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Antimicrobial Activity of Centaura derderiifolia, Stachys aleurites and Anthemis aciphylla

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ABSTRACT

The antimicrobial activity of the ethanol, aseton, ethyl asetate and chloroform extracts from *Centaurea derderiifolia* Wagenitz (*Asteraceae*) *, Stachys aleurites* Boiss. (Lamiaceae) and *Anthemis aciphylla* Boiss. var.*aciphylla* (*Asteraceae*) were investigated by agar-well diffusion assay. These extracts were tested in vitro against 26 bacterial and 3 yeast species. Etyhl acetate and acetone extracts of *C. derderiifolia***,** *S. aleurites* and *A. a.* var. *aciphylla* showed antimicrobial activity in varying degrees on all the microorganisms tested. However, chloroform and ethanol extracts of all the plants were not active on some microorganisms tested. Ethyl asetate extracts of the samples exhibited stronger and broader spectrum of antimicrobial activity as compared to ethanol, aseton and chloroform extracts. Therefore, minimal inhibitory concentration (MIC) of only ethyl acetate extracts of the samples was determined for seven bacteria chosen from agar-well diffusion assay. MIC values of the extracts were between 250-125 µg/ml. In conclusion, ethyl asetate extract of *C. derderiifolia* was the most active against *Aeromonas hydrophila* both in agar-well diffusion and MIC assays.

Key Words: Antimicrobial activity, *Centaura derderiifolia***,** *Stachys aleurites* and *Anthemis aciphylla* var. *aciphylla*

INTRODUCTION

The antiseptic qualities of aromatic and medicinal plants and their extracts have been recognized since antiquity, while attempts to characterize these properties in the laboratory date back to the early 1900s [1]. Plant based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials needs to occur. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of enfectious diseases while simultaneously migitating many of the side effects that are often associated with synthetic antimicrobials [2].

Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, much recent attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine [3].

Many species of genus *Centaurea* L. (*Asteraceae*) have traditionally been used for the treatment of various ailments [4,5]. The genus *Centaurea* has been the subject of many antimicrobial activity studies [4,6-14].

A few species of *Anthemis* are important as herbal medicine and dyes in some regions. *A. wiedemannia* is folk remedy for cough and cold. *A. altissima* and *A. cotula* are used for treatment of hepatitis [15]. Sesquiterpene lactones are isolated from *A. wiedemannia* [16]. Flavonoids of *A. austriaca* , *A. cotula*, *A. pseudocotula*, *A. cretica* ssp. *pontica*, *A. chia* and *A. tinctoria* var. tinctoria are used for yellow coloration of fabric material [17].

Species from *Stachys* genera belong to Lamiaceae are used in folk medicine [18]. Leaves and flowers from *S. lavandulifolia*, *S. recta*, *S. officinalis*, *S. palustris* ve *S. sylvatica* are used as tea and they are useful in treating tumor, asthma, bronchitis, dysentery, epilepsy, stomach inflamation, neuropaty, cancer (mouth), common cold, laryngitis, respiratory ailments, tonsilitis [19,20]. Flavonoids, phenyl ethanoid glycosides, phenolic acids, iridoids, monoterpenes, sesquiterpenes, diterpenes, and triterpene saponins have been reported to be present in different *Stachys* species [21-26]. Essential oil analysis and antimicrobial activity of eight *Stachys* species from Greece have been reported by Skaltsa et al. [27].

Grujic-Jovanovic et al. [28] studied the composition and antibacterial activity of the essential oil of six *Stachys* species from Serbia and they proved that the essential oil of *S. officinalis* was the most active. Flamini et al. [29] reported the essential oils of *Stachys aleurites,* Delazar et al. [30] isolated acylated flavanoid glycosides from *S. bombycina*. Petrovic et al., [31] studied the composition and antimicrobial activity of essential oil of *S. plumosa* and reported antimicrobial activity against the bacteria and yeasts tested except *Staphylococcus aureus.*

The genus *Anthemis* L. (*Asteraceae*) comprises ca. 210 species and chemical studies showed that sesquiterpene lactone patterns are systematically important in the genus such as *A. plutonia* [32], *A. carpatia* [33], *A. cotula* [34], *A. wiedemannia* [16], and *A. arvensis* [35]. However, there is a few study on the antimicrobial activity of *Anthemis* sp. [36-39].

