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Antimicrobial Activites of the Extracts of Various Plants (valex, mimosa bark, gallnut powders, *Salvia sp.* and *Phlomis sp.*)

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Abstract: The antimicrobial activities of valex (an extract of valonia), the extracts of mimosa bark, gallnut powders, *Salvia aucheri var. aucheri and Phlomis bourgei* were studied. The antimicrobial efficiency of the above plants was evaluated according to the disk diffusion method by using *Bacillus brevis* FMC 3, *Bacillus subtilis* IMG 22, *Bacillus cereus* EÜ, *Escherichia coli* DM, *Pseudomanas aeruginosa* DSM 50071, *Staphylococcus aureus* Cowan 1, *Listeria monocytogenes* A, *Micrococcus luteus* LA 2971, *Klebsiella pneumoniae* FMC 5, *Mycobacterium smegmatus* RUT, *Proteus vulgaris* FMC 1 bacteria , and *Alternaria alternata* MDC 97, *Penicillium italicum* MDC 101, *Fusarium equisetii* C, and *Candida albicans* fungi. The findings indcated that mimosa bark extracts having an inhibition zone of 11-31 mm had the maximum antibacterial efficiency, followed by valex, gallnut powders, *Salvia aucheri var. aucheri* and *Phlomis bourgei* extracts, respectively. Furthermore, it was found that gallnut powders and the extracts of mimosa barks only, containing high amounts of tannins, showed the antifungal efficiency, However the others did not.

Key Words: Valex; Mimosa bark; Gallnut powders; *Salvia aucheri var. aucheri; Phlomis bourgei;* Antimicrobial activity

Bazı Bitki Ekstraktlarının (Akasya kabuğu, palamut ekstraktı, mazı tozu, *Salvia sp.,* ve *Pholmis sp.*) Antimikrobiyal Aktivitesi

Özet: Palamut ekstraktı (valonia ekstraktı), akasya kabuğu, mazı tozu, *Salvia aucheri var. aucheri* ve *Phlomis bourgei* ekstraktlarının antimikrobiyal aktivitesi araştırılmıştır. Çalışmada, belirtilen ekstraktların antimikrobiyal aktivitesi disk difüzyon metodu ile *Bacillus brevis* FMC 3, *Bacillus subtilis* IMG 22, *Bacillus cereus* EÜ, *Escherichia coli* DM, *Pseudomonas aeruginosa* DSM 50071, *Staphylococcus aureus* Cowan 1, *Listeria monocytogenes* A, *Micrococcus luteus* LA 2971, *Klebsiella pneumoniae* FMC 5, *Mycobacterium smegmatus* RUT, *Proteus vulgaris* FMC 1 bakterileri ve *Alternaria alternata* MDC 97, *Penicillium italicum* MDC 101, *Fusarium equisetii* C, *Candida albicans* fungusları kullanılarak test edildi. Araştırma sonucunda akasya kabuklarının en fazla antimikrobiyal aktiviteye sahip olduğu görüldü (11-13 mm inhibisyon zonu). Palamut ekstraktı, mazı tozu, *Salvia aucheri var.*

aucheri ve *Phlomis bourgei* sırasıyla en fazla antimikrobiyal aktivite gösteren türler olmuştur. Ayrıca fazla miktarda tanen içeren mazı tozu ve akasya kabuk ekstarktının antifungal aktivite gösterdiği belirlendi. Diğer ekstraktların antifungal etkilerinin olmadığı da tespit edildi.

Anahtar Sözcükler: Valeks; Akasya kabuğu, Mazı tozu, Palamut ekstraktı, *Salvia aucheri var. aucheri, Phlomis hourgei;* Antimicrobial activity.

Introduction

Recently, we have conducted several studies on the inhibitory effects of various agricultural or forestry plant extracts on the growth of many bacteria in culture (1-4). However, the antimicrobial activities of the extracts of various vegetable materials, e.g., valonia, mimosa bark, garden sage, *Phlomis bourgei*, and gallnut, have not been investigated. First of all, valonia extracts, commercially known as "valex", are rich in tannin and are widely used in many fields, e.g., essentially, in the leather trade, pharmaceutics and painting (5). Valex produced by the extraction of valonia (a fruit of valonia oak, maturally and widely grown in Turkey as well as in Greece, Albania, Itlay, Syria, Palestine, and Jordan) is used in the leather industry as a filler material (6).

Valonia is utilized either in the form of its extract or direct powder. In the tanning of leather, valonia can be used either alone or mixed with the other tanning materials (e. g., the barks of pine, oak or spruce) to increase its penetration into leather. The valonia extracts are able to stabilize the proteins in leather, thus protecting the leather against microorganisms which causes decay. In this process, valonia fills in the pores remaining after the fats and hairs are removed (5). However, the antimcirobial activities of valonia have not as yet been studied in detail.

