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Antibacterial, antifungal and antiviral bioactivities of selected *Helichrysum* species

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ABSTRACT

Helichrysum species are used widely to treat various medical conditions. In the present study, 14 extracts obtained from 7 *Helichrysum* species such as *H. araxinum* Takht. ex Kirp., *H. armenium* DC, *H. arenarium*, *H. pallasii*, *H. stoechas*, *H. sanguineum*, and *H. graveolens* (Asteraceae) were screened for their *in vitro* antibacterial, antifungal, and antiviral activities. Antibacterial and antifungal activities were evaluated against both standard and isolated strains of Gram negative (*E. coli*, *P. aeruginosa*, *P. mirabilis*, *K. pneumoniae*, *A. baumanni*) and Gram positive (*S. aureus*, *E. faecalis*) bacteria, as well as fungi (*Candida albicans*, *C. parapsilosis*) by microdilution method. We performed susceptibility testing according to the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS). As human pathogens both *Herpes simplex* virus Type-1 (*HSV*-1), a DNA virus, and *Parainfluenza*-3 virus (*PI*-3), an RNA virus, were employed for antiviral assessment of the water and ethanol extracts of *Helichrysum* species by using Madin-Darby Bovine Kidney and Vero cell line. All the extracts showed more potent antibacterial activity against Gram positive bacteria (ranging between 16 and 64 μg/mL) than Gram negative (8–64 μg/mL), while showing considerable antifungal activity at 8 μg/mL concentration. Antifungal activities of the extracts were found to be close to antifungal agents in routine clinical use. In particular, all of the ethanolic extracts were found to show better inhibition against *S. aureus* with a MIC value of 8 μg/mL. In the meantime, ethanolic extracts of *H. arenarium* and *H. armenium* DC showed notable antiviral activity against both *HSV*-1 and *PI*-3; at a range of 2–32 and 4–64 μg/mL, respectively, by the absence of cytophatic effect. All activities were evaluated at nontoxic concentrations. *Helichrysum arenarium* and *H. armenium* DC were found to be the most remarkable species among the tested extracts in terms of bioactivity.

1. Introduction

The name *Helichrysum* is derived from Greek "helios" meaning sun and "chrysos" meaning gold, referring to the appearance of many of the flowers defined in thisenus. Members of this genus may be annuals, herbaceous perennials or shrubs and some species can grow up to 90 cm tall. There are over 600 species of *Helichrysum* worldwide; occurring in Africa, Madagascar, Australasia, and Eurasia. Belonging to Asteraceae (Compositae) family, *Helichrysum* species are widely acknowledged in these regions as traditional medicinal plants and represented by 20 species in Turkey, while more than 244 species were identified in South Africa alone. Decoctions of Various *Helichrysum* species have been widely used in Turkish and South African folk medicine. Traditional preparations mainly consist of decoctions for their stomachic, diuretic, and antiasthmatic properties as well as activities against kidney stones. Capitulums are powdered, mixed with barley flour and used as a pomade for wound healing (Cosar and Cubukcu, 1990; Maffei Facino et al., 1990; Sezik et al., 1991, 2001; Fujita et al., 1995).

Many screening efforts have been made to find new antimicrobial and antiviral agents from extracts of medicinal plants in order to discover new drug candidates for the treatment of various infectious diseases caused by bacteria, fungi, or viruses (Abad et al., 2000; Esquenazi et al., 2002; Ozcelik et al., 2010, 2011, 2013). Also, during the past several years, emergence of microorganisms that are resistant to nearly all classes of commercially available antimicrobial agents has become a motive for the discovery of new drug candidates (Bossche et al., 1994; Gold and Moellering, 1996).

Traditional healers have long used plants to prevent or cure infectious diseases. A number of these agents appear to have structures and modes of action distinct from those of the antibiotics currently in use. Therefore, it is worthwhile to study plants and plant products for activity against microorganisms (Ye et al., 2002; Chu et al., 2003; Ibrikci et al., 2003). Also, plants can produce antimicrobial compounds to protect themselves from biotic attacks, which is essential for resistance against microbial infections. Plants are also well known to be rich sources of biologically active compounds. Since not many people

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have access to professional health services, particularly in rural areas of undeveloped and developing countries, plants with antimicrobial properties are still widely used to treat infections.

