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Biochemical, antibacterial and antifungal activity of extracts from *Achillea fragrantissima* and evaluation of volatile oil composition

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ABSTRACT

Essential oils of the air-dried aerial parts of Achillea fragrantissima were analyzed by GC-MS chromatography. The results show that 48 components were identified in the oils, and main compounds of oils were 4-terpineol (15.65%), Linalool (11%), carvone (9.42%), β-phellandrene (6.2%), γ-terpinene (5.6%), β-pinene (4.55%), verbenone (4.42%), cedrol (3.0%) and ρ-cymene (2.95%). The antimicrobial activity of various extracts from A. fragrantissima was evaluated against 5 fungi and 5 bacteria. Water-soluble extract method I and II from aerial dried powder were inactive against all tested fungi and bacteria. The other extracts were active against the five used fungi except ethanol extract was inactive against all tested fungi. The crude saponin from aerial dried powder was inactive against all tested bacteria. Proteus mirabilis was the only bacterium that was inhibited by the most of the extracts from A. fragrantissima. The only one extract of fresh flowers soaked in ethanol showed activity against all tested bacteria.

Key words: *Achillea fragrantissima*; Essential oils; Extracts; Antimicrobial; Antifungal.

INTRODUCTION

Six species of the genus *Achillea* are widely distributed in Jordan. *Achillea fragrantissima* is considered the most important species and is thoroughly studied. This plant is used by natives in the region to relieve gastrointestinal pains and cure bronchiolitis and by Bedouins for the preparation of antidiuretic drinks, for treatment of stomach ailments and various infections, among them infection of the eye [1]. The common name of the plant is Lavender Cotton (English) and Al-Qisum (Arabic). The plant has a relatively high essential oil content (1.0%, w/v) that gives the plant a characteristic, pleasant aroma [2]. Earlier research indicated the essential oil has antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Staphyloccocus aureus* and *Candida albicans* [3].

We reported here on the essential oil composition of *Achillea fragrantissima* obtained from the medicinal and aromatic plant garden of the Hashemite University, Zarqa, Jordan. The composition determined by means of gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) techniques. Additionally, various plant extracts were tested for its potential to inhibit *in vitro* the growth of various bacterial and fungi strains.

MATERIALS AND METHODS

Plant- *Achillea fragrantissima* was harvested from the medicinal and aromatic plant garden of the Hashemite University, Zarqa, Jordan. The taxonomic identity of the plant was confirmed by Prof. A. Al-Oqlah, Department of Biological Sciences, Faculty of Sciences, Yarmouk University, Irbid, Jordan. A voucher specimen from the study plant had been deposited at the Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan.

The plant material was shed dried under laboratory conditions for few days and then ground to pass through a sieve of 0.25 mm in a Wiley grinder, then they were kept in a glass jar and stored at 5^{o}C until further use.

Microorganisms- The microorganisms used in all antimicrobial assays were Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Proteus mirabilis*), molds (*Penicilium spp., Fusarium spp., Aspergillus spp.* and *Rhizopus spp.*) and yeast (*Candida albicans*)

Soxhlet extraction method- Dried aerial parts of the plant were extracted in a soxhlet extraction apparatus with petroleum ether for separation of crude phenolic compounds and with a methanol for preparation of a crude saponin fraction.

Successive extraction of 20 g dried aerial parts of the plant were extracted continuously for 24 hr in a soxhlet extraction apparatus with three solvents, starting with 200 ml n-hexane to separate lipids and terpenoids, with 200 ml ethyl acetate for separation of more polar compound, and then using 200 ml ethanol for separation of the polar compounds. Each extract was concentrated at 40-45^{o}C under vacuum until a dry residue is obtained. The residue was weighed and stored in a freezer until the time of testing.

Organic solvent extraction method (Cold perculation)- A fifty gram of fresh and dried aerial parts or flowers of *A. fragrantissima* were soaked separately in pure ethanol in glass jars and kept under laboratory conditions for 3 weeks. The mixture was homogenized and the supernatant was taken and concentrated at 40-45^{o}C under a vacuum until a dry residue was obtained. The residue was weighed and stored in a freezer until the time of testing.

