



Full Length Article

Antibacterial Activities of *Marrubium catariifolium* and *Phlomis pungens* Var. *Hirta* Grown Wild in Eastern Anatolia, Turkey

Z. ULUKANLI¹ AND A. AKKAYA[†]

Department of Biology, Science and Arts Faculty, Osmaniye Korkut Ata University, Osmaniye, Turkey

[†]Department of Biology, Science and Arts Faculty, Kafkas University, Kars, Turkey

¹Corresponding author's e-mails: zeynepulukanli@osmaniye.edu.tr; zeynepulukanli@hotmail.com

ABSTRACT

The antibacterial activity of aerial parts of *Marrubium catariifolium* and *Phlomis pungens* var. *hirta* were evaluated by disc diffusion method. The hexane, acetone and methanol extracts were tested against nine bacteria. Two concentrations of hexane extracts of *M. catariifolium* and *P. pungens* var. *hirta* revealed various levels of antibacterial activity, but methanol and acetone extracts of those plants had no activity towards any bacteria examined in this work. The results found in this study showed that increasing concentrations of hexane extracts from *M. catariifolium* and *P. pungens* var. *hirta* appeared to be more active against bacteria. © 2011 Friends Science Publishers

Key Words: *Marrubium catariifolium*; *Phlomis pungens* var. *hirta*; Antibacterial; Plant extracts

INTRODUCTION

Medical plants are widely used in the treatment of various diseases in today's world. Plant extracts and their various formulations in the treatment and/or alleviation of several disease in folk medicine have been dated back to the ancient times. Besides, some natural products also exist in vegetables, fruits and beverages (Oz, 2010). The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the classical antibiotics led researchers to investigate the antimicrobial activity of several medicinal plants (Al-Bakri & Afifi, 2007). Therefore, reports of antimicrobial activity of many plant extracts have been published from many regions in the world. It is estimated, however, that of the 250,000–500,000 species found on Earth, only 1% have been studied for their pharmaceutical potential (Melendez & Capriles, 2006). Lamiaceae (Labiatae) is a well known family and represented by approximately 250 genera and 3000 species within the flowering plants. *Phlomis* L. has more than 100 species of herbs or shrubs distributed in Euro-Asia and North Africa. It has recently been documented that 52 taxa including 6 varieties, 12 natural hybrids and 34 endemic taxa of *Phlomis* are growing in Turkey (Kyriakopoulou *et al.*, 2001; Demirci *et al.*, 2006). Flora of Turkey is rich and diverse with well over 11000 flowering taxa recorded (Baser, 2002). *Phlomis* L. is a genus of the family Lamiaceae and recognized by local names as a *Ballık otu*, *Şalvar otu*, *Çalba* or *Şalba* in Turkey (Baytop, 1999). In traditional Turkish folk medicine, flowers and/or leaves of *Phlomis* species have been commonly used as herbal teas

(Dağcay), as tonic, carminative, appetizer and stimulants and painkiller for stomachache (Baytop, 1999). Other folk medicine applications include healing of wound and treatment of stomach disorders (Tamaro & Xepapadakis, 1986; Ozcelik, 1987; Bucar *et al.*, 1998; Gurbuz *et al.*, 2003). Over the past decade, significant progress has been made in establishing the pharmacological mechanisms of *Phlomis* sp. and the individual constituents responsible for them. Phytochemical investigations of the genus *Phlomis* have revealed that they include iridoids, flavonoids, phenylpropanoids, phenylethanoids, lignans, neolignans, diterpenoids, alkaloids and essential oils (Kamel *et al.*, 2000; Saracoglu *et al.*, 2003; Zhang & Wang, 2008). Besides conventional usage, *Phlomis* sp. have been shown to possess antidiabetic (Sarkhail *et al.*, 2007), ulcerogenic (Gurbuz *et al.*, 2003), antimicrobial (Digrak *et al.*, 1998; Couladis *et al.*, 2000; Demirci *et al.*, 2009), anti-inflammatory, antinociceptive, antimutagenic (Sarkhail *et al.*, 2003; Sarkhail *et al.*, 2004), immunosuppressive (Saracoglu *et al.*, 1995) and free radical scavenging properties (Ismailoglu *et al.*, 2002; Kyriakopoulou *et al.*, 2001; Zhang & Wang, 2009).

