COMPOSITION AND ANTIMICROBIAL ACTIVITY OF *ACHILLEA FRAGRANTISSIMA* ESSENTIAL OIL USING FOOD MODEL MEDIA

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Abstract

The emergence of food-borne disease combines with consumer demands for avoiding artificial food preservatives shifted the research interest to natural food preservatives such as essential oils isolated from medicinal plants which have antimicrobial activity. The aim of this study was to evaluate the efficiency of *Achillea fragrantissima* essential oils (E.O.) as natural food preservative in four different food model media (cheese, meat, milk, and tomato) against two gram-positive and two gram-negative bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) and three different food spoilage fungi using agar well diffusion method. The results showed that the maximum activity of *Achillea fragrantissima* E.O. was against *Bacillus cereus* in tomato media with relative inhibition zone diameter (RIZD) 180%, the most sensitive food spoilage fungi was *Aspergillus* spp. in tomato media with RIZD 150%. The results showed that there was inhibitory effect of *Achillea fragrantissima* E.O. against most of the tested species in different food model media which will lead to control food pathogen organisms by using E.O. as food preservative and flavoring agents.

Keywords: Achillea fragrantissima, Essential oils, Antibacterial, Antifungal, Food preservatives

Introduction

Protection of different food products are needed against microbial spoilage to increase food shelf life. There are growing demands of consumers for safe and natural products, without chemical preservatives; therefore, there has been increasing interest from researchers to assess the feasibility of mild preservation techniques and to improve the microbial quality and safety of products, and to replace chemical preservatives with natural, effective and nontoxic compounds. (Goñi *et al*., 2009).

Essential or volatile oils (EOs) can be defined as an aromatic oily liquids obtained from any of plant materials (flowers, leaves, roots, fruits and seeds). Essential oils can be obtained by different methods such as expression, fermentation, extraction and steam distillation, but the most commonly used method is steam distillation method which used for commercial production of essential oils (Van de Braak and Leijten, 1999; Burt, 2004). In the European Union (EU), Eos are used in food, perfumes and pharmaceuticals. Also little more than 2% of the total markets are the well-known use of essential oil in aromatherapy (Burt, 2004).

Essential oils and their components have antibacterial properties which exploited in different commercial products such as dental root canal sealers, antiseptics and feed supplements. A few of commercially available preservatives contain essential oils. One of these food preservative is ‘DMC Base Natural’ which is produced by DOMCA S.A., Alhendı´n, Granada, Spain which contain rosemary, sage and citrus essential oils (50%) and glycerol (50%) (Mendoza-Yepes *et al*., 1997). Another safe food additive in the US is Protecta One and Protecta Two which are blended herb extracts produced by Bavaria Corp. Apopka, FL, USA. Sodium citrate and sodium chloride are used respectively to disperse extracts which probably contain one or more essential oils, although the precise contents are not made known by the manufacturer (Cutter, 2000). Further essential oils effects are using them in widely differing products such as commercial potato bud suppressants and insect repellents (Burt, 2004).

The genus *Achillea* L. (Asteraceae) is commonly known as yarrows and represented by about 115 species found in the Northern Hemisphere, mostly in Europe and Asia (Radulovic *et al*., 2010; Benedek *et al*., 2008; Nemeth and Bernath, 2008, Motavalizadehkakhky *et al*., 2013). The *Achillea* L. species belong to the oldest medicinal plants that are used in folk medicine and for pharmaceutical purposes. Plants which used in folk medicine contain a complex of different pharmacological compounds, such as, alkaloids, bitters, flavonoids, lignans, tannins, terpenes etc. (Aburjai and Hudaib, 2006). *Achillea* species are diuretic, emmenagogue agents, used for healing wound, curing stomachache, diarrhea and antichloristic antispasmodic, antiseptic and infection preventing properties, and have also been used to reduce sweating and to stop bleeding (Motavalizadehkakhky *et al*. 2013). Different researches have been previously studied the antimicrobial, antioxidant, antitumor, antiinflammatory, antidiabetic activity, and cytotoxic effects of different *Achillea* species (Benedek *et al*., 2008; Kupeli-Akkol *et al*., 2009; Demirci *et al*., 2009; Konyalioglu and Karamenderes, 2005; Iscan *et al*., 2006; Karamenderes and Apaydin, 2003).

