Pakistan J. Zool., vol. 45(2), pp. 475-482, 2013.

Comparsion of Antimicrobial Activities of Essential Oil and Solvent Extracts of Endemic *Phlomis oppositiflora* Boiss. & Hausskn. from Turkey

Sevil Toroğlu[*1] and Menderes Çenet[2]
[1]*Biology Department, Science and Art Faculty, Kahramanmaras Sütcü Imam University, 046100 Kahramanmaras, Turkey.*
[2]*Biology Department, Science and Art Faculty, Osmaniye Korkut Ata University, 80000 Osmaniye, Turkey*

Abstract.- Antimicrobial activity of methanol, ethanol, ethyl acetate extracts and essential oil of *Phlomis oppositiflora* was investigated against eight bacteria and three fungi by disc diffusion method. The methanol, ethanol and ethyl acetate extracts of *P. oppositiflora* showed antibacterial activity with 8-15 mm, 7-11 mm 8-9 mm inhibition zones) and antifungal activity with 9-13 mm and 8-15 mm and 9-12 mm inhibition zones at 30 μl^{-1}. The essential oil of *P. oppositiflora* shows antibacterial activity with 7-10 mm inhibition zone at 1 μl^{-1}; and antifungal activity with 9-12 mm inhibition zone at 2 μl^{-1}. There is no statistical difference between 2 μl^{-1} essential oil and methanol extract of *P. oppositiflora* standard antibiotics (V30 and E15) in respect to antibacterial effectiveness in *E. coli*. The essential oil, however, had a more significant antibacterial effect on *E. coli* than did the methanol extract (f= 81,905; df= 8; p‹ 0,0001). To conclude *P. oppositiflora* contains antimicrobial components against various microorganisms, which could be important in various pharmaceutical preparations.

Key Words: *Phlomis oppositiflora*, antimicrobial plant extracts, essential oil.

INTRODUCTION

Flora of Turkey is rich and diverse with well over 11000 flowering taxa recorded (Başer, 2002). The genus *Phlomis* L. has more than 100 species of herbs or shrubs scattered in Europe, 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 (Demirci *et al*., 2006). In Anatolian folk medicine, some *Phlomis* species have been generally used as botanical teas (Dağçayı) as tonic, carminative, appetizer and stimulants and painkiller for stomachache and diuretics, and for curing of ulcers and haemorrhoids (Baytop, 1994, 1999). Phytochemical studies of the *Phlomis* genus have been described that they involve iridoids, flavonoids, phenylpropanoids, phenylethanoids, lignans, neolignans, diterpenoids, alkaloids and essential oils (Saracoglu *et al*., 2003; Calis *et al*., 2005a; Zhang and Wang, 2008). Different classes of glycosides comprising the phenylpropanoids and phenylethanoids, iridoids, monoterpenoids and diterpenoids as well as a caffeic acid ester have been isolated from Turkish *Phlomis* species (Ersöz *et al*., 2002). Moreover, it has been reported that new natural products, including a new phenylethanoid glycoside and along with a new neoglinanglucoside have been shown in the aerial parts of *P. oppositiflora* (Calis *et al*., 2005b). Some of the *Phlomis* species are locally known as Ballık otu, Şalvar otu, Çalba or Şalba in Turkey (Baytop, 1999; Harput *et al*., 2006). *P. oppositiflora* is an endemic variety among *Phlomis* species in Turkey, distribution on calcareous slopes and steppe at 910-1500 meters. To the best of our knowledge, there has been no information available about antimicrobial activities of *P. oppositiflora*. Therefore, the preliminary assay was undertaken to study the antibacterial and antifungal activities of different extracts and essential oil of *P. oppositiflora*.

MATERIALS AND METHODS

Plant sample and morphological properties

Aerial parts of *Phlomis oppositiflora* Boiss. & Hausskn. (Lamiaceae) were collected by Dr. E. Toroğlu from a step area in Aladağlar Mountain (Kayseri–Turkey) at an altitude of 1500m during the

* Corresponding author: storoglu@ksu.edu.tr
0030-9923/2013/0002-0475 $ 8.00/0

flowering stage in July 2004. The plant was identified by Dr. M. Çenet. A voucher specimen was deposited in the Herbarium of the Department of Biology, Osmaniye Korkut Ata University, Osmaniye, Turkey (Voucher no: 1830). The plants were dried in the shade at ambient temperature. Morphological properties of *P. oppositiflora* were determined using stereo microscope (Nikon SMZ 1000 model) and the present results were compared with the previous data from the flora of Turkey (Davis *et al*., 1982).

