Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
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Asian Journal of Plant Sciences 6 (4): 692-695, 2007
ISSN 1682-3974

Chemical Constituents and Antimicrobial Activity of *Helichrysum stoechas*

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Abstract: Chemical constituents and antimicrobial activity of the essential oils and crude ethanol extract from apical part of *Helichrysum stoechas* were investigated. The chromatographic and spectrophotometric analysis revealed that the major components of essential oil were alpha-pinene (59%), limonen (16.7%), alpha-bisabolol (9.6%) and beta-carophyllene (4%). The major components of ethanolic extract were 3 isomers of caffeoylquinic acid, 2 isomeric dicaffeoylquinic acids, a pigenin glucosides, quercetin and kaempferol. Both essential oils and ethanolic extracts had significant antimicrobial activity on *Staphylococcus aureus*, *Staphylococcus epidermis* and *Klebsiella pneumonae* in addition to some pathogenic fungi as *Candida albicans*.

Key words: *Helichrysum stoechas*, chemical constituents, essential oils

INTRODUCTION

Members of the genus *Helichrysum* (*Asteraceae*) are usually aromatic, perennial shrubs, having dense leaves with hardy flower heads that are distributed allover. The Mediterranean (Jafri and Gadi, 1980; Tutin *et al*., 1980). Folk remedies including *Helichrysum* have been used to treat wounds, topical infections and respiratory ailments (Meyer and Dilika, 1996; Mathekga and Meyer, 1998; Stafford *et al*., 2005). Plants of the genus *Helichrysum* are prolific producers of a host of secondary metabolites and essential oils (Sala *et al*., 2002; Van Vurren, 2006). These are presumably responsible for the remarkable antiviral, antifungal, antimicrobial and anti-inflammatory properties showed by extracts from various *Helichrysum* species (Sala *et al*., 2003; Van Vurren, 2006).

The increasing antibiotic resistance of some pathogens that are associated with diseases has increased the interest in the development of new types of effective and nontoxic antimicrobial compounds. Plant essential oils and secondary metabolites have gained popularity in recent years as natural antimicrobial and antioxidant agents. Despite the great interest in the genus *Helichrysum*, little is known about *Helichrysum stoechas*. The objective of the present study is the determination of the chemical constituents and antimicrobial activity of the essential oils and ethanol extract of *Helichrysum stoechas* (L.) D.C. collected from Green Mountain region of Libya.

MATERIALS AND METHODS

Fresh *H. stoechas* (150 plants) were collected from Green Mountain area in Lybia during spring of 2006 then transferred to the laboratory of Floriculture and Medicinal Plants Department, Faculty of Agriculture, Moshtohor, Benha University, Benha, Egypt. Plants were dried and their apical parts were prepared for extraction and investigations.

Extraction of essential oils: The essential oils were extracted from apical area of plants by steam distillation method for 2½ h (Blazques *et al*., 1990).

Preparation of ethanol extract: Fifty gram of dried apical parts of plant were soaked in ethyl alcohol 95% for 48 h, then the preparation was squeezed through double layers of muslin and the supernatant was transferred to glass funnel with Whatman filter paper No. 1. The ethanolic extract was concentrated under vacuum (Karam *et al*., 1999).

Chemical constituents: The chemical components of essential oils and ethanolic extract of *H. stoechas* were determined and separated using Shimadzu UV-260 spectrophotometer, infrared spectrophotometer Pye Unicam SP-1000 and Silica gel (70-230 mesh, Merck) was used for thin liquid chromatography.

Antimicrobial activity: Antimicrobial activity of essential oils and ethanolic extract were tested in duplicate with some gram-positive and gram-negative pathogenic bacteria, i.e., *Staphylococcus aureus*, *Staphylococcus epidermis*, *Staphylococcus citrus*, *Sterptococcus pneumonae*, *Escherchia coli*, *Enterobacter coleacae*, *Klebsiella pneumonae*, *Pseudomonas aeruginosa* and three types of fungi i.e., *Candida albicans*, *Candida*

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tropicolis and *Torulopsis glabrata*. The tested bacteria were kindly supplied by Diary Research Laboratory, Food Science Department, Faculty of Agriculture, Moshtohor, Benha University, while the tested fungi were supplied by Plant Pathology Laboratory, Agriculture Botany Department, Faculty of Agriculture, Moshtohor, Benha University.

Central wells were made using sterilized cork porer (ϕ 6 mm) in the center of each plate and filled with the tested plant extract (200 μmL) and then inoculated under sterilized conditions with both tested bacteria and fungi. Bacterial plates were incubated at 37°C for 72 h and fungal plates were incubated at 25°C for full growth of control plate (5-7 days).

The anti-microbial activity of each test solution was estimated by measuring the zone of inhibition (clearing) around the central well. The diameter of the disc was subtracted from the measured clear zone (Levy, 2001).

RESULTS AND DISCUSSION

Chemical constituents

Essential oils: The essential oils isolated from flowers and leaves of *H. stoechas* were obtained in yield 0.7% (v/w). The major components in oil were alpha-pinene (59%), limonen (16.7%), alpha-bisabolol (9.6%), beta-carophyllene 4% and alpha-humlene (2.5%) (Table 1). These results were in agreement with that reported by Rios *et al.* (1991), Vermin and Poite (1998). Tsoukatou *et al.* (1999), Ascensao *et al.* (2001) and Carini *et al.* (2001).

