Research Article

Anti-inflammatory activity and chemical compositions of essential oil of Achillea fragmmentissma

Eman Elsharkawy[1,2]

[1]Department of Environment and plant pasture, Desert Research Center, Mathef El-Mataria, Egypt.
[2]Department of Chemistry, Science Faculty, Girls section, Northern Border University-Arar, North Region, Saudi Arabia.

Correspondence to: Eman Elsharkawy, E-mail: elsharqawyeman@hotmail.com

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Abstract

Background: At present, there are considerable scientific evidences to suggest that nutritive and non-nutritive plant-based dietary factors can inhibit the process of carcinogenesis effectively. **Aims and Objective:** The aim of the present study was to use essential oils extracted by different methods from *Achillea fragrantissima* (hexane extract [HE] and oil extract [OE]) in the manufacture of soap, which can be used as anti-inflammatory agent. **Materials and Methods:** The anti-inflammatory effect of volatile oil was studied using carrageenan induced paw edema method at 100 mg/kg dose orally. Furthermore, it also studies the chemical composition of *Achillea* extracts by GC-MS. **Results:** This study demonstrated that extracts of *Achillea fragrantissima* have excellent anti-inflammatory properties. Of the two extracts analyzed by gas chromatography-mass spectrometry (GC-MS), there were differences in the chemical composition of essential oil of two extracts. Bisabolol, bisabolol oxide, menthol, and β-caryophyllene are the major constituents of the *Achillea* extract, some compounds were found in OE and not found in HE. **Conclusion:** Our observations supports *Achillea fragrantissima* which is used by many people in the northern region for medicinal uses, it is safe and it can be used as anti-inflammatory drug.

KEY WORDS: Anti-inflammatory; Natural Soap;

Introduction

Natural products once served humankind as the source of all drugs, and higher plants provided most of these therapeutic agents.

Plants provided most of these therapeutic agents. Now, natural product derivatives still represent over 50% of drugs in clinical use, with higher-plant-derived natural products representing 25% of the total.[1] The World Health Organization estimates that 80% of the people in developing countries of the world depend on traditional medicine for their primary health care, and about 85% of traditional medicines involve the use of plant extracts. This means that about 3.5 to 4 billion people in the world depend on plants as herbal drugs.[2]

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The genus *Achillea*, consisting of 140 perennial herbs, which is used traditionally in Middle Eastern countries, for treating digestive problems, diseases of liver and gallbladder, menstrual irregularities, cramps, fever, and for healing wounds.[3]

Achillea fragrantissima, family Asteraceae, is a common plant in the Mediterranean region and easily found growing in fields and on roadsides. It contains high percentage of flavonoids, tannins, volatile oils, sterols, triterpenes, unsaturated amides, and sesquiterpene lactones. *Achillea* was highly valued as a medicinal plant for its antiseptic properties. It was used to cover cuts and sores and hasten scar tissue formation; the clinical use of *Achillea fragrantissima* is not described.[4]

Achillea fragrantissima grows in dry areas, steppe, and desert. People collect it in the Badia region, where it is common. It is the last species of *Achillea* to flourish, it has

a strong smell and an astringent taste, for this reason it is usually mixed with sugar, but the flowers can be eaten alone, for diabetes, stomach (alone, or mixed with *Teucrium polium*), muscular rheumatism (fumigation), cough (drinking in the morning with sugar).[5]

Chemical analysis revealed that *Achillea* genus contains volatile oils, mainly azulene, it also contains several known anti-inflammatory compounds such as flavonoids.[6]

The anti-inflammatory effect of volatile oil extracted by hydrodistillation of *Achillea fragrantissima* was studied using carrageenan-induced paw edema. Essential oil (100 mg/kg and 200 mg/kg) was tested; the plant shows high inhibition at 200 mg/kg than 100 mg/kg after 4 h.[7]

Pervious review shows the importance of known medicinal plant used by many people in the northern region, the used to drink it with a tea (Alqausom in Arabic). *Achillea fragrantissima* is used by Bedouins in the treatment of fever and for wound healing, so the study of anti-inflammatory effect of the plant extract is important for discovering new drug. The aim of present study, are extraction of volatile oil by two different methods and, differentiate quantitative and quantitative between these methods we use the oil extract (OE) in manufacture of (soap) to used it as anti-inflammatory drug.