Preparation of extracts

P. oppositiflora used was dried and broken into small parts under sterile conditions, and 20 g of this plant was extracted with 150 ml of methanol, ethanol and ethyl acetate (Merck, Darmstadt) for 24 hr by Soxhlet apparatus (Khan *et al*., 1988; Alzoreky and Nakahara, 2003). Most of the solvents of methanol, ethanol and ethyl acetate extracts were evaporated in vacuo at 30^{o}C using a rotary evaporator until 1ml. The disc assay described by Bauer *et al*. (1966) was used. All of the extracts individually were injected into empty sterlized antibiotic discs having a diameter of 6 mm (Schleicher and Schül No:2668, Germany) in the quantity of 30µl. Discs injected with pure methanol, ethanol and ethyl acetate served as negative controls.

Preparation of essential oil

P.oppositiflora was dried, broken into parts using blender and was exposed to hydrodistillation for 2.5 h, using a Clevenger-type equipment, according to the method recommended by the European Pharmacopoeia (1975) to produce essential oils.

The essential oil obtained was individually injected into empty sterilized antibiotic discs in 6 mm diameter (Schleicher and Shull No: 2668, Germany). 0.5, 1, 2 µl of essential oil were saturated to antibiotic discs for determination of inhibition zones (Brooks *et al*., 1995; Toroglu, 2007, 2011). In addition, standard antibiotic discs were also used as positive controls.

Microorganisms

The tested microorganisms were supplied from the culture collections of the Microbiology Laboratory of the Science and Art Faculty of the University of Kahramanmaras Sutcu Imam, in Kahramanmaras Turkey. Test microorganisms include *Escherichia coli* ATCC 8739, *Staphylococcus aureus* 6538 P, *Klebsiella pneumonia* 13883, *Mycobacterium smegmatis* CCM 2067, *Pseudomonas aeruginosa* ATCC 27859, *Enterobacter cloaca* ATCC 13047, *Bacillus megaterium* NRS, *Micrococcus luteus* LA 2971 bacteria, and *Rhodotorula rubra, Candida albicans, Kluyveromyces marxianus* fungi, Vancomycin (30µg/disc), Erytromycin (15µg/disc), Nystatin 100 Units (10µg/disc) discs were used as standard antibiotics.

Antimicrobial assays

The bacteria were incubated in Nutrient Broth (NB) (Difco) at 37±0.1^{o}C for 24 h, and the yeasts in Sabouraud Dextrose Broth (SDB) (Difco) at 25±0.1^{o}C for 24 h. The bacteria and yeasts were inoculated into petri dishes (9 cm) in the amount of 0.01 ml (10^{6} ml-1 for the bacteria and 10^{5} ml-1 for the fungi) (NCCLS, 2000), 15 ml of Mueller Hinton Agar (MHA, Oxoid) and Sabouraud Dextrose Agar (SDA) (sterilized in a conical flask and cooled to 45-50^{o}C) were homogenously dispersed onto the sterilized petri dishes (Collins *et al*., 1989; Bradshaw, 1992). Sterilized blank paper discs in 6 mm diameter were saturated with 0.5, 1 and 2 µl of essential oil by microinjector (Hamilton) and with 30µl of methanol, ethanol and ethyl acetate extracts per disc.

Standard antibiotic discs were saturated with 30 µl of the extracts and saturated with 0.5, 1 and 2 µl of essential oil per disc then placed onto petri dishes which had previously been inoculated with the above microorganisms (with the bacterium or the fungus). The petri dishes with bacterium or fungus culture were left at 4^{o}C for 2 h, and then the petri dishes inoculated with bacterium culture were incubated aerobically at 37±0.1^{o}C for 24 h, the petri dishes inoculated with fungus culture were incubated aerobically at 25±0.1^{o}C for 48 h (Collins *et al*., 1989; Bradshaw, 1992). At the end of the period, the diameter of inhibition zones were measured in mm. These studies were made in triplicate.

Statistical analysis

Data from treatments for each plant were subjected to analysis of variance (ANOVA) (one-way ANOVA) using the SPSS 17.0 for Windows to find out the most effective plant extract and the most sensitive test microorganisms. Means were separated at the 5% significance level by the least significant difference (LSD) test (SPSS 17.0 commercial software, SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

As shown in Table I, *in vitro* antibacterial and antifungal activities of essential oil and solvent extracts of *P. oppositiflora* and the inhibition zones were constituted by standard antibiotic discs. Methanol, ethanol and ethyl acetate used as controls did not show antimicrobial activity against all the microorganisms. All plant based solvent extracts and essential oils used in this study revealed to have lower antimicrobial effect compared to standard antibiotics.

