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Essential Oil Composition and Antibacterial Activity of Flowers of *Achillea filipendulina*

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ABSTRACT

Achillea filipendulina is an aromatic plant of family Asteraceae with bright yellow colour flowers. The objective of the study was to extract the essential oil from the flowers of the selected plant using Clevenger apparatus, to analyse the chemical composition of the oil using GC-MS and to screen the essential oil for its antibacterial property against both gram-positive and gram-negative bacteria. GC-MS results revealed that the main chemical constituents present in the oil were trans 2,7 Dimethyl 4,6 octadien-2-ol (27.93%), borneol (21.44%) and santolina triene (7.13%). The results of agar well diffusion assay revealed that the oil showed antibacterial activity against all the tested bacteria except *Pseudomonas aeruginosa*. Minimum inhibitory concentration (MIC) of oil was determined against the sensitive bacteria. MIC values against *Staphylococcus aureus* and *Escherichia coli* were similar (0.312% v/v) and for *Bacillus cereus*, MIC value was found to be (0.156%). Results indicated the great antibacterial potential of oil of this plant against gram positive and gram negative bacteria.

Keywords: Essential oil, GC-MS, Antibacterial.

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INTRODUCTION

Infectious diseases cause serious problems to health and are one of the main reasons for increased death rate worldwide. [1] Food-borne illness is caused by pathogens like *Staphylococcus aureus*, *Clostridium perfringens*, *Bacillus cereus*, *Escherichia coli* etc. [2-3] *Staphylococcus aureus* causes endocarditis, osteomyelitis and is present on the skin and nasal passages in humans. [4] *Escherichia coli* is present in the intestine of humans and causes infections of urinary tract. [5]

Various antibiotics are available in the market to fight against bacterial infections. The use of synthetic chemicals has negative effect on environment and health of living organisms as their decomposition rate is very slow. [6] Also, many bacteria have developed resistance against the already present antibiotics. [7] Therefore, there is a need for more potent and plant based antibacterial agents as they are less expensive and ecofriendly.

About 80% of the population in Asia, Africa and Latin America use herbal plants as main remedy to treat various diseases. [8] These plants also have minimum side effects. Essential oils from medicinal and aromatic plants are already known to possess antibacterial, antifungal and antioxidant properties. [9-11] Plant essential oils are secondary metabolites that are produced in plants as a result to fight against various abiotic stresses, microbial pathogens and predators. The chemical composition of essential oils depends on the plant genotype, geographic conditions and various environmental factors. [12]

The genus *Achillea* is a member of family Asteraceae which includes wide range of various aromatic plants of economic importance. The plants of this family are distributed throughout the world. There are around 85 species of *Achillea* distributed in Europe, Asia and in North America. The healing power of various species of plants is due to the presence of proazulenes in them. The local name for most species of this genus in Persian language is Bumadaran. *Achillea* species has great medicinal value. [13] The aerial parts of different *Achillea* species possess properties such as antioxidant, anti-inflammatory and antimicrobial. [14]

Achillea filipendulina is one of the species which flowers in June to September. The leaves of this plant are feather shape. The flowers grow in clusters and are yellow in colour. Decoctions of this plant have been used to treat various diseases like arthritis, gastrointestinal problems, congestions and malaria etc. The whole plant of *Achillea filipendulina* is aromatic due to the presence of essential oils. The chemical constituents present in large amount in the essential oil of flowers of the plant are limonene, carvacrol, borneol, 1,8 cineole etc. [15] Hippocrates known as the 'father of medicine' sometimes prescribed the persons for perfume fumigations. The Ancient Egyptians used the essential oils of various aromatic plants as antibacterial agents. [16]

Very few studies have been done on the essential oils of this species of *Achillea*. The present study was designed to extract the essential oils from flowers of *Achillea filipendulina*, analysing the chemical composition of the oil using GC-MS technique and screening the essential oil of the plant for its antibacterial potential for both gram-positive and gram-negative bacteria.

MATERIALS AND METHODS

Collection and identification of the plant material

The flowers of the plant were collected in the month of July from Chail, Himachal Pradesh and identified from Regional Horticultural Research and Training Section Mashobra.

Extraction and analysis of essential oil

About 1 Kg flowers were collected and cut in to fine pieces. The essential oil was extracted by steam distillation using Clevenger apparatus. The oil obtained was pale yellow in colour. The extracted essential oil was analysed for its various chemical constituents

using GC-MS technique in which the mass spectral fragmentation patterns of the compounds were compared with those reported in the literature and in the MS library.

Antibacterial screening

The antibacterial activity of the essential oil was checked against both gram-positive and gram-negative bacteria by agar well diffusion method. Tested bacteria were *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. MIC was determined using microdilution technique for those bacteria showing zones of inhibition in well diffusion assay.