Material and Methods

Plant Material

The sample (*Achillea fragrantissima*) plant was collected from north region, Arar-turaif road, in spring season where the plant was flowering; plant sample was identified in the Botany Department, Faculty of Science, Northern Border University.

Preparation of Extracts

Oil extract: The plant sample was collected from north region, 0.6 kg of arial part of the plant was cleaned and grounded to a fine powder, percolated in 1000 mL of olive oil for 15 days. After 15 days, the mixture was filtered, then the filtrate was kept till used.

Hexane extract: The plant powder was filtered and solvent was evaporated under reduced pressure.

Analysis

The constituents of the volatile oils obtained from OE and HE were analyzed by gas chromatography-mass spectrometry (GC-MS) as reported by El-Shazly et al.[8] Compounds were identified by comparison of their retention indices (RI), (C9–C24 *n*-alkane mixture) and mass spectra as mentioned in literature.[9–12]

Anti-inflammatory: The method developed by Agbaje,[13] was employed. Albino Wistar rats of both sex (120–130 g) were randomized to four groups. Animals were deprived of food for 12 h before the experiment and only water was given ad-libitum.

- First group (control group) received 1 mL of distilled water (10 mL/kg)
- Second group received indomethacin (10 mg/kg) suspended in distilled water
- Third group received HE at a dose of 100 mg/kg orally dissolved in distilled water
- Fourth group received OE at a dose of 100 mg/kg orally dissolved in distilled water

After 1 h of administration of the treated extracts, carrageenan suspension (0.1 mL of 1% w/v suspended in 0.9% saline solution) was injected into the subplantar region of the left hind paw of the animals. Directly, the paw volume was measured; initial paw volume was measured using plethysmometer (UGO Basile, 21025 Comerio, Italy) before carrageenan injection. Thereafter, the paw volume was measured after 1, 2, 3, and 4 h after carrageenan administration. The difference between initial and subsequent readings gave the change in edema volume for the corresponding time. Edema volume of control (V_c) and volume of treated (V_t) were used to calculate percentage (%) inhibition and percentage edema volume using Eq. (1).

$$\% \text{ Inhibition} = [1-(V_t/V_c)] \times 100 \quad (1)$$

Statistical Analysis

Data were subjected to SPSS (ver. 8.0). $P < 0.05$ was regarded as significant.

Soap Manufacturing Process

The soap was produced from OE according to the method by Selinger.[14,15]

Soaps and detergents are widely used in our society, So, can use this soap for the treatment of skin inflammation. Soap is the product of reaction between a fat and sodium hydroxide:

$$\text{Fat} + 3\text{NaOH} \rightarrow \text{glycerine} + 3 \text{ soap}$$

Soap is produced industrially in four basic steps.

Step 1—Saponification

OE of *Achellia fragrantissima* is treated with sodium hydroxide (160 g) and the soap produced is the salt of a long-chain carboxylic acid.

Step 2—Glycerine removal

Glycerine is more valuable than soap, so most of it is removed. Some is left in the soap to help make it soft and smooth.

Step 3—Soap purification

Any remaining sodium hydroxide is neutralized with a weak acid such as citric acid and two-thirds of the remaining water removed.

Step 4—Finishing

Additives such as preservatives, color can added and mixed in with the soap then is shaped into cups.