Phenolic compounds extraction method- Isolation and purification of phenolic compounds were carried out by the same procedure mentioned by Tharib and El-Migirab [4] and Al-Charchafchi *et al*. [5].

Saponin fraction extraction method- Isolation and purification of saponin fraction were carried out by the same procedure mentioned by Tharib and El-Migirab [4] and Al-Charchafchi *et al*. [6].

Water extraction methods- Water extraction of the plant was done by two different methods. Method one was done according to Ann *et al*. [7]. Water extract was prepared by pouring boiling distilled water (50 ml) on 5 g of dried powder. The mixture was allowed to stand for 15-20 min. Then the mixture was filtered through one layer of cheesecloth and the resulting filtrate was centrifuged at 6000 rpm for 15 min. The supernatant was taken then filtered through microfilter Millipore (0.2 µm). The sterilized filtrate concentrated at 40-45^{o}C under vacuum until a dry residue is obtained. The residue was weighed and stored in a freezer until the time of testing.

Method two was carried out as described by Al-Charchafchi *et al*. [8]. Fifty ml of boiling distilled water was poured on 5 g of dried powder. The mixture was boiled for 10 min. Then the mixture was filtered through one layer of cheesecloth and the resulting filtrate was centrifuged at 6000 rpm for 15 min. The supernatant was taken then filtered through microfilter Millipore (0.2 µm). The sterilized filtrate concentrated at 40-45^{o}C under vacuum until a dry residue is obtained. The residue was weighed and stored in a freezer until the time of testing.

GC/MS analysis- The GC analyses were accomplished with a HP-5890 Series II instrument equipped with HP-WAX and HP-5 capillary columns (30 m × 0.25 mm, 0.25 μm film thickness), set to the following conditions: temperature program of 60◦C for 10 min, followed by an increase of 5◦C/min to 220◦C; injector and detector temperatures at 250◦C; carrier gas nitrogen (2 mL/min); detector dual FID; split ratio 1:30; injection of standards of 0.5 μL). For both the columns, identification of the chemicals was performed by comparing their retention times with those of pure authentic samples and by means of their Linear Retention Indices (LRI) relative to the series of *n*

-hydrocarbons. The relative proportions of the individual constituents, expressed as percentages, were obtained by FID peak-area normalization (mean of three replicas).

GC/EIMS analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (both 30 m × 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. The analytical conditions included: injector and transfer line temperatures at 220 and 240◦C respectively; oven temperature was programmed from 60◦C to 240◦C at 3◦C/min; carrier gas helium at 1 mL/min; injection of 0.2 μL (10% hexane solution); split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their Linear Retention Indices relative to the series of n-hydrocarbons, and by computer matching against commercial (NIST 98 and ADAMS 95) and home-made library mass spectra built from pure substances and components of known essential oils [9, 10].

Disc agar diffusion method- Sterile discs were impregnated with the various plant extracts and allowed to dry, then kept inside sterile plate within 1 hr after pouring.

Minimum inhibitory concentration method- Determination of the minimum inhibitory concentration (MIC) was carried out according to the method described by NCCLS (2003) with some modifications. Dilution series of the extracts were prepared from 2.5 to 0.5 mg/mL in test tubes and then transferred to the broth in 96-well microtiter plates. Final concentrations in the medium were 25 to 250 µg/mL. Before inoculation of the test organisms, the bacteria strains and yeast strain were adjusted to 0.5 McFarland standards and diluted 1:100 (v/v) in Mueller–Hinton broth and Sabouraud dextrose agar. Plates were incubated at 35°C for 18–24 h and at 30°C for 48 h for the yeast. All the tests were performed in broth and repeated twice. The MIC was defined as the lowest concentration that showed clear against a black background (no visible growth). Samples from clear wells were subcultured by plotting on to Mueller–Hinton agar. Ampicillin, streptomycin, and line solid were used as standard antibacterial agents, whereas nystatin was used as a standard antifungal agent. All antibiotics were purchased from Sigma Aldrich Chemical Co. (StLouis, MO, USA), and dilutions were prepared at concentrations ranging from 0.25 to 128 µg/mL in microtiter plates.

