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Investigation of Antibacterial and Antifungal Properties of *Acanthophyllum acerosum* and *Acanthophyllum microcephalum*

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Abstract
Turkey is located in breakpoint of Iranian-Turan, Anatolia-Siberia and Mediterranean regions, for this reason, it is among the few countries in the World with regard to plant diversity. *Acanthophyllum* species is used in many areas of soap and detergent production due to the saponin material in the content of it. In this study, the antibacterial and antifungal properties of the different concentration of *Acanthophyllum acerosum* and *Acanthophyllum microcephalum* extract was determined against fourteen bacteria and one fungi using minimum inhibition concentration (MIC) and disc diffusion method. As a result, *A. acerosum* has been shown better antibacterial properties than *A. microcephalum* in both methods. It was found that both plants had no effect against *Candida albicans*.

Keywords: *Acanthophyllum,* Antifungal, Antimicrobial, Caryophyllaceae.

INTRODUCTION

Turkey has an important place in terms of flora as a result of its location at the junction of three continents Asia, Europe and Africa. Turkey is the meeting place of three phyto-geographical regions: Euro-Siberian, Mediterranean and IranoTuranian. Further has extensive floral diversity due to its various climate and different geological zones [8; 9]. There are 11707 plant species in our country that are defined up to now. Among these, 3649 taxon are endemic in Turkey [7; 8; 12].

According to the WHO, 80% of the worlds population rely on plant-derived medicines for their healthcare. Basically one quarter of all medical prescriptions, formulations are based on the substances derived from plants or plant-derived synthetic analogs [3; 4; 11].

Antimicrobials are presumedly one of the most successful forms of chemotherapy in the history of medicine. Bacterial resistance to antibiotics has been a recognized reality almost since the beginning of the antibiotic era, but exclusively within the past twenty years has the rise of dangerous, resistant strains occurred with a worrying regularity [10].

Caryophyllaceae is a large family is known for its ornamental plants and saponin compounds. [17]. Saponins demonstrated that haemolytic, anti-inflammatory, molluscicidal, antifungal, antibacterial, antiparasitic, cytotoxicity, anti-tumor and antiviral activities [19]. *Acanthophyllum* rendered cytotoxic effects of crude extracts, *Acanthophyllum microcephalum* showed activity against MCF-7 and MDBK cell lines. In addition, *Acanthophyllum bracteatum* showed cytotoxic effect against MCF-7 [17]. The genus *Acanthophyllum* (Caryophyllaceae) is represented by 5 species in Turkey and one of which are endemic to Turkey [2].

This study aimed to investigate the antibacterial activities of species of *Acanthophyllum microcephalum* Boiss. and *Acanthophyllum acerosum* Sons. and its usefulness as a herbal drug.

MATERIALS and METHODS

Microorganisms

In order to analyse the antimicrobial activity of plant extracts 19 microorganisms namely *Enterobacter aerogenes* ATCC 13048, *Listeria monocytogenes* ATCC 7644, *Klebsiella pneumoniae*, *Pseudomonas fluorescens* P1, *Pseudomonas aeruginosa* DSMZ 50071, *Enterococcus faecalis* ATCC 29212, *Listeria innocua*, *Salmonella enteritidis* ATCC 13075, *Enterococcus durans*, *Salmonella typhimurium* SL1344, *Enterococcus faecium*, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* DSMZ 20044, *Bacillus subtilis* DSMZ 1971, *Escherichia coli* CFAI, *Escherichia coli* ATSS 2592, *Salmonella infantis*, *Salmonella kentucky* and *Candida albicans* ATCC 10231 were used.

Plant Extract

Acanthophyllum microcephalum Boiss. (collector number, 6758) and *Acanthophyllum acerosum* Sons. (collector number, 6759) that were collected by Armağan [5]. Samples were grounded into fine powder by liquid nitrogen and shaken in 80% ethyl alcohol. Then, it was filtrated through Whatman No. 1 filter paper and treated in the heater for several hours to remove the solvent. The resulting extract was then removed to -20°C until use analysis.