Antifungal and insecticidal activity of five *Achillea biebersteinii* essential oil compositions from central Turkey had also been investigated (Tabanca *et al*., 2011; Motavalizadehkakhky *et al*. 2013).

Higher concentrations of essential oils are needed in food to achieve the same effect of essential oil when used against food-borne pathogens and spoilage microorganisms when in vitro tests are conducted (Burt, 2004; Hulin, *et al*., 1998). Few studies have been proposed to minimize essential oil concentrations which will reduce the sensory effect. Essential oils are generally recognized as safe, because of this reinforcing their natural antimicrobial effects by the addition of small amounts of other natural preservatives may be possible also may be a way of attaining a balance between sensory acceptability and antimicrobial efficiency (Goñi *et al*., 2009).

The aim of the present study was to investigate the potential role of *Achillea fragrantissima* essential oils for their antibacterial and antifungal activities using an in vitro different food model media (cheese, meat, and tomato).

Materials and methods

Plant material

Achillea fragrantissima samples were collected from North Badia, Mafraq, Jordan, during April to July 2013. Essential oils from fresh aerial parts were obtained by steam distillation.

Essential oil extraction

Aerial parts of *A. fragrantissima* were subjected to steam distillation for 3.0 to 4.0 h and yielded essential oils from 1.0 % (v/w) of plant. The obtained essential oils were dried after decanting over anhydrous sodium sulfate and then stored in refrigerator at -4°C until needed.

Antimicrobial assay of *A. fragrantissima* essential oil.

Antimicrobial assay of *A. fragrantissima* essential oil were carried out according to agar wells diffusion method (Ruberto *et al*., 2000; Burt, 2004). Antimicrobial activity of the oil was tested against two gram positive bacterial strains (*Bacillus subtilis* and *Staphylococcus aureus)*; two gram negative bacterial strains (*Escherichia coli and Pseudomonas auregnosa)* and three fungal species (*Aspergillus niger*, *Penicillium* sp. and *Rhizopus* sp.). Food model media agar was used as the bacteriological and fungal medium. The oils were diluted in 5% (v:v) dimethylsulphoxide (DMSO). Then the microbial suspensions were streaked over the surface of food model media using a sterile cotton swab to ensure the confluent growth of the organism. Aliquots of 100 µl of the diluted oil were added to agar well. The plates were incubated at 37°C for 24 h and fungi were incubated at 28°C for 72 h, zones of inhibition were observed and the diameters of inhibition zones were measured. Cefaclor (5 mg/ml) and fluconazole (10 mg/ml) were used as standard control of the tested bacteria and fungi, respectively.

Analysis of essential oils using Gas chromatography-Mass spectrometry (GC-MS)

The chemical compounds and the ratio of each in the essential oil were determined by using Gas Chromatography Mass Spectrometry (GC-MS). Varian chrompack CP-3800 GC/MS/MS-200 equipped with split-splitless injector and DB-5 GC column (5% diphenyl 95% dimethyl polysiloxane), (30 m × 0.25 mm ID, 0.25 μm film thickness) was used. The injector temperature was set at 250 C^o with a split ratio of 1:10. Detector and transfer-line temperatures were 160 C^o and 230 C^o respectively. A linear temperature program was used to separate the different oil components.

Food model media preparation

200 g of tomato, meat, handmade cheese and milk were added to 100 ml of sterile distilled water and shaken for 1 min. The suspension was mixed and homogenized by a blinder then filtered using cotton gauze. In order to obtain a solid media, 1.5% agar was added to food model media. The media were then autoclaved at 121ºC for 15 min.

