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 pigment glucosides, quercetin and kaempferol. Both essential oils and ethanolic extracts had significant antimicrobial activity on Staphylococcus aureus, Staphylococcus epidermis and Klebsiella pneumoniae 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 hardly flower heads that are distributed all over the Mediterranean (Jafari 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; Mathikga 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 Vuren, 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 Vuren, 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 Libya during spring of 2006 then transferred to the laboratory of Floriculture and Medicinal Plants Department, Faculty of Agriculture, Moshothor, 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 citrinus, Streptococcus pneumoniae, Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae, Pseudomonas aeruginosa and three types of fungi i.e., Candida albicans, Candida

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692
trocolis and Torulopsis glabrata. The tested bacteria were kindly supplied by Diary Research Laboratory, Food Science Department, Faculty of Agriculture, Boshtohor, Benha University, while the tested fungi were supplied by Plant Pathology Laboratory, Agriculture Botany Department, Faculty of Agriculture, Boshtohor, Benha University.

Central wells were made using sterilized cork pokers (ϕ 6 mm) in the center of each plate and filled with the tested plant extract (200 µL) 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%), limonene (16.7%), alpha-bisabolol (9.6%), beta-caryophyllene 4% and alpha-humulene (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 camphene 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 narigenin glucosides (2.5%) and tetrahydroxycalcone-glucoside (1%) (Table 1). These results agreed with those reported by Merieli et al. (1992) and Ali-Shtayeh et al. (1998). The chemical analysis revealed the presence of other constituents which couldn’t be quantified.

<table>
<thead>
<tr>
<th>Tested bacteria</th>
<th>Inhibition activity</th>
</tr>
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<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>++ (14 mm)</td>
</tr>
<tr>
<td>Staphylococcus epidermis</td>
<td>+++ (21.5 mm)</td>
</tr>
<tr>
<td>Staphylococcus citrullus</td>
<td>-</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>++ (15 mm)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>+++ (22.5 mm)</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>++ (5.5 mm)</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>++ (24 mm)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>++ (22 mm)</td>
</tr>
</tbody>
</table>

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

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

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 Staphylococcus aureus, Staphylococcus epidermis and Klebsiella pneumoniae (zone of inhibition varied from 21.5-24 mm), while oil had moderate inhibitory activity on Escherichia coli (zone of inhibition 15.5 mm). Mild inhibitory activity of essential oil was observed on Enterobacter cloacae (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 pneumoniae, Enterobacter cloacae, Pseudomonas aerugenos and Escherichia coli (zone of inhibition 22-25 mm). On the other hand, the ethanolic extract had moderate bacteriostatic activity on Staphylococcus 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 ethanolic 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.

**REFERENCES**


