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Antimicrobial Activity of *Silene cariensis* subsp *cariensis* and *Silene pungens* from Turkey

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ABSTRACT

The antimicrobial activities of the extracts ofethanol, methanol, etyl aceetate, propanol*S. cariensis* subsp *cariensis* and *S. pungens* were studied by disc diffusion method. These extracts were tested against five bacteria and one fungus, which revealed various levels of antimicrobial activity. The ethanol extracts of *S.cariensis* subsp *cariensis* leaves and branches showed the best antibacterial activity against *E.coli*(14mm). The methanol extracts of *S.cariensis* subsp *cariensis* leaves and branches displayed the best antibacterial activity against *E.coli* and *A.hydrophila*(10mm). The ethyl acetate extracts of *S.cariensis* subsp *cariensis* leaves and branches showed the best antibacterial activity against *E.coli* (10mm). The ethanol extracts of *S.pungens* leaves and branches showed the best antibacterial activity against *B.cereus* (12mm). The methanol extracts of *S.pungens* leaves and branches displayed the best antibacterial activity against *E.coli* and *A.hydrophila* (15mm).The ethanol extracts of *S.cariensis* subsp *cariensis*leaves and branches showed the best antibacterial activity (0.8 mg/ml) against *S. aureus* compared to the MIC results of erithromycin (1.6 mg/ml). The ethanol extracts of *S.cariensis* subsp *cariensis* leaves and branches showed the best antifungal activity (0.4 mg/ml) against *C.albicans* compared to the MIC results of nystatin (3.2 mg/ml). Similarly, the methanol extracts of *S.pungens* leaves and branches showed same activity (3.2 mg/ml) against *C.albicans* compared to the MIC results of nystatin (3.2 mg/ml).

KEYWORDS: *Silene cariensis* subsp *cariensis*, *Silene pungens*, antimicrobial activity, chemical content

INTRODUCTION

Plants have been used for thousands of years to flavour and conserve food, to treat health disorders and to prevent diseases including epidemics. The knowledge of their healing properties has been transmitted over the centuries within and among human communities. Active compounds produced during secondary vegetal metabolism are usually responsible for the biological properties of some plant species used throughout the globe for various purposes, including treatment of infectious diseases. Data on the antimicrobial activity of numerous plants, so far considered empirical, have been scientifically confirmed, concomitantly with the increasing number of reports on pathogenic microorganisms resistant to antimicrobials [1]. The emergence and spread of multidrug-resistant (MDR) bacterial pathogens have substantially threatened the current antibacterial therapy. MDR bacterial infections often lead to increased mortality, longer length of stays in hospitals, and higher cost of treatment and care [2,3]. Because of its geographical location, climate, and topographical and geological structure, Turkey's rich flora is one of the most significant in the world. The main research into the flora of Turkey is Flora of Turkey and the East Aegean Islands, which was published from 1965 to 1988 and in 2000.

There are 135 taxa belonging to *Silene* L. in 31 sections in Flora of Turkey; this has increased to 165 with the addition of new taxa[4-15].

The antimicrobial effect of some species of *Silene* has been studied in previous researchers[16-19]. As far as we know, this is the first study, the antimicrobial activity of *Silene cariensis* subsp *cariensis* and *Silene pungens* four different extracts against to five bacteria and one fungi were reported in this study from Turkey.

MATERİAL AND METHODS

Plant collection and preparation of extracts:

Silene cariensis subsp. *cariensis* was collected from Denizli: Babadağ, Başalan plateo, at 925-950 m altitude, in 30.vii.2014 and *Silene pungens* was collected from Erzincan, northern Erzincan, near Kolçekmez pass, at 2045 m altitude, in 21.vii.2011. Voucher specimens of the plants are kept at the herbarium of Celal Bayar University, Faculty of Science and letters. The plant parts used were dried and broken into small pieces under sterile conditions, and 20 g of each plant was extracted with 150 mL of ethanol, methanol, etyl aceetate, propanol extracts (Merck, Darmstadt) for 24 h by Soxhlet appartaus .Prepared extracts were dried at 30^{o}C using a rotary evaporator until amount of each extracts was 1 mL.

