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RESEARCH NOTE

Evaluation of the Antimicrobial Activity of Some Commercial Vegetable Extracts Used in Tanneries

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Abstract
The aim of this study is to determine the antimicrobial activity of eight different commercial vegetable tanning extracts used in the leather industry against bacterial and fungal isolates from tannery processes such as beamhouse, tanning, and post-tanning processes. Two different concentrations (1% and 5%) of these extracts were used in order to determine their antimicrobial activity. The antimicrobial activity of the extracts was assessed by the disc diffusion method. Nutrient Agar and Malt Extract Agar were used for growing the bacterial and fungal isolates respectively, each containing 0%, 5%, and 10% NaCl. It was observed that all of the extracts were more effective against the bacterial isolates than the fungal isolates. It was also seen that extracts IV, VII, and II were more effective against bacterial isolates growing on 0%, 5%, and 10% NaCl Nutrient Agar plates, respectively. Extracts I and II were found to be more effective against the fungal isolates growing on the Malt Extract Agar plates containing 10% NaCl, while no extracts had any antifungal effect on the NaCl-free and 5% NaCl plates.
Keywords: Antimicrobial activity, isolate, leather, natural biocide, and vegetable tanning agent.

Deri İşletmelerinde Kullanılan Bazı Ticari Bitkisel Ekstraktların Antimikrobiyal Aktivitelerinin Değerlendirilmesi

Özet
Bu çalışmada, deri endüstrisinde kullanılan sekiz farklı ticari bitkisel tabaklama maddesinin; tabaklama öncesi, tabaklama ve tabaklama sonrası proseslerden izole edilen bakteriyal ve fungal izolatlara karşı antimikrobiyal aktivitelerinin belirlenmesi amaçlanmıştır. Ekstraktların antimikrobiyal aktivitelerini tespit etmek için onların iki farklı konsantrasyonu (%1 ve %5) kullanılmıştır. Ekstraktların antimikrobiyal aktiviteleri disk difüzyon yöntemi ile belirlenmiştir. %0, %5 ve %10 NaCl içeren Nutrient agar ve Malt Ekstrakt agar besi yerleri sırasıyla bakteriyal ve fungal izolatların gelişiminde kullanılmıştır. Bütün ekstraktların bakteriyal izolatlara karşı fungal izolatlardan daha etkili olduğu gözlenmiştir. Ayrıca IV, VII ve II numaralı ekstraktların sırasıyla %0, %5 ve %10 NaCl içeren Nutrient agar besi yerlerinde bakteriyal izolatlara karşı daha etkili olduğu gözlenmiştir. I ve II numaralı ekstrakt %10 NaCl içeren Malt ekstrakt agar besi yerlerinde gelişen fungal izolatlara karşı daha etkili olurken, hiçbir ekstrakt NaCl içermeyen ve %5 NaCl içeren besi yerlerinde herhangi bir antifungal etki göstermemiştir.
Anahtar Kelimeler: Antimikrobiyal aktivite, izolat, deri, doğal biyosid, bitkisel tabaklama maddesi.

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INTRODUCTION

Tanning refers to the process of chemically treating raw skin to make it stronger, more flexible and resistant to microorganism attacks. In leather making today, a wide variety of tanning methods and materials can be used. One of the oldest tanning methods is vegetable tanning, which is extensively used alone for heavy or vegetal leather production. The vegetable tanning method is also used in manufacturing shoe upper leather, belt leather, bag leather, wallet leather, etc.

The leather industry is one of the prominent industries in the world, and 30% of its annual demand for tanning agents is provided by vegetable extracts (Şen et al. 2006). Vegetable tanning agents are extracted from barks, woods, fruits, leaves, roots, and galls of higher plants (Kılıçarislan and Özgünay 2012). Vegetable tanning agents are also known as "tannin". Plants tend to generate a complex mixture of tannins, and the many types of tannins have

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different characteristics (Lupini et al. 2009). Tannins and natural polyphenols, are the most plentiful secondary metabolites produced by plants, and their structure has an important effect on biochemical activities (Barbehen and Constabel 2011). In many instances, these kinds of substances serve as plant defense mechanisms against microorganisms, insects, and herbivores (Cowan 1999). They may be classified as hydrolysable tannins and condensed tannins (Özgünay et al. 2007). Today commonly used vegetable tanning agents are extracts of mimosa, quebracho, mangrove, pine, acorn, myrobalan, oak, chestnut, and sumac (Bickley 1999). The significant tanning agents produced in Turkey are acorns, galls, oak, sumac leaf, and pine bark. Acorns, the most commonly produced among these, are processed and sold under the brand name "Valex" (Şen et al. 2006).

Due to their organic structure, vegetable tanning agents are considered less harmful to the environment than some other mineral tanning agents such as chrome and aluminum (Adıgüzel Zengin et al 2012). At the present time, the number of studies focusing on the environment and humans is increasing, and there is also research on the use of vegetable tanning agents. In addition, some research suggests that vegetable tanning agents possess antimicrobial properties owing to such ingredients as tannin and flavonoid (Haslam 1989, Scalbert 1991 and Eke Bayramoğlu et al. 2012). It has also been stated in such studies that tannic acid, another of these ingredients, can be used in the soaking (Mentes-Çolak 2006) and pickling processes (Menteş-Çolak et al. 2010) to protect skins against microorganisms.

A wide variety of antimicrobial agents can be used to prevent microbial activities in leather making, but many of these have an adverse effect on the environment, and these agents are also the most expensive among the many chemicals employed. Therefore, it is important to reveal naturally based compounds with antimicrobial activity which can be used in the leather industry. Hence, this study aimed to investigate the antimicrobial properties of certain commercial vegetable tanning agents produced in Turkey against test microorganisms isolated from tannery processes.

MATERIALS AND METHODS

Commercial Tanning Agents

Eight different commercial vegetable tanning agents produced in Turkey were chosen to investigate their antimicrobial properties. Tanning agents I, II, III and IV, used in the research, were chestnut, mimosa, tara, and quebracho-based, respectively. Tanning agent V was a vegetable-based product containing various special synthetic auxiliary ingredients. Tanning agents VI and VII were acorn-based and known respectively as valonia and sulphited valonia extracts. Tanning agent VIII was a combination of various solvents and tannins. Two different aqueous solutions (1% and 5%) of these tanning agents were used to determine their antimicrobial activities.

Test Microorganisms

In this study 15 bacterial and 15 fungal isolates were used as test microorganisms. These microorganisms were isolated from various leather manufacturing stages using two types of media containing 0%, 5%, and 10% NaCl (Table 1). In order to obtain more realistic results, the bacterial and fungal isolates were chosen from different tannery processes such as beamhouse, tanning, and post-tanning processes. Some phenotypic characteristics (shape, Gram stain reaction, catalase test, and oxidase test) of bacterial isolates were also determined (Table 2).

Media and Solutions

Nutrient Agar (NA) and Malt Extract Agar (MEA) were used to determine the antimicrobial properties of tanning agents against bacterial and fungal isolates respectively. The media that were used for the bacterial and fungal isolates which could grow in different NaCl concentrations were modified by adding 0%, 5%, and 10% NaCl (Bitlisli et al. 2004). Vegetable tanning agent solutions of 1% and 5% were prepared for use in the antimicrobial activity studies and impregnated on sterile standard empty discs.

Preparing Discs and Microorganism Cultures

The disc diffusion method was used in the present study to determine antimicrobial activity. Samples solutions of 25 µl of the prepared tanning agents were impregnated on sterile standard empty discs of 6 mm diameter.

The bacterial cultures were activated on Nutrient Broth at 37±0.1°C for 24 hours, and the fungal cultures were activated on Malt Extract Broth at 27 ±0.1°C for 48 hours. The counts of bacterial

Table 1. Isolate numbers of the microorganisms with regard to NaCl contents.

NaCl (%)	Medium	0	5	10
Bacterial isolate number	Nutrient Agar	1, 2, 3, 4, 5	6, 7, 8, 9, 10	11, 12, 13, 14, 15
Fungal isolate number	Malt Extract Agar	16, 17, 18, 19, 20	21, 22, 23, 24, 25	26, 27, 28, 29, 30

Table 2. Some phenotypic characteristics of bacterial isolates and their inhibition zone diameters (mm) in media containing different amounts of NaCl.

	Bacterial isolates growing on media without NaCl										Bacterial isolates growing on media containing 5 % NaCl										Bacterial isolates growing on media containing 10 % NaCl									
Isolates	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
Shape*	B		C		B		B		C		C		C		C		C		C		C		B		C		C		C	
Gram Reaction	p		p		p		p		p		p		p		p		p		p		p		p		n		p		p	
Catalase	p		p		p		p		p		p		p		p		p		p		p		p		p		p		p	
Oxidase	p		n		p		n		n		n		p		n		p		n		n		p		n		n		n	
CVE**	Concentration of Extract										Concentration of Extract										Concentration of Extract									
	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%
I	-	9.0	-	10.3	-	10.3	-	11.8	-	32.5	-	24.0	-	12.0	8.0	13.5	8.0	13.3	9.8	13.3	10.8	14.0	12.3	15.3	9.0	15.0	10.0	11.8	11.3	13.3
II	7.8	10.0	-	11.5	7.0	10.8	11.0	11.3	-	24.0	-	17.0	-	17.0	9.3	12.0	10.8	14.3	10.0	15.0	12.5	13.8	12.0	15.5	10.0	15.3	11.3	13.3	11.0	15.3
III	-	-	-	8.8	-	8.0	8.0	8.0	-	28.0	-	10.0	-	12.0	-	10.3	12.8	13.0	-	11.0	7.5	14.5	-	11.0	-	11.0	9.8	8.0	-	10.0
IV	9.5	12.0	-	13.0	-	11.3	10.8	11.0	8.8	20.0	-	15.0	-	11.0	10.0	11.0	8.3	13.3	8.5	13.8	9.5	13.3	12.0	13.5	10.0	13.8	11.0	11.3	11.3	13.5
V	-	8.0	-	-	-	7.0	-	8.0	-	30.0	-	22.0	-	14.0	-	10.0	-	12.8	-	15.8	9.5	13.3	7.5	10.3	9.0	10.0	8.0	8.3	10.5	11.0
VI	-	8.0	-	-	-	8.0	-	11.8	9.3	29.0	-	14.0	-	20.0	7.0	13.5	12.3	13.0	10.5	12.3	12.0	14.3	12.0	13.5	11.0	12.5	11.8	14.8	11.0	11.0
VII	8	8.5	-	-	-	8.0	9.3	12.5	9.0	28.0	-	17.0	-	20.0	10.5	17.0	11.5	15.8	11.5	12.0	13.3	17.0	12.5	12.5	11.0	12.0	12.5	18.3	11.0	14.0
VIII	-	8.0	-	-	-	7.0	-	7.0	-	28.0	-	13.0	-	13.0	10.3	10.3	11.0	11.8	10.0	11.0	7.0	11.0	9.9	12.0	8.0	11.0	7.0	8.0	11.0	11.5
P***	19.3		13.5		15.3		25.0		50.0		50.0		50.0		21.0		26.0		31.5		32.3		31.0		25.5		25.0		25.3	
A****	18.8		7.0		17.3		21.3		22.0		46.0		40.0		18.5		24.8		27.0		25.5		19.3		21.0		21.3		19.3	

*: B; Bacil; C; Coccus, **: Commercial Vegetable Extracts, ***: Penicillin G (10μg), **** : Ampicillin (10μg), - : Not detected, p:positive, n:negative

cultures were adjusted to 7-8 log cfu/mL using the standard McFarland counting methods, while the counts of fungal cultures were adjusted to 5-6 log cfu/mL using the Thoma slide counting methods. The test microorganisms (0.1 mL) were then inoculated with a sterile micropipette and spread on the surface of the appropriate solid media on the plates. The agar plates inoculated with the bacterial and fungal cultures were held for 1 h before placing an extract-impregnated paper disc on the plates. The bacterial plates were incubated at 37 ± 0.1°C for 48 h while the fungal plates were incubated at 27 ± 0.1°C for 72 h (Pelczar et al. 1993). When the incubation was over, the diameters of the inhibition zones were measured. Because the diameters of the discs used were 6 mm, values equal to and exceeding 6 mm were considered to be inhibition zones. Hyphens (-) in the tables mean, that the relevant tanning agents showed no antimicrobial effect against the microorganisms. Penicillin G (10 µg/disc), ampicillin (10 µg/disc), nystatin discs (100 units), and ketoconazole (50 µg/disc) were used as positive controls. Experiments were conducted in three replications and the results were calculated from the averages of these.

RESULTS

Antimicrobial Activity of Vegetable Tanning Agents against Bacterial Isolates

In Table 2, the inhibition zone diameters determined on the nutrient agar plates are given together with some phenotypic characteristics of bacterial isolates like cell shape, Gram stain reaction, and tests of catalase and oxidase. It is clear from the findings that all isolates were Gram positive except isolate 13. Additionally, all were found to exhibit a catalase positive reaction. Five bacterial isolates were determined as oxidase positive, while the remaining ten were oxidase negative.

It is obvious from the data that the effects of the different vegetable tanning agents on microorganisms were varied. It was found that 5% solutions of commercial vegetable tanning agents are generally more effective against bacterial isolates than the 1% solutions. This result was determined by testing in both NaCl-free and NaCl-added media. Also, 1% concentrations of vegetable tanning agents exhibited no antibacterial effectiveness against isolate 2 on NaCl-free media or against

Table 3. Inhibition zone diameters (mm) of fungal isolates in media containing different amounts of NaCI.

CVE*	Fungal isolates growing on media without NaCl										Fungal isolates growing on media containing 5 % NaCl										Fungal isolates growing on media containing 10 % NaCl									
	16		17		18		19		20		21		22		23		24		25		26		27		28		29		30	
	Concentration of Extract										Concentration of Extract										Concentration of Extract									
	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%
I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.0	-	-	-	8.0	-	-	-	9.0
II	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.0	-	13.5	-	10.0	-	-	-	-
III	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9.0
V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9.5	-	-	-	-
VI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VII	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10.0	-	-	-	-	-	-	-	-
VIII	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.0	-	-	-	-
N**	28.0		30.0		30.8		27.5		30.8		38.5		25.8		30.3		33.3		32.8		38.5		34.0		39.5		25.0		34.3	
K***	47.0		44.5		47.5		53.0		47.0		34.8		44.5		45.8		42.3		38.8		40.8		41.3		42.3		21.3		43.3	

* : Commercial Vegetable Extracts, **: Nystatin (100 units), *** : Ketoconazole (50μg), - : Not detected

isolates 6 and 7 on media containing 5% NaCl.

When isolates grown on NaCl-free media were considered, it was found that 5% solutions of tanning agents were more effective against isolate 5. The inhibition zone diameters of this isolate were determined to be from 20 mm to 32.5 mm. For this isolate, a zone diameter of 32.5 mm was obtained from tanning agent I, and 50.0 mm and 22.0 mm from the antibiotics Penicillin G and Ampicillin respectively. On the other hand, the antimicrobial activity of the tanning agents at the same concentrations was found to be lower against isolates 1, 2, 3, and 4.

In the media modified with 5% NaCl, 1% solutions of all tanning agents proved ineffective against isolates 6 and 7. In addition, tanning agent V displayed no antibacterial activity against any of the isolates (6, 7, 8, 9, and 10). On the other hand, 5% solutions of tanning agents exhibited inhibition zones of 10.0 mm to 24.0 mm for the same isolates. When results obtained from the control antibiotics (Penicillin G: 21-50 mm; Ampicillin: 18.5-46 mm) and from the 5% solutions of tanning agents are compared, it can be suggested that the tanning agents showed considerable antimicrobial effectiveness against these five isolates. The 1% solutions of vegetable tanning agents were found to be less effective than the 5% solutions in NaCl-free media as well as in media containing 5% NaCl.

On media containing 10% NaCl, almost all of the 1% solutions of tanning agents (except for isolates 12, 13, and 15 for the tanning agent III) exhibited inhibition zone diameters of 7.0-13.3 mm for all the bacterial isolates. Besides, as at other NaCl concentrations, 5% solutions of vegetable tanning agents showed higher antibacterial activity than did the 1% solutions. A 5% solution of tanning agent VII showed the highest inhibition zone diameter, 18.3 mm, against isolate 14 (Table 2).

Antimicrobial Activity of Vegetable Tanning Agents against Fungal Isolates

Solutions of 1% and 5% of all the commercial vegetable tanning agents used in the study were found to have no antifungal effect against fungal isolates (16-25) growing on the NaCl-free and 5% NaCl-added MEA plates. Inhibition zone diameters of control antibiotics against the same isolates were measured as 25.8-38.5 mm for Nystatin and 34.8-53.0 mm for Ketoconazole (Table 3).

In the media containing 10% NaCl, 1% solutions of only the vegetable tanning agents V and VIII showed inhibition zones of 9.5 mm and 12.0 mm respectively against isolate 29, while other tanning agents showed no effect against isolates 26, 27, 28 and 30. The 5% solutions of tanning agents proved effective against some isolates in media with the same NaCl concentration. For instance, inhibition zone diameters obtained from tanning agent I were 12.0 mm, 8.0 mm, and 9.0 mm for isolates 26, 28, and 30 respectively. Inhibition zone diameters obtained from tanning agent II were 12.0 mm, 13.5 mm and 10.0 mm for isolates 26, 27, and 28 respectively. Tanning agents IV, V, VII, and VIII displayed antifungal activity against different isolates.

When zone diameters occurring with tanning agents showing their activity against fungal isolates grown in media containing 10% NaCl are

considered, the most effective tanning materials were determined to be tanning agents I and II (Table 3). However, when these findings were compared with the control antibiotics Nystatin and Ketoconazole used against fungi, it can be asserted that the antifungal activities of these tanning agents were quite limited.

DISCUSSION

When an overall evaluation of the findings was carried out, it was found that commercial vegetable tanning agents commonly used in leather making displayed considerable antibacterial effect against the bacterial isolates isolated from tannery processes, but not remarkable antifungal activity against the fungal isolates (Tables 2 and 3).

In a study in which the antimicrobial activities of extracts of valonia, mimosa bark, and gall powder, Salvia aucheri Bentham var. aucheri and Phlomis bourgaei Boiss were investigated, it was found out that mimosa bark extract had the highest antibacterial activity, followed by extracts of valonia, gall powder, *Salvia aucheri* var. *aucheri*, and *Phlomis bourgei* (Dığrak et al. 1999). Moreover, gall powder containing high amounts of tannins was also revealed to exhibit antifungal activity. The findings of our study, such as the remarkable level of antimicrobial activity of 5% solutions of commercial mimosa (II) and valonia extracts (VI, VII) against bacteria and the effectiveness of the mimosa extract against three of the five fungi isolates, are similar to the results of these researchers.

Menteş Çolak et al. 2010) used the tannic acid found in the vegetable tanning agents in the pickling process at a ratio of 0.1%, 0.5%, 1%, 2%, and 3%, and showed the antimicrobial properties of the leather samples against 16 test microorganisms by the disc diffusion method. It was determined that tannic acid displayed high antibacterial activity against all tested bacteria. Increasing tannic acid concentrations displayed more effective results, which is in line with our findings.

Elizondo et al. (2010) determined the antimicrobial activity of quebracho and chestnut tannins. In their comparative analyses, they revealed that the inhibitory capacity of quebracho tannins was higher than that of chestnut tannins. Besides, it was found that the antimicrobial activity of quebracho tannins increased by 20 fold when 25% chestnut tannins were added, and by 85 fold with an addition of 75% chestnut tannins. The antimicrobial activity of the commercial product (a mixture of the two tannins) was revealed to be 50 times higher than the quebracho tannins alone.

In the abovementioned studies, it was found that extracts of chestnut, quebracho, mimosa, and valonia displayed varied antimicrobial activities against various microorganisms. In terms of antimicrobial activity, extracts of sulphited valonia and mimosa proved more effective against bacteria, and extracts of chestnut and mimosa were more effective against fungi. From this perspective, it can be said that the findings of the researchers are in line with ours. We also found that the inhibition zone diameters obtained from the commercial vegetable tanning agents that were used in our study were similar to or even higher than those found by these researchers.

In our previous study on the activity of some bactericides used in the leather industry, it was determined that two different bactericides proven to be effective totally controlled the halophilic and halotolerant bacteria in the floats of the soaking process (M. Yapıcı et al. 2004). In the present study, halophilic and halotolerant bacteria growing on media containing NaCl were more susceptible to commercial vegetable tanning agents, whereas, bacteria growing on NaCl-free media turned out to be more resistant to these agents. These results were noted as significant findings.

The main target of our research was to assess the antimicrobial activity of commercial vegetable tanning agents against various bacterial and fungal isolates from tannery processes, and it is considered that the study yielded realistic and practical results for the leather industry.

CONCLUSION

In an overall evaluation of our findings, it was noted that commercial vegetable tanning agents widely used in the leather industry displayed a notable activity against bacterial isolates which have reactions gram-positive or -negative and shape of coccus, but not against fungal isolates. In addition, it was found that 5% solutions of vegetable tanning agents were more effective against bacteria and fungi isolates than 1% solutions. It was found in the present study that tanning agents IV (commercial quebracho extract), VII (commercial sulphited valonia extract), and II (commercial mimosa extract) were effective against bacterial isolates growing in an NaCl-free media, and in media containing 5% NaCl

and 10% NaCl. The highest inhibition zone diameters against bacterial isolates were measured as 32.5 mm for tanning agent I in an NaCl-free media against isolate 5, 24.0 mm for tanning agent I in a media containing 5% NaCl against isolate 6, and 18.3 mm for tanning agent VII in a media containing 10% NaCl against isolate 14.

Bacterial isolates growing in a media containing 10% NaCl were shown to be more affected by the tanning agents than those growing in NaCl-free and 5% NaCl media. It was found that fungal isolates growing in media containing 10% NaCl were slightly susceptible to tanning agents, and fungal isolates growing in NaCl-free and 5% NaCl media were not affected by the tanning agents. As a result, it was found that as the salinity concentration of the media increased, so did the susceptibility of the halophilic and halotolerant bacteria to tanning agents. In other words, halophilic and halotolerant bacteria growing in media containing 5% and 10% NaCl were found to be more susceptible to commercial vegetable tanning agents. Thus, it can be advised that commercial vegetable tanning materials could be alternatively added to soaking floats that predominantly contain halotolerant or halophilic bacteria.

In conclusion, it was revealed in the present study that vegetable tanning agents can also serve as alternative agents to both environmentally hazardous and more expensive conventional antimicrobial agents used to control microorganism activities.

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