ISSN : 0974 - 7435 Volume 11 Issue 4

BioTechnology
An Indian Journal

FULL PAPER

BTAIJ, 11(4), 2015 [121-125]

Composition of *Helichrysumarenarium* essential oil and antimicrobial activity against some food-born pathogens

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ABSTRACT

The aim of this study was to determine the composition and antimicrobial effect of *Helichrysumarenarium L.* essential oil in "in-vitro" condition. For this purpose, the chemical composition of the essential oil which obtained by hydro-distillation was examined by GC/MS and the antimicrobial effect was studied on the growth of threefood born pathogens including *Serratiamarcescens*, *Streptococcus agalactiae* and *Staphylococcus aureus* using micro-dilution method. The minimum inhibitory concentration (MIC) and minimum bactericidal were determined for the essential oil at ten concentrations. Chemical composition analysis of the essential oil identified a total of 38 compounds. The main components of essential oil were a-pinene (32%), 1,8-cineole (16%), α-humulene (15%), β-caryophyllene (8%). Other separated components accounted for less than 29% of the oil. Results of antimicrobial analysis showed MIC values of 406, 812 and 812µg/ml respectively for *Serratiamarcescens*, *Streptococcus agalactiae* and *Staphylococcus aureus*but the MBC was observed respectively at 812, 3250 and 3250µg/ml for the mentioned bacteria.

KEYWORDS

Helichrysumarenarium;
Antimicrobial activity;
Essential oil composition;
Minimum inhibitory concentration.

INTRODUCTION

Food borne disease mediated by pathogenic microorganisms or microbial toxins is an important global public health problem because they take a huge toll on human health and mortality[1]. It has been estimated that as many as 30% of people in the industrialized countries suffer from food borne diseases each year caused by microbes[2]. Food additives have been used for centuries in the food processing practices for several purposes including the prevention of microbial growth and increase in the food shelf lives[3]. Due to the excessive use of food preservativeswhich some of them are doubtful to be carcinogenic and teratogenic and also increasing consumer demand to natural foods with a long shelf life and without chemical preservatives, food producers trend to replace chemical preservatives with natural forms such as oils and herbal extracts as antibacterial additives[4-6]. In the recent years, efforts have been devoted to find new antimicrobial materials from natural resources for food preservation[7]. Reports indicated that many extracts and essential oils of edible plants

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had properties to prevent against a wide range of fungal contamination of foods[4,7-12].

Essential oils are liquid, volatile, natural, limpid and rarely coloured, lipid soluble and soluble in organic solvents with a generally lower density than that of water and formed by aromatic plants as secondary metabolites[13]. They have been used in many cases because of their natural properties such as antifungal, antibacterial and insecticidal activities. An estimated 3000 Essential oils are known, of which about 300 are commercially important especially for the pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries[13,14].

Helichrysumarenarium L. (popularly known as everlasting, immortal flower or fadeless flower) belongs to asteraceae family[15]. It is a perennial herbaceous plant of height 15–40 cm that flowers in June- August[16] with yellow to reddish-orange or even brown inflorescences of various colour intensity[17] and broadly distributed in Europe, western Siberia, and central Asia[16,18]. *Helichrysumarenarium* are used for the treatment of kidney stones, uro-genital disorders, stomach pain, jaundice, diarrhea, asthma[15], gall-bladder and gastricdisorders, cystitis, and arthritis[17]. For coughs and colds, a tea is prepared or the leaves are boiled in milk. For pain relief, leaves are burned and the smoke is inhaled. Leaves are widely used on wounds to prevent infection[19].

The aims of the present study were to evaluate the composition and potential antimicrobial activities of essential oil of *Helichrysumarenarium*L collected from Iran on the growth of some food born pathogens.

MATERIAL AND METHODS

Plant material and extraction of essential oil

Aerial parts of the *Helichrysumarenarium L*. plant were collected in 2012 from KhorasanRazavi Province (the northeast of Iran). The plant confirmed by Medicinal Plants Institute, Ferdowsi University, Mashhad, Iran. The essential oil of aerial parts of the *H.arenarium*L. was extracted with water steam distillation using a clevenger apparatus according to the method of British Pharmacopoeia. The distilled essential oils were dried with anhydrous sodium sulfate and stored in the sterilized vial at 4°C until use[4,5,20].