The genus *Marrubium* includes about 400 species, which grow mainly along the Mediterranean area and in the central and southeastern Europe as well as Anatolia and Asia (Rigano *et al.*, 2007; Hennebelle *et al.*, 2007). In Turkish folk medicine, aerial parts of some species of *Marrubium* have been called as *Bozotu*, *itsineği*, *Köpekotu*, and *Kukasotu*. It has been used as carminative and insect repellent (Baytop, 1999). Aerial part of many *Marrubium* sp. have also been used by the European and Middle East

native populations in many instances to treat cough, urinary tract infections, hypoglycaemics (diabetes), febrifuges (malaria), antispasmodics (colics), neurosedative, antiinflammatory activities and have external applications against snake bites and as cicatrizants of wounds (Rigano *et al.*, 2007). Phytochemical studies indicated that many species of *Marrubium* included flavonoids and phenylethanoids, diterpenoids, phenolic compounds and essential oil (Hennebelle *et al.*, 2007; Rigano *et al.*, 2007). Over the years, some of the medicinal properties attributed to the *Marrubium* have also been investigated by pharmacological assays covering antibacterial (Keles *et al.*, 2001; Hernandez *et al.*, 2003; Molina-Salinas *et al.*, 2006; Rigano *et al.*, 2007; Castillo-Juárez *et al.*, 2009; Gonzalez & Marioli, 2010), antispasmodic (Schlemper *et al.*, 1996), antinociceptive (De Souza *et al.*, 1998), antiinflammatory (Rigano *et al.*, 2006) and antioxidant activities (Sarikurkcu *et al.*, 2008; Cigremis *et al.*, 2010). To the best of our knowledge, there has been no information available about *M. catariifolium* and *P. pungens* var. *hirta* used in this work. Therefore, the preliminary assay was undertaken to study the antimicrobial activities of extracts of these plants.

MATERIALS AND METHODS

Plant species and the extraction: *M. catariifolium* and *P. pungens* var. *hirta* were collected from Kars region (an altitude of 1750 m) in eastern part of Turkey. Plants were identified by Dr. A. Ilcim, followed by deposition of the voucher specimens (*P. pungens* var. *hirta* (A. ILCIM KSUH 742) and *M. catariifolium* (A. ILCIM KSUH 751) at the herbarium of Faculty of Science and Arts, Kahramanmaraş Sutcu Imam University, Kahramanmaraş, Turkey. The aerial parts of plants were dried in the shade at room temperature, powdered using Waringer blender and loaded to soxhlet apparatus. The extraction was carried out using three solvents such as purified hexane, acetone and methanol for 8 to 10 h. The resulting mixture was then filtered and concentrated under vacuum at 40°C (Buchi, Rotavapor R-210, Labortechnik, AG, Flavil, Switzerland). Hexane, methanol and acetone solution of extracts was filter-sterilized and pipetted to antibiotic assay discs at two concentrations (500 & 1000 µg). The filter-sterilized solvents without extracts were used as negative controls.

Antimicrobial assay: Bacterial strains used in this work: *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 6538 P, *Staphylococcus aureus* (meat isolate), *Bacillus subtilis* ATCC 6633, *Listeria monocytogenes* NCTC 96040, *Enterobacter cloacae* ATCC 13047, *Staphylococcus epidermidis* ATCC 12228 and *Enterococcus faecalis* ATCC 29212. Test organisms were kindly provided by Prof. M. Digrak (Department of Biology, Kahramanmaraş Sutcu Imam University, Turkey) and by Prof. Zihni Demirbag (Department of Biology, Karadeniz Technical University). All bacterial species were subcultured into 10 mL of Nutrient Broth (Difco) and

incubated for 24 h at 37°C before being used for testing. Bacterial suspension was prepared in sterile 0.85% saline corresponding in an optical density of 0.5 McFarland standards corresponding to 10⁸ cfu/mL. A 100 µL from each culture was transferred onto the Mueller Hinton Agar. Antibiotic susceptibility discs (6 mm in diameter, Oxoid) impregnated with each plant extract solution placed onto agar media. Each plate also received control discs containing filter sterilized solvent. Ampicillin, 10 µg (Oxoid) and Gentamycine 10 µg (Oxoid) were used as positive controls. The plates were inverted and preincubated at 4°C for 2 h to allow uniform diffusion into the agar medium, followed by incubation at 37°C for 18-24 h. Inhibition distances were measured for each disc at four equidistant locations from the disc edge to the limit of bacterial growth. The experiments were conducted twice.

