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RESEARCH ARTICLE

Antimicrobial Activity of Extracts of Some Plants from Amasya (Turkey)

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ABSTRACT

The study is to test in vitro the antimicrobial efficacy of chloroform extracts of 10 plant taxa from Turkey and four of them are endemic. The antimicrobial activity from the extracts of various plants was determined according to the disc diffusion method by using Escherichia coli ATCC 25213, Escherichia coli ATCC 35218, Klebsiella pneumoniae ATCC 27736, Proteus vulgaris RSKK 96026, Yersinia enterocolitica RSKK 1501, Salmonella enteritidis RSKK 171, Pseudomonas aeruginosa NRLL B-23, Proteus vulgaris RSKK 96026, Listeria monocytogenes Li6, Staphylococcus aureus ATCC 25923, S. aureus Cowan I, Bacillus cereus RSKK 863, B. subtilis ATCC 6633, Candida albicans ATCC 10231 and C. tropicalis (clinical isolate). 10 extracts showed antibacterial activity against all test bacteria. Four plants namely Phlomis pungens var. pungens, P. pungens var. hirta, P. armeniaca, Tanacetum argenteum subsp. canum var. canum, demonstrated broad spectrum anticandidal activity. It is shown that there is no anticandidal activity of Astragalus densifolius subsp. amasiensis, A. angustifolius subsp. angustifolius var. angustifolius, Achillea biebersteinii, A. setacea, A. teretifolia, A. phrygia. B. subtilis and B. cereus were the most sensitive bacteria to all plant extracts. On the contrary, P. aeruginosa were the most resistant microorganism.

KEYWORDS: *Amasya, antimicrobial activity, crude extracts, endemic plants, Turkey*

INTRODUCTION

Plant extracts, for the treatment of various ailments, were highly regarded by the ancient civilizations [1]. Even today, plant materials remain an important resource for combating illnesses, including infectious diseases, and many of these plants have been investigated for novel drugs or templates for the development of new therapeutic agents[2]. Nowadays, the development of resistance by a pathogen to many of the commonly used antibiotics provides an impetus for further attempts to search for new antimicrobial agents to combat infections and overcome the problems of resistance and side effects of the currently available antimicrobial agents [3]. The treatment of infectious diseases with antimicrobial agents continues to present problems in modern-day-medicine with many studies showing a significant increase in the incidence of bacterial resistance to several antibiotics [4, 5]. Multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation forced scientists for searching new antimicrobial substances from various sources which are the good sources of novel antimicrobial chemotherapeutic agents [6-8].

This research was focused on the antimicrobial activity of 10 different plant taxa; *Phlomis pungens* Willd. var. *pungens, P. pungens* Willd. var. *hirta* Velen., *P. armeniaca* Willd. (endemic/Irano-Turanian), *Tanacetum argenteum* (Lam.) Willd subsp. *canum* (C. Koch) Grierson var. *canum*, *Astragalus densifolius* Lam. subsp. *amasiensis* (Freyn) Aytaç&Ekim (endemic/Irano-Turanian), *A. angustifolius* Lam. subsp. *angustifolius* var. *angustifolius, Achillea biebersteinii* Afan., *A. setacea* Waldst.&Kit, *A. teretifolia* Willd (endemic/Irano-Turanian)., *A. phrygia* Boiss.&Ball (endemic/Irano-Turanian).

MATERIALS AND METHOD

Plants materials

In the current work, 10 plant taxa from Amasya (Turkey) were selected. Mature plants were collected from several sites in the Amasya region (Turkey), during the spring and summer seasons (AprilJune) of 2005. Davis [9] and other related studies for example; Cansaran and Aydoğdu [10], Cansaran [11] were utilized in the identification of the specimens. When the authors of the plant taxa were being written, "Authors of Plant Names" was used [12]. Plants were deposited in the Department of Biology, Faculty of Science, Pamukkale University, Denizli (Turkey).

Preparation of the crude extracts

The dried plant samples were powdered in a blender. Then about 200 g of dry powdered plant material was extracted individually with chloroform (Merck) in a soxhlet apparatus (6 h for each plant) at 62 °C. The extracts were evaporated under reduced pressure and dried using rotary evaporator. Dried extracts were stored in labeled sterile screw capped bottles at -20 °C.