Analysis of the essential oil

The chemical composition of the essential oil was analyzed using GC–MS technique. The mass spectrometer was Agilent 6890 N GC/5973MSD-SCAN (Agilent Technologies, Palo Alto, CA, USA) in the electron impact (EI) ionization mode (70ev) and HP- 5MS (bonded and cross-linked 5% phenyl-methylpolysiloxane,30 mm-0.25 mm, coating thickness 0.25 mm) capillary column (Restek, Bellefonte, PA). Injector and detector temperatures were set at 220°C. The oven temperature was held at 50°C for 30 min, then programmed to 240°C at rate of 3°C/min. Helium (99.99%) was the carrier gas at a flow rate of 1 ml/min. Diluted samples (1/100 in hexane, v/v) of 1.0 were injected manually. The identification of the components was based on the comparison of their retention times and mass spectra with the data given in the literature, National Institute of Standard and Technology (NIST), Wiley and our own created library[21].

Organisms and inoculation conditions

The test organisms used in this study included *Serratiamarcescens (*PTCC 1187), *Streptococcus agalactiae*(PTCC 1321)and*Staphylococcus aureus*(PTCC29213)which were obtained from Persian Type Culture Collection (PTCC), Iran.

To prepare microbial suspension, bacterial species were cultivated on nutrient agar (Merck, Germany) slant at 37°C for 24 h while yeasts and fungal species were cultivated on PDA (Merck, Germany) slants and incubated at 25°C for 48 h. Finally, suspensions were adjusted to 0.5 McFarland standard turbidity[22,23,24,25,26]. The yeasts and fungal suspensions were adjusted to make a conidial or spores concentrations of 10^6 cell or spore/ml via counting with a hemacytometer[10,20,27,28]. Bacterial suspensions were standardized to concentrations of 1.5×10^8 CFU/ml[22,24].

Minimum inhibitory concentration (MIC) test

H.arenarium essential oil dissolved at 5% dimethyl sulfoxide (Aplichem, Germany) and Then, it diluted to the highest concentration (60000 µg/ml), and then serial twofold dilutions were made in a concentration range from 50.75 to 30000 µg/ml.

MIC values of essential oil against microbial strains were determined based on a microwell dilution method.

Ninety five µl of Mullerhinton broth (Merck, Germany) was dispended in to each 96 wells. 100 µl of stock solution of *H.arenarium*essential oil was added in to the first wells. Then 100 µl from their serial dilutions was transferred in to other consecutive wells except the well number 11 as positive control. Then 5 µl of the microbial suspension was added to each well except well number 12 as negative control. Contents of each well were mixed on a plate shaker at 300 rpm for 20 s and then incubated at 25°c for 48 h for yeasts and fungi and 37°c for 24 h for bacterial strains. Microbial growth was determined by detecting the absorbance at 630 nm using the ELX808 Elisa reader (Biotek Instrument Inc, USA). The MIC of essential oil was taken as the lowest concentration that showed no growth[22,24,25,29,30].

RESULTS

Chemical composition of *H.arenarium* essential oil

Chemical composition analysis of the essential oil identified a total of 38 compounds. The main components of essential oil were a-pinene (32%), 1,8-cineole (16%), α-humulene (15%), β-caryophyllene (8%). Other separated components accounted for less than 29% of the oil.

Effect of essential oil of *Helichrysumarenarium L* on microbial species

Antimicrobial activity of essential oil of *Helichrysumarenarium* L. was determined via the microwell dilution method at 10 concentrations against threefood born pathogens including *Serratiamarcescens*, *Streptococcus agalactiae* and *Staphylococcus aureus*. The results of in vitro antimicrobial activity assay showed that the essential oil possessed antimicrobial activity against the microorganisms tested.