Table 1: Chemical constituents of essential oil extracts of (*Achillea fragrantissima)* by GC-MS

Chemical compound	Structure	AF Hexane extract	AF Oil extract
Eucalyptol	C10H18O	0.28	–
Santolina Alchol	C10H18O	–––	0. 3
Camphor	C10H16O	4.8	––
Viridiflorol	C15H26O	2.95	–––
Oxazolidine	C3H7NO	0.93	–––
Benzyl alcohol	C7H8O	0.46	1.6
Limonene oxide	C10H16O	2.93	–––
Limonene	C10H16	2.9	1.62
Limonene dioxide	C10H16O2	–––	1.52
β-Caryophyllene	C15H24	2.0	0.87
Caryophllene oxide	C15H22O2	1.54	1.84
Globulol	C15H26O	6.51	––––
Thunbergol	C20H34O	8.95	––––
α-Selinene	C15H24	1.2	–––
α-Humulene	C15H24	0.28	0.67
Flacarinol	C17H24O	1.35	––
α-Phellandrene	C10H16	–	0.38
Thujol	C10H18O	0.39	–––
Thujone	C10H16O	–	0.30
Lanceol	C18H36O	0.86	0.86
Bergamotol	C15H24O	––	0.86
Cederne	C15H24	–	0.36
α-Cubebene	C15H24	–	0.36
α-Bisabolol	C15H26O	–	1.95
Geramcerene B		–	0.1
Azulene	C10H8	1.36	0.67
Spathulenol	C15H24O	–	0.08
Farnesol	C15H24O	–	0.27
Gerinol	C10H18 O	–	0.12
α-Tocopherol	C29H50 O	–	0.08
¥-Tocopherol	C29H50 O	–	0.08
Bisbolone epoxide	C15H24 O	–	1.54
Menthol	C10H20 O	1.35	1.5
Phorbol	C20H28O6	–	1.2
Isochiapin-B	C19H22O6	1.0	2.0
β-Sesquiphellandrene	C15H24	–	0.4
Coumarin	C9H6O2	–	0.9

Results

Anti-inflammatory study of two extracts of Achillea plant collected from north region and extracted by two methods, HE and OE, revealed that OE showed good result in anti-inflammatory assay by carrageenan method.

The extracts have a yellow color and a pleasant odor. Most of their components could be identified by GC-MS., by comparison (mass fragmentation and retention index) with literatures. The identified compounds are a mixture of oxygenated monoterpenes and sesquiterpenes, *A. fragrantissima* oil is rich with sesquisabinene hydrate, bisabolene epoxide, camphor, and caryophyllene oxide; also limonene, menthol, azulene, and thujone are found in moderate amount, while santolina alcohol, lanceol, cedrene, and granny geraniol are found in a minor amounts (Table 1). The essential oil in the OE differs from that in the HE; qualitatively some compounds such as azulene, alpha-bisabolol, and bisbolene epoxide hydrate are found in OE and not in HE, while oxazolidine, viridiflorol, camphor, and thujol are found only in HE; quantitatively some little difference found as in α-humulene and limonene increased in Hexane Extract (HE) than in Oil Extract (OE).

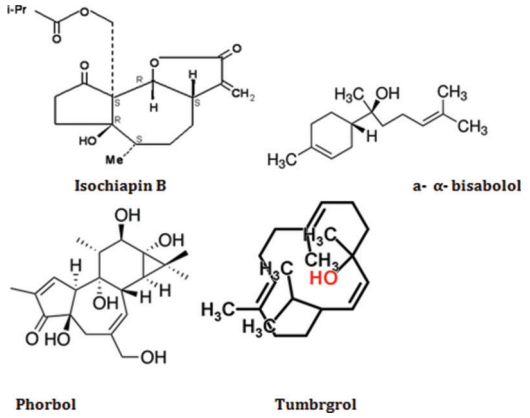

Figure 1: Structures of some compounds identified from *Achillea fragrantissima.*

Anti-inflammatory Activity

Anti-inflammatory activity of extracts (OE and HE) was screened in vivo by inhibition of carrageenan-induced rat paw edema method at aa oral dose of 100 mg/kg. Results are presented in Table 2 as percent of inhibition and percent edema, increase at the right hind paw.