This study was conducted to investigate antimicrobial properties of ethanol, aseton, ethyl asetate and chloroform extracts of 3 endemic plant taxa namely, *Centaurea derderiifolia* Wagenitz*, Stachys aleurites* Boiss. and *Anthemis aciphylla* Boiss. var. *aciphylla*, collected from Turkey against a panel of microorganisms (bacteria and yeasts) both clinical and food borne by using agar-well diffusion assay. Minimal inhibition concentration (MIC) of ethly acetate extracts of the plants were also evaluated for some bacteria selected from previous response obtained by agar-well diffusion assay.

MATERIALS AND METHODS

Plant materials and extraction procedure

Centaura derderiifolia, *Stachys aleurites* and *Anthemis aciphylla* taxa of Turkish samples [40,41] are endemic and they were collected from original (typical) localities in Turkey by Celik and Uysal. *C. derderiifolia* Wagenitz was collected from Başkale in Van (July 2003, 1850 m)*, S. aleurites* Boiss. was collected Konya Alti Varyant in Antalya (May 2003, 10 m) and *A. aciphylla* Boiss.var. *aciphylla* was collected from Söğüt in Eskişehir (June 2003, 950 m). The specimens collected were identified with the help of *Flora of Turkey and East Aegean Islands* [42-44]. Voucher specimens were deposited in the Herbarium of Faculty of Science and Arts, Canakkale Onsekiz Mart University (COMU). Collector numbers were given by Celik and Uysal. The plants previously were air-dried, and then aerial parts (stem, leaf, flower, and fruit) were ground with the help of a Waring blender. Ground samples (20 gr) were extracted with 150 ml of ethanol, aseton, ethyl asetate and chloroform solvent (Merck, Darmstadt, Germany) for 24 h by using Soxhlet equipment. The extracts were filtered using Whatman filter paper (no.1) and then concentrated in *vacuo* at 70 °C. The residues were stored in a refrigerator until subsequent use. The extracts were prepared by suspending the residues in the same solvents (ethanol, aseton, ethyl asetate, and chloroform) at a concentration of 10 mg/ml for the bioassay.

Microorganisms

A total of 29 microbial genera including 26 bacteria and 3 yeast species were used in this study. Table 1 shows the isolates used in the study and their source of origin.

Table 1. Strains used in the study and their source.

Strain	Source
	USDA, Agricultural Research Service, Peoria, IL.US
Bacillus cereus NRRL B-3711	
Bacillus subtilis NRRL 744	
Salmonella typhimurium NRRL B-4420	
Micrococcus luteus NRRL B-4375	
Proteus vulgaris NRRL B-123	
Xanthomonas campestris pv. *campestris* NRRL-B1459	
Enterobacter aerogenes NRRL B-3567	
Streptococcocus feacalis NRRL B-14617	
Rhodotorula rubra NRRL Y-2505	
	Ege University, Faculty of Science, Department of Biology, İzmir, Turkey
Escherichia coli ATCC 25922	
Staphylococcus aureus ATTC 6538 T	
	Ankara University, Faculty of Veterinary, Ankara, Turkey
Aeromonas hydrophila	
Listeria monocytogenes	
Klebsiella pneumoniae	
Yersinia enterocolitica	
	J. B. Jones, University of Florida, IFAS, Plant Pathology Department
Pseudomonas fluorescens B 130	
Bacillus pumilus B122	
Pseudomonas syringae pv. *tomato* 32	
Pseudomonas tobacco 8	
Pseudomonas lachrymans	
Pseudomonas syringae -1	
	Vidaver, A. K. University of Nebraska Lincoln, Institute of Agricultural and Natural Resources
Pseudomonas syringae pv. *glycine* PG1-T	
	J. G: Turner, University of East Anglia, UK
Pseudomonas syringae pv. *phaseolicola* NCPPB 52	
Pseudomonas gingeri 3146	
Pseudomonas gladioli pv. *agricola* RR3	
	Osmangazi University Medical Faculty
Brucella spp.	
Pseudomonas maltophila	
Candida albicans	
Candida globrata	

