

## ANTIBACTERIAL AND ANTIFUNGAL EFFECTS OF VARIOUS COMMERCIAL PLANT EXTRACTS

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### ABSTRACT

*The antimicrobial activities of valex (the extract of valonia), the extracts of mimosa bark, gallnut powders, Salvia aucheri Bentham var. aucheri and Phlomis bourgei Boiss were studied. The antimicrobial activity of the above plants was evaluated by the disk diffusion method using 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 equiseti C, Candida albicans CCM 314 fungi. The results indicated that mimosa bark extracts had the greatest antibacterial activity, followed by the valex, gallnut powders, Salvia aucheri var. aucheri and Phlomis bourgei extracts, respectively. Furthermore, it was found that gallnut powders and the extracts of mimosa bark contained high amounts of tannins and showed antifungal activity.*

### INTRODUCTION

Recently, the inhibitory effect of various agricultural or forestry plant extracts on the growth of many bacteria in culture have been studied in our laboratory (İlçim et al., 1997a, b, 1998; Dıġrak et al., 1997). 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 studied. First, valonia extracts, commercially called “valex”, are rich in tannins and widely used in many areas, e.g., in the leather trade, pharmacy, and painting (Armaġan, 1988). Valex produced by the extraction of valonia (a fruit of valonia oak, naturally and largely grown in Turkey as well as in Greece, Albania, Italy, Syria, Palestine, and Jordan) are used in the leather industry as a filler material (Bozkurt & Göker, 1986).

Valonia is utilized either in the form of an 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 bark of pine, oak or spruce) to increase its penetration into leather. The valonia extracts are able to stabilize proteins in leather, thus protecting the leather against microorganisms decaying the leather. In this process, valonia fills in the pores remaining after taking the fats and hairs out of leather (Armaġan, 1988). However, the antimicrobial activities of valonia have not been studied in detail.

Valonia includes hydrolyzable tannins, e.g., castalagin and vescalagin (Toptaş, 1993). The components of valex determined by a filter method are as follows: Tannins, 68–70%; non-tannins, 25–25.5%; undissolved materials, 1.10–1.15%; moisture: 4.05–5.50%. It was also found that the quality of the valex is comparable to the world standard (Anon., 1984).

In addition, the antibacterial activities of mimosa bark obtained from *Acacia mollissima* species grown in the Southern Africa was studied. Mimosa bark is sold either as sticks (20–25 cm), chopped bark (2–5 cm) or ground bark. Mimosa trees reach a length of 2–3 m within one year and form a bark with a thickness of 6–11 mm; the bark can be rich in tannins within 5–6 years (Browning, 1967).

*Keywords:* Antimicrobial activity, Gallnut powders, Mimosa bark, *Phlomis bourgei*, *Salvia aucheri* var. *aucheri*, Valex.

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As with valonia, mimosa bark has largely been used in the leather trade as filler/antibacterial materials. Mimosa bark can speedily tan leather and give a reddish color. Varying its color between red and violet, mimosa bark includes 25–35% tannins and give a bright section while being cut. The extracts obtained from mimosa bark by using hot water or solvents (e.g., methanol and acetone) can also be used together with other tanning materials, e.g., valonia (Browning, 1967). The tannins of mimosa bark are condensed, e.g., 7,3',4'-tri- and 7,3',4',5'-tetrahydroxy-flavan-3,4-diols (Hathway, 1975).

The third material examined in this study is the nodules occurring on the leaves of *Quercus infectoria*, which is also known as Turkish tannin or gallotannin (commercial name). It can directly be used after grinding (i.e., in the form of powder). Since 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 open colored and is ideal tanning material. Its tannins are also included to the hydrolyzable tannin groups and consists of polygallol and *m*-digalloyl derivatives of glucose or polysaccharides (Bozkurt & Göker, 1986).

Gallnuts consist of gallic tannin (50–75%), gallic acid (2–3%), and ellagic acid (2%), and glucose, starch, and etheric fats (3%) (Huş, 1969). Since tannins of gallnuts are very valuable, it can be used in the production of ink, textile painting (as a fixator), and blue dye, rather than in processing leather. Among the materials having tannins, the highest amounts of tannins (64.18 and 69.70%) are found in gallnuts (Bozkurt & Göker, 1986).