Agar well diffusion assay

Fresh cultures of bacterial strains were grown for 24 hours and then next day O.D. was adjusted at 600 nm to 0.4. Muller Hinton Agar medium was prepared and poured on sterilised petri plates. Bacterial cultures swabbed on MHA and different concentrations of oil were added to wells made on MHA. After this, the petri plates were incubated at 37°C for 24 hours and zone of inhibition around each well was measured.

Microdilution method

Minimum Inhibitory Concentration was determined using broth microdilution technique stated in CLSI. [17] In this case bacteria cultures were grown overnight and O.D. was adjusted at 600 nm to give bacterial count of 10^5 cfu/ml. To a sterilised microtitre plate, 100µl of nutrient broth was added in each well. 200µl of nutrient broth was added in last well of each row which served as negative control and 100µl of media and 100µl of culture was added to second last well of each row which served as positive control. To the first well of each row, 100µl of stock concentration (10%) of essential oil of *Achillea filipendulina* was added. Essential oil was then serially double diluted from 1 to 10^{th} well. 100µl of adjusted culture was then added to all the wells except negative control. The microtitre plate was then covered and incubated at 37°C. After incubation, to each well 10µl of resazurin dye (0.01% w/v) was added including controls. The lowest concentration at which the colour of resazurin changed was labelled as the MIC value.

RESULTS AND DISCUSSION

Steam distillation of fresh flowers of *Achillea filipendulina* yielded 0.1% v/w essential oil. The oil obtained was pale yellow in colour. A total of 11 compounds were identified in the oil by means of GC-MS technique which comprises 75% of the plant essential oil (Fig. 1). The main components in the floral essential oil were trans 2,7 Dimethyl 4,6 octadien-2-ol (27.93%), borneol (21.44%) and santolina triene (7.13%) comprising about 56.5% of the essential oil (Table 1). Other compounds present were camphor, α -terpineol, α -pinene, yomogi alcohol etc. The results obtained are in accordance as per work of Rahimmalek *et al.* [18] They also reported 2,7 Dimethyl 4,6 octadien-2-ol to be the major compound present in the floral essential oil of *Achillea filipendulina*. Besides this, borneol, bornyl

acetate, germacrene D and small amounts of 1,8 cineole were also reported by them in the plant essential oil.

Antibacterial activity

The essential oil was screened for its antibacterial activity by agar well diffusion assay. Results showed that all the tested bacteria were sensitive to the oil except *P. aeruginosa* which did not show any zone of inhibition. Zone of inhibition ranged from 1 cm to 3.5 cm (Table 2).

Resazurin dye was added to the microtitre plate containing bacteria and oil. Pink colour was noticed where there was bacterial growth indicating no inhibition of bacterial growth at that particular concentration of oil. The appearance of blue colour indicated the absence of bacterial growth, meaning the bacteria were susceptible to the oil at that particular concentration of essential oil. MIC for *S. aureus* and *E. coli* were same 0.312% v/v and for *B. cereus* it was 0.156% v/v (Table 3). Results revealed that *Achillea filipendulina* floral essential oil was more effective for gram positive *B. cereus* than the rest of the tested bacteria.

Asteraceae is the largest family of angiosperms and most of the plants are used as folk medicines. [19] The extracts of plant *Elephantopus scaber* (Asteraceae) possess antibacterial activity. [20] The essential oils and extracts of various species of *Achillea* have already been reported to possess antimicrobial activity. Unlu *et al.* [21] reported the antimicrobial activity of *Achillea setacea* and *Achillea teretifolia*. Major compounds responsible for the antimicrobial effect were camphor, eucalyptol and borneol. The essential oil of *Achillea fragrantissima* has terpinen-4-ol as the main component for its bacteriosidic effect. [22] Kiyanpour *et al.* [23] reported the antimicrobial activity of essential oil of leaves and flowers of *A. filipendulina* against both gram-positive and gram-negative bacteria and noticed high antibacterial activity. Jianu *et al.* [24] reported

antimicrobial and antioxidant activity of the essential oil of *Achillea collina* and found the essential oil to be effective against food related bacteria.

Table 1: GC-MS results of essential oil from flowers of *Achillea filipendulina*

S. No	Compound	Percentage
1.	Trans 2,7dimethyl 4,6 -octa-dien-2-ol	27.93
2.	Borneol	21.44
3.	Santolina triene	7.13
4.	Camphor	7.08
5.	α -pinene	1.58
6.	Camphene	1.52
7.	Pinocarveol	1.33
8.	Bornyl acetate	1.33
9.	Yomogi alcohol	3.34
10.	α -terpineol	1.39
11.	Carene	1.00

Table 2: Zone of inhibition against bacteria in agar well diffusion assay

Concentration of oil	Zone of inhibition (cm)			
	<i>B. cereus</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
20%	1.9	2.0	-	-
40%	2.0	2.2	-	-
80%	2.2	2.5	-	1.0
100%	3.0	3.5	-	1.8