Antimicrobial activity

The antimicrobial activities of the ethanol, aseton, ethyl asetate, and chloroform extracts of *C. derderiifolia*, *S. aleurites* and *A. a.* var. *aciphylla* were evaluated by means of the agar-well diffusion assay [45,46] with some modifications. Twenty milliliters of the specified molten agar (45 °C) was poured into 9 cm sterile Petri dishes. A suspension (100 µl) containing 10^8 cfu/ml bacteria and 10^6 cfu/ml yeasts was spread on the plates of Nutrient agar (Merck) and Mueller-Hinton agar (Oxoid) respectively. Once the plates were dried aseptically, 6 mm wells were bored using a sterile cork borer. Extracts (50 µl) were placed into the wells, and the plates were incubated for 37 °C for 24 h for bacterial strains and 48 h for yeasts. Chloramphenicol (100 µg/ml) for bacteria and ketakonozol (100 µg/ml) for yeast were used as standard antibiotics. The tests were carried out in triplicate. Antimicrobial activity was evaluated by measuring zone of inhibition against the test organism.

Microdilution assay

The minimal inhibition concentration (MIC) values were also studied for some of the microorganisms which were determined as sensitive to the extracts in agar-well diffusion assay [12,47,48]. Stock solution of the etyhl asetate extract which gave the best inhibition in the agar-well diffusion test was prepared in dimethylsulfoxide (DMSO, Carlo-Erba, France). Dilution series using sterile distilled water were prepared from 2000 µg/ml to 3.9 µg/ml in test tubes which were then transferred to 96 well microtiter plates. Overnight grown bacterial suspensions in double strength Mueller Hinton Broth (MHB) were standardized to 10^8 cfu/ml using McFarland No:0.5 standart solution. Microorganism suspension (100 µl) was then added into the wells. The last-well chain without microorganism was used as a negative control. Sterile distilled water and the medium served as a positive growth control. After incubation at 37 °C for 18 h the first well without turbidity was determined as the minimal inhibitory concentration (MIC). Chloramphenicol was used as standart antibacterial agent.

RESULTS AND DISCUSSION

The antimicrobial activities of ethanol, acetone, ethyl asetate and chloroform extracts of *C. derderiifolia*, *S. aleurites* and *A. a.* var. *aciphylla* against microorganisms were examined in the current study and their potency were qualitatively assassed by the presence or absence of inhibition zones and zone diameter (Table 2).

Listeria monocytogenes, Pseudomonas gingeri, Pseudomonas syringae pv. *glycine, Pseudomonas syringae* pv. *tomato, Proteus vulgaris* and *Pseudomonas fluorescens.* Chloroform extracts of *S. aleurites* was not inhibitory to *Staphylococcus aureus, Yersinia enterocolitica, Listeria monocytogenes, Pseudomonas gingeri, Pseudomonas syringae* pv. *glycine, Pseudomonas syringae* pv. *tomato, Escherichia coli* and *Candida globrata.* Chloroform extracts of *A. a.* var *aciphila* was not inhibitory to *Klebsiella pneumoniae, Pseudomonas gladioli pv. agricola, Staphylococcus aureus, Pseudomonas lachrymans, Aeromonas hydrophila, Yersinia enterocolitica, Salmonella typhimurium, Pseudomonas syringae, Proteus vulgaris and Candida albicans.* Interestingly, chloroform extract of *S. aleurites* was the most inhibitory against yeast *Rhodotorula rubra.*

Ethanol extracts of *S. aleurites* and *A. a.* var. *aciphyla* were not inhibitory to *Pseudomonas gingeri, Pseudomonas lachrymans* and *E. coli,* respectively (Table 2).