Test microorganisms

The following strains of bacteria were used: *Escherichia coli* ATCC 25213, *E. coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 27736, *Yersinia enterecolitica* RSKK 1501, *Salmonella enteritidis* RSKK 171, *Pseudomonas aeruginosa* NRRL B-23, *Proteus vulgaris* RSKK 96026, *Listeria monocytogenes* Li6, *Staphylococcus aureus* ATCC 25923, *S. aureus* Cowan I, *Bacillus cereus* RSKK 863, *B. subtilis* ATCC 6633, *Candida albicans* ATCC 10231 and *C. tropicalis* (clinical isolate) were used as test microorganisms.

Determination of antimicrobial activity

The antimicrobial activities of the plant extracts were determined by the disc-diffusion method [13]. All the microorganisms mentioned above were incubated at 37 ± 0.1 °C (30 ± 0.1 °C for only *L. monocytogenes*) for 24 h by inoculation into Nutrient broth. *Candida albicans* and *C. tropicalis* were incubated Yeast Extract Peptone Dextrose (YEPD) in broth at 28 ± 0.1 °C for 48 h. The culture suspensions were prepared and adjusted by comparing against 0.5 Mc Farland turbidity standard tubes. Nutrient Agar and YEPD Agar (20 ml) were poured into each sterilized Petri dish after injecting cultures (100 l) of microorganisms and distributing medium in Petri dishes homogeneously. For the investigation of the antibacterial and anti-candidal activity, the dried plant extracts were dissolved in dimethylsulfoxide (DMSO) to a final concentration of 10% and sterilized by filtration through a 0.22 m membrane filter [3, 14]. The discs (6 mm) were impregnated with 30 µl of the prepared extracts at the concentration of 10 mg/ml and placed on the inoculated agar. At the end of the incubation period, inhibition zones formed on the medium were evaluated in mm. Studies were performed in duplicate and the inhibition zones were compared with those of reference discs. Reference discs used are as follows: Ampicillin (10 µg), Gentamicin (10 µg), Streptomycin (10 µg), and Nystatin (50 µg). Inhibitory activity of DMSO was tested by preparing a concentration of 10 % DMSO in water and inoculating with 30 µl on Petri dishes after injecting cultures of microorganisms.

Statistical analysis

All antimicrobial experiments were done in seven times. The comparisons of the recorded data were made by ANOVA General Linear Model.

RESULTS

In the present study, the antimicrobial activity of chloroform extracts of *P. pungens* var. *pungens, P. pungens* var. *hirta, P. armeniaca* (endemic), *T. argenteum* subsp. *canum* var. *canum*, *A. densifolius* subsp. *amasiensis* (endemic), *A. angustifolius* subsp. *angustifolius* var. *angustifolius, A. biebersteinii, A. setacea, A. teretifolia* (endemic) and *A. phrygia* (endemic) was determined against some Gram (-) and Gram (+) which bacteria pathogenic and yeasts, *C. albicans* and *C. tropicalis*.

The results of the antimicrobial screening of the crude extracts of all species of plants are shown in Table 1. The inhibition zone diameter of the reference antibiotics and antifungal to the microorganisms are shown in Table 2. The chloroform extracts of this plants (4 of them are endemic) inhibited the growth of bacteria and yeasts. The antimicrobial activity of plant extracts was variable according to the species, subspecies or variety. The inhibition zones ranged between 4.5 and 33 mm diameter. The final concentration of DMSO in the assays did not interfere with the microbial growth. Thus, we may conclude that the antibacterial activity in this assay is exclusively due to plant extracts.

In general, among tested microbial strains, bacteria were found to be more sensitive, except *P. aeruginosa*, to many of the tested plants than fungi. Four plants namely *P. pungens* var. *pungens* (9 mm for *C. albicans* and 8 mm for *C. tropicalis*) *P. pungens* var. *hirta* (13 mm for *C. albicans* and 11 mm for *C. tropicalis*), *P. armeniaca* (15 mm for *C. albicans* and 13 mm for *C. tropicalis*), *T. argenteum* subsp. *canum* var. *canum* (12 mm for *C. albicans* and 10 mm for *C. tropicalis*) demonstrated broad spectrum anticandidal activity. It is shown that there is no anticandidal activity of *A. densifolius* subsp. *amasiensis*, *A. angustifolius* subsp. *angustifolius* var. *angustifolius, A. biebersteinii, A. setacea, A. teretifolia, A. phrygia*. *B. subtilis* and *B. cereus* were the most sensitive bacteria to all plant extracts. On the contrary, *P. aeruginosa* were the most resistant bacteria. Some organisms exhibited only slight susceptibility. Generally, the plants showed better antimicrobial activity against the Gram (+) bacteria than Gram (-) bacteria. Gram (+) bacteria were most susceptible to all plants with inhibition zones between 11 and 37 mm. Gram-negative bacteria were most resistant to plants with inhibition zones between 4.5 and 25 mm. The diameters of growth inhibition zones in *P. armeniaca* extract ranged from 29 to 34 mm with the highest inhibition zone values observed against the medically important pathogens such as *S. aureus* ATCC 25923 and *S. aureus* Cowan I (32 mm), *L. monocytogenes* (29 mm), *B. cereus* (34 mm) and *B. subtilis* (33 mm). The extract of *T. argentum* subsp. *canum* var. *canum* showed significant inhibition of *S. aureus* ATCC 25923, *S. aureus* Cowan I, *L. monocytogenes*, *B. cereus* and *B. subtilis* (diameter inhibition zones; 25, 23, 32, 36 and 37 mm, respectively).