RESULTS

Chemical composition- The constituents of the air dried aerial parts of essential oils of *A. fragrantissima* obtained from the aromatic plant garden of the Hashemite University, Jordan, are listed in order of their elution on the HP-Wax and HP-5 columns (Table 1).

In total of 48 volatile compounds, representing 94.1% of the total composition, were identified in the air dried aerial oils. Monoterpene hydrocarbons were found to be the major one being 4-terpineol (15.65%), linalool (11%), carvone (9.42%), β-phellandrene (6.2%), γ-terpinene (5.6%), β-pinene (4.55%), verbenone (4.42%), cedrol (3%) and *p*-cymene (2.95%).

Percentages of A. fragrantissima extracts- The percentages of *A. fragrantissima* extracts are shown in Table 2. The percentage of crude phenolic compound that extracted from flowering aerial part was 5.5%, the crude saponin extracts from flowering aerial part gave 4% while crude saponin fraction extracts from vegetative aerial part was 2.6%. Ethyl acetate extract from flowering aerial parts resulted in 3.2%. On the other hand, ethanolic extract from flowering aerial parts produced 6.1%.

The percentages of water extracts obtained from the soaking of flowering aerial parts in boiling water (method I) and from the boiling of plant material with water (method II) were 1.6% and 1.0%, respectively. Soaking of plant materials in ethanol found to produce 10.2% of ethanolic extract from the flowering aerial parts and 9.3% for the flowers, while soaking of flowering aerial parts in methanol gave 18.0% of methanol extract.

Antibacterial activity of the plant extracts using agar diffusion method- Crude extracts from the tested plant were screened for their antibacterial activity against 5 bacteria by the agar diffusion method (Table 3). The phenolic extract tested and showed activity against *P. mirabilis* (16 mm) and no activity against the other bacteria in comparison with the positive control. The saponin extract of flowering aerial part showed no activity on all tested bacteria. On the other hand, the saponin extract of vegetative aerial part was considered to be active against *S. aureus* (16 mm) only. The data also revealed that ethyl acetate extract exhibited activity against only *P. mirabilis*

(15 mm). The extract from soaking of fresh aerial part in ethanol showed activity against *S. aureus* (18 mm) and moderate activity on *P. mirabilis* and *Psd. aeruginosa* (14 mm and 11.8 mm, respectively). While extract from soaking of fresh flowers in ethanol exhibited the largest zone of inhibition against *S. aureus* (20 mm) and also showed activity against *E. coli* (13.9 mm), *P. mirbilis* (14 mm) and *K. pneumonia* (13.5 mm) but no inhibitory effect was exerted on the growth of *Psd. aeruginosa*. Extracts from soaking of aerial parts in methanol exhibited antibacterial activity against *S. aureus* (14.6 mm) and *P. mirabilis* (14 mm). Water extracts by either method I or method II showed no inhibition activity against all tested bacteria.

MIC of A. fragrantissima extracts against bacteria- The results through the determination of MIC (Table 3) were revealed variability in the MIC of each extract against the given bacteria. *S. aureus* was sensitive to extract concentrations ranging from 3.125 to 25 mg/ml. The strongest effect of the extract (MIC 3.125mg/ml) was observed for vegetative aerial part, saponin extract and extracts from both fresh flowers soaked in ethanol and aerial part soaked in methanol. The highest value of MIC (25 mg/ml) was estimated for aerial part soaked in ethanol.

Growth of *E. coli* and *K. pneumonia* were inhibited by extract from flowers soaked in ethanol with MIC 12.5 mg/ml. The growth of *P. mirabilis* was inhibited by plant extract concentration ranging from 3.125 to 25 mg/ml. The strongest effect against *P. mirabilis* was reported for extract from the fresh aerial part soaked in ethanol and methanol, 3.125 and 6.25 mg/ml, respectively. The highest MIC value (25 mg/ml) was estimated for phenolic extracts, while the moderate MIC value (12.5 mg/ml) was recorded for ethyl acetate extracts, ethanolic extracts and extract from fresh soaked in ethanol. All the tested plant extracts showed no effect against *Psd. aeruginosa*.