RESULTS AND DISCUSSION

The results of antimicrobial screening of two medicinal species *M. catariifolium* and *P. pungens* var. *hirta* are shown in Fig. 1 and Fig. 2, respectively. Hexane, acetone and methanol used as controls did not show antibacterial activity against the all bacterial species. Acetone and methanol extracts of *M. catariifolium* and *P. pungens* did not show any inhibitory effects towards any test bacteria. Therefore, data were excluded from the Fig. 1 and 2. All plant based solvent extracts used in this study revealed to have lower antibacterial effect compared to standard antibiotics (Fig. 3).

Unlike acetone and methanol extracts, hexane extracts of *M. catariifolium* and *P. pungens* displayed activity against a number of bacteria. Antibiotics assay discs containing 500 to 1000 µg of hexane extract of *M. catariifolium* revealed activity against most of the gram positive bacteria except *E. faecalis*. When hexane extracts applied onto assay discs at a ratio of 500 µg, *S. aureus* ATCC 29213 (12 mm) and *B. subtilis* (10 mm), *S. aureus* ATCC 6538 (9 mm) and *S. epidermidis* (9 mm) were the most sensitive organisms among Gram-positive bacteria. An increasing concentration (1000 µg per disc) of *M. catariifolium* hexane extract significantly inhibited the growth of *S. aureus* ATCC 29213 (13 mm), *S. aureus* ATCC 6538 P (13 mm), *B. subtilis* (12 mm) and *S. epidermidis* (10 mm). As shown in Fig. 1, the antibacterial effect towards *S. aureus* (meat isolate), *L. monocytogenes*, revealed by two fold concentration of *Marrubium* hexane extract applied onto discs was of the similar magnitude as that of 500 µg. Additionally, concentration as high as 1000 µg per disc revealed an inhibition zone against one gram negative bacterium *E. cloacae*.

The antibacterial properties of members of the *Marrubium* genera have been documented in earlier studies. The ethanol extract of *Marrubium parviflorum* showed activity towards *Klebsiella pneumoniae* (8 mm), *S. aureus* (12 mm) using disc diffusion assay, but the same extract did

not reveal any activity towards *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, *Salmonella enteritidis*, *Salmonella gallinarum*. MIC values were reported as 1 mg/mL for *S. aureus* and 4 mg/mL for *K. pneumoniae* (Keles *et al.*, 2001). All aqueous, hexane, acetone and methanol and essential oil extracts of *Marrubium vulgare* against *Mycobacterium tuberculosis* had inhibitory effect over 200 µg/mL (Molina-Salinas *et al.*, 2006). The minimal inhibitory concentration (µg/mL) of the methanolic extracts of the *Marrubium globosum* showed various activities against *S. epidermidis* (8), *E. faecalis* (16), *S. aureus* (16), *E. cloacae* (32), *E. coli* (32) and *B. subtilis* (128) (Rigano *et al.*, 2007).

Anti-*Helicobacter pylori* activity of aqueous and methanol extracts of *M. vulgare* were reported as >1000 and 31.2 µg/mL, respectively (Castillo-Juárez *et al.*, 2009). The essential oil of *M. vulgare* revealed no inhibitory activity towards several strains of *Paenibacillus* larvae, but, the decoction fraction of the same species revealed inhibitory activity towards the same group of bacterial strains using the disc diffusion assay (Gonzalez & Marioli, 2010).

The acetone and methanol solvent extracts of *M. catariifolium* displayed no activity towards bacteria tested in this work, which is not consistent to those for the methanol extracts of *Marrubium* species such as *M. vulgare* (Molina-Salinas *et al.*, 2006; Castillo-Juárez *et al.*, 2009) and *Marrubium globosum* (Rigano *et al.*, 2007). Several scientific reports have described the inhibitory effect of plants on a variety of microorganisms, although considerable variation for resistance of different microorganisms to a given plant and of the same microorganisms to different plants (Arora & Kaur, 1999). Differences in the activity of many species may be explained due to variations in the nature and combinations of phytochemicals present in the solvent extract, strain sensitivity, antimicrobial procedure adopted in tests, or may be largely depending on the plant species and/or geographical sites (Dupont *et al.*, 2006; Ozturk & Ercisli, 2007; Al-Zoreky, 2009). The extraction product also varied in terms of quality, quantity and composition according to climate, soil composition, plant organ, age etc., (Bakkali *et al.*, 2008).