The antibacterial of the extracts of some additional plant species, i.e., *Salvia aucheri* var. *aucheri* and *Phlomis bourgei*, were also investigated. *Salvia aucheri* var. *aucheri* is generally known as “garden sage” in Turkey. It has been used externally as an antiseptic (especially in the ear), gas remover, power-giving, and stimulator. It can be taken internally either in the form of an indusion (1–5%) or as a chemical component of gargle (Hathway, 1975). The *Salvia* species used in this study are mainly camphor and 1,8-cineol (Baytop, 1984). *Phlomis bourgei* species have traditionally been used for curing stomach ache in the city of Isparta-Turkey (Baytop, 1984). However, there is not any information about its chemical contents.

Based on these uses, we have studied the antibacterial activities of extracts of these vegetable materials and to extend their usages to new areas such as the wood industry. Therefore, as reported in this study, we

determined the antimicrobial activities of extracts of valonia, mimosa bark, *Salvia aucheri* var. *aucheri*, *Phlomis bourgei*, and gallnut powders, using a variety of common bacteria which have not been evaluated previously.

## MATERIALS AND METHODS

### Materials

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

Furthermore, standard antibiotic discs such as penicillin G, ampicillin, cefataxime, vancomycine, ofloxacin and tetracycline used for comparision were provided by the Microbiology Division of Medicine Faculty of Firat 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 equiseti* C, *Candida albicans* CCM 314 fungi were also provided by the Microbiology Division of Science Faculty of Firat University in Elazığ-Turkey. In addition, choloroform was used as solvent for the extraction of the plants.

### Extraction of the Plants

The extraction method used in the production of valex is a classic reverse current method. Valonia was firstly broken into small pieces 2–6 mm by using a cylindric crusher. Then, the valonia pieces were extracted in hotwater at 60–70°C by using an extractor made of copper resistant to oxidation. The obtained valonia solution was acidic (i.e., pH 3–3.5). The solution with tannin was transfered into a diffusion tank, mixed

Table 1. Evaporation conditions as a function of stages.

Stage	Vacuum (Atm)	Temperature (°C)
1 <sup>st</sup>	0.0–0.12	75–85
2 <sup>nd</sup>	0.6–0.53	65–75
3 <sup>rd</sup>	0.4–1	42–46

with distilled water and filtered to remove inert materials. On the other hand, in order to increase the quality of valex, sodium bisulphide was added to the solution. The solution thus collected was indirectly heated by steam in a tank, charged into evaporation tanks (Armağan, 1988), and evaporated at three stages as summarized in Table 1.

Next, the concentrated solutions were filtered and transferred into the upper section of a drying tower. The solution was sprayed toward the middle of the tower in a thin form. Solution sprayed in pulverized form became powder after air drying at 185–200°C.

Finally, the dried valex was ground in a mill to get smaller particle sizes and collected in small plastic bags. On the other hand, mimosa bark directly provided from the company had been extracted by using water at 100°C (Armağan, 1988).

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 by using a Soxhlet equipment (Erol & Tuzlacı, 1996; Dülger et al., 1997).

### 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 a control. All the bacteria mentioned above were incubated at 30±0.1°C for 24 h by inoculation into Nutrient Broth (Difco), and the fungi studied were incubated in Malt Extract Broth (Difco) for 48 h. Mueller Hinton Agar (oxid) sterilized in a flask and cooled to 45–50°C was distributed to sterilized Petri dishes having a diameter of 9 cm, by using pipettes in the amount of 15 ml after injecting cultures of bacteria prepared as mentioned above and mold for 24 h in the amount of 0.01 ml (10<sup>5</sup> bacteria per ml and 10<sup>3</sup> fungi spores per ml), and providing the distribution of food medium in Petri dishes homogeneously. Dishes injected with extracts were located on the solid agar medium by pressing slightly

(Özçelik, 1992; Collins et al., 1989; Bradshaw, 1992). After Petri dishes so obtained were placed at 4°C for 2 h, plates inoculated with fungi were incubated at 25 ± 0.1°C for 7 days. At the end of the period, inhibition zones formed on the food medium were evaluated in millimeters. These studies were performed in triplicate.

### RESULTS AND DISCUSSION

Table 2 shows the *in vitro* antibacterial and antifungal activities of the extracts of valonia, mimosa bark, gallnut, salvia, and phlomis. In addition, the inhibition zones formed by standard antibiotic discs are indicated in Table 3.

As can clearly be seen from Table 2, the extracts of mimosa bark have the greatest antibacterial efficiency, followed by valex, the extracts of *Salvia sp.*, gallnut, and *Phlomis sp.*, respectively. Moreover, it was determined that the extracts of gallnut and mimosa bark have antifungal effects, while the others (e.g., valex and phlomis) do not (Table 2).