Disk Difussion Method

Acanthophyllum microcephalum Boiss., and *Acanthophyllum acerosum* Boiss., samples were extracted under sterile conditions and 1 µg/ml, 5 µg/ml and 10 µg/ml concentration of extractions were prepared. 20 µl extraction of determinated concentrations were loaded in sterile antibiotic disks. Disks were left to dry overnight at room temperature in sterile conditions. Microorganisms and fungus were respectively inoculated into petri dishes that contain Mueller Hinton agar and Potato Dextrose Agar (PDA). After that, the plates were allowed to dry for 5 min

at room temperature in aseptic conditions. Piperacillin were used for positive control of antibacterial test and oceral (Roche) that is an antifungal drug were used for positive control of antifungal test. Petri dishes containing bacterial strains and yeast strains were respectively incubated at 37ºC for 24 hours and at 25ºC for 48 hours; the inhibition zone diameters were observed in milimeters [6].

Minimum Inhibitory Concentration (MIC)

A broth microdilution MIC test was performed as mentioned by Göger, 2016. Two-fold dilutions of the extracts were prepared ranging from 10 mg/ml to 0.3375 mg/ml by using 96-well microtitraion plates. Each well was inoculated with an inoculum prepared as mentioned. The microtitration plates of bacterail strains were incubated at 37ºC for 24 hours and the that of fungus were incubated at 27ºC for 48 h and measured the each well by OD_{600} nm. The MIC value was determined as the lowest concentraiton of extract that completely inhibited growth of the organism.

Minimum Bacteriastatic/Bacteriacidal/Fungicidal Concentration (MBC/MFC) Method

The wells in which no visual growth were observed in MIC test were used for further test called MBC and MFC. According to these tests, the results obtained by MIC applied to draw conclusions about whether the activity showed by MIC values comprises bacteriacidal/fungicidal activity or bactaeriostatic/fungistatic activity. The wells where no visible growth was observed were inoculated into plates that contain Mueller Hinton Agar and these were incubated at 37ºC for 24 h for bacteria and at 27ºC for 48 h for fungus [20].

RESULTS

In this study, the extract of *Acanthophyllum acerosum* and *Acanthophyllum microcephalum* was applied fourteen bacteria using disc diffusion (Table 1, 2) and MIC (Table 3, 4) methods to determine antimicrobial activity. Also, the antifungal activity of these plants was investigated on *Candida albicans* by diffusion method.

A. acerosum extract prove to exhibit good antibacterial activity against *Klebsiella pneumoniae*, *Salmonella enteritidis* ATCC 13075, *Salmonella typhimurium* SL1344, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* DSMZ 20044, *Escherichia coli* CFAI, *Salmonella infantis*, *Salmonella kentucky* and *Pseudomonas aeruginosa* DSMZ 50071 by disc diffusion method. It was found to have the highest effect against *K. pneumoniae* and *S. aureus* (Table 1). According to MIC assay, *A. acerosum* had inbition effect to *K. pneumoniae*, *S. epidermidis* DSMZ 20044, *E. coli* CFAI, *S. kentucky*, *Enterococcus faecalis* ATCC 29212 and *Pseudomonas fluorescens* P1 with its 10 mg/ml concentration (Table 3).

A. microcephalum extract got antibacterial effect against *K. pneumoniae*, *S. enteritidis* ATCC 13075, *S. typhimurium* SL1344, *S. epidermidis* DSMZ 20044 and *S. kentucky* in spite of no effect other microorganisms. The highest antimicrobial effect was observed to *S. typhimurium* and *S. epidermidis* (Table 2). There was no result MIC assay result of *A. microcephalum* (Table 4). As a result of inhibiton zones of both plant, *A. acerosum* showed more antibacterial activity. Both plant showed no result against *C. albicans.*

Table 1. Inhibiton zones (mm) of *Acanthophyllum acerosum* extract

Microorganism	Inhibition zones (mm)*		
	A. acerosum 1 mg/ml	*A. acerosum* 5 mg/ml	*A. acerosum* 10 mg/ml
K. pneumoniae	6,67	6,17	6,17
S. aureus	5,42	6,33	6,67
E. coli CFAI	4	4	2
S. epidermidis	6	4	6,33
P. aeruginosa	1,83	1,83	1,83
L. monocytogenes	-	-	-
E. durans	-	-	-
S. kentucky	2	3,83	2
S. infantis	6	2	6,33
P. fluorescens	-	-	-
E. faecalis	-	-	-
L. innocua	-	-	-
S. enteritidis	2	2	2
S. typhimurium	4,67	2	1,83
C. albicans	-	-	-

[(-) no zone, * three replicaiton]

The highest effect of 10 mg/ml concentration of *A. acerosum* extract was seen on *S. epidermidis* with 6,33 mm and also at its 1 mg/ml and 5 mg/ml concentrations formed 4 mm diameter zone on *E. coli* CFAI (Figure 1, Table 1).