In this work, *P. pungens* hexane extract (500 µg per disc) had activity against *S. aureus* (meat isolate), with inhibition diameter of 9 mm; whereas, it revealed weak activity towards *S. aureus* ATCC 6538, *S. epidermidis*, *L. monocytogenes* and *B. subtilis* as shown in Fig. 2. Unlike other bacterial strains, the same test concentration did not exhibit any inhibitory activity against *S. aureus* ATCC 29213, *E. cloacae* and *E. faecalis*. *P. pungens* hexane extract (1000 µg per disc) possessed significant activity against *S. aureus* ATCC 6538, *S. aureus* (meat isolate) and *B. subtilis*, with inhibition diameter of 10 mm. However, it revealed poor activity against *S. aureus* ATCC 29213, *S. epidermidis*, *L. monocytogenes*, *E. cloacae* and *E. faecalis*, with inhibition zones of 8 mm.

Fig. 1: Inhibitory diameter of hexane extracts of *M. catariifolium*

a) *S. aureus* ATCC 29213, b) *S. aureus* ATCC 6538 P, c) *S. aureus* (meat isolate), d) *B. subtilis* ATCC 6633, e) *L. monocytogenes* NCTC 96040, f) *E. cloacae* ATCC 13047, g) *S. epidermidis* ATCC 12228, h) *E. faecalis* ATCC 29212

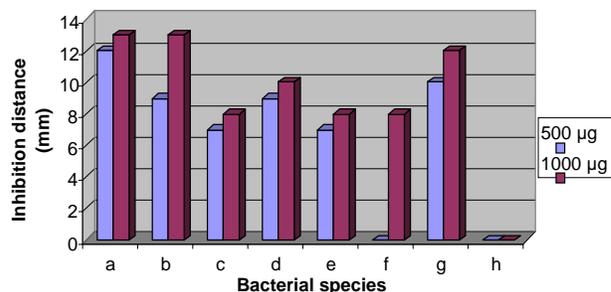


Fig. 2: Inhibitory diameter of hexane extracts of *Phlomis pungens*. var. *hirta*

a) *S. aureus* ATCC 29213, b) *S. aureus* ATCC 6538 P, c) *S. aureus* (meat isolate), d) *B. subtilis* ATCC 6633, e) *L. monocytogenes* NCTC 96040, f) *E. cloacae* ATCC 13047, g) *S. epidermidis* ATCC 12228, h) *E. faecalis* ATCC 29212

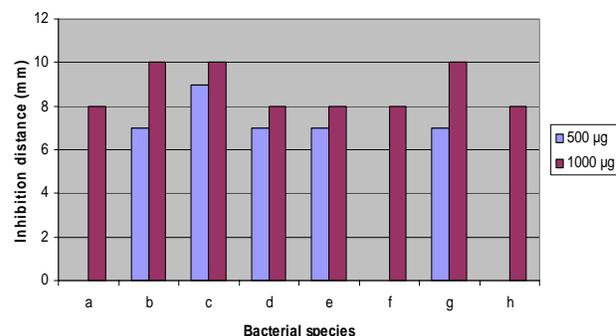
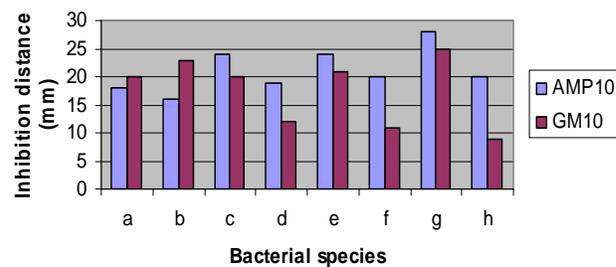


Fig. 3: Inhibitory diameter of standard antibiotics against bacteria



Hexane extract of *M. catariifolium* and *P. pungens* displayed sensitivity towards only gram positive bacteria. The greater sensitivity of gram positive bacteria to plant extracts has been reported earlier (Kelmanson *et al.*, 2000; Palombo & Semple, 2001). These observations are likely to be the result of the differences in cell wall structure between gram-positive and gram negative bacteria, with the gram negative outer membrane acting as a barrier to many environmental substances, including antibiotics (Palombo & Semple, 2001).