Antifungal activity of the plant extracts using agar diffusion method- Crude extracts from the tested plant were screened for their antifungal activity against 5 fungi by the agar diffusion method (Table 4). The phenolic extract tested and showed activity against *Aspergillus, Candida albicans, Fusarium* and *Rhizopus* (16.0, 15.2, 15 and 15 mm, respectively). The saponin extract of flowering aerial part showed effect on *Aspergillus, Fusarium* and *Rhizopus* (15 mm). On the other hand, the saponin extract of vegetative aerial part was considered to be active against *Rhizopus* and *Aspergillus* (16 and 15.2 mm, respectively). The data also revealed that ethyl acetate extract exhibited activity against all tested fungi (15.0 mm). The extract from soaking of fresh aerial part in ethanol showed moderate activity on *Rhizopus, Aspergillus, C. albicans* and *Fusarium* (24.0, 18.0, 16.0and 16.0 mm, respectively). While extract from soaking of fresh flowers in ethanol exhibited the largest zone of inhibition against *Rhizopus, Fusarium, Aspergillus, C. albicans* and *Penicillium* (24.0, 22.0, 22.0, 20.3, 20.0 mm, respectively). Extracts from soaking of aerial parts in methanol exhibited antifungal activity against *Rhizopus* (15.0 mm). The extracts by ethanol and by water either by method I or method II showed no inhibition activity against all tested fungi.

MIC of A. fragrantissima extracts against fungi- All the plant extracts with the exception of ethanolic and water-soluble (method I and II) extracts, exhibited fungicidal effect against all tested fungi, the MIC of the above plant extracts ranging from 3.125 to 12.5 mg/ml (Table 4).

The strongest MIC value (3.125 mg/ml) was estimated for extracts of aerial dried powder (with flowers) of crude phenolic against *Aspergillus* spp. and *Rhizopus* spp., of crude saponin against *Candida* albicans, *Penicilium* spp. and *Rhizopus* spp. and of ethyl acetate extracts against *Candida albicans*, *Penicilium* spp. and *Aspergillus* spp.; finally fresh flowers soaked in ethanol against *Penicilium* spp. only.

The moderate MIC value (6.25 mg/ml) was estimated for extracts of aerial dried powder (with flowers) of crude phenolic against *Fusarium* spp. only, of crude saponin against *Candida albicans*, *Aspergillus spp.* and *Fusarium* spp. and of ethyl acetate extracts against *Fusarium spp.* and *Rhizopus* spp.; soaked in methanol against *Penicilium* spp. and *Rhizopus* spp.; aerial dried powder (without flowers) of crude saponin against *Candida albicans*, *Penicilium* spp. and *Fusarium* spp.; finally fresh flowers soaked in ethanol against *Candida albicans, Fusarium* spp., *Aspergillus* spp. and *Rhizopus* spp..

The slight effect of MIC (12.5 mg/ml) was estimated for three extracts of aerial dried powder (with flowers) of crude phenolic against *Candida albicans* and *Penicilium* spp., soaked in methanol against *Candida albicans* spp. and *Aspergillus* spp.; finally aerial dried powder (without flowers) of crude saponin against *Aspergillus* spp. and *Rhizopus* spp..

DISCUSSION

Chemical composition

Aromatic and medicinal plants of *Achillea* produce a wide variety of volatile terpene hydrocarbons (aliphatic and cyclic) and their corresponding oxygenated isoprenoid derivatives and analogues. Mixtures of these substances, which are known as essential oil, can be isolated from diverse parts of plants by steam distillation [11]. The volatile constituents of some *Achillea* species have been analyzed using GC/MS, Twenty- five components making up 81.6% of the oil were characterized with camphor, myrcene, 1,8-cineole, beta-caryophyllene and linalool being the major constituents [12].