As it can obviously be seen from Table I, the essential oil of *P.oppositiflora* has antibacterial and antifungal activities. Although, the essential oil of *P.oppositiflora* did not show inhibition zones against tested all bacteria at low quantity (0.5 µl), it showed inhibition zones against tested all bacteria at high quantity (1 and 2 µl). But, the essential oil of *P. oppositiflora* showed inhibition zones against tested all fungi, both at the low and at the high quantity.

In the present study, the essential oil of *P. oppositiflora* showed antibacterial activity (7-9 mm 0.5 μl^{-1} inhibition zone; 7-10 mm 1 μl^{-1} inhibition zone; 8-13 mm 2 μl^{-1} inhibition zone) to the listed bacteria. The essential oil of *P.oppositiflora* showed antifungal activity (8-9 mm 0.5 μl^{-1} inhibition zone; 9-12 mm 1 μl^{-1} inhibition zone; 10-13 mm 2 μl^{-1} inhibition zone) to the listed fungi.

When *P.oppositiflora* essential oil applied onto assay discs at a ratio of 0.5 µl, *Rhodotorula rubra* (9mm) and *Candida albicans* (9mm), among tested microorganisms. When *P. oppositiflora* essential oil applied onto assay discs at a ratio of 1 µl, *Candida albicans* (12mm), *Rhodotorula rubra* (11mm) and *Klebsiella pneumonia* (10mm). An increasing concentration of *Phlomis oppositiflora* essential oil (2 µl) significantly inhibited the growth of *Mycobacterium smegmatis* (13mm), *Bacillus megaterium* (12mm), *Rhodotorula rubra* (12mm), *Candida albicans* (13mm). The antimicrobial activity of essential oil of *P. oppositiflora*, the 2 μl^{-1} essential oil showed the best antibacterial activity against *M. smegmatis* (13 mm 2 μl^{-1} inhibition zone), antifungal activity against *C. albicans* (13 mm 2 μl^{-1} inhibition zone). When compared with antimicrobial standards, antimicrobial activity of *P. oppositiflora* essential oils (0.5 µl to 2 µl/disc) were observed ranging from weak to moderate.

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. Aligiannis *et al.* (2004) evaluated the MIC (mg/mL) values of the essential oils of three Phlomis species. *P.cretica, P. samia* and *P. fructicosa* were active on *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. cloacae*, *K. pneumoniae*, and *E. coli*.

In a recent study by Demirci *et al.* (2008), who reported that *P. russeliana* (Sims.) Bentham and *P. grandiflora* H.S. Thompson var. *grandiflora* essential oils were tested in vitro against prevalent food borne bacteria and an anaerobic pathogen bacteria. When compared with antimicrobial standards, antimicrobial activity of these plants esential oils observed changed from weak to moderate (Demirci *et al*., 2008), which partially support our findings.

In the present study, as likened among different extracts (methanol, ethanol, and ethyl acetate extracts 30 μl^{-1}) of *P.oppositiflora*, antimicrobial activities of methanol and ethanol extracts of it showed higher than ehyl acetate extracts of it. Although methanol and ethanol extracts displayed antimicrobial activity towards all tested microorganisms, ethyl acetate extract did not show antimicrobial activity towards all tested microorganisms.

Table I.- Antimicrobial activities of essential oil and different extracts of *Phlomis oppositiflora* Boiss. & Hausskn.

Microorganisms	Inhibition zone (mm)*									
	Phlomis oppositiflora						Antibiotics (µg/disc)			Controls (A,B,C)
	Extracts (30µl/disc)			Essential oils (µl/disc)						
	A	B	C	0.5	1	2	V30	E15	N10	
Escherichia coli ATCC 8739	8_b	7b	0_a	7_b	7_b	10_c	11_c	10_c	NT	0_a
Staphylococcus aureus 6538 P	8_b	10_c	0_a	0_a	7_b	8_b	15_d	16_d	NT	0_a
Klebsiella pneumonia 13883	9_b	9_b	0_a	9_b	10_{bc}	11_c	21_e	18_d	NT	0_a
Mycobacterium smegmatis CCM 2067	11_c	10_c	0_a	0_a	7_b	13_d	22_e	27_f	NT	0_a
Pseudomonas aeruginosa ATCC 27859	9_d	7_b	0_a	0_a	7_b	8_{bc}	17_e	35_f	NT	0_a
Enterobacter cloaca ATCC 13047	15_e	11_d	9_c	0_a	7_b	8_{bc}	27_f	28_f	NT	0_a
Bacillus megaterium NRS	10_c	8_b	0_a	8_b	9_{bc}	12_d	16_e	25_f	NT	0_a
Micrococcus luteus LA 2971	14_d	9_c	8_{bc}	7_b	8_{bc}	9_c	21_e	34_f	NT	0_a
Rhodotorula rubra.	13_d	12_{cd}	9_b	9_b	11_c	12_{cd}	NT	NT	18_e	0_a
Candida albicans	9_b	15_d	12_c	9_b	12_c	13_c	NT	NT	18_e	0_a
Kluyveromyces marxianus	0_a	8_b	0_a	8_b	9_{bc}	10_c	NT	NT	14_d	0_a