Percentage Relative Inhibitory Zone Diameter (RIZD) for Bacteria and Fungi

The antimicrobial activity expressed as percentage relative inhibition zone diameter (RIZD) was calculated according to Perez *et al*., (1990) as follows:

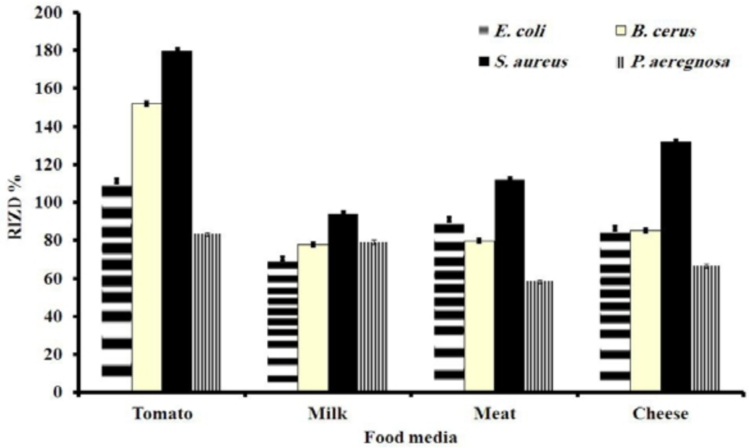
$$\%\,\mathrm{RIZD}=\left(\frac{\mathrm{IZD\ sample}-\mathrm{IZD\ negative\ control}}{\mathrm{IZD\ antibiotic\ standard}}\right)\times 100$$

Where RIZD is the percentage of relative inhibition zone diameter and IZD is the inhibition zone diameter (mm).

Results

The antibacterial activities of *A. fragrantissima* essential oil are given in figure (1). The results show that the essential oil possessed significant antibacterial activity comparing to the standard antibiotic. The essential oil possessed antibacterial activity against all used bacterial strains with all type of food media models. The highest antibacterial activity with relative inhibition zone diameter (RIZD) 180 ± 9 % was observed against *S. aureus* using tomato media, while the essential oil shows the lowest antibacterial activity with RIZD 58 ± 7 % was observed against *P. aureginosa* using meat media.

Figure 1: Antibacterial activity of *A. fragrantissima* essential oil against four bacterial species.



The antifungal activities of essential oils of *A. fragrantissima* are given in figures (2). The results showed that the essential oils showed significant antifungal activity comparing to the standard antifungal. The antifungal activities of *A. fragrantissima* essential oil against *Penicillium* sp. using meat media showed the highest RIZD (152± 9 %) comparing to standard antifungal, while the lowest RIZD of the essential oil (82±7%) was against *Rhizopus* sp. using cheese model media.

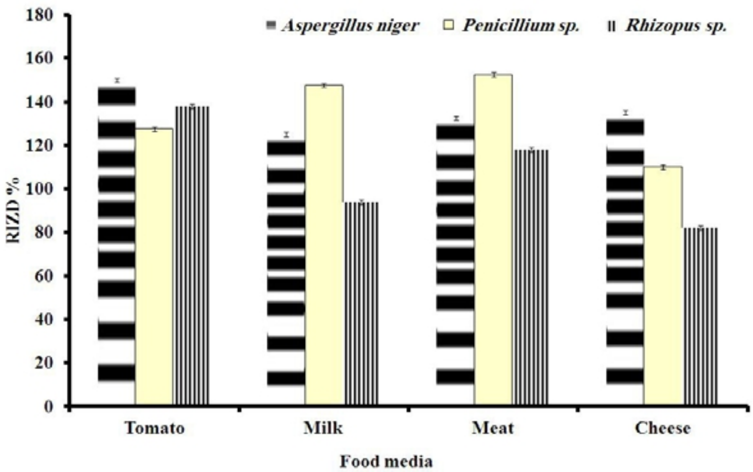


Figure 2:

Antifungal activity of *A. fragrantissima* essential oil against three isolated fungal species

Table (1) the chemical composition of *A. fragrantisma* essential oil. The GC-MS analyses indicated that 15 compounds (87.09%) were identified. As shown in table (1), Artemisia ketone, β-Sesquiphellandrene and carvacrol are the major observed compounds with ratio 19.87, 14.57 and 13.44 %, respectively.

