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Comparision of Antimicrobial Activity of *Silene montbretiana* Boiss. five different Extracts from Turkey

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Abstract: The antimicrobial activities of the extracts of ethyl acetate, chloroform, methanol, ethanol and acetone *S. montbretiana* Boiss. was studied by disc diffusion method. These extracts were tested against eight bacteria and two fungi, which revealed various levels of antimicrobial activity. The ethyl acetate extracts of *S.montbretiana* (SM) showed the best antibacterial activity against *R.rubra* and *E.cloacae* (15mm 25 μl^{-1}). The ethyl acetate extracts of SM showed the best antibacterial activity against *S.aureus* (21mm 50 μl^{-1}). While the chloroform extracts of SM displayed the best antimicrobial activity against *R.rubra* (17mm 50 μl^{-1}). The methanol extracts of SM showed the best antibacterial activity against *E.cloacae* (24mm 50 μl^{-1}). And also the ethanol extracts of SM showed the best antimicrobial activity against *R.rubra* (16mm 50 μl^{-1}).

Key words: Antimicrobial activity; Plant extracts; *Silene montbretiana* Boiss.

INTRODUCTION

In the 1930s the first compounds with antimicrobial activity were found (Goodman *et al*.,1991). Since that period, the development and use of these substances has increased, especially with the appreance of resistant strains (Zihener & Mear,1972). In Turkey, many rural people use plants for the treatment of several diseases, including microbial infections. Researchers have been interested in biologically active compounds isolated from plant species for the resistance that microorganisms have built against antibioticts (Essawi &Srour,2000).

Silene is one of the larger genera of flowering plants in the world, comprising ca. 750 species, of which approximately half occur in the Mediterranean area. The southern part of the Balkan Peninsual and SW Asia are two main centres of diversity (Greuter.,1995). *Silene* in Turkey was revised by Coode & Cullen (1967). Many researchers have been reported about species of *Silene* in *Flora of Turkey and the East Aegean Islands* (Davis, Mill & Tan, 1988; Tan & Vural, 2000; Duran & Menemen, 2003; Özgökçe *et al*., 2005; Aksoy *et* al., 2008).

The antimicrobial effect of some species of *Silene* has been studied in previous researchers (Mahesh and Satish., 2008; Borchardt *et al*., 2008, Bajpai *et al*, 2008 Ertürk *et al*, 2006).

As far as we know, this is the first study, the antimicrobial activity of *Silene montbretiana* Boiss. five different extracts against to eight bacteria and two fungi were reported in this study from Turkey.

MATERIALS AND METHODS

Plant Collection and Preparation of Extracts:

S. montbretiana was collected from Osmaniye, Zorkun Yaylasi, Keldazi Tepesi at 9.vii.2006. 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 ethyl acetate, chloroform, methanol, ethanol and acetone extracts (Merck, Darmstadt) for 24 h by Soxhlet appartaus (Khan *et al*., 1988). Prepared extracts were dried at 30^{o}C using a rotary evaporator until amount of each extracts was 1 mL.

Microorganisms and Media:

Eight bacteria (*Escherichia coli* ATCC 8739, *Staphylococcus aureus* Cowan 1, *Klebsiella pneumonia* 13883, *Mycobacterium smegmatis* CCM 2067, *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter cloacae* ATCC 13047, *Bacillus megaterium* DSM 32, *Micrococcus luteus* LA 2971) were obtained from the Biology Department of KSU, 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 (*Rhodotorula rubra* and *Candida albicans*). Cultures of these fungi were grown in Sabouraud Dextrose Broth (SDB) (Difco) at 25±0.1^{o}C for 24 h.

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Antibacterial Activity:

The disc assay described by Bauer *et al.* (1966) 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 ethyl acetate, chloroform, methanol, ethanol and acetone 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} (NCCLS, 2000)] into petri dishes containing homogenously distributed 15 mL of sterilized Muller-Hinton agar (MHA, Oxoid) (Collins *et al*.,1989). Disc injected with extracts were applied on the solid agar medium by pressing slightly. The treated petri disheswere placed at 4^{o}C for 1-2 h and then the injected plates with bacteria were incubated at 37±^{0}.1oC for 18-24 h, (Collins *et al*., 1989; Bradshaw, 1992; Toroglu, 2007;2011). Vancomycin (30 µg/disc), Erythromycin (30µg/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 (Bauer *et al*., 1966). The respective fungal cultures were inoculated [10^{5} mL-1 (NCCLS, 2000)] into petri dishes containing homogenously distributed sterilized Saboraud Dextrose Agar (SDA) (Collins *et al*.,1989). 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.