0.5 (µl/disc), 1 (µl/disc), 2 (µl/disc) of Concentrations of the Oil
V30: Vancomycin (30 µg/disc), E15: Erytromycin (15 µg/disc), N10: Nystatin 100 Units (10 µg/disc)
NT: Not tested discs were used as standard antibiotics
A: Methanol; B: Ethanol; C: Ethyl acetate
*****Values, including diameter of the filter paper disc (6.0 mm), are means of three replicates.
(a-g)Values specified in the same letters in the same row are not statisticaly significant.

When it comes to antimicrobial activity of different extracts of *P. oppositiflora*, the methanol extract of *P. oppositiflora* showed antibacterial activity (8-15 mm 30 μl^{-1} inhibition zone) to the listed bacteria and presented antifungal activity (9-13 mm 30 μl^{-1} inhibition zone) to the listed fungi. The ethanol extract of *P. oppositiflora* displayed antibacterial activity (7-11 mm 30 μl^{-1} inhibition zone) to the listed bacteria and showed antifungal activity (8-15 mm 30 μl^{-1} inhibition zone) to the listed fungi. The ethyl acetate extract of *P.oppositiflora* showed antibacterial activity (8-9 mm 30 μl^{-1} inhibition zone) to the listed bacteria and presented antifungal activity (9-12 mm 30 μl^{-1} inhibition zone) to the listed fungi.

Methanol extract of *P. oppositiflora* revealed activity against all of the tested bacteria and fungi, except *Kluyveromyces marxianus*. When methanol extract applied onto assay discs at a ratio of 30 µl, *Enterobacter cloaca* (15mm), *Micrococcus luteus* (14mm) and *Rhodotorula rubra* (13mm) were the most sensitive organisms among tested microorganisms. Ethanol extract of *P.oppositiflora* revealed activity against all of the tested bacteria and fungi. When ethanol extract applied onto assay discs at a ratio of 30 µl, *Candida albicans* (15mm), *Rhodotorula rubra* (12mm) and *Enterobacter cloaca* (11mm) were the most sensitive microorganisms. Among the extracts, ethanol extract displayed activity against *Kluyveromyces marxianus* in fungi. Unlike methanol and ethanol extracts, ethyl acetate extract of *P.oppositiflora*, did not displayed activity against six bacteria, namely *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Mycobacterium smegmatis, Pseudomonas aeruginosa, Bacillus megaterium.* When *P.oppositiflora* ethyl acetate extract applied onto assay discs at a ratio of 30 µl, *Candida albicans* (12mm) was the most sensitive microorganisms. The methanol extract of *P. oppositiflora* showed the best antibacterial activity against *E. cloaca* (15 mm 30 μl^{-1} inhibition zone). The ethanol extract of *P. oppositiflora* showed the best antifungal activity *C.albicans* (15 mm 30 μl^{-1} inhibition zone). If the extraction solvent type considered, methanol and ethanol extraction were more suitable than ethyl acetate extraction for higher inhibition zones.

In this study, there is no statistical difference between 2 μl^{-1} essential oil and standard antibiotics

(V30 and E15) in respect to antibacterial effectiveness in the investigated *E. coli*. The 2 μl^{-1} essential oil was as effective as standard antibiotics (V30 and E15) with respect to antibacterial activity against *E. coli*. The methanol extract of *P. oppositiflora* showed antibacterial activity against *E. coli* close rate to standard antibiotics (V30 and E15). Moreover, 2 μl^{-1} essential oil had a more significant antibacterial effect on *E. coli* than did the methanol extract of *P. oppositiflora* (f= 81,905; df= 8; p‹ 0,0001).