The antimicrobial effect of essential oil against the microorganisms is shown in TABLE1. Results obtained from the microdilution method, followed by measurements of MIC and MBC indicated that essential oil of *H.arenarium* L. exhibited antibacterial activity against tested bacteria and the sensitivitywas as follows:*Serratiamarcescens*>*Streptococcus agalactiae*=*Staphylococcus aureus.*

TABLE 1 : Minimum inhibitory concentration (µg/ml) and minimum bactericidal concentration (µg/ml) of essential oil of *Helichrysumarenarium*

Microorganisms	MIC (µg/ml)	MBC (µg/ml)
Serratiamarcescens	406	812
Streptococcus agalactiae	812	3250
Staphylococcus aureus	812	3250

- The values in the table are an average of 3 experiments

DISCUSSION

Chemical composition of *H.arenarium* essential oil

Different studies have been done in other regions on chemical composition of the essential oil of different species of *Helichrysum.*Ramanoelina et al., (1992) analysed*Helichrysumbracteiferum* oil of fresh leaves collected in Madagascar Island and reported that 1, 8-cineole (18%), α-humulene (11.6%) and β-caryophyllene (9.6%) were the main components[32]. Torabbeigi et al., (2011) found that the essential oil of aerial parts of *HelichrysumAucheri* from Iran contained a-pinene (39.6 %), 1,8-cineole (19.7 %) and β-caryophyllene (7.3 %) as the main constituents[33]. EI-Olemy studied on aerial parts of *Helichrysumforsskahlii*, indemic to southern Saudi Arabia, and reported selina-5,11-diene (45.3%), δ-3-carene (7.8%), 1,8-cineole (4.2%) and β-caryophyllene (4.9%) as main constituents[34]. Baser et al. (2002) analyzed chemical composition of four commercially available Helichrysum oils obtained from, *H. bracteiferum* (DC.) Humbert, *H. cordifolium* DC.*H. hypnoides* (DC.) R. Vig et Humbert and *H. rusillonii*Hochr., of Madagascar origin. They found that *Helichrysumbracteiferum* oil was found to be rich in β-pinene (10.3%), 1,8-cineole (24.8%) and α-humulene (10.1%), whereas *H. cordifolium* oil contained β-caryophyllene (46.4%) and α-humulene (10.9%), as major components. *Helichrysumhypnoides* oil contained 1,8-cineole (51.5%) and *H. rusillonii* oil was rich in 1,8-cineole (11.7%) and β-caryophyllene (29.5%)[35]. Jidzentiene&Butkiene[36] studied on essential oil composition of *Helichrysumarenarium* (L.) Moench.of natural populations from eastern Lithuania and found

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that the principal constituents were: β-caryophyllene), δ-cadinene, octadecane and heneicosane. Monoterpenes and oxygenated monoterpenes made up 4.0–13.9%, aliphatic hydrocarbons 0.4–35.3%, and sesquiterpenes 24.7–71.2% of the oils[36]. Wande et al.[37] analyzed essential oil of the flowers of *Helichrysumodoratissimum* (L.) Less growing wild in Kenya and detected nineteen compounds in which the major constituents being α-pinene (43.4%), (E, E)-farnesol (16.8%) and α-humulene (14.6%)[37].

Effect of essential oil of *Helichrysumarenarium L* on microbial species

The antimicrobial activities of different Helichrysumspecies have been studied by different researchers[38-44]but, there is not enough data about the antimicrobial activity of essential oil of the *Helichrysumarenarium*.

Albayrak et al[40] showed that methanolic extract of *H.arenarium* inhibited growth of *E.coli* and *S.aureus* while they did not find a significant influence on *B.subtilis*[40]. Also antibacterial activity of methanolic extract of two subspecies of *H.arenarium* on *B.cereus* and *S.aureus* was demonstrated by Albayrak et al[41] but any antibacterial activity was specificated for *B.subtilis*[41]. Aslan et al in their research showed that petroleum ether and ethanol extracts of *H.arenarium* had antibacterial activity on *S.aureus* but had no effect on *E.coli*[38].The differences observed in activity of essential oil or extraction products in other researches[37,39]may be due to their different quality and quantity of active compounds in essential oil and extract. It is clear that essential oil of plant is more active than its extract[22] furthermore, the extraction product can vary in quality, quantity and in composition according to climate, soil composition, plant organ, age and vegetative cycle stage[13,14].

CONCLUSION

The results of the present study indicateed that *Helichrysumarenarium L* essential oil had antimicrobial activity; therefore, it can be used as a natural preservation to increase the shelf life of food products.

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