Valonia includes hydrolyzable tannins, e.g., castalagin and vescalagin (7). The components of valex determined by the filder method are as follows: tannins , 68-70%; non-tannins, 25-25%; undissolved materials, 1.10-1.15%; moisture: 4.05-5.50%. It was also found that the quality of the valex is up to world standards (8).

In this study, the second natural material whose antibacterial activities were studied, is mimosa bark obtained from *Acacia mollissima* species grown in South Africa. The mimosa barks aro sold either in stick (20-25 cm), chopped bark (2-5 cm) or ground bark. The mimosa trees reach a length of 2-3 m within one year and form a bark with a thickness of 6-11 mm, which can be rich in tannin within 5-6 years (9).

As in valonia, mimosa barks have long been largely used in the leather trade as filler/antibacterial materials. Mimosa bark can tan leather speedily, giving it a reddish color. With a color ranging between red and violet, mimosa bark includes 25-35% tannins, yielding a bright section when cut (10). Extracts from mimosa bark obtained by hot water or solvents (e.g., methanol and acetone) can also be used together with other tanning materials, e.g., valonia (10). The tannins of mimosa barks are condensed tannins, e.g. 7, 3, 4-tri-and 7, 3, 4, 5-tetra-hydroxy-flavan-3, 4-diols (11).

The third material evaluated in this study was a nodule occuring on the leaves of *Quercus infectoria,* which is also known as Turksih tannin or gallotannin (commercial name). It can be

used directly after grinding (i.e., it is in the form of powder). Because it contains high amounts of tannic acids such as gallic and egallic acids and is expensive, it is exported and used in the production of tannic acid. It makes leather light-colored and is an ideal tanning material. Its tannins are also included in the hydrolyzable tannin groups and consist of polygallol and m-digallolyl derivatives of glucose or polysaccharides (6).

Gallnuts consist of gallic tannin (50-75%), gallic acid (2-3%), ellagic acid (2%), and glucose, starch, and etheric fats (3%). Since the tannis of gallnuts are very valuable, they can also be used in the production of ink, textile painting (as fixator), and blue dye, in addition to processing leather. Of materials containing tannins, the highest amuonts (64.18 and 69.70%) have been found for gallnuts (6).

Additionally, in this study the antibacterial activities of the extracts of some plant species, i.e., *Salvia aucheeri* var. *aucheri* and *Phlomis bourgei,* were investigated. *Salvia aucheeri* var *aucheri* is generally known as "garden sage" in Turkey. It has externally been used as an antiseptic (especially in the nose and ear), antiflatulent, and stimulant. It can be taken internally either in the form of an infusion (1-5%) or a gargle (11). The salvia species used in this study are mainly camphor and 1, 8-cineol (12). So far, *Phlomis bourgei* species have traditionally been using for curing stomach-ache in the city of Isparta, Turkey (12). However, no information on its chemical contents exists.

It is therefore important to study the antibacterial activities of the extracts of these plant materials and to extend their usage into new areas such as the wood industry. Therefore, our aim was to determine the antimicrobial activities of extracts of valonia, mimosa bark, *Salvia aucheri var. aucheri, Phlomis bourgei,* and gallnut powders, through the us of a variety of common bacteria *in vitro*, which have not yet been evaluated in detail.

Material and Methods

Materials

The materials tested as bactericides in this study were valex obtained from the extraction valonia, an essential fruit of valonia oak (*Quercus macrolepis Ky-Q. aegilops L.*), grown in the western Anatolian region (Turkey). Valonia consists of gland (camata; fruit in cupola), hoof (*trillo;* sharp and stubby points covered with cupola), and cup (*cupula*; outside of valonia). The extracts of mimosa barks from *Acacia mollissima* species were donated by Sümerbank Co. ın Turkey, and gallnut powder was provided by *Quercus infectoria* grown in the Southern Anatiola (Turkey). Furthermore, *Salvia aucheri* Bentham var. *aucher* (Adana-Pozantı) and *Phlomis bourgei* Boiss were collected in the city of K. Maraş, Turkey.

In addition, standard antibiotic discs such as Penicillin G, Ampicillin, Cefataxime, Vancomycine, Oflaxacin and Tetracyclin used for comparision were provided by the Microbiology Division of the Faculty of Medicine at Fırat University in Elazığ, Turkey. *Bacillus brevis* FMC 3, *Bacillus subtilis* IMG 22, *Bacillus cereus* EÜ, *Escherichia coli* DM, *Pseudomonas aeruginosa* DSM 50071, *Staphylococcus aureus* Cowan 1, *Listeria monocytogenes* A, *Micrococcus luteus* LA 2971, *Klebsiella pneumoniae* FMC 5, *Mycobacterium smegmatus* RUT, *Proteus vulgaris* FMC 1 bacteria, and *Alternaria alternata* MDC 97, *Penicillium italicum* MDC 101, *Fusarium equisetii* C,

and *Candida albicans* fungi were donated by the Microbiology Division of the Faculty of Science at Fırat University in Elazığ, Turkey. In addition, choloroform was used as solvent for the extraction.