The 2 μl^{-1} essential oil of *P. oppositiflora* showed antibacterial activity against *B. megaterium* close rate to standard antibiotics (V30 and E15) (f= 266,476; df= 8; p‹ 0,0001). The methanol and the ethanol extracts and the 2 μl^{-1} essential oil of *P. oppositiflora* showed antifungal activity against *R. rubra* close rate to standard antibiotic (N10) (f= 170,667; df= 7; p‹ 0,0001). The ethanol extracts and the 2 μl^{-1} essential oil of *P. oppositiflora* showed antifungal activity against *C. albicans* close rate to standard antibiotic (N10) (f= 187,429; df= 7; p‹ 0,0001). The 1 μl^{-1} and 2 μl^{-1} essential oils of *P. oppositiflora* showed antifungal activity against *K. marxianus* close rate to standard antibiotic (N10) (f= 176,600; df= 7; p‹ 0,0001).

Previous 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.

Ristic *et al.* (2000) reported that ethanol extracts of *P. fruticosa* showed antimicrobial activity against *S. aureus* and *B. Subtilis***,** they didn't show *P. aeruginosa*, *E. coli*, *S.faecalis*, *K. pneumoniae* and *M. luteus*. In another study, Calis *et al.* (2005a) isolated phenyl ethanoid glycosidic compounds from *P.viscosa* and tested against different bacteria, namely *S. aureus*, *E faecalis*, *E. coli* and *P. aeruginosa*. Some of tested compounds showed very weak activity against two gram positive bacteria, but all compounds were inactive towards all gram negative bacteria. Kyriakopoulo *et al*. (2001) reported that a phenylethanol glycoside (called as samioside) from *P.samia* showed activity on *S. aureus*, *S.epidermidis*, *E. cloacae*, *E. coli*, *K. pneumoniae* and *P. aeruginosa*.

Former antimicrobial activity studies on diverse *Phlomis* species from different localities demonstrated inhibitory activity against a broad spectrum of microorganisms such as human, animal and plant pathogen microorganisms (Couladis *et al*., 2000; Ristic *et al*., 2000; Tsitsimi, *et al.*, 2000; Kyriakopoulou *et al*., 2001; Aligiannis, *et al.*, 2004; Demirci *et al.*, 2008), which partially support our findings.

Our data similar to the earlier studies reported above. Differences in the activity of *P. oppositiflora* used in this work may be due to variations in the phytochemicals in the extracts of the plant species.

Plants employed a significant reference of potentially useful structures for the development of new chemotherapeutic medicines. The primary step towards this target is the in vitro antibacterial activity experimentations (Tona *et al*., 1998). The differences in composition is probably due to climate, soil composition, altitude and age as well as species (Bakkali *et al*., 2008).

Some researchers stated that there is a relationship between the chemical structures of the most plentiful compounds in the tested extracts or essential oils of plants and the antimicrobial activity (Farag *et al*., 1989; Deans and Svoboda, 1989).

Noteworthy progress has been made in establishing the pharmacological mechanisms of *Phlomis* sp. and the individual constituents responsible for them. Phytochemical composition studies have revealed that iridoids, flavonoids, phenylpropanoids, phenylethanoids, lignans, neolignans, diterpenoids, alkaloids, β-caryophyllene, α-pinene, germacrene D, limonene and linalool, bicyclogermacrene and essential oils are the main components of *Phlomis* species (Kamel *et al*., 2000; Couladis *et al*., 2000; Saracoglu *et al*., 2003; Celik *et al.*, 2005; Zhang and Wang, 2008, 2009). In another study, Calis *et al.* (2005a) isolated phenyl ethanoid glycosidic compounds from *P. viscosa.* Kyriakopoulo *et al*. (2001) reported that a phenylethanol glycoside (called as samioside) from *P. samia.*

Some of these constituents varying in quantity and composition were also reported from *Phlomis* species by other researchers (Tsitsimi *et al*., 2000; Ghassemi *et al*., 2001; Sokovic *et al*., 2002; Mirza and Nik, 2003). Antimicrobial activity can be based on these compounds.