Microorganisms and media:

Five bacteria (*Staphylococcus aureus* ATCC 6538/P, *Bacillus cereus* CCM 99, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Aeromonas hydrophila* ATCC 19570) were obtained from the Biology Department of Mugla University, Science and Art Faculty. Cultures of these bacteria were grown in Nutrient Broth (NB) (Difco) at 37±0.1^{o}C for 24 h. One fungus (*Candida albicans*). Cultures of these fungi were grown in Sabouraud Dextrose Broth (SDB) (Difco) at 25±0.1^{o}C for 24 h.

Antibacterial activity:

The disc assay described by Bauer *et al.* [20] was used for antimicrobial activity. All of the extracts individually were injected into empty sterlized antibiotic discs having a diameter of 6 mm (Schleicher & Schül No:2668, Germany) in the amount of 25 µL and 50 µL. Discs injected with pure ethanol, methanol, etyl aceetate and propanol served as negative controls. The bacteria were incubated in Nutrient Broth (NB) (Difco) at 37±0.1^{o}C for 24h, and then inoculated (10^{6} mL-1 [21]into petri dishes containing homogenously distributed 15 mL of sterilized Muller-Hinton agar (MHA, Oxoid) [22]. Disc injected with extracts were applied on the solid agar medium by pressing slightly. The treated petri dishes were placed at 4^{o}C for 1-2 h and then the injected plates with bacteria were incubated at 37±0.1^{o}C for 18-24 h, [22-25]. Ampicillin (10 µg/disc) and erythromycin (10µg/disc) discs were used as standard antibiotics (as positive control). After incubation, all plates were observed for zones of growth inhibition, and the diameters of these zones were measured in millimeters. The experiments were conducted three times.

Antifungal Activity:

Antifungal assay was performed using disc diffusion method [20]. The respective fungal cultures were inoculated (10^{5} mL-^{1})[21] into petri dishes containing homogenously distributed sterilized Saboraud Dextrose Agar (SDA) [22]. Discs injected with extracts were applied on the solid agar medium by pressing slightly. The treated petri dishes were placed at 4^{o}C for 1-2 h and then the injected plates with fungi were incubated at 25±0.1^{o}C for 48 h. Nystatin 100 Units (10 µg/disc) discs were used as positive control. Different plant extracts were used to saturate the disc and placed on the seeded plates. Respective solvents act as a negative controls. After incubation period, the antifungal activity was evaluated by measuring the zone of inhibition against test organisms. The experiments were conducted three times.

GC/MS analysis:

The steam-distilled components were analysed by GC/MS. A HP 6890 gas chromatograph equipped with a HP-PTV and a 0.32mX0.60m HP-Innowax capillary column (0.5µm coating) was employed for the GC analysis. GC/MS analysis was performed on a HP-5973 mass selective detector coupled with a 6890 gas chromatograph, equipped with a HP 6890 gas chromatograph, equipped with HP-1capillary column. The column temperature was programmed from an initial temperature of 60 ^{o}C to a final temperature of 250 ^{o}C at 15 ^{o}C/min. The carrier gas was helium (14.1mL/min). Identification of the individual components was performed by comparison of mass spectra with literature data and by a comparison of their retention time (Rt) relative to a C_{8}-C_{32} *n*-alkanes mixture [26]. A computerized search was carried out using the Wiley 7n.l GC/MS library and ARGEFAR GC/MS library created with authentic samples.

RESULTS AND DİSCUSSİON

The ethanol extracts of *S.cariensis* subsp *cariensis* leaves and branches showed the best antibacterial activity against *E.coli* (14mm). The methanol extracts of *S.cariensis* subsp *cariensis* leaves and branches displayed the best antibacterial activity against *E.coli* and *A.hydrophila* (10mm). The ethyl acetate extracts of *S.cariensis* subsp *cariensis* leaves and branches showed the best antibacterial activity against *E.coli* (10mm). The propanol extracts of *S.cariensis* subsp *cariensis* leaves and branches showed the best antibacterial activity against *E.coli* and *A.hydrophila* (9mm) . The ethanol extracts of *S.pungens* leaves and branches showed the best antibacterial activity against *B.cereus* (12mm). The methanol extracts of *S.pungens* leaves and branches displayed the best antibacterial activity against *E.coli* and *A.hydrophila* (15mm). The ethyl acetate extracts of*S.pungens* leaves and branches showed the best antibacterial activity against S.*aureus, B.cereus* and *E.coli* (8mm). The propanol extracts of *S.pungens* leaves and branches showed the best antibacterial activity against *E.coli* (9mm).