Methods

Extraction

The extraction method used in the production of valex was the classic reverse current method. Valonia was firstly broken into small pieces 2-6 mm in size with a cylinder crasher. The pieces of valonia were then extracted in hot water at 60-70°C with an extractor made of oxidation-resistant copper. The obtained valonia solution was acidic (i.e., pH: 3-3.5) and its concentration was 6 Be. The solution with tannin was transferred into a diffusion tank, mixed with distilled water and filtered to remove inert materials. In order to increase the quality of valex, sodium disulfide was added to the solution, which significantly decreased the particular size of the valex and lightened its color remarkably. The solution thus collected was indirectly heated by steam in tank, charged into evaporation tanks (5), and evaporated in three stages, as shown in Table 1.

Next, the concentrated solutions were again filtered and transferred into the upper section of the drying tower. The solution was sprayed towards the middle of the tower in pulverized form, turning to powder after hot-air drying at 185-200°C.

Finally, the dried valex was ground in a valex mill and collected in small plastic bags. On the other hand, mimosa barks directly provided by the company were extracted with hot water at 100°C (5).

The collected *Salvia* and *Phlomis* species and gallnut were identified and broken into pieces under sterile conditions. The pieces (20 g) were extracted with chloroform (150 ml) (Merck, Darmstadt) for 24 h with soxhlet equipment (15).

Preparation of Microorganism Culture

All the extracts thus obtained and the standard antibiotics were injected into empty sterilized antibiotic discs having a diameter of 6 mm (Schleicher & Shüll No: 2668, Germany) in the amount of 20 µl. The discs injected with only chloroform were used as control. All the bacteria mentioned above were incubated at 30±0.1 for 24 h by inoculation into Nutrient Borth (Difco) for 48 h. Mueller Hinton Agar (oxoid) sterilized in a flask and coolled to 45-50°C was distributed by pipette to the sterilized petri dishes having a diameter of 9 cm, in the amount of 15 ml after injecting cultures of bacteria prepared as stated above and mold for 24 h in the amount of 0.01 ml (10^5 bacteria per ml and 10^3 mold spore per ml), and providing the distribution of food medium in petri dishes homogenously. Dishes injected with extracts were placed in the solid agar meduim by pressing slightly (16-18).

Table 1. Evaporation conditions as a function of stages.

Stages	Vacuum (Atm)	Temperature (°C)
1st	0.0-0.12	75-85
2nd	0.6-0.53	65-75
3rd	0.4-1	42-46

Table 2. Antimicrobial activities of various plant extracts.

	Inhibition Zone (mm)*					
Microorganisms	A	B	C	D	E	F
Bacillus brevis	12	23	11	-	12	-
Bacillus subtilis	23	20	19	7	10	-
Bacillus cereus	7	14	12	-	8	-
Escherichia coli	15	18	7	-	-	-
Pseudomonas aeruginosa	11	12	9	-	10	-
Stapylococcus aureus	18	22	18	21	12	-
Listeria monocytogenes	12	31	12	-	-	-
Micrococcus luteus	15	12	7	-	10	-
Klebsiella pneumoniae	10	11	-	-	11	-
Mycobacterium smegmatus	-	15	10	-	8	-
Proteus vulgaris	18	24	14	9	10	-
Alternaria alternata	-	21	15	-	-	-
Penicillium italicum	-	-	-	-	-	-
Fusarium equisetii	-	-	-	-	-	-
Candida albicans	-	-	-	-	-	-

A: Valex, B: Mimosa bark C: Gallnut D: *Salvia aucheri* var. *aucheri*
E: *Phlomis bourgei,* F: Control (chloroform)
Inactive (-); moderately active (7-13); highly active (>14).
* İncludes diameter of disc (6 mm).

After petri dishes so obtained were kept at 4°C for 2 h, placks inoculated with mold were incubated at 25±0.1°C for 7 days. At the end of the period, inhibition zones formed on the food medium were measured in millimeters (mm). Tests were conducted in three parallel parts.

Results and Discussion

Table 2 shows *in vitro* antibacterial and antifungal activities of the extracts of three biometrials, e.g., valonia, mimosa bark, gallnut, *Salvia*, and *Phlomis*. In addition, the inhibition zones formed on some of the bacteria and fungi listed in Table 2 by standard antibiotic discs are indicated in Table 3 for comparison.