The extracts obtained from valex inhibits considerably the growth of *L. monocytogenes*, *P. vulgaris* and *B. brevis*, having an inhibition zone of 22–31 mm. The growth of *S. aureus* and *B. subtilis* is inhibited by the whole extracts used in the study and an inhibition zone varying between 12 and 26 mm is formed. When the results obtained with valex were compared to those of standard antibiotics, it was determined that *K. pneumoniae* is more resistant, *P. aeruginosa*, *M. luteus*, and *B. cereus* are similarly resistant, and the other species is more susceptible to the valex. On the other hand, *S. aureus* and *B. subtilis* are more susceptible, *L. monocytogenes*, *B. cereus* and *P. vulgaris* are similarly resistant, and the others (except for *K. pneumoniae*) are more resistant to the gallnut extracts, as compared to VA 30, OFX 5 and TE 30 standard antibiotics.

*B. brevis* and *S. aureus* are similarly resistant and the other bacteria species are seen to be much more resistant to *Phlomis* extracts when compared to penicillin G. All the bacteria are found to be more susceptible to the extracts obtained from mimosa bark when compared to the standard SAM 20 and CTX 30 antibiotics. Similarly, in comparison to VA 30 standard, it was observed that *P. vulgaris* is more resistant and *S. aureus* is more susceptible to *Salvia* extracts.

Recently, İlçim et al. (1998) reported that the extracts of *Parmelia furfuraceae*, *Myrtus communis* subsp. *communis* and *Eugenia coryophyllata* showed antibacterial activities. Especially, *E. coryophyllata*

Table 2. Antibacterial and antifungal activities of various commercial plant extracts.

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

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).

\*Includes diameter of disc (6 mm).

Table 3. Antimicrobial activities of some standard antibiotics.

Microorganisms	Inhibition Zone (mm)					
	P10	SAM20	CTX30	VA30	OFX5	TE30
<i>Bacillus brevis</i>	15	14	16	19	30	25
<i>Bacillus subtilis</i>	12	15	12	15	29	24
<i>Bacillus cereus</i>	13	14	14	17	34	28
<i>Escherichia coli</i>	16	10	11	24	33	27
<i>Pseudomonas aeruginosa</i>	9	10	60	8	49	32
<i>Staphylococcus aureus</i>	11	16	10	10	22	26
<i>Listeria monocytogenes</i>	10	12	15	24	34	33
<i>Micrococcus luteus</i>	32	34	32	32	25	–
<i>Klebsiella pneumoniae</i>	18	17	11	21	32	28
<i>Mycobacterium smegmatis</i>	16	19	13	23	34	28
<i>Proteus vulgaris</i>	9	14	19	20	29	24
Antifungal Nystatin, (30 µg)						
<i>Alternaria alternata</i>	14					
<i>Penicillium italicum</i>	19					
<i>Fusarium equisetii</i>	15					
<i>Candida albicans</i>	18					

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

was found to be very effective with all the bacteria tested (except for *K. pneumoniae* and *E. aerogenes*). In a similar study, *B. megaterium* was determined to be similarly 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.

Also, Dıđrak et al. (1997) found that extracts of *Ajuga orientalis*, *Smyrinum olusatrum*, *Astragalus*

*schizopterus*, and *Salvia viridis* inhibited the growth of some bacteria and fungi. In particular, it was found that *A. schizopterus* was effective against *Bacillus* species with an inhibition of 23 mm and it inhibited the growth of *Enterobacter aerogenes*. The findings obtained from this study are similar to those stated above.

The extract of mimosa bark and gallnut powder inhibited the development of *A. alternata*, having inhibition zones of 21 and 15 mm, respectively. However, it was also determined that they were effective on the other fungi (*P. italicum*, *F. equiseti*, *C. albicans*). When

the results were compared to the standard antifungal nystatin, the extracts of mimosa bark and gallnut powder were found to be more effective.

It is not surprising that there are differences in the antibacterial effects of plant groups, due to phytochemical properties and differences among species. For the evaluation of plants which are naturally grown in Turkey and are potential useful resources, additional studies will be beneficial from medicinal and economic standpoints. In conclusion, whole extracts, especially the extracts of mimosa bark, valex, and gallnut powders can be used for protection against bacteria and, in some cases, against fungi.

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