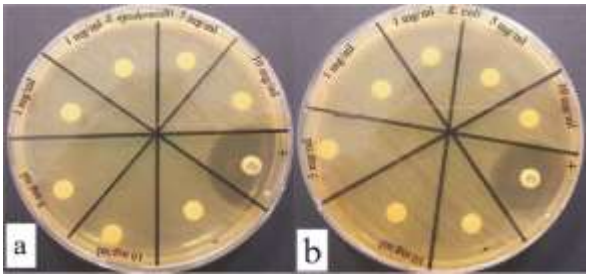

Figure 1. Inhibition zone of *A. acerosum S. epidermidis* (a) and *E. coli CFAI* (b) [(+) control (PRL 100 antibiotic), (-) control (sterile dH_2O)]

Table 2. Inhibition zones of *Acanthophyllum microcephalum* extract (mm)

Microorganisms	Inhibition zones (mm)*		
	A. microcephalum 1 mg/ml	*A. microcephalum* 5 mg/ml	*A. microcephalum* 10 mg/ml
K. pneumoniae	6	-	6
S. aureus	-	-	-
E. coli CFAI	-	-	-
S. epidermidis	6	7	7
P. aeruginosa	-	-	-
L. monocytogenes	-	-	-
E. durans	-	-	-
S. kentucky	6	6	7
S. infantis	-	-	-
P. fluorescens	-	-	-
E. faecalis	-	-	-
L. innocua	-	-	-
S. enteritidis	6	-	-
S. typhimurium	6	7	7
C. albicans	-	-	-

[(-) no zone; * three replication]

Table 3. MIC results of *Acanthophyllum acerosum*

Microorganisms	Minimum Inhibitory Concentration (MIC)					
	A. acerosum 10 mg/ml	*A. acerosum* 5 mg/ml	*A. acerosum* 2,5 mg/ml	*A. acerosum* 1,25 mg/ml	*A. acerosum* 0,625 mg/ml	*A. acerosum* 0,3125 mg/ml
K. pneumoniae	+	-	-	-	-	-
S. aureus	+	-	-	-	-	-
E. coli CFAI	+	-	-	-	-	-
S. epidermidis	+	-	-	-	-	-
P. aeruginosa	-	-	-	-	-	-
L. monocytogenes	-	-	-	-	-	-
E. durans	-	-	-	-	-	-
S. kentucky	+	-	-	-	-	-
S. infantis	-	-	-	-	-	-
P. fluorescens	+	-	-	-	-	-
E. faecalis	+	-	-	-	-	-
L. innocua	-	-	-	-	-	-
S. enteritidis	-	-	-	-	-	-
S. typhimurium	-	-	-	-	-	-

[(+) minimum inhibition, (-) no effect]

Table 4. MIC results of *Acanthophyllum microcephalum*

Microorganisms	Minimum Inhibitory Concentration (MIC)					
	A. microcephalum 10 mg/ml	*A. microcephalum* 5 mg/ml	*A. microcephalum* 2,5 mg/ml	*A. microcephalum* 1,25 mg/ml	*A. microcephalum* 0,625 mg/ml	*A. microcephalum* 0,3125 mg/ml
K. pneumoniae	-	-	-	-	-	-
S. aureus	-	-	-	-	-	-
E. coli CFAI	-	-	-	-	-	-
S. epidermidis	-	-	-	-	-	-
P. aeruginosa	-	-	-	-	-	-
L. monocytogenes	-	-	-	-	-	-
E. durans	-	-	-	-	-	-
S. Kentucky	-	-	-	-	-	-
S. infantis	-	-	-	-	-	-
P. fluorescens	-	-	-	-	-	-
E. faecalis	-	-	-	-	-	-
L. innocua	-	-	-	-	-	-
S. enteritidis	-	-	-	-	-	-
S. typhimurium	-	-	-	-	-	-

[(+) minimum inhibition, (-) no effect]

About MIC and disc diffusion method results for *A. microcephalum* and *A. acerosum,* the antimicrobial effect of plants support each other.