RESULTS AND DISCUSSION

Disc diffusion methods are extensively used to investigate the antimicrobial activity of natural substances and plant extracts. These assays are based on the use of discs as reservoirs containing solutions of substances to be examined. All the concentrations of the plant extracts showed strong activity against all the test organisms on concentration dependent manner in Table I. In this study, we compared two different amount of *S. montbretiana* Boiss with 25 and 50 μl^{-1}. The ethyl acetate, chloroform, methanol, ethanol and acetone used as negative controls did not show antimicrobial activity against the all tested microorganisms.

The ethyl acetate extracts of *S.montbretiana* (SM) showed the best antibacterial activity against *R.rubra* and *E.cloacae* (15mm 25 μl^{-1}). The chloroform extracts of SM displayed the best antifungal activity against *R.rubra and C.albicans* (9mm 25 μl^{-1}). The methanol extracts of SM displayed the best antimicrobial activity against *R. rubra* (12mm 25 μl^{-1}). The ethanol extracts of SM the best antimicrobial activity against *R.rubra* (15mm 25 μl^{-1}). The acetone extracts of SM showed the best antimicrobial activity against *R.rubra* (10mm 25μl^{-1}).

Table 1: Antimicrobial activity of five different solvent extracts of *S. montbretiana* Boiss.

Microorganisms	Inhibition Zone (6mm/disc)													
	25µl					50µl					Antibiotics			Controls
	A	B	C	D	E	A	B	C	D	E	V30	E15	N10	ABCDE
E.coli	7	0	9	7	7	17	10	17	9	8	11	10	NT	0
S.aureus	10	7	8	7	8	21	12	17	11	11	15	16	NT	0
K.pneumoniae	12	7	8	7	7	12	8	17	11	8	21	18	NT	0
M.smegmatis	9	7	7	7	8	17	12	15	8	8	22	27	NT	0
P.aeruginosa	7	7	0	7	7	8	7	8	8	9	17	35	NT	0
E.cloacae	15	9	11	8	7	17	12	24	8	9	27	28	NT	0
B.megaterium	9	8	0	9	7	11	10	11	9	8	16	25	NT	0
M.luteus	9	8	8	0	7	11	10	17	8	8	21	34	NT	0
C.albicans	10	9	10	0	0	10	9	9	9	9	NT	NT	18	0
R.rubra	15	9	12	15	10	20	17	17	16	13	NT	NT	18	0

A:Ethyl acetate B:Chloroform C: Methanol D:Ethanol E:Acetone

V30: Vancomycin (30 µg/disc), E15: Erytromycin (15 µg/disc), N10: Nystatin 100 Units (10 µg/disc), NT: Not tested

When we compared to antimicrobial activity of SM, the ethyl acetate extracts of SM showed the best antibacterial activity against *S.aureus* (21mm 50 μl^{-1}). While the chloroform extracts of SM displayed the best antimicrobial activity against R.rubra (17mm 50 μl^{-1}). The methanol extracts of SM showed the best antibacterial activity against *E.cloacae* (24mm 50 μl^{-1}). And also the ethanol extracts of SM showed the best antimicrobial activity against *R.rubra* (16mm 50 μl^{-1}). The acetone extracts of SM showed the best antimicrobial activity against *R.rubra* (13mm 50 μl^{-1}).

Plant extracts have been studied against bacteria and fungi for years, but in a more intensified way in the last three decades. During this period, a lot of antimicrobial screening evaluations have been published based on the traditional use of Chinese, African and Asian plant drugs (Forestiere *et al*.,1988; Vlientick *et al*.,1995). There has been considerable interest in essential oils and extracts of medicinal and edible plants, herbs and spices for the development of alternative food additives, in order to prevent the growth of food borne pathogens or to delay the onset of food spoilage (Marino *et al*.,2001; Baydar *et al*., 2004, Rota *et al*., 2004, Sokmen *et al*., 2004; Oke *et al*.,2009). Recently our group and various publications have documented the antimicrobial activity of essential oils and plant extracts including clove, lavander, spices, walnut (Keskin *et al*., 2012; Keskin *et al.,* 2010, Keskin &Toroglu., 2011; Sivasankaridevi *et al*., 2013; Bisignano *et al*.,2013).

There are a few study about antimicrobial activity of *Silene* species (Mahesh and Satish., 2008; Borchardt *et al*., 2008, Bajpai *et al*, 2008 Ertürk *et al*, 2006).

The antimicrobial efects of plants are mostly due to the essential oils present in their composition. High phenolic compounds, flavonoids, aldehydes, ketones, saponins, and alcohols causes antimicrobial activity (Azzouz & Bullerman .,1982; Shelef.., 1983; Akgul.,1989; Sindhu &Manorama.,2012; Sengul *et al*.,2011). It is known that these compounds including Caryophyllaceae family.

Conclusıon:

It can be suggested that degree of antimicrobial activity depends on type of extracts and amount of extracts and chemical properties of compounds. In our study, all of the extracts showed antimicrobial activity against all tested microorganisms. Inhibition zones of some of the organisms close to zones of antibiotics especialy 50 µL.

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