Effect against Gram-positive bacteria

The growth of *S. aureus* was inhibited very effectively by *A. Fragrantissima*. Crude saponin extracts showed the strongest effect (MIC 3.125 mg/ml). This observation was consistent with the result in which saponin inhibited the growth of several strains of *Staphylococci* [13]. The mode of action of antibacterial effect of saponin seems to be on membrane properties. This explanation was in agreement with that obtained by Killeen *et al.* [14]. They reported that the mode of action of antibacterial effect of saponin seems to involve membranolytic properties, rather than altering the surface tension of extracellular medium. The crude phenolic extracts showed no effect on *S. aureus*. The variation in the degree of antibacterial effect of phenolic extracts might be related to the diversity and variability among different forms of phenolic compounds or related to the number of OH group present in phenolic compound. If the inhibitory activity of phenolic extract resulted from increasing hydroxyl group number, then the extract activity of the present investigation was in agreement with the idea that more highly oxidized phenols are more inhibitory to microorganisms [15, 16], and this finding disagree with the result obtained by Chabot *et al*. [17] Which mentioned that that less hydroxyl groups number was more toxic to microorganisms. It was found that group of phenolic extract has been reported to exhibit antibacterial activity against *S. aureus* [18].

Both ethyl acetate and ethanol extracts showed no effect against *S. aureus*. The mechanism thought to be responsible for ethanolic inhibition could be by disruption of microbial membrane [19]. The crude ethanolic extract exerted slight to moderate activity against *S. aureus* [20, 21]. This activity might be due to the presence of sesquiterpene lactones in their fractions [22]. It was found that the ethanol soluble fraction yields terpenoid which showed excellent activity against *S. aureus.*

Ethanolic and methanolic extracts of *A. fragrantissima* had been shown slightly and highly effect respectively against *S. aureus*. The differences in their activities might be related to different substances dissolved in ethanol and methanol. Several investigations have been conducted to determine the activities of methanolic extract and indicated the inhibitory action in the development of *S. aureus* [23].

No activities were shown for water extracts (method I and Method II) from *A. fragrantissima* against *S. aureus*.

Effect against Gram-negative bacteria

Escherichia coli, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* are resistant to all various plant extracts. The exception was water soluble extract method II against *E. coli* and *K. pneumonia*, the results showed slight effect against both above bacteria. This finding was in contrary with various previous works. Sen *et al*. [24] reported that saponin extracts can inhibit *in vitro* growth of *E. coli*. Kazmi *et al*. [25] described bacteriostatic effect of some phenolic compound against *Psd. aeruginosa*.

Crude phenolic extracts from *A. fragrantissima* showed slight effect against *P. mirabilis* (MIC 25 mg ml^{-1}). This result is in agreement with Nishino *et al*. [18]; they recorded that group of phenolic extract had been exhibited antibacterial activity against *P. mirabilis*.

Crude saponin extract showed no activity against *P. mirabilis*. This resistance might be due to its phospholipids membrane, since gram-negative bacteria having an outer phospholipids membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic substance [26].

A moderate effect of ethyl acetate and ethanolic extracts of the tested plant observed against *P. mirabilis*. This might be related to the different kind of compounds dissolved in ethyl acetate; and might be related to the diversity and variability among different kind of ethanolic extracts compounds.

In this study ethanolic and methanolic extracts of *A. fragrantissima* had been shown strong effect (MIC 3.125 and 6.25 mg ml^{-1}, respectively) against *P. mirabilis*. These identical activities might be related to identical substances dissolved in ethanol and methanol. These results were in contrast with others [23]. They indicated that less inhibitory action was exerted by methanolic extract on the development of *P. mirabilis*. This variation might be related to the different component of methanolic extract.