Flavonoid compounds are reported to have

antimicrobial property (Hymete *et al*., 2005). Phytochemical combinations with high flavonoid ingredient have also been reported to show antibacterial activity (Quarenghi *et al*, 2000; Rauha *et al*., 2000). Karou *et al*. (2006) stated that the alkaloids from *Sida acuta* displayed good antimicrobial activity against several test microorganisms. Similarly, Aljadi and Yusoff (2003) reported that phenolic acids and the phenylpropanoid compounds have showed an important antibacterial activity and biological activity and are therapeutic agents of crude drugs. Two kaurane diterpenes isolated from *Aspilia foliacea* are reported to have antimicrobial property against various oral pathogens (Ambrosio *et al*., 2008). Also, Barbary *et al.* (2010) reported that lignans are important plant phenolic compounds and lignan extracts showed antimicrobial activity against various microorganisms. Lee *et al.* (1986) reported that aucubin, an iridoid glycoside displayed antimicrobial activity, and some researchers did similar studies (Yang *et al*., 2006; Modaressi *et al*., 2009).

Essential oils are sufficiently complex combinations, which are well known to have varying in vitro and in vivo antimicrobial actions. They generally show selective toxicity against different pathogen microorganisms and are relatively safe both to animals and humans (Cowan, 1999; Burt, 2004).

CONCLUSIONS

Essential oils and solvent extracts of plants and their active components, possessing antimicrobial activity besides several biological activities, can be supplied instead of expensive antibiotics in the effective control of different pathogen microorganisms. Phlomis essential oils evaluated in this study showed varying antimicrobial activity against all tested bacteria and fungi. It is worthwhile also to test other sections for their antimicrobial and biological activity functions and mechanisms of actions.

ACKNOWLEDGEMENT

The authors thank Dr. E. Toroğlu for collecting plant used in this study.

REFERENCES

ALIGIANNIS, N., KALPOUTZAKIS, E., KYRIAKOPOULOU, I., MITAKU, S., AND CHİNOU, I. B., 2004. Essential oils of *Phlomis* species growing in Greece: Chemical composition and antimicrobial activity. *Flavour Fragr. J.,* **19**: 320–324.

ALJADI A.M. AND YUSOFF, K.M., 2003. Isolation and identification of phenolic acids in Malaysian honey with antibacterial properties. *Turk. J. med. Sci*., **33**: 229-236.

ALZOREKY, N.S. AND NAKAHARA, K., 2003. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *Int. J. Fd. Microbiol.,* **80**: 223-230.

AMBROSIO, S.R., FURTADO, N.A.J.C., OLIVEIRA, D.C.R, COSTA, F.B., MARTINS, C.H.G., CARVALHO, T.C., PORTO, T.S. AND VENEZIANI, R.C.S., 2008. Antimicrobial activity of Kaurane Diterpenes against oral pathogens. *Z. Naturforsch*., **63c**: 326-330.

BAKKALI, F., AVERBECK, S., AVERBECK, D. AND IDAOMAR, M., 2008. Biological effects of essential oils. *Fd. Chem. Toxicol.,* **46**: 446–475.

BARBARY, O.M., EL-SOHAIMY, S.A., EL-SAADANI, M.A. AND ZEITOUN, A.M.A., 2010. Antioxidant, antimicrobial and anti-HVC activities of lignan extracted from flaxseed. *Res. J. Agric. biol. Sci.,* **6**: 247-256,

BAŞER, K.H.C., 2002. Aromatic biodiversity among the flowering plant taxa of Turkey. *Pure appl. Chem.,* **74**: 527–545

BAUER, A.W., KIRBY, W.M.M., SHERRIS, J.C. AND TURCK, M., 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. clin. Pathol*., **45**: 493–496.

BAYTOP, T., 1994. Türkçe Bitki Adları Sözlüğü (*The Vocabulary of Turkish Plant Names),* Turk Dil Kurumu Publication no. 578. Turk Tarih Kurumu Press, Ankara.

BAYTOP, T., 1999. *Therapy with Medicinal Plants in Turkey, Past andPresent (Türkiye'de Bitkiler İle Tedavi, Geçmişte Ve Bugün*), 2nd edition. Nobel Tıp Basımevi, Istanbul, Turkey.

BRADSHAW, L.J., 1992. *Laboratory of microbiology*, 4th edn, Saunders College Publishing, USA, pp. 435.

BROOKS, G.F., BUTEL, J.S., ORNSTON, L.N., JAWETZ, E., MELNICK, J.L. AND ADELBERG, E.A., 1995. *Medical microbiology*. Appleton and Lange, Connecticut, p. 656.

BURT, S., 2004. Essential oils: Their antibacterial properties and potential applications in foods – a review. *Int. J. Fd. Microbiol.*, **94**: 223–253.