Table 1: Chemical composition of *A. fragrantisma* essential oil

#	Rt	% content	CI	AI	KI	Compound	Identification
1	8.790	2.1	995	999	999	Yomogi alcohol	RI-MS
2	9.954	1.24	1026	1020	1024	p-Cymene	RI-MS
3	10.138	2.26	1031	1034	1040	Santolina alcohol	RI-MS
4	11.219	19.87	1058	1056	1062	Artemisia ketone	RI-MS
5	12.12	0.91	1080	1080	1083	Artemisia alcohol	RI-MS
6	13.352	12.36	1110	1101	1102	α-Thujone	RI-MS
7	13.873	3.91	1121	1112	1114	β-Thujone	RI-MS
8	14.767	1.48	1143	1137	1142	trans-Sabinol	RI-MS
9	15.666	6.06	1163	1169	1173	Artemisyl acetate	RI-MS
10	21.301	3.62	1290	1289	1290	trans-Sabinyl acetate	RI-MS
11	21.857	13.44	1305	1298	1299	Carvacrol	RI-MS
12	29.561	1.55	1486	1484	1485	Germacrene D	RI-MS
13	31.178	14.57	1527	1521	1522	β-Sesquiphellandrene	RI-MS
14	36.432	2.66	1660	1652	1653	α-Eudesmol	RI-MS
15	37.725	1.06	1694	1685	1685	α-Bisabolol	RI-MS
		87.09				Total	

Discussion

The antibacterial activity of essential oils was performed against four bacterial species. Two gram negative bacteria, *E. coli* and *P. auregenosa* and the other two species are gram positive, *B. subtilis* and *S. aureus*. The essential oil showed antibacterial activity and inhibit the growth of all tested bacterial species.

The essential oil showed variation in antibacterial activity against different bacteria species using different type of food model media(Fig. 1). The essential oil showed higher activity against *S. aureus* comparing to standard antibiotic (RIZD = 180%) using tomato media, while essential oil showed significantly higher RIZD (111 %) using tomato media comparing to other media against *E. coli*. The variations in bacterial response to essential oils may refer to the structural difference of bacteria and the mode of action of the essential oils against these bacterial species.

The antifungal activity of *A. fragrantisma* essential oil was performed against three fungal species that were isolated from food samples. These species were chosen according to their ability as food contaminants and they were isolated from spoiled food samples. The tested essential oil (fig. 2) possessed antifungal activity and inhibit the growth of all tested fungal species. It was observed that the effect of essential oil against *Aspergillus niger* using tomato media was significantly higher than the effect of other tested fungi, this may due variation in resistance of the fungal species.

It is very difficult to comparison between results reported about antibacterial properties of different essential oils due to the variation in methods of antimicrobial effects evaluation, source of essential oil and different species of used microbes (Arab and Ettehad, 2008).

As mentioned in some studies about antimicrobial effects of plants some of essential oil components are responsible for antimicrobial properties. Kim *et al*. (1995) studied the antibacterial activity of carvacrol on *Salmonella typhimurium* and its rifampin resistant genus. It was found that carvacrol showed powerful bactericidal effect against rifampin-resistant genus. Another study taht showed powerful bacteriostatic effect of *Thymus revolatus* essential oil on *Staphylococcus aureus* (Karman *et al*., 2001) . Karman *et al*. (2001) illustrated high amount of carvacrol in essential oil which may be possible reason of the powerful bacteriostatic effects.