Carrageenan is a useful method for studying new drugs uesd in reducing inflammation. Carrageenan-induced rat paw edema is a nonspecific inflammatory agent resulting from a complex of diverse mediators. Edema of this type is more sensitive to nonsteroidal anti-inflammatory drugs.[13]

The results showed significant anti-inflammatory activity, with inhibition in edema in the range of 71%–79.9% after 4 h. The standard drug indomethacin has shown 89.5% inhibition after 4 h., the plant shows high inhibition at a concentration 100 mg/kg, OE shows high inhibition than HE, this may be due to the higher amount of some volatile oil compound present in OE than in HE, such as bisbolene epoxide, alpha-bisabolol, and azulene, these compounds are used as anti-inflammatory agents.

Discussion

Essential oils isolated from aromatic plants have wide applications in perfumery, flavor, cosmetic, and pharmaceutical industries. They have been used since ancient times, and despite many of them being substituted by synthetic ones, the

Table 2: Anti-inflammatory activity of *Achillea fragrantissima* extracts

	% Edema				% Inhibition			
Time (H)	**1st**	**2nd**	**3rd**	**4th**	**1st**	**2nd**	**3rd**	**4th**
Control	54.70 ± 2.9	67.11 ± 4.4	79.58 ± 4.1	83.22 ± 4.7				
Indomethacin	49 ± 4.2	37 ± 4.1*	23 ± 3.0*	8.6 ± 0.9*	10.5	44	70	89
AF1	58 ± 2.7	49.5 ± 2.3*	30 ± 3.8*	23. ± 2.3*[a]	0.78	32	54.6	71.9
AF2	50 ± 2.3	42.8 ± 1.9*	28 ± 2.4*	17 ± 1.7*	2.3	36	58.6	79.4

AF1: Hexane extract; AF2: oil extract

demand for essential oils obtained from natural sources is increasing,[16] so if the plant contains many important compounds as in *Achillea* plant, it is very important to study the pharmacological activity of the plant. Menthole is a volatile aromatic compound, it is the major constituent of many plants such as eucalyptus, lemongrass, palmarosa and peppermint. The reports proved it have antibacterial and antifungal activity, the study confirmed that, (-)menthol can inhibit the growth of rat liver epithelial tumor cells.[17] Laboratory studies have verified that, menthol can inhibit *N*-acetyltransferase activity in human hepatoma cells.[18]

The LD50 of α-bisabolol was studied in previous review and it was found it (13.14 g/kg) of body weight orally in adult mice and rats,[19] also other studies show it is known to possess anti-inflammatory and antibiotic,[20,21] gastroprotective,[22] and antioxidant effects.[23] In addition, recent studies showed that α-bisabolol inhibits the growth and survival of glioblastoma, prostate cancer, breast cancer, and liver cancer in vitro,[24] wherever α-bisabolol showed no toxicity against normal cells.[25] So the presence of α-bisabolol in high percent in plant confirmed its important anti-inflammatory activity.

GC-MS analysis revealed the presence of terpenoid compounds (Phorbol, Isochiapin B, stigmasterol acetate, and β-sitosterol). These compounds are well known for their biological activities as anti-insect and antitumor agents.

Studies on α-bisabolol and α-bisabolol oxide found in flower of chamomile plant in 1%–2% of volatile oils showed anti-inflammatory and antiphlogistic activity.[26,27]

The bisabolol, bisabolol oxide, mentho, menthol, and β-caryophyllene are the major constituents of the *Achillea* extract so concern the discusses the role of these compounds as antitumor and anti-inflammatory agent.

The results suggested that the excellent performance of essential oil might be attributable to the effects of major constituents or synergistic effects among the constituents. To decipher the mechanism of oil in anti-inflammatory action need more investigation and research.

Conclusion

The aim of the present study was to use essential oils extracted by different methods from *Achillea fragrantissima* (HE and OE) in the manufacture of soap, which can used as anti-inflammatory agent. The two extracts were analyzed by GC and GC-MS. There are differences in the chemical component of volatile oil of the two extracts.

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