CALIS, I., KIRMIZIBEKMEZ, H., BEUTLER, J. A., DÖNMEZ, A. A., YALCIN, F. N. AND KILIC, E., 2005a. Secondary metabolites of *Phlomis viscosa* and their biological activities. *Turkish J. Chem.,* **29**: 71–81.

CALIS, I., BEDIR, E., KIRMIZIBEKMEZ, H., ERSÖZ, T.,

DÖNMEZ, A. A. AND KHAN, I.A., 2005b. Secondary metabolites from *Phlomis oppositiflora*. *Nat. Prod. Res.*, **19**: 493–501.

CELIK, S., GOKTURK, R. S., FLAMINI, G., CIONI, P.L. AND MORELLI, I., 2005. Essential oil of *Phlomis leucophracta, Phlomis chimerae* and *Phlomis grandiflora* var. *grandiflora* from Turkey. *Biochem. Syst. Ecol.*, **33**: 617–623.

COLLINS, C.H., LYNE, P.M. AND GRANGE, J.M., 1989. *Microbiological methods*. 6th edn, Butterworths, London, pp. 410.

COULADIS, M., TANIMANIDIS, A., TZAKOU, O., CHINOU, I.B. AND HARVALA, C., 2000. Essential oil of Phlomis lanata growing in Greece: Chemical composition and antimicrobial activity. *Planta Medica*, **66**: 670–672.

COWAN, M.M., 1999. Plant products and antimicrobial agents. *Clin. Microbiol. Rev.*, **12**: 564–582.

DAVIS, P.H., MILL R.R. AND TAN, K., 1982. *Flora of Turkey and the East Aegean Islands*., Vols. 7. Edinburgh University Press., Edinburgh, UK, pp. 117.

DEANS, S.G. AND SVOBODA, K.P., 1989. Antimicrobial activity of summer savory (*Satureja hortensis* L.) essential oil and its constituents. *J. Horticul. Sci.*, **88**: 308-316.

DEMIRCI, B., BASER, K.H.C. AND DADANDI, M.Y., 2006. Composition of the essential oils of *Phlomis rigida* Labill. and *P. samia* L.. *J. Essent. Oil Res.*, **18**: 328–331.

DEMIRCI, F, GUVEN, K. DEMIRCI, B., DADANDI, M.Y. AND BASER, K.H.C., 2008. Antibacterial activity of two *Phlomis* essential oils against food pathogens. *Food Contr.*, **19**: 1159–1164.

ERSÖZ T., SARACOĞLU, I., HARPUT, U.S., CALIS, I. AND DÖNMEZ, A.A., 2002. Iridoid and phenylpropanoid glycosides from *Phlomis grandiflora* var. *fimbrilligera* and *Phlomis fruticosa. Turk. J. Chem.*, **26**: 171-178.

EUROPEAN PHARMACOPOEIA, 1975. Maissonneuve SA, Sainte-Ruffine, Vol. 3. p. 68.

FARAG, R.S., DAW, Z.Y., HEWEDI, F.M., G.S.A. AND EL-BAROTY, 1989. Antimicrobial activity of some Egyptian spice essential oils. *J. Fd. Prod.*, **52**: 665-667.

GHASSEMI, N., SAJJADI S.E., AND LAME, M.A., 2001. Volatile constituents of *Phlomis olivieri* Benth. Daru. *J. Fac. Pharm. Tehran Univ. Med. Sci.*, **9**: 48-50.

HARPUT, U.S., CALIS, I., SARACOĞLU I., DÖNMEZ, A. A. AND NAGATSU, A., 2006. Secondary metabolites from *Phlomis syriaca* and their antioxidant activities. *Turk. J. Chem*., **30**: 383 -390.

HYMETE, A., IVERSEN, T.H., ROHLOFF, J. AND ERKO, B,, 2005. Screening of *Echinops ellenbeckii* and *Echinops longisetus* for biological activities and chemical constituents. *Phytomedicine*, **12**: 675-79.

KAMEL, M.S., MOHAMED, K.M., HASSANEAN, H.A., OHTANI, K., KASAI, R., AND YAMASAKI, K., 2000. Iridoid and megastigmane glycosides from *Phlomis aurea*. *Phytochemistry*, **55**: 353–357.

KAROU, D., SAVADOGO, A., CANINI, A., YAMEOGO, S., MONTESANO, C., SIMPORE, J., VITTORIO COLIZZI, V. AND TRAORE, A.S., 2006. Antibacterial activity of alkaloids from *Sida acuta. Afr. J. Biotech.*, **5**: 195-200.