The highest antimicrobial activities were obtained with ethyl acetate extract of *S. aleurites* and *A. a.* var. *aciphyla* against *Xanthomonas campestris* pv. *campestris. Aeromonas hydrophila* was highly inhibited by ethyl acetate extract of *C. derderiifolia.* Other microorganisms tested were inhibited by different plant extracts in varying degree of inhibition (Table 2).

Table 2 Antimicrobial activity of *Centaura derderiifolia* **,** *Stachys aleurites* and *Anthemis aciphylla* var. *aciphylla* extracts against the bacterial, yeast and fungal strains tested based on agar well diffusion method.

	Inhibiton zone in diameter (mm/sensitive strains)														
	C. derderiifolia				*S. aleurites*				*A. a.* var. *aciphylla*						
Microorganisms	A	B	C	D	A	B	C	D	A	B	C	D	Standard Antibiotic		
Klebsiella pneumoniae	13	9	-	15	14	14	8	14	14	14	-	8	29		
P. gladioli pv. *agricola*	18	15	9	14		14	18	9	12		13	11	-	10	28
Staphylococcus aureus	18	12	8	16	12	10	-	10	13	14	-	8	35		
Bacillus pumilus	13	11	9	17		14	10	8	10		14	9	8	9	28
Pseudomonas lachrymans	13	15	12	16		13	15	8	11		12	13	-	-	23
Aeromonas hydrophila	24	16	-	19	18	16	8	21	17	15	-	18	31		
Xanthomonas campestris pv. *campestris*	18	15	-	15		25	19	12	13		38	18	12	13	38
Bacillus cereus	19	18	14	17	17	12	10	12	20	13	9	18	33		
Yersinia enterocolitica	13	13	8	14	14	18	-	24	19	19	-	10	29		
Salmonella typhimurium		20	13	8	19		17	20	10	20		23	20	-	18	35
Pseudomonas syringae		18	15	8	14		14	14	8	11		12	15	-	20	36
Listeria monocytogenes		10	12	-	11		12	20	-	13		12	10	8	13	32
Pseudomonas gingeri		20	17	-	12		10	15	-	-		14	17	10	16	42
Pseudomonas syringae pv. *glycine*		17	15	-	11		15	22	-	13		12	17	-	17	39

	Inhibiton zone in diameter (mm/sensitive strains)														
	C. derderiifolia				*S. aleurites*				*A. a.* var. *aciphylla*						
Microorganisms Standard Antibiotic	A	B	C	D	A	B	C	D	A	B	C	D			
Pseudomonas syringae pv. *tomato*	17	12	-	10		15	12	-	20		16	18	9	16	41
Brucella spp.	15	11	8	9		13	14	9	9		12	14	9	10	30
Bacillus subtilis	14	11	8	20		13	14	9	10		13	19	11	15	39
Enterobacter aerogenes	15	12	8	10		16	10	10	12		13	14	12	15	34
Proteus vulgaris	8	10	-	10		8	11	8	10		8	12	-	11	25
Pseudomonas mallophila	19	10	8	10		15	12	8	9		14	18	8	10	37
Escherichia coli	13	12	8	10		12	11	-	8		8	8	10	-	39
Pseudomonas tobacco	17	12	9	12		14	12	8	16		20	16	9	16	39
Micrococcus luteus	10	11	10	8		10	10	8	8		16	11	10	12	44
Pseudomonas fluorescens	12	12	-	21		12	17	8	20		12	14	9	10	26
Streptococcocus feacalis	12	10	8	9		11	12	9	10		12	10	8	10	29
P. s. pv. *phaseolicola*	10	8	10	10		12	11	8	8		8	12	8	8	42
Candida albicans	12	11	8	10		12	10	7	9		11	9	-	11	29
Candida glabrata	14	10	9	10		12	10	-	9		12	10	9	10	29
Rhodotorula rubra	9	12	8	8		13	10	26	8		10	10	8	20	37

A:, ethyl acetate extract; B, Acetone extract; C, Chloroform extract and D, ethanole extract.