Table 1. Antimicrobial activities [a] of chloroform extracts from selected plants of Amasya, Turkey

[b]

E.c.(1)	E.c.(2)	K.p.	Y.e.	S.ent.	P.a.	P.v.	L.m.	S.a.(1)	S.a.(2)	B.c.	B.s.	C. a	C.t.
12 ± 0	13± 0	11± 0	17± 0	21± 1	-	24± 0	29± 0	28± 0	27± 0	30± 0	31± 0	-	-
11± 0	12± 0	10± 0	18± 0	22± 0	-	26± 0	27± 0	29± 0	24± 0	30± 0	32± 0	-	-
5± 1	9± 1	7± 1	16± 0	18± 0	-	19± 0	21± 1	19± 0	15± 0	25± 0	27± 0	-	-
6± 0	11± 0	9± 0	15± 3	16± 0	-	21± 0	20± 0	22± 0	13± 1	22± 0	25± 0	-	-
4.5± 0.5	10± 0	11± 0	17± 0	19± 0	-	23± 1	25± 0	21± 1	12± 0	19± 0	23± 0	-	-
7.5± 0.5	12± 0	8± 0	14± 0	21± 1	-	20± 0	24± 0	20± 0	11± 0	21± 1	22± 0	-	-
6± 0	8± 0	11± 0	17± 0	21± 1	-	18± 0	23± 0	31± 0	29± 0	32± 0	30± 0	9± 1	8± 0
8± 0	13± 0	14± 0	19± 0	23± 0	-	25± 0	27± 0	28± 0	30± 0	32± 0	31± 0	13± 0	11± 1
7.5± 0.5	12± 0	13± 0	21± 1	25± 0	-	28± 0	29± 0	32± 0	32± 0	34± 0	33± 0	15± 0	13± 0
18± 0	17± 0	21± 1	23± 0	25± 0	-	29± 0	32± 0	25± 0	23± 0	36± 0	37± 0	12± 0	10± 0

[a]Diameter in mm of the zone of inhibition; (-)= no inhibition, E. c.(1) = *E. coli* ATCC 25213, E. c.(2) = *E. coli* ATCC 35218, K. p. = *K. pneumoniae* ATCC 27736, Y. e. = *Y. enterocolitica* RSKK 1501, S. ent. = *S. enteritidis* RSKK 171, P. a. = *P. aeruginosa* NRRL B-23, P. v. = *P. vulgaris* RSKK 96026 L.m.= *L. monocytogenes* Li6, S.a.(1)= *S. aureus* ATCC25923, S.a.(2)= *S. aureus* Cowan I, B. c. = *B. cereus* RSKK 863, B.s. = *B. subtilis* ATCC 6633, C. a.= *C. albicans* ATCC 10231, C.t.= *C. tropicalis* (clinical isolate). Plants name[b]; *A. densifolius* subsp. *amasiensis* (A.d.), *A. angustifolius* subsp. *angustifolius* var. *angustifolius* (A.a.), *A. biebersteinii* (A.b.), *A. setacea* (A.s.), *A. teretifolia* (A.t.), *A. phrygia* (A.p.), *P. pungens* var. *pungens* (P.p.1), *P. pungens* var. *hirta* (P.p.2), *P. armeniaca* (P.a.), *T. argentum* subsp. *canum* var. *canum* (T.a.).