Earlier studies on the members of the genera *Phlomis* showed antimicrobial activity associated with solvent based extracts or fractions isolated from solvent extracts or essential oils. The chloroform extract of *Phlomis bourgei* showed inhibition only towards *Bacillus brevis*, *B. subtilis*, *P. aeruginosa*, *S. aureus*, with inhibition diameters of 12, 10, 10, and 12 mm, respectively but not for *E. coli* and *L. monocytogenes* (Digrak *et al.*, 1999). In another study, Calis *et al.* (2005) isolated phenyl ethanoid glycosidic compounds (n=10) from *Phlomis viscosa* and tested against *S. aureus*, *E. faecalis*, *E. coli* and *P. aeruginosa*. Of the tested compounds, four (MIC=500 µL) and two compounds (MIC=1000 µL) showed very weak activity against two gram positive bacteria, but all compounds were inactive towards all gram negative bacteria (MIC >1000). The effects of ethanol extract of *Phlomis fruticosa* against microorganisms were evaluated by Ristic *et al.* (2000), who reported that ethanol extracts (20 & 100 µg per disc) showed activity against *S. aureus* (12 & 16 mm, respectively) and *B. subtilis* (12 & 17 mm, respectively) but not for *P. aeruginosa*, *E. coli*, *S. faecalis*, *K. pneumoniae* and *M. luteus*.

Previous studies on the members of the genera *Phlomis* showed antimicrobial activity associated with the essential oil to be low and/or high levels compared with the current studies. Ristic *et al.* (2000) demonstrated that essential oil of *P. fruticosa* did not show any activity towards *P. aeruginosa* and *Streptococcus faecalis*. In those work, significant activity towards *S. aureus*, *E. coli*, *B. subtilis*, *K. pneumoniae* and *Micrococcus luteus* were also observed depending on the increasing concentration.

Kyriakopoulou *et al.* (2001) reported that MIC (mg/mL) of a phenylethanol glycoside (called as samioside) from *Phlomis samia* showed activity on *S. aureus* (0.46), *S. epidermidis* (0.48), *E. cloacae* (0.89), *E. coli* (0.52), *K. pneumoniae* (0.79) and *P. aeruginosa* (0.85). Aligiannis *et al.* (2004) evaluated the MIC (mg/mL) values of the essential oils of three *Phlomis* species. *Phlomis cretica* were active on *S. aureus* (14.87), *S. epidermidis* (11.23), *P. aeruginosa* (7.78), *E. cloacae* (15.45), *K. pneumoniae* (9.27), and *E. coli* (5.37). *P. samia* were active on *S. aureus* (>20), *S. epidermidis* (11.23), *P. aeruginosa* (7.80), *E. cloacae* (>20), *K. pneumoniae* (15.76) and *E. coli* (6.43). *Phlomis fruticosa* was also active on *S. aureus* (14.34), *S. epidermidis* (10.86), *P. aeruginosa* (8.75), *E. cloacae* (>20), *K. pneumoniae* (12.78) and *E. coli* (7.28).

In a recent study by Demirci *et al.* (2008), who reported that the MIC (µg/mL) values of the essential oils of the *Phlomis russeliana* had activities on *E. coli* H7:O157 (1000), *Aeromonas hydrophila* (250), *L. monocytogenes* (500), *Salmonella typhimurium* (1000), *S. aureus* (1000), *B. cereus* (250), *Yersinia enterocolitica* (500), *P. aeruginosa* (500), *Clostridium perfringens* (125). Also, essential oils of the *Phlomis grandiflora* var. *grandiflora* showed activity on *Escherichia coli* H7:O157 (1000), *A. hydrophila* (500), *L. monocytogenes* (250), *S. typhimurium* (250), *S. aureus* (500), *Bacillus cereus* (250), *Y. enterocolitica* (125), *P.*

aeruginosa (500), *C. perfringens* (125). Our data differ from the earlier studies reported above. Differences in the activity of *P. pungens* used in this work may be due to variations in the phytochemicals in the extracts of the plant species.

CONCLUSION

This study has shown that hexane of extracts of two medicinal plants compared to the acetone and methanol extracts exhibit antibacterial activity against Gram-positive bacteria. Hexane seemed to be better solvent for extracting the antibacterial substances from two medicinal plants used in this work. Unlike *M. catariifolium*, *P. pungens* revealed activity against gram positive and only one gram negative bacterium included in the study. Further assessment of the antibacterial properties of these extracts against a wide range of microorganisms, specifically towards gram negative bacteria and elucidation of the components responsible for the biological activities seems to be imperative for more comprehensive studies.

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