Ethyl asetate extracts of the samples exhibited stronger and broader spectrum of antimicrobial activity as compared to ethanol, aseton and chloroform extracts. Therefore in the determination of minimal inhibitory concentration (MIC) only the ethly asetate extracts of *C. derderiifolia***,** *S. aleurites* and *A. a.* var. *aciphylla* were used against seven bacteria, namely *Pseudomonas lachrymans, Bacillus cereus, Bacillus pumilus, Aeromonas hydrophila, Salmonella typhimurium, Pseudomonas syringae pv. glycine* and *Xanthomonas campestris pv. campestris*, chosen from agar-well diffusion assay. MIC values of the extracts were between 250-125 µg/ml (Table 3).

Table 3. The MIC values of C. derderiifolia**,** S. aleurites and A. a. var. aciphylla extracts against the microorganisms tested in microdilution assay (MIC in µg/ml).

	Ethyl acetate extracts			
Microorganisms	*C. derderiifolia*	*S. aleurites*	*A. a.* var. *aciphylla*	standard antibiotic
Pseudomonas lachrymans	250	250	250	250
Bacillus cereus	250	250	250	125
Bacillus pumilus	250	250	125	125
Aeromonas hydrophila	125	250	250	31.25
Salmonella typhimurium	250	250	250	15.62
Pseudomonas syringae pv. *glycine*	125	250	250	15.62
X.anthomonas campestris pv. *campestris*	125	250	250	7.81

At present, quick and effective management of plant diseases and microbial contamination in several agricultural commoditites is generally achieved by the use of synthetic pesticides [49]. However, the wide and indiscriminate application of these chemical pesticides has caused health hazards in animals and humans due to their residual toxicity. They have undesirable attributes such as high and acute toxicity, long degradation periods, accumulation in the food chain and an extension of their power to destroy both useful and harmful pests [50]. Many pathogenic microorganisms have developed resistance against chemicals. Therefore, there is an urgent need for alternative agents, for the management of plant pathogenic bacteria. The ethyl acetate extract of *A. a.* var. *aciphyla* against *Xanthomonas campestris* pv. *campestris* was same with the standard antibiotic chloramphenicol therefore, this extract have the potential of an alternative antimicrobial substance for the pathogen.

Quarengi et al. [36], studied the antimicrobial activity of methanolic extracts of *Anthemis cotula* flower and found activity against six of the bacteria tested except *Streptococcus pneumoniae* and *Salmonella* sp. In our study, we found moderate activity against *Salmonella typhimurium.* Methanol extract of *Anthemis melanolepsis* was tested for anti-*Helicobacter* activity and a moderate activity was obtained [37]. In a study of Uzel et al. [38], chemical composition and antimicrobial activity of the essential oils of *Anthemis xylopoda* O. Schwarz from Turkey was invesitigated and siginificant antimicrobial activities was observed against 13 of bacteria and yeasts tested. Anti-*Candida* activities of *Anthemis nobilis* and *Stachys byzantina* were studied by Duarte et al. [39] and ethanol extracts were found to be inactive however, the essential oils of *A. nobilis* had a notable activity.

Stachys annua (L.) subsp. *annua* Ic.and *Stachys pumilia* Banks & Sol. antimicrobial activities were studied by Digrak et al. [51] and it was recorded that *B. subtilis* IMG 22, *B. cereus* FMC 19, *E. coli* DM and *C. albicans* CCM were not inhibited by any of the chloroform extract of the plants. No antimicrobial activity against *E. coli* was observed with the *S. aleurites* chloroform extract in our study. Dulger and Gonuz [52] studied the antimicrobial activity of endemic *Stachys sivasica*, *Stachys anamurensis* and *Stachys cydni* methanol extracts and found that the extracts were effective against bacteria but not the yeasts tested.

In conclusion this study showed the antimicrobial properties of three plants namely, *C. derderiifolia***,** *S. aleurites* and *A. a.* var. *aciphylla* to be used as antimicrobials against many bacteria of plant pathogenic, food and cliinical origin and yeasts. More study should be carried to investigate the ethyl acetate extracts of the plants to reveal the animicrobially active compounds of the plants. In this study, no attempt was done to carry out to reveal the chemical composition and active compounds of plant extracts. However, future studies should be carried out to investigate these points.

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