerate the development of new and safe anti-inflammatory drugs.

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