International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.7, No.1, pp 41- 46, 2014-2015

Chemical Composition and Antimicrobial Activity of the Essential Oils of *Achillia falcata* L. (Asteraceae)

Nawras Al- Abrass[1] Rasha Aleid[2] Lina Al –Amir[2] Rudwan Badr Al-Deen[2]

[1]Department of medicinal plants in National Commission for Biotechnology (NCBT),Syria.
[2]Department of Food and Industrial Biotechnology in (NCBT). Syria

Abstract: *Achillia falcata L.* is an important genetic resource in medicinal plants and is widely distributed in Qalamoun highest (Syria). Plant aerial parts were collected during the full flowering stage, fresh and dried samples were hydrodistillated.
The yield of essential oil was 0.48 ml / 100g fresh material and 0.76 ml / 100 g dried material.
GC/MS analysis resulted in the identification of 18 compounds. The main components identified were camphor (30.06 %), and 1,8-cineole (28.82 %). The essential oil showed antibacterial activity against three pathogenic bacterial species (*Escherchia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*). Three isolates of each species were tested to determine the minimal inhibitory concentration (MIC) of the essential oil using the micro dilution method. MIC range values of essential oil of *A. falcata L.* was 3.15- 3.6 µg/µl for *E.coli*, 1.35-1.8 µg/µl for *S. aureus* and 1.8 -4.5 µg/µl for *K. pneumonia.*
Key word: *Achillia falcata L.* Essential oil, *Escherchia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, Syria.

Introduction:

The genus *Achillea* belongs to the family Asteraceae. It comprises more than 200 species of aromatic plants that are used for their medicinal, cosmetic, agricultural and fragrance properties[1,2]. Eight wild species are widely distributed in Syria. [3]

Achillia falcate is one of the four species of *Achillea* widely distributed in Qalamoun region and also on the eastern mountains from which the tested samples were collected [4].

Chemical composition and biological activity of *Achillea* spp. were investigated by many researchers. Most of the *Achillea* essential oils contain large amounts of 1,8-cineole[5, 6], camphor[5, 7] and borneol[8, 9], while other oils have as major components camphene[10] piperitone[11], thujone, Artemisia ketone and Santolina alcohol [12]. Several researchers revealed that the major active components in *Achillea spp*. that grow in Turkey are: 1,8-cineol, piperitone and ascaridole [13,14,15,16].

Literature survey shows that extracts of *Achillea spp.* exhibit antimicrobial properties against human and plant pathogenic bacteria, fungi and yeasts [17,18]. Some studies revealed that essential oils from *Achillea spp*. may be used as antimicrobial agents in various medical applications [2 ,19,20] .

The ethanol extract of *Achillea falcata* showed a lower inhibitory effect on *Staphylococcus* that causes Clinical Mastitis in cow compared with the extract from *Thymus vulgaris* [21].

A Jordanian study showed that the highest yield of oil (0.6% w/w) came from the dried parts of *Achillea falcata* [22].

In Turkey, the hexane extracts from flowers of *Achillea falcata* did not affect *Pseudomonas aeruginosa, S.aureus* and *E. coli*, whereas the methanol extract affected just two strains of *S.aureus* [23].

Materials and Methods

Plant Material

Samples of aerial parts of wild *Achillea falcata* at the full flowering stage were collected from Nabek region (Qalamoun, Syria) during June 2011. Samples were indentified by the Department of Biodiversity in the National Commission of Biotechnology (NCBT) /Syria.

Oil distillation:

Essential oils were obtained from fresh and dried aerial parts by hydrodistillation using a Clavenger-type apparatus. Distillation was performed using 50 g of plant material in 500 mL distilled water for 3 hrs. Each treatment was repeated four times. The oil obtained was dried over anhydrous sodium sulfate and stored in a dark glass bottle at 4°C until analysis [4].

GC/MS Analyses:

The essential oil was analysed by GC/MS type Mass Spectrometer(Agilent), using hexane as organic solvent. The GC/MS instrument was equipped with a capillary column (DB-I,30 m × 250 µm × 0.20 µm film thickness) and helium (He) was used as the carrier gas with a flow rate of 1.0 mL/min.

Operating conditions were as follows: injector and detector temperature; 250 °C, oven temperature programme at 4 min isothermal at 60 °C, subsequently rising at 1 °C/min to 64 °C, then raised at a rate of 2.5 °C/min to 155°C the final rising reached to 250 °C at a rate of 5 °C/min then held isothermally at 250 °C for 4 min.