Table 1: Antimicrobial activity of *S. cariensis* subsp *cariensis* and *S. pungens* leaf and branchces extracts against test microorganisms by disc diffusion method

Microorganisms	Plants								Antibiotics		
	S.cariensis subsp *cariensis*				*S.pungens*						
	E	M	EA	P	E	MEA	P		Ert	Amp	Nis
S.a	10	9	8	8	8	12	8	-	14	12	NT
B.c	11	8	7	7	12	14	8	8	20	15	NT
E.c	14	10	10	9	11	15	8	9	10	12	NT
P.a	13	9	-	-	8	14	-	8	13	10	NT
A.h	13	10	8	9	9	15	-	-	21	10	NT
C.a	10	8	-	-	-	11	-	7	NT	NT	16

(-): No İnhibition, NT:Not tested, E: Ethanol, M: Methanol, EA: Etyl aceetate, P: Propanol *S.a: S. aureus* ATCC 6538/P, B.c: *B. cereus* CCM 99, E.c: *E. coli* ATCC 35218, P.a: *P. aeruginosa* ATCC 27853, *A.h: A.hydrophila* ATCC 19570, C.a: *C. albicans* ATCC 10239, Ert:Eritromicin, Amp: Ampicillin Nis: Nystatin

The ethanol extracts of *S.cariensis* subsp *cariensis* leaves and branches showed the best antibacterial activity (0.8 mg/ml) against*S. aureus* compared to the MIC results of erithromycin (1.6 mg/ml). The ethanol extracts of *S.cariensis* subsp *cariensis* leaves and branches showed the best antifungal activity (0.4 mg/ml) against*C.albicans* compared to the MIC results of nystatin (3.2 mg/ml). Similarly, the methanol extracts of *S.pungens*leaves and branches showed same activity (3.2 mg/ml) against *C.albicans* compared to the MIC results of nystatin (3.2 mg/ml).

Table 2: MIC values of *S. cariensis* subsp *cariensis* and *S. pungens* leaf and branchces extracts, ampicillin, erithromycin and nystatin against test microorganisms

Microorganisms	Plants Antibiotics				
	Silene cariensis subsp *cariensis* Etanol	*Silene pungens* Metanol	Ert	Amp	Nis
S.a	0.8	6.4	1.6	0.8	N.T
B.c	3.2	3.2	1.6	0.8	N.T
E.c	6.4	6.4	3.2	3.2	N.T
P.a	1.6	3.2	6.4	3.2	N.T
A.h	12.8	6.4	1.8	3.2	N.T
C.a	0.4	3.2	N.T	N.T	3.2

(-): No İnhibition, NT:Not tested, E: Ethanol, M: Methanol, EA: Etyl aceetate, P: Propanol *S.a: S. aureus* ATCC 6538/P, B.c: *B. cereus* CCM 99, E.c: *E. coli* ATCC 35218, P.a: *P. aeruginosa* ATCC 27853, *A.h: A.hydrophila* ATCC 19570, C.a: *C. albicans* ATCC 10239 Ert:Eritromicin, Amp: Ampicillin Nis: Nystatin

Many researchers were studied antimicrobial activity of some *Silene* species in Table 3.