The chemical composition of plant essential oils was performed using MS-GC analysis (table 1). The ratio of identified compounds range between 0.91% for Artemisia alcohol and 19.87 % for Artemisia ketone. This result showed variations in the *Achillea fragrantissima* chemical composition from previous studies. El-Shazly *et al*. (2004) found that the major component of Achillea fragrantissima essentai oil was *cis*-thujone which form 29.48 %., also Alsohaili and Oran (2011) found that the perecnte of these two compound (Artemisia alcohol and Artemisia ketone) was 0.73% and 23.98 % respectivley. The different in ratio of some molecule found in plants essentail oils affected by different factors scuh as time of plant collection, humidity, climate and other environmental conditions

The ratio of the components present in essential oils varies greatly. Major constituents can form up to 85% of the essential oils, while the remaining components can be present in only trace amounts (Miguel, 2010). Other study showed that simultaneous application of thymol and ρ-cymen resulted in a greater antibacterial effect compared to using the seperated compounds (Delgado *et al*., 2004). Therefore, the presence of some chemical compounds in the essential oil suc as (thymol, carvacrol and ρ-cymene) in plant essential oils, could be the reason for antimicrobial effects of the medicinal plant.

Conclusion

From the above discussion it is cleared that *Achillea fragrantissima* essential oil in all type of food model media showed significant high antimicrobial activity against all tested microorganisms compared with standard antibiotics such as Cefaclor (5 mg/ml) and antifungal such as fluconazole (10 mg/ml). The RIZD was higher within bacterial species compared to fungal species. The chemical composition of *A.fragrantisma* essential oil showed that E.O contains many phytochemicals such as Carvacrol which may responsible for antimicrobial activity of *A.fragrantisma* essential oil. It can be concluded that this essential oil may be used as natural food preservatives.

References:

Aburjai, T., & Hudaib, M. (2006). Antiplatelet, antibacterial and antifungal activities of *Achillea falcata* extracts and evaluation of volatile oil composition. *Pharmacog. Mag*. **2**:191-198.

Alsohail, S. & Oran, S. (2011) Assement of antimicrobial and antioxidant activity of essential oils extracted from some selected aromatic Jordanian medicinal plnts.*Unpublished PhD. Thesis*. The university of Jordan.

Arab, A., & Ettehad, G. H.(2008). Effects of *Zataria multiflora* Boiss on common pathogenic grame-positive cocci and gram negative bacilli. *J. Animal Vet. Adv*. **7** (6) :695–7.

Benedek, B., Rothwangl-Wiltschnigg, K., Rozema, E., Gjoncaj, N., Reznicek G., Jurenitsch J., Kopp B., & Glasl S. (2008). Yarrow (*Achillea millefolium* L): Pharmaceutical quality of commercial samples. *Pharmazie*. 63:23-26.

Burt, S. (2004). Essential oils: their antimicrobial properties and potential application in foods-A review. *International Journal of Food Microbiology*. 94: 223-253.

Cutter, C.N. (2000) Antimicrobial effect of herb extracts against *Esherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium* associated with beef. *Journal of Food Protection* 63: 601-607.

Delgado, B., Palop, A., Fernández, P. S., & Periago, P. M.(2004). Combined effect of thymol and cymene to control the growth of *Bacillus cereus* vegetative cells. *Eur. Food Res.Technol*. 218 (2): 188 - 93.

Demirci, F., Demirci, B., Gurbuz I., Yesilada, E., & Baser, K. H. C. (2009). Characterization and biological activity of *Achillea teretifolia* Willd. and *A. nobilis* L. subsp. *neilreichii* (Kerner) Formanek essential oils. *Turk. J. Biol*. 33:129-136.

El-Shazly, A. M. Hafez, S. S. & Wink M. (2004) Comparative study of the essential oils and extracts of *Achillea fragrantissima* (Forssk.) Sch. Bip. and *Achillea santolina* L. (Asteraceae) from Egypt. *Pharmazie* 59: 226–230

Goñi, P.; López, P.; Sánchez, C.; Gómez-Lus, R.; Becerril, R., & Nerín, C.(2009). Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. *Food Chem.,* 116: 982–989.

Hulin, V., Mathot, A. G., Mafart, P., & Dufosse, L. (1998). Antimicrobial properties of essential oils and flavour compounds. *Sciences Des Aliments*, 18(6): 563–582.