The oils also contain other components in small amounts as geraniol and camphen which has bacteriostatic activity against gram positive and gram negative bacteria. This result was similar to that reported by Rios *et al.* (1990) and Chinou *et al.* (1997). Phloroglucinol and acetophenone derivatives were also identified from oil isolated from flowers and leaves of plants.

Ethanol extract: The chemical analysis of ethanolic extract obtained from *H. stoechas* revealed the identification of ten constituents in the extract including the three naturally occurring isomers of caffeoylquinic acid (37%), 2 isomeric dicaffeoylquinic acids (26.3%), 2 isomeric naringenin glucosides (2.5%) and tetrahydroxychalcone-glucoside (1%) (Table 1). These results agreed with those reported by Mericli *et al.* (1992) and Ali-Shtayeh *et al.* (1998). The chemical analysis revealed the presence of other constituents which couldn't be quantified.

Table 1: Chemical analysis of essential oil and ethanolic extract isolated from *Helichrysum stoechas*

Essential oils	Ethanolic extract
Alpha-pinine 59.0%	Isomers of caffeoylquinic acid 37.0%
Limonen 16.70%	Isomers of dicaffeoylquinic acids 26.3%
Alpha-bisabolol 9.60%	Isomers of narigenin glucosides 11.7%
Beta-carophyllene 4.00%	Querectin 8.8%
Alpha-humlene 2.50%	Kaempferol 3.7%
Geraniol	Apigenin glucosides 2.5%
Camphen	Tetrahydroxychalcone glucosides 1.0%
Phloroglucinol derivatives	
Acetophenone derivatives	

Table 2: Antimicrobial activity of essential oils extracted from *Helichrysum stoechas*

	Inhibition activity	
Tested bacteria	Essential oil	Ethanolic extract
Staphylococcus aureus	-	++ (14 mm)
Staphylococcus epidermis	+++ (21.5 mm)	-
Staphylococcus citrus	-	-
Streptococcus pneumonae	-	-
Escherchia coli	++ (15 mm)	+++ (22.5 mm)
Enterobacter coleacae	+ (5.5 mm)	+++ (24 mm)
Klebsiella pneumonae	+++ (24 mm)	+++ (22 mm)
Pseudomonas aeruginosa	-	++ (25 mm)
Tested fungi		
Candida albicans	++ (14.5 mm)	-
Candida tropicalis	-	-
Torulopsis glabrata	-	-

- = no effect, + = active with diameter of inhibition zone of 5-12 mm, + + = inhibition zone 13-20 mm, + + + = inhibition zone more than 20 mm

Antimicrobial activity

Essential oils: Data in Table 2 revealed that the essential oils extracted from leaves and flowers of *H. stoechas* had different inhibitory activities against some pathogenic bacteria and fungi. The essential oils had potent inhibition activity on *Staphyllococcus aureus*, *Staphyllococcus epidermis* and *Klebsiella pneumonae* (zone of inhibition varied from 21.5-24 m), while oil had moderate inhibitory activity on *Eschershia coli* (zone of inhibition 15.5 mm). Mild inhibitory activity of essential oil was observed on *Enterbacter coleaca* (5.5 mm), while it had no effect on other pathogenic organisms. The essential oil had moderate antifungal activity on *Candida albicans* only without any effect on other tested fungi.

These results agreed with that reported by Tsoukatou *et al.* (1999) and Roussis *et al.* (2002). The antimicrobial activity of essential oil extracted from *H. stoechas* might be attributed to that, the oil contain phloroglucinol and acetophenone derivatives which had antimicrobial activity against some types of pathogenic bacteria and some pathogenic fungi (Tomas *et al.*, 1990).

The essential oils recorded in the present study were comparable with those of Lourens *et al.* (2004). The occurrence of terpenes such as α-pinene and

sesquiterpenes such as β-caryophyllene exhibited activity in the *in vitro* 5-lipoxygenase assay (Baylac and Racine, 2003). In addition, administration by inhalation suggests that the volatile aromatic compounds may play a role in anti-infective therapy and several studies indicate significant antimicrobial properties for *Helichrysum* oils (Hutchings and Van Staden, 1994; Roussis *et al*., 2000; Van Vuuren *et al*., 2006).

Ethanol extract: The antimicrobial activities of ethanolic extract extracted were illustrated in Table 2. The ethanolic extract had potent inhibition activity on most gram-negative tested bacteria i.e., *Klebsiella pneumonae*, *Enterobacter coleacae*, *Pseudomonas aerugenosa* and *Escherchia coli* (zone of inhibition 22-25 mm). On the other hand, the ethanolic extract had moderate antibacterial activity on *Staphyllococcus aureus* while it had no effect on other tested bacteria and pathogenic fungi. These results were similar to that reported by Ali-Shtayeh *et al.* (1998).

The constituents of ethanol extract in present study were previously recorded in aqueous extract from *H. stoechas* (Carini *et al*., 2001) and other *Helichrysum* species (Tepe *et al*., 2005; Lall *et al*., 2006). These constituents were shown to display radical scavenging properties, with potency comparable to that of Trolox, the water-soluble analogue of vitamin E since many respiratory conditions and skin ailments are associated with inflammation (and hence release of free radicals) the presence of these anti-oxidant agents could explain the effectiveness of *H. stoechas* in the treatment of these conditions.

In conclusion, the present investigation indicates the potency of *H. stoechas* as antimicrobial and antioxidant plant. However, the observation that some organisms tolerate essential oils while were susceptible to ethanol extract (vice versa) robustly supports the traditional medicinal uses of *H. stoechas* as the whole crude extract.

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