As per literature, the gram-negative bacteria are less susceptible to plant essential oils. [25] Even the marine alga *Gracilaria coticata* possess high antibacterial activity against gram-positive bacteria. [26] The gram-negative bacteria are more resistant due to the presence of an outer membrane which surrounds a thin underlying peptidoglycan layer that restricts the diffusion of essential oils through lipopolysaccharide. The antibacterial property of the essential oils may be attributed to their hydrophobicity due to which they can breakdown lipids of bacterial cell membrane, interfere with the cell structures. [27] As a result, leakage of ions and molecule from the bacterial cells lead to the death. [28]

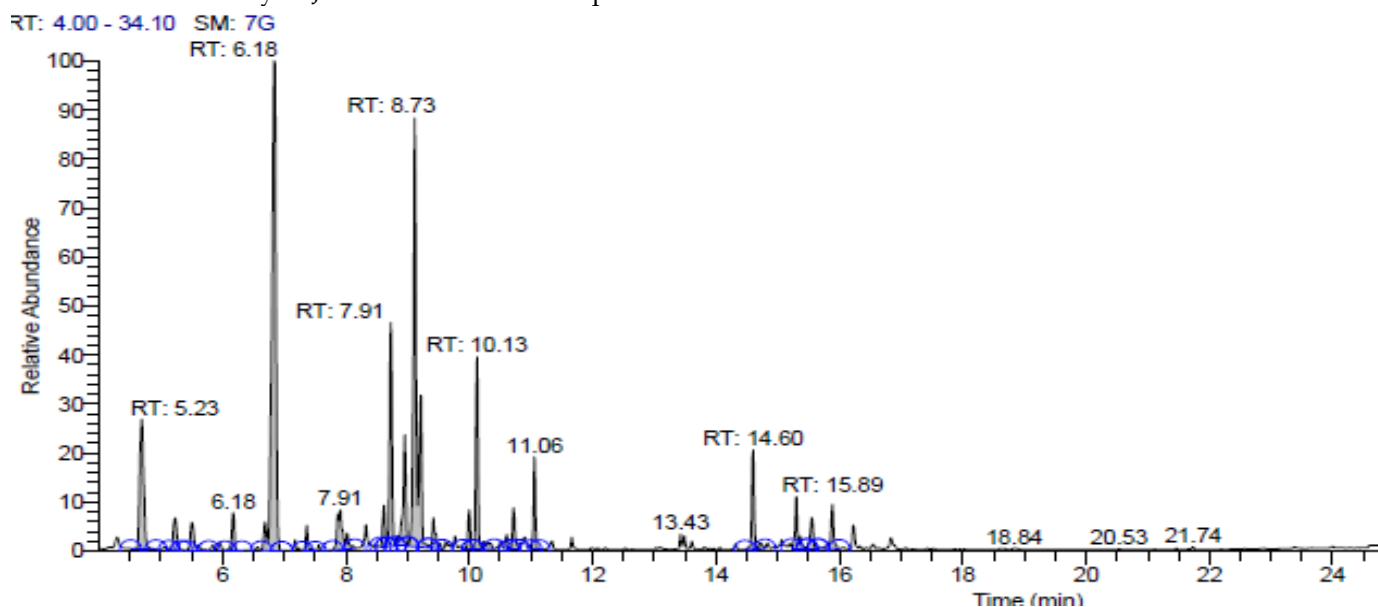


Fig. 1: Chromatogram of essential oil from flowers of *Achillea filipendulina*

Table 3: Determination of MIC using broth microdilution assay

Bacteria	Percentage of oil										Positive control	Negative control
	10	5	2.5	1.25	0.62	0.312	0.156	0.018	0.039	0.019		
<i>S. aureus</i>	-	-	-	-	-	-	+	+	+	+	+	-
	-	-	-	-	-	-	+	+	+	+	+	-
	-	-	-	-	-	-	+	+	+	+	+	-
	-	-	-	-	-	-	+	+	+	+	+	-
<i>B. cereus</i>	-	-	-	-	-	-	-	+	+	+	+	-
	-	-	-	-	-	-	-	+	+	+	+	-
	-	-	-	-	-	-	-	+	+	+	+	-
	-	-	-	-	-	-	-	+	+	+	+	-
<i>E. coli</i>	-	-	-	-	-	-	+	+	+	+	+	-
	-	-	-	-	-	-	+	+	+	+	+	-
	-	-	-	-	-	-	+	+	+	+	+	-
	-	-	-	-	-	-	+	+	+	+	+	-

The effectiveness of the essential oil is linked with its chemical composition and is dose dependent. [29] The antibacterial potential of some chemical constituents such as borneol [30-31], α -terpineol [32], camphor [33-36] is already reported. All of these compounds are present in the floral essential oil of *Achillea filipendulina*. This justifies that the antibacterial property of *A. filipendulina* essential oil may be due to the presence of such chemical compounds. Therefore, the plant essential oil has great antibacterial potential and may be used in future as food preservatives against some food borne bacteria and to treat infections caused by them.

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