As clearly seen in Table 2, the extracts of mimosa bark had the highest antibacterial efficiency, followed by valex, *Salvia sp.,* extracts and gallnut, and *Phlomis sp.* extracts respectively. Moreover, it was determined that the extracts of gallnut and mimosa bark an antifungal effect, while the others (e.g., valex and *Phlomis*) do not (Table 2).

The extract obtained from valex considerably inhibited the growth of *L. moncytogenes, P. vulgaris* and *B. brevis,* having an inhibition zone of (22-31 mm). The growth of *S. aureus* and *B. subtilis* were inhibited by the whole extracts used in the study, and an inhibition zone ranging

Table 3. Antimicrobial activities of some standard antibiotics

	Inhibition Zone (mm)*					
Microorganisms	P	SAM	CTX	VA	OFX	TE
	10	20	30	30	5	30
Bacillus brevis	15	14	16	19	30	25
Bacillus subtilis	12	15	12	15	29	24
Bacillus cereus	13	14	14	17	34	28
Escherichia coli	16	10	11	24	33	27
Pseudomonas aeruginosa	9	10	60	8	49	32
Stapylococcus aureus	11	16	10	10	22	26
Listeria monocytogenes	10	12	15	24	34	33
Micrococcus luteus	32	34	32	32	25	-
Klebsiella pneumoniae	18	17	11	21	32	28
Mycobacterium smegmatus	16	19	13	23	34	28
Proteus vulgaris	9	14	19	20	29	24
Antifungal Nystatin, (30 μg)						
Alternaria alternata	14					
Penicillium italicum	19					
Fusarium equisetii	15					
Candida albicans	18					

P10: Penicillin G (10 unit) SAM 20: Ampicillin 10 μg CTX 30: Cefatoxime 30 μg
VA 30: Vancomycin 30 μg OFX 5: Oflaxacin 5 μg TE 30: Tetracyclin 30 μg

from 12 to 26 mm is formed. When the results obtained from valex were compared with those of standard antibiotics, it was determined that *K. pneumoniae* was more resistant, *P. aeruginosa, M. luteus,* and *B. cerus* were mid-resistant, and the other species were more susceptible to valex. On the other hand, *S. aureus* and *B. subtilis* were more susceptible, *L. moncytogenes, B. cerus* and *P. vulgaris* were mid-resistant and the others (except for *Klebsiella pneumoniae*) were mode resistant to the gallnut extracts than were VA 30, OFX 5 and TE 300 standard antibiotics.

B. brevis and *S. aureus* were mid-resistant and the other bacteria species were much more resistant to phlomis extracts than was Penicillin G. All the bacteria were found to be more susceptible to the extracts obtained from mimosa bark than were the standard SAM 20 and CTX 30 antibiotics. Similarly, in comparison to VA 30 standard, it was observed that *P. vulgaris* was more resistant and *S. aureus* was susceptible to *Salvia* extracts.

İlçim et al. (3) reported that the extracts of *Parmelia furfuraceae, Myrtus communis* subsp. *communis* and *Eugenia coryphyllata* showed antibacterial activities. In particular, *E.*

caryophyllata was found to be very effective on all the bacteria (except for *K. pneumoniae* and *E. aerogenes*). In a similar study, *B. megaterium* was found to be mid-resistant, and the other bacteria (except for *K. pneumoniae* and *S. aureus*) were resistant to the extracts of *Morus nigra,* showing an inhibition zone of 7-9 mm. In addition, the extracts of *Juniperus drupacea* inhibited the growth of some bacteria at different ratios.

Dığrak et al. (4) found that the extracts of *Ajuga orientalis, Smyrinum olusatrum, Astragalus schizopterus,* and *Salvia viridis* inhibited the growth of some bacteria and mould. In particular, they found that *A. shizopterus* was effective on *Bacillus* species, with an inhibition of 23 mm, and it inhibited the growth of *Enterobacter aerogenes.* The findings obtained this study were almost the same as those stated above.

The extracts of mimosa bark and gallnut powder inhibited the development of *A. alternata,* having an inhibition zone of 21 and 15 mm, respectively. However it was also determined that they were effective on the other fungi (*P. italicum, F. equisetii, C. albicans*). When the results were compared to standard antifungal Nystatin, the extracts of mimosa bark and gallnut powder were found to be more effective.

It is natural that there are differences among various antibacterial effects of plant groups. According to the phytochemical properties of plants and to whether the plants are of antimicrobic material or not, there are differences among the species. Therefore, it is thought that the differences observed from the findings result from the factors mentioned above. Furthermore, for the evaluation of plants, which are naturally grown in Turkey and are potential rich sources, studies will be very beneficial from medicine and economic standpoints.

In conclusion, it may be said that whole extracts, especially the extracts of mimosa bark, valex, and gallnut powders, can be used for protection against bacteria and fungus in some cases.

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