Appendino and colleauges reported that *Helichrysum italicum* was well documented in terms of ethnobotanical use and clinical efficiency and performed a systematical evaluation on the plant as a source of new pharmaceuticals. It was reported that acetone extract of *H. italicum* ssp. *microphyllum* collected from Sardinia, Italy contained the phloroglucinol α-pyrone arzanol as a potent NF-κB inhibitor, which was found to have dose-dependent activity against HIV-1 viral replication (Appendino et al., 2007). Venditti et al. collected specimens of *Helichrysum microphyllum* ssp. *tyrrhenicum* from La Maddalena Island in Italy and discovered a new glycosidic phtalide, 6-*O*-β-(D-glucopyranosyl)-4-methoxy-1(3*H*)-benzofuranone (Venditti et al., 2016). In another study, during a large-scale isolation for the heterodimeric phloroglucinyl pyrone arzanol from *H. italicum* subsp. *microphyllum* collected from Arzana, Italy, several new phenolics as well as an unusual class of lipids named santinols have been characterized. Arzanol and other selected phenolics were screened for their antibacterial activities and heterodimers were found to have potent antibacterial action against multidrug-resistant *Staphylococcus aureus* isolates (Taglialatela-Scafati et al., 2012). *Helichrysum microphyllum* Cambess. subsp. *tyrrhenicum* Bacch., Brullo e Giusso (Asteraceae) in a littoral location of La Maddalena Archipelago was investigated and its oil containing monoterpene ester neryl acetate, the oxygenated sesquiterpene 5-eudesmen-11-ol, the sequiterpene hydrocarbons δ-cadinene and γ-cadinene showed a strong inhibitory activity on human malignant melanoma cells A375 (IC_{50} of 16 μg/mL) (Ornano et al., 2015). Les et al. performed phytochemical analysis on the methanolic extracts of *Helichrysum stoechas* Moench collected from Villanueva de Gállego (Zaragoza, Spain) and identified 10 constituents as 2 heterodimeric phloroglucinols including arzanol, 1 homodimeric α-pyrone (helipyrone), 3 phenolic acids (*p*-hydroxybenzoic, caffeic and *neo*-chlorogenic acids), 1 polymethoxylated and 2 glycosidic flavonoids (5,7-dihydroxy-3,6,8-trimethoxyflavon, isoquercitrin and quercetagetin-7-*O*-glucopyranoside) together with santinol B. Bioassays of these metabolites showed significant antioxidant, antiproliferative, anti-α-glucosidase, anti-dipeptidyl peptidase-4 activities as well as inhibiton of MAO-A, AChE, and TYR enzymes (Les et al., 2017).

Two new acylated styrylpyrones, one 5-methoxy-1(3*H*)-isobenzofuranone glucoside and a hydroxymethyl-orcinol derivative, along with 16 known aromatic metabolites, including lignans, quinic acid derivatives low-molecular weight phenol glucosides, have been isolated from the methanol extract of *Helichrysum italicum* collected in Nature Reserve of Castel Volturno (Caserta, Italy). Selected compounds were evaluated for their anti-biofilm properties against *Pseudomonas aeruginosa* and styrylpyrones, hydroxydihydrobenzofuran glycosides, chlorogenic acid, and dicaffeoylquinic acid were found to have significant antibiofilm activities (D'Abrosca et al., 2013). Rigano et al. reported that a new acetophenone derivative named gnaphaliol 9-*O*-propanoate was isolated from the chloroform fraction of EtOH extract of *H. italicum* ssp. *italicum* flowers collected in Southern Italy along with the five known acetophenones 12-acetoxytremetone, 13-(2-methylpropanoyloxy)toxol, [2,3-dihydro-2-[1-(hydroxymethyl) ethenyl]-5-benzofuranyl]-ethanone, 1-[2-[1-[(acetyloxy) methyl] ethenyl]-2,3-dihydro-3-hydroxy-5-benzofuranyl]-ethanone and gnaphaliol. Upon further biological assays on human colonic epithelial cells showed that 12-acetoxytremetone possessed antioxidant effects reducing reactive oxygen species production (Rigano et al., 2014).