DISCUSSION

Emergence of multi-drug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin. For the study of plant extracts, the number of microbial strains was reduced in accordance with their known function as human pathogenic and food destroying agents. *P. pungens* var. *pungens, P. pungens* var. *hirta, P. armeniaca* (endemic), *T. argenteum* subsp. *canum* var. *canum*, *A. densifolius* subsp. *amasiensis* (endemic), *A. angustifolius* subsp. *angustifolius* var. *angustifolius, A. biebersteinii, A. setacea, A. teretifolia* (endemic) and *A. phrygia* (endemic) were selected based on their relevant ethno-medical use. The antimicrobial activity profile of all species of plants against the tested strains indicated that Gram (+) bacteria were the most susceptible bacteria group of all the bacterial test strains. *P. aeruginosa* was the most resistant strain of all the bacteria used in this study. In fact, Gram (-) bacteria especially *P. aeruginosa* are frequently reported to have developed multi drug resistance to many of the antibiotics [15]. Therefore, it is not surprising to learn that *P. Aeruginosa* is the least responding bacterial strain to the tested plant extracts. These results are similar to those of previous reports in the literature indicating that Gram (-) bacteria are more resistant to essential oils than Gram (+) bacteria.

Table 2. Standards and inhibition zones diameters (mm).

Microorganisms	Ampicilin (10 µg)	Gentamicin (10 µg)	Streptomycin (10 µg)	Nystatin (50 µg)
E.coli ATCC 25213	S	ND	ND	ND
E. coli ATCC 35218	S	ND	ND	ND
K. pneomoniae ATCC 27736	ND	ND	IS	ND
Y. enterocolitica RSKK 1501	R	ND	ND	ND
S. enteritidis RSKK 171	S	ND	ND	ND
P. aeruginosa NRLL B-23	ND	IS	ND	ND
P. vulgaris RSKK 96026	ND	ND	S	ND
L. monocytogenes Li6	ND	ND	S	ND
S. aureus ATCC 25923	ND	ND	S	ND
S. aureus Cowan I	ND	ND	S	ND
B. cereus RSKK 863	ND	ND	R	ND
B. subtilis ATCC 6633	ND	ND	S	ND
C. albicans ATCC 10231	ND	ND	ND	IS
C. tropicalis (clinical isolate)	ND	ND	ND	IS

ND: not determined, S: Sensitive, R: Resistant, IS: Intermediate Sensitivity

This resistance has been attributed to the presence of cell wall lipopolysaccharides, which can screen out the essential oils, the lipids are thus prevented from accumulating on the transporting cell membrane, and from entering the cells [16]. As known *S. aureus*, *L. monocytogenes*, and *Bacillus* species especially *B. cereus* is agents of food poisoning. The most interesting area of application for plant extracts is the inhibition of growth and reduction in numbers of the more serious foodborne pathogens such as *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes* [17]. The diameters of growth inhibition zones in *P. armeniaca* extract ranged from 29 to 34 mm with the highest inhibition zone values observed against the medically important pathogens such as *S. aureus* ATCC 25923, *S. aureus* Cowan I, *L. monocytogenes*, *B. cereus* (34 mm) and *B. subtilis*. Baytop [18, 19] reported that Turkish *Phlomis* species are used as herbal teas (dağ çayı), as tonic, carminative, appetizer and stimulants in the folk medicine and recognized by local names as "ballıkotu, calba, çalba or şalba". Some medicinal usages of *P. grandiflora* as treatment for stomach disorders were documented by Gurbuz *et al*.[20] and Ozcelik [21]. In our study, while *Achillea biebersteinii, A. setacea, A. teretifolia* and *A. phrygia* extracts were inactive against *Candida albicans* and *C. tropicalis*, these extracts showed antibacterial activity against all of bacteria, except *P. aeruginosa*. Similar results were reported with *Achillea* L. shown that chloroform extract of *A. teretifolia* exhibited antibacterial activity against *S. aureus* (MORSA), *S. epidermidis*, and *S. typhimurium* and all of the extracts were inactive against *C. albicans* [22].

Among the plant species, significant differences were found in terms of inhibition zones ($F=145.48$, $P<0.0001$). Also among the microorganisms, significant statistical differences were found in terms of testing these plants on 14 different microorganisms ($F=784.74$ $P<0.0001$).

When comparing the antimicrobial activity of the tested samples to that of reference antibiotics, the inhibitory potency of tested extracts could mostly be considered as important (Table 2). This is due to the fact that medicinal plants are of natural origin, which means more safety for consumers, and are considered that they are being low risk for resistance development by pathogenic microorganisms.

Enterotoxins produced by *Escherichia coli*, *Staphylococcus aureus* and *Yersinia* species are responsible for toxicity in the intestinal tract causing diarrhea, etc. in humans [23]. In present study,

plant extracts inhibited the growth of these bacteria that cause some diseases and thus plant extracts can be a good

antibacterial for medicated some infections care formulations.
In summary, it might be said that these plants which are used in our study, may be used or protection against bacteria and *Candida* in ethno-medicine and future work is needed to focus the research on the chemical identification of the antimicrobial ingredients.

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