Injection split mode volume: (1 µl) with a spilt Ratio of 1:80.

The mass detector was set to scan ions between 30-450 m/z using full-scan fixed mode electron impact (EI, 70eV). Components of the oil were identified by matching their recorded spectra with the data bank mass spectra (Wiley and NIST library databases) provided by the instrument software. Mass spectrometry parameters were as follows: ion source temperature 230ºc, quadrapole temperature 150ºC, scanning range 30-450[24], ion source voltage 70 e.v.

Tested bacterial species :

Three species of pathogenic bacteria of each of *E.coli* ($E_{36} - E_{94} - E_{103}$) of *K. pneumoniae* ($K_{25}$ - $K_{29} - K_{37}$) and of *S. aureus* ($S_{176} - S_{180} - S_{185}$) were tested. These strains were provided by the Department of Food and Industrial Biotechnology in NCBT.

Antimicrobial activity assay was carried by the micro dilution method using Muller Hinton broth (MHB) and Tween 20 [25].

Antimicrobial Assay

The antibacterial activity was evaluated by determining the minimum inhibitory concentration (MIC) of oil extracts. Bacteria were grown on nutrient agar; Mueller Hinton Broth was used for the antimicrobial tests. In order to facilitate the dispersion of oil in the aqueous nutrient medium, it was emulsified with Tween 20 (0.1%). Each strain was tested with essential oil samples that were serially diluted in broth to obtain concentrations ranging from 120 µl /ml(108 mg/ml) to 1 µl/ml (0.9 mg/ml).Over night cultures of the tested bacteria incubated at 37 ° C were diluted in MHB to (10^{-3}). Positive and negative controls were prepared using pathogenic bacteria in MHB only and MHB with Tween 20, respectively [24, 25].

Statistical analysis:

Experiments were carried out using a completely randomized design with 3 replicates. Statistical analysis was performed according to the SPSS 17 software.The data were analyzed using analysis of variance (ANOVA), LSD test was applied at $P < 0.01$.

Results and Discussion

Results revealed that high yields of essential oils were obtained from fresh plants with a peak at flowering stage compared to samples taken from air dried plants for 3 weeks with significant differences using T test. Significant differences were observed between fresh and dried samples (at P≤ 1), the loss in essential oil was 20.8% of fresh weight in dried samples.

The amount of essential oil in *Achillea falcata* obtained from 100g of dried aerial parts was 0.76% (w/v). It was higher than the amount (0.6% w/v) obtained by Aburjai and Hudaib in Jordan[22]. however, it was lower than the amount obtained by Senatore, et al in Lebanon[26] at the peak of the flowering stage (0.69% w/v=0.62% w/w).

Composition of essential oil

Table1. Composition of the essential oil from aerial parts of *Achillea falcata*L.

Compound	Of compound%	retention time
Santolinatriene	1,199	4,633
Tricyclene	0,250	5,073
α-Pinene	1,029	5,422
Camphene	3,802	5.842
β-Phellandrene	1,995	6,685
β-Pinene	0,819	6,794
1,8-Cineole	28,82	9,299
γ-Terpinene	0,375	10,651
Thujone	1,517	13,093
Camphor	30,063	14,520
Borneol	5,528	16,008
Cis- verbenol	1,661	16,147
{+}-4-Terpineol	1,540	16,711
α-Terpineol	2,653	17,396
Bornyl acetate	0,656	22,700
Eugenol	0,499	25.906
β-Cubebene	3.260	33.047
Ledol	0,588	34,909
Total	86,254	

When comparing the present results (table 1) of essential oil composition with that obtained in Jordan [22], we noticed that the major components in Syrian *A. falcata* were camphore and 1,8-cineol (30.06 and 28.8 % respectively), while their percentage in Jordan was 17.03 and 15.90% respectively, and the percentage of p-cymene and β-thujone was 11.32 and 9.84% respectively, while the percentage of β-thujone was approximately 1.5%, and p-cymene was absent in the current study.

As for the other components γ-terpinene, camphene, α-pinene, phellandrene, β-pinene, santolinatriene, borneol and α-terpineol the percentage in our study was 0.38, 3.8, 1, 2, 0.8, 1.2, 5.5 and 2.7 % respectively, while in the Jordanian study[22] it was 1.1, 0.3, 0.5, 0.20, 2.3, 0.1, 1.02 and 2.58 % respectively.