In another study, *Helichrysum italicum* (Roth) G. Don collected in Acciaroli (Salerno, Italy) was found to contain 14 compounds of flavonoid, phenylpropanoid and acylbenzofuran structures along with one identified novel sesquiterpen (Mari et al., 2014).

It is widely stated in the literature that variability of chemical compositions of the plants are linked to genetic, geographic and climatic factors.

In this study, antibacterial, antifungal, and antiviral properties of water and ethanol (80%) extracts of seven *Helichrysum* species were assessed. They were tested against *E. coli*, *P. aeruginosa*, *P. mirabilis*, *K. pneumoniae*, *A. baumannii, E. faecalis*, *S. aureus*, *C. albicans*, and *C. parapsilosis* as well as *Herpes simplex* virus Type-1 and *Parainfluenza*-3 virus.

2. Materials and methods

2.1. Plant materials

Seven *Helichrysum* species native to Anatolia were collected from different regions of Turkey. The collection sites and dates of plants are shown in Table 1. The voucher specimens, reported at Table 1, were authenticated by Dr. M. Aslan and deposited at the Herbarium of the Faculty of Pharmacy, Gazi University. Species were identified as *H. araxinum* Takht. ex Kirp. *H. armenium* DC, *H. arenarium* L. (Moench), *H. pallasii* (Sprengel) ledep, *H. stoechas* (L.) Moench, *H. sanguineum* (L.) Kostel. and *H. graveolens* (Bieb) Sweet.

2.2. Preparation of extracts

Prior to extraction, plant material was dried at room temperature in shade and consequently powdered in a mechanical grinder. After weighing 10 g of the capitulums of each species, materials were independently extracted with distilled water (H_2O) and ethanol (EtOH) and the resultant extracts were obtained by evaporating to dryness under vacuum by a rotary evaporator. Extraction yields (w/w) were given as follows: H_2O: 4.80%, EtOH: 13.32% for *H. araxinum*, H_2O: 4.60%, EtOH: 12.01% for *H. armenium* DC, H_2O: 6.12%, EtOH: 14.51% for *H. arenarium*, H_2O: 4.59%, EtOH: 17.76% for *H. pallasii*, H_2O: 8.46%, EtOH: 9.01% for *H. stoechas*, H_2O: 11.76%, EtOH: 17.88% for *H. sanguineum*, H_2O: 12.86%, EtOH: 15.10% for *H. graveolens*.

2.3. Bioactivity assays

2.3.1. Test materials

Fourteen stock solutions of extracts (water and ethanol extracts of seven *Helichrysum* species) were prepared by dissolving in dimethylsulphoxide using ultrasonic bath and vortexer. Stock solutions

Table 1
Collection sites and dates of the *Helichrysum* species growing in Turkey.

Species	Herbarium codes	Collection sites	Collection dates
H. araxinum	GUE 2349	Skirts of Palandoken Mountain, Erzurum	July, 2000
H. armenium DC	GUE 2350	Skirts of Palandoken Mountain, Erzurum	August, 2000
H. arenarium	GUE 2351	Skirts of Palandoken Mountain, Erzurum	August, 2000
H. pallasii	GUE 2352	Skirts of Palandoken Mountain, Erzurum	August, 2000
H. stoechas	GUE 2353	Vicinity of St. Pierre Church, Antakya	July, 2001
H. sanguineum	GUE 2354	Vicinity of St. Pierre Church, Antakya	July, 2001
H. graveolens	GUE 2356	Ilgaz Mountain, near skiing slope, Kastamonu	August, 2002