Iscan, G., Kirimer, N., Kurkcuoglu, M., Arabaci, T., Kupeli, E., & Baser, K.H.C. (2006). Biological activity and composition of the essential oils of *Achillea schischkinii* Sosn. and *Achillea aleppica* DC. subsp. aleppica. *J. Agric. Food Chem*., 54:170-173.

Karamenderes, C. & Apaydin, S. (2003). Antispasmodic effect of *Achillea nobilis* L. subsp. sipylea (O. Schwarz) Bässler on the rat isolated duodenum. *J. Ethnopharmacol*., 84:175-179.

Karman, S., Digark M., Ravid V. & Ilcim A. (2001). Antibacterial and antifungal activity of essential oil of *Thymus revolatus* Celak from Turkey. *J. Ethnopharmacol*., 76: 183- 186.

Kim, J., Marshall, M.R., & Wei C. I. (1995). Antibacterial activity of some essential oil components against five foodborne pathogens. *Journal of Agricultural and Food Chemistry*, 43: 2839–2845.

Konyalioglu, S., & Karamenderes C. (2005). The protective effects of *Achillea* L. species native in Turkey against H2O2-induced oxidative damage in human erythrocytes and leucocytes. *J. Ethnopharmacol*., 102:221-227.

Kupeli-Akkol, E., Koca, U., Pesin, I., & Yilmazer D. (2009). Evaluation of the wound healing potential of *Achillea biebersteini*, Afan. (Asteraceae) by *in vivo* excision and incision models. Evid-Based Complement. *Altern. Med*. 6:1-7.

Mendoza-Yepes, M. J., Sanchez-Hidalgo, L. E., Maertens, G., & Marin-Iniesta, F.(1997). Inhibition of *Listeria monocytogenes* and other bacteria by a plant essential oil (DMC) in Spanish soft cheese. *J. Food Safety,* 17: 47-55.

Miguel, G.M. (2010). Antioxidant and Anti-Inflammatory Activities of Essential Oils: A Short Review. *Molecules* 15(12): 9252-9287.

Motavalizadehkakhky, A.,Shafaghat, A., Zamani, H. A., Akhlaghi, H., Mohammadhosseini M., Mehrzad, J., & Ebrahimi, Z. (2013). Compositions and the *in vitro* antimicrobial activities of the essential oils and extracts of two *Achillea* species from Iran. *Journal of Medicinal Plants Research*, 7: 1280-1292.

Nemeth, E., & Bernath, J. (2008). Biological activities of yarrow species (*Achillea* spp.). *Curr. Pharm. Des*., 14:3151-3167.

Perez, C., Poly, M., & Bazevque, P. (1990). An antibiotic assay by the agar well diffusion method. *Acta Biologiae et Medicine Experimentalis*, 15:113-115.

Radulovic, N.S., Blagojevic, P.D., Skropeta, D., Zarubica, A.R., Zlatkovic, B.K., & Palic R.M. (2010). Misidentification of Tansy, *Tanacetum* *macrophyllum*, as yarrow, *Achillea grandifolia*: a health risk or benefit. *Nat. Prod. Commun*., 5:121-127.

Ruberto, G., M.T. Baratta, S.G. & Dorman, H.J. (2000). Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essential oils. *Planta Med*., 66: 687-693.

Tabanca, N., Kirimer, N., Demirci, B., Demirci F., & Baser, K.H.C. (2011). Composition and antimicrobial activity of the essential oils of *Micromeria cristata* sub sp. *phyrgia* and the enantiomeric distribution of borneol; *J. Agric. Food Chem.,* 49:4300-4303.

van de Braak, S.A.A.J., & Leijten, G.C.J.J.(1999). *Essential Oils and Oleoresins: A Survey in the Netherlands and Other Major Markets in the European Union;* CBI, Centre for the Promotion of Imports from Developing Countries: Rotterdam, The Netherlands, p. 116.