As recorded for *E. coli* and *Psd. aeruginosa*, no activities were shown for water soluble extracts (method I and method II) from extracts of tested plant against *P. mirabilis.*

Table 1. Chemical composition of *A. fragrantissima* essential oil

Components	LRI [a]	LRI [b]	%	Identification
santolina triene	910	1010	1.1	MS, RI*e*
α-thujene	932	1016	1.45	MS, RI, ST
α-pinene	941	1029	1.45	MS, RI, ST
α-fenchene	953	1054	tr[c]	MS, RI
camphene	955	1071	tr	MS, RI, ST
benzaldehyde	963	1491	tr	MS, RI, ST
sabinene	978	1112	2.35	MS, RI, ST
β-pinene	981	1126	4.55	MS, RI, ST
2,3-dehydro-1,8-cineole	992	–	tr	MS, RI
yomogi alcohol	997	1401	1.0	MS, RI
α-terpinene	1020	1183	2.4	MS, RI, ST
p-cymene	1028	1274	2.95	MS, RI, ST
limonene	1033	1198	1.0	MS, RI, ST
santolina alcohol	1035	1413	2.15	MS, RI
β-phellandrene	1035	1199	6.2	MS, RI, ST
1,8-cineole	1039	1204	0.65	MS, RI, ST
γ-terpinene	1064	1252	5.6	MS, RI, ST
cis -sabinene hydrate	1070	–	tr	MS, RI, ST
cis -linalool oxide	1075	–	tr	MS, RI
linalool	1101	1547	11.0	MS, RI, ST
trans -sabinene hydrate	1103	–	tr	MS, RI, ST
α-thujone	1106	1428	2.4	MS, RI, ST
β-thujone	1106	1446	1.6	MS, RI, ST
myrcenol	1118	1588	1.4	MS, RI
fenchol	1123	1584	1.05	MS, RI
chrysanthenone	1125	–	tr	MS, RI
cis-p-menth-2-en-1-ol	1127	–	tr	MS, RI
trans-p-menth-2-en-1-ol	1142	–	0.5	MS, RI
camphor	1145	1522	1.6	MS, RI, ST
cis -chrysanthenol	1164	–	tr	MS, RI
borneol	1175	1796	1.7	MS, RI, ST
4-terpineol	1182	1607	15.65	MS, RI, ST
p-cymen-8-ol	1185	1838	0.25	MS, RI
α-terpineol	1193	1698	2.0	MS, RI, ST
methyl chavicol	1197	–	0.32	MS, RI, ST
verbenone	1208	1716	4.42	MS, RI, ST
carvone	1246	1741	9.42	MS, RI, ST
isobornyl acetate	1285	1582	1.0	MS, RI
thymol	1292	2187	0.32	MS, RI, ST
carvacrol	1298	2219	tr	MS, RI, ST
α-terpinyl acetate	1350	–	tr	MS, RI, ST
β-caryophyllene	1419	1604	tr	MS, RI, ST
germacrene D	1491	1691	1.42	MS, RI
bicyclogermacrene	1496	1493	0.5	MS, RI
δ-cadinene	1524	1731	tr	MS, RI, ST
caryophyllene oxide	1578	2071	0.5	MS, RI, ST
cedrol	1603	2143	3.0	MS, RI
β-eudesmol	1651	–	tr	MS, RI
Total identified			94.1	

[a]*linear retention indexes (apolar column)*
[b]*linear retention indexes (polar column)*
[c]*trace amounts < 0.1*
[e]*identification: MS=mass spectrometry, RI=retention index, ST=pure reference compound*

Effect against fungi

There was fluctuation in the activity of different extracts of the tested plant against all tested fungi. The activity of crude phenolic extract against *Aspergillus* spp. and *Rhizopus spp*. was strong (MIC 3.125 mgml^{-1} for both fungi). The mode of antifungal action of phenolic extract might be related to their ability to inactive adhesions, enzymes, cell envelope transport proteins [27] or related to the number of OH groups [17].

Crude saponin extracts exhibited strong activity against *Candida albicans*, *Penicillium* spp. and *Rhizopus spp*. (MIC 3.125 mg ml^{-1} for both fungi). This observation was consistent with the result in which saponin inhibited the growth of several *Candida species* [28]. The mechanism of the antifungal activities of saponins seems to be through disruption of fungal membranes. This finding was consistent with other investigation recorded by Keukens *et al*. [29]. They reported that the major mechanism of the antifungal activities of saponins apparently involved their ability to complex with sterols in fungal membranes and cause loss of membrane integrity. It was found that saponin derivatives inhibited the growth of *C. albicans* [30].