Table 3: Name of *Silene* species detected antimicrobial activity by authors

Plant Name	Author Name and year	Publication Name
S.aegyptiaca	Tawaha *et al*.,2007[27]	*Food Chemistry*
S.alba ssp.*divaricata*	Taskin and Bitlis.,2013[28]	*Spatula DD*
S.alba ssp *calycina*	Tosun *et al*.,2005[29] Quave *et al*.,2008[30].	*Pharm Biology* *J Ethnopharmacol*
S.arguta	Tosun *et al*.,2005[29]	*Pharm Biology*
S. brachuica	Mamadalieva *et al*.,2010a[29]	*Chemistry of Natural Compounds*
S.chlorifolia	Tosun *et al*.,2005[29] Fazly Bazzaz and Haririzadeh.,2003[32]	*Pharm Biol* *Pharm. Biol*
S.conoidea	Fazly Bazzaz and Haririzadeh.,2003[32]	*Pharm. Biol*
S.conoidema	Meymand *et al.*,2009[33]	*Journal of Rafsenjan University of Medical sciences,*

S.coronaria	Souri *et al*.,2004[34]	*Iranian J of Pharmaceutical Res*
S.cserei	Borchardt *et al*.,2009[18]	*JMed Plants Research*
S.dichotoma ssp *sibthorpiana*	Tosun *et al*.,2005[29]	*Pharm Biol*
S.guntensis	Mamadalieva *et al*.,2010b[35] Mamadalieva *et al*.,2011[36]	*Chem Nat Comp* *Z Naturforsch C.*
S.gynodioca	Karamian and Ghasemlou., 2013[37]	*Int J Agri Crop Sciences*
S.italica	Ferrazzano*et al*.,2013[38]	*Evidence-Based Comp Alter Med*
S. latifolia	Fazly Bazzaz and Haririzadeh.,2003 [32] Ferrazzano*et al*.,2013[38] Gillitzer*et al*.,2012[39]	*Pharm. Biol* *Evidence-Based Comp Alter Med* *J Med Plants Res*
S.montbretiana	Toroglu *et al*.,2013a[40]	*J ASA*
Silene laxa Boiss. & Kotschy	Toroglu *et al*.,2013b[41]	*JPA M*
Silene caramanica Boiss. & Heldr	Toroglu *et al*.,2013b[41]	*JPA M*
S.multifida	Erturk *et al*.,2006[16]	*Turk J Biol,*
S.nutens	Quave *et al*.,2008[30]	*J Ethnopharmacol*
S. parishii	Hoffmann*et al*.,1993	*Int J Pharmacognosy*
S.salsuginea	Basyemenici.,2012[43]	*Master thesis*
S.swertiifolia	Karamian and Ghasemlou., 2013[37] Fazly Bazzaz and Haririzadeh.,2003[32]	*Int J Agri and Crop Sci* *Pharm. Biol*
S.spergulifolia	Karamian and Ghasemlou., 2013[37]	*Int J Agri and Crop Sci*
S.uralensis	Singh *et al*.,2012[44]	*Polar Biology*
S.virginica	Frey and Meyers.,2010[45]	*BMC Compl and Alter Med*
S.viridiflora	Mamadalieva *et al*.,2010a[31]	*Chem Nat Compounds*
S.vulgaris	Tosun *et al*.,2005 [29] Borchardt *et al*.,2009[18] Orhan *et al*.,2009 [46] Kucukboyaci *et al*.,2010[47] Ferrazzano*et al*.,2013 [38] Dagdelen *et al*.,2014 [48] Simopoulos.,2004[49] Vanzani *et al*., 2011[50]	*Pharm Biology* *J Med Plants Research* *Food Chem* *Chem. of Natural Comp* *Evidence-Based Compl Alter Medicine* *J Food Process Preserv,* *Biol Res.* *J. Food Sci*

According to GC-MS results, ethanol and acetic acid was detected 96.89% and 0.11 respectively, the ethanolic extracts of *Silene cariensis* subsp *cariensis*, but any of the essential oil was detected. It can be suggested that amount of the essential oil was very little.

From the above studies it can be concluded that the ethanol extracts of both leaf and branches extract of *Silene cariensis* subsp *cariensis* exhibit significant antibacterial activity against pathogenic bacteria. Therefore this*Silene cariensis* subsp *cariensis*both leaf and branches may be act as another source of natural antibiotic. This study reaffirms the ethanomedicinal property of*Silene cariensis* subsp *cariensis.*

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