DISCUSSIONS

In this study, the antimicrobial activity of *A. microcephalum* and *A. acerosum* was investigated on fifteen microorganism and *A. acerosum* showed more antimicrobial effect than *A. microcephalum*.

Rabe and Staden (1997) have observed the antimicrobial effect of methanol and water extract of twenty-one plant used in traditional medicine. They have reported that plant extracts are usually effective on Gram (+) bacteria, but not against *Klebsiella pneumoniae* on Gram (-) bacteria. Also, they showed that only methanol extract of plants inhibited *E. coli* growth. In this study, *A. acerosum* extract had the antimicrobial effect on *Klebsiella pneumoniae.*

Moghimipour et al. (2015) have observed the antimicrobial activity of the saponin of *Glycyrrhiza glabra, Acanthopyllum squarrusom, Quillaja saponaria* against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis* and they reported that *Glycyrrhiza glabra* saponin has shown a stronger antimicrobial effect than other plant saponin. *Acanthopyllum squarrusom* saponin showed the most effect in *E. coli* and formed 11.00 ± 0.17 mm zone and inhibition values on *E. coli, S. aureus, P. aeruginosa* microorganisms were higher than the inhibition values of *A. acerosum* and *A. microcephalum* extracts we used.

Yücel and Yaylı (2018) have studied that the antimicrobial activity of essential oils of *Dianthus carmelitarum* Reut. ex Boiss. and *Dianthus calocephalus* Boiss. that are in Caryophyllaceae familia against *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 43288, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* 702 Roma, *Mycobacterium smegmatis* ATCC607 and *Candida albicans* ATCC using MIC assay. Essential oils of both plant has shown antimycotic effect at 668 µg/mL and 1041 µg/mL concentrations while they has shown no antibacterial effect. *A. acerosum* extract has antibacterial effect against *E. coli*, *S. aureus* and *E. faecalis.*

In other study (Albayrak and Aksoy, 2010) reported that the antimicrobial effect of methanol and water extract of *Paronychia mughlaei* Chaudhri (Caryophyllaceae) were investigated against fifteen microorganisms. The methanol extract of that has showed weak antimicrobial effect against *Aeromonas hydrophila* (Gram+), *Bacillus brevis*, *Bacillus cereus*, and *Bacillus subtilis* (Gram-) and also the water extract of that has demonstrated the poor effect *B. brevis*. Like this study, both extract had no antifungal effect.

Mukherjee et al. (1997) studied that *Drymaria cordata* (Caryophyllaceae) with different solvents (benzene, chloroform, methanol and water) extracts prepared against microorganisms (*Staphylococcus aureus* ATCC 29737, *Escherichia coli* ATCC 10536, *Bacillus subtilis* ATCC 6633, *Bacillus pumilis* ATCC 14884 and *Pseudomonas aeruginosa* ATCC 25619) have tested for antibacterial activity and the extracts showed significant antimicrobial activity on all microorganisms. *A. acerosum* extract had an impact on *S. aureus*, *E. coli* and *P. aeruginosa*.

Khaledi et. al (2017) have declared that the hydroalcoholic extracts of *Dianthus orientalis*, *Ziziphora clinopodioides*, *Euphorbia* sp. and *Acanthophyllum glandulosum* Bunge ex Boiss. investigated the antibacterial effects on *Staphylococcus aureus* and *Acinetobacter baumanii* using MIC and MBC assay. *D. orientalis* and *A. glandulosum* extract had the highest activity against both bacteria.

As a result, the effect of the plant species we use on the tested microorganisms is more effective than some plant species when compared to the studies. It can be seen that the plant extracts used in this study can be used in the production of drugs or supplementary foods, considering the antimicrobial activities. In our study, it is thought that the antimicrobial properties of two species belonging to Caryophyllaceae family can be useful for other studies.

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