KHAN, N.H., NUR-E KAMAL M.S.A. AND RAHMAN, M., 1988. Antibacterial activity of *Euphorbia thymifolia* Linn. *Indian J. med. Res.*, **87**: 395-397.

KYRIAKOPOULO, I., MAGIATİS, P., SKALTOUNIS, A.L., ALIGIANNIS, N. AND HARVALA, C., 2001. Samioside, a new phenylethanoid glycoside with free-radical scavenging and antimicrobial activities from *Phlomis samia*. *J. Nat. Prod.*, **64**: 1095–1097.

LEE, E.S., AHN, J.W., MAR, W. AND CHANG, I.M., 1986. Pharmacology of Iridoid: Antimicrobial activities of aucubin. *Kor. J. Pharmacogn.*, **17**: 129-133.

MIRZA, M. AND NIK, Z.B., 2003. Volatile constituents of *Phlomis olivieri* Benth. from Iran. *Flavour Fragr. J.*, **18**: 131-132.

MODARESSI, M., DELAZAR, A., NAZEMIYEH, H., FATHI-AZAD F., SMITH E, RAHMAN, M.M., GIBBONS, S., NAHAR, L. AND SARKER, S.D., 2009. Antibacterial iridoid glucosides from *Eremostachys laciniata. Phytother. Res.*, **23**: 99-103.

NCCLS National Committee for Clinical Laboratory Standarts. 2000. *Performance standarts for antimicrobial disc susceptility tests*; 7 ed. Approved Standart M2-A7 NCCLS, Pennsylvania.

QUARENGHI, M.V., TERESCHUK, M.L., BAIGORI, M.D. AND ABDALA, L.R., 2000. Antimicrobial activity of flowers from *Anthemis cotula*. *Fitoterapia*, **71**:710–2.

RAUHA, J.P., REMES, S. AND HEINONEN, M., 2000. Antimicrobial effects of finnish plant extracts containing flavonoids and other phenolic compounds. *Int. J. Fd. Microbiol.*, **56**:3–12.

RISTIC, M. D., DULETIC-LAUSEVIC, S., KNEZEVIC-VUKCEVIC, J., MARIN, D. P., SIMIC, D., VUKOJEVIC, J., JANACKOVIC, P. AND VAJS, V., 2000. Antimicrobial activity of essential oils and ethanol extract of Phlomis fruticosa L. (Lamiaceae). *Phytother. Res.*, **14**: 267–271.

SARACOGLU, I., VAREL, M. AND CALIS, I., 2003. Flavonoid, phenylethanoid and iridoid glycosides from *Phlomis integrifolia. Turk. J. Chem.*, **27**: 739–747.

SOKOVIC, M.D., MARIN, P.D., JANACKOVIC, P., VAJS, V., MILOSAVLJEVIC, S., DOKOVIC, D., TESEVIC, V. AND PETROVIC, S., 2002. Composition of the essential oils of *Phlomis fruticosa* L. (Lamiaceae). *J. Essent. Oil Res.*, **14**: 167-168.

TONA, L., KAMBU, K., NGIMBI, N., CİMANGA, K. AND VLIETINCK, A.J., 1998. Antiamoebic and phytochemicalscreening of some Congolese medicinal

plants. *J. Ethnopharmacol*., **61**: 57-65.

TOROGLU, S., 2007. *In vitro* antimicrobial activity and antagonistic effect of essential oils from plant. species. *J. environ. Biol.*, **28**: 551-559.

TOROGLU S., 2011. *In-vitro* antimicrobial activity and synergistic/antagonistic effect of interactions between antibiotics and some spice essential oils. *J. environ. Biol*., **32**: 23-29.

TSITSIMI, E., LOUKIS, A. AND VERYKOKIDOU, E., 2000. Composition of the essential oil of the flowers of Phlomis fruticosa L. from Greece. *J. Essent. Oil Res.*, **12**: 355–356.

YANG, X.P., YUAN, C.S. AND JIA, Z.J., 2006. Two new antibacterial iridoid from *Patrinia rupestris*. *Chinese Chem. Lett.*. **17**: 337-340,

ZHANG, Y. AND WANG, Z.Z., 2008. Comparative analysis of essential oil components of three *Phlomis* species in Qinling Mountains of China. *J. Pharmaceut. Biomed. Anal.*, **47**: 213–217

ZHANG, Y. AND WANG, Z.Z., 2009. Phenolic composition and antioxidant activities of two *Phlomis* species: A correlation study. *Comp. Rend.Biol.*, **332**: 816–826

(Received 23 August 2012, revised 16 Novemebr 2012)