The results of chemical composition of the current study differed from that obtained in Lebanon [26]. In the latter, the major components were grandisol and fragranol, and their percentages were 21.4 and 16.8%, respectively.

The last two components were not noticed in both Syrian and Jordanian studies. The amounts of 1,8 cineol, camphor and α-terpinel were slightly higher than in Lebanese samples, especially the first two components (1,8 cineol and camphor); while the components α-pinene, tujone, cis-verbenol and bornyl acetate were found in low percentages and they were rather lower than in Lebanese samples. The percentages of β-cubebene, {+}-4-terpineol, ledol and tricyclene were 3.26, 1.54,0.58 and 0.25% respectively in this study but they were not found in both of Jordanian [22] and Lebanese [26] studies.

It is of importance to mention that all the plants included in this study and in Jordanian and Lebanese studies were at the full flowering stage.

In a study performed in Turkey[27] on the same species, the major components of the essential oil were 1,8-cineol (14-24%), camphor (2-24%). The results were compatible with ours where these two components were dominant. However the component α-pinene in our study was 1.029% while it was 2-12% in the Turkish study.

We can conclude by comparing the results of the three neighboring countries (Jordan, Lebanon, and Syria) that there were differences among the samples. The similarity in chemical composition of essential oils, especially for major components, is expected, since we are working on the same species. Though an exception appeared in the Lebanese study. The differences in the quantity of the major components and lower percentage of some components, or the absence of others may be due to environmental, geographical and genotypic differences of the same species in different countries.

Bioactivity of essential oil against bacteria

Table 2. Antimicrobial activity of *Achillea falcata* essential oil.

Bacteria species	Strains	MIC values (µg/µl) (means of 3 replicates)
Escherichia coli	E94	3.6
	E36	3.6
	E103	3.15
Staphylococcus aureus	S176	1.8
	S180	1.58
	S185	1.35
Klebsiella pneumoniae	K25	4.5
	K29	2.7
	K37	1.8

The bioactivity of essential oil obtained from *Achillea falcata* through the determination of minimal inhibitory concentration (MIC) against three species of bacteria *Escherichia coli*, *Staphylococcus aureus* and *Kelbsiella pneumoniae* is shown in Table (2), using the broth dilution method [25, 26]. A strong effect of the essential oil was found against both Gram-positive and Gram-negative bacteria. Results revealed that the bioactivity was higher against gram-positive than gram-negative bacteria. This result is relatively close to that obtained by Senatore, et al[26]. Values of MIC for *Escherichia coli* and *Staphylococcus aureus* differed in the present study where they appeared to be similar in another study [26]. In the present study the range of MIC values for three isolates of the same species was narrow in *E. coli* and *S.aureus* (3.15 - 3.6 µg/µl),(1.35 - 1.8µg/µl) respectively. On the other hand, MIC values for the three strains of *K. pneumoniae* were distant (1.8 - 4.5 µg/µl). this may be due the genetic properties of this bacteria and the chemical composition of their cell wall.

In the study performed in Jordan to estimate the bioactivity of the essential oil of *A. falcata* using the disk diffusion method, the essential oil exhibited higher antibacterial activity against both gram positive and gram-negative bacteria. However, it exhibited a weak activity only against resistant strains of *S. aureus* [22].

Antibacterial activity of the ethanol extract and water extract prepared from *A. falcata* have also been studied in Jordan[28]. All gram positive bacteria showed sensitivity to hydro-alcoholic extract, while in Turkey[23], the hydro-alcoholic extract showed mild to low antibacterial activity against tested species.

Differences between our results and others in neighboring countries may be due to biodiversity tested bacteria. This difference in composition and amount of essential oil may be attributed to different environmental conditions like soil, temperature, rainfall, day-length and altitude. Last factors are important in affects in plant growth and development of secondary metabolites. The obtained results indicate a high potential of application for the *Achillea falcata* essential oil, to be used in health industry and food preservation.

Conclusion

A. Falcata essential oils coud be used in complimentary or alternative medicine, but it needs evaluation for testing its side effects. It was found that the best period for essential oil extraction was at the fresh full flowering stage. The highest antimicrobial activity of the essential oil was exhibited towards *S.aureus*.

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