A strong activity was recorded from extract of aerial dried powder by ethyl acetate from the tested plant against *Candida albicans*, *Penicilium* spp. and *Aspergillus* spp. (MIC 3.125 mg ml^{-1} for three fungi).The mode of action of ethyl acetate could be related to their ability to alter membrane properties. The mechanism thought to be responsible for ethanolic inhibition could be by disruption of microbial membrane [31]. Also this activity might be due to the presence of sesquiterpene lactones in their fractions [22].

Penicilium spp. was strongly inhibited by fresh flowers soaked in ethanol (MIC 3.125 mg ml^{-1}). This strong activity might be related to the nature of substances dissolved in ethanol or due to susceptibility of the fungi to the plant species.

Table 2. Various extracts of *Achillea fragrentissima*. Weight of aerial parts and flowers was 20 g

Part of plant tested		*Type of extract*	*Weight of extract (%)*
Aerial dried powder (with flowers)	1)	Crude phenolic	5.5
	2)	Crude saponin	4.0
	3)	Ethyl acetate extracts	3.2
	4)	Ethanol extracts	5.4
	5)	Soaked in methanol	18.0
	6)	Water-soluble extract method I	1.6
	7)	Water-soluble extract method II	1.0
Aerial dried powder (without flowers)	8)	Crude saponin	2.6
Fresh Aerial (with flowers)	9)	Soaked in ethanol	10.2
Fresh flowers	10)	Soaked in ethanol	9.3

Table 3. Antibacterial activity of various extracts of *Achillea fragrantissima* by agar diffusion and MIC methods

Type of Extract	*Gram-positive*		*Gram-negative*							
	St. aureus		*E.coli*		*P. aeruginosa*		*P. mirabilis*		*K. pneumonia*	
	ADM	MIC	ADM	MIC	ADM	MIC	ADM	MIC	ADM	MIC
1	-	-	-	-	-	-	16	25	-	-
2	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	18	12.5	-	-
4	-	-	-	-	-	-	15	12.5	-	-
5	14.6	3.125	-	-	-	-	14	6.25	-	-
6	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-
8	16.0	3.125	-	-	-	-	-	-	-	-
9	18.0	25	-	-	-	-	14	3.125	-	-
10	20.0	3.125	13.9	12.5	11.8	-	14	12.5	13.5	12.5
+ve control	12.0	-	13.0	-	13.0	-	13	-	13.0	-
-ve control	-	-	-	-	-	-	-	-	-	-

Where, ADM: Agar diffusion method; MIC: Minimal inhibition concentration

Table 4. Antifungal activity of various extracts of *Achillea fragrantissima* by agar diffusion and MIC methods

Type of extract	*Candida albicans*		*Penicilium spp.*		*Fusarium spp.*		*Aspergillus spp.*		*Rhizopus spp.*	
	ADM	MIC	ADM	MIC	ADM	MIC	ADM	MIC	ADM	MIC
1	15.2	12.5	14.0	12.5	15.0	6.25	16.0	3.125	15.0	3.125
2	14.3	6.25	12.0	3.125	15.0	6.25	15.0	6.25	15.0	3.125
3	15.0	3.125	15.0	3.125	15.0	6.25	15.0	3.125	15.0	6.25
4	-	-	-	-	-	-	-	-	-	-
5	14.0	12.5	13.0	6.25	14.0	6.25	14.0	12.5	15.0	6.25
6	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-
8	14.2	6.25	13.0	6.25	14.0	6.25	15.2	12.5	16.0	12.5
9	16.0	3.125	15.2	3.125	16.0	3.125	18.0	3.125	24.0	3.125
10	20.3	6.25	20.0	3.125	22.0	6.25	22.0	6.25	24.0	6.25
+ve control	12	-	12	-	12	-	12	6.25	12	-
-ve control	-	-	-	-	-	-	-	-	-	-

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