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IN VITRO ANTIMICROBIAL ACTIVITY OF DRIED AND FRESH LEAF EXTRACTS OF OLD AND YOUNG APRICOT TREES (PRUNUS ARMENIACA)

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Abstract

This work was undertaken to investigate the antimicrobial activity of water extracts of apricot leaves collected from old (>25 years) and young trees (<8 years old) of Erevani cultivar. Agar well diffusion assay was used for in vitro antibacterial and antifungal screening. The antimicrobial activities of the extracts were determined by measuring the respective zone of growth inhibition in cm.

Water extracts of apricot leaves were tested against 9 gram-positive and 2 gram-negative bacteria (Staphylococcus aureus 205, Staphylococcus citreus, Escherichia coli M 17, Salmonella typhimurium, Brevibacterium flavum 14067, Bac. megatherium, Bac. subtilis 1759, Bac. subtilis 205, Bac. mycoides, Bac. mesentericus) and fungi (Candida guillermondii and Candida albicans).

The water extract showed broad spectrum of antimicrobial activity against 9 gram-positive and 2 gram-negative strains. A large zone of inhibition was observed against pathogenic bacteria Staphylococcus citreus (1.73-2.73 cm), Salmonella typhimurium (2.0-2.1 cm) and against Bacillus subtilis 1759 (1.83-1.93 cm).

In antifungal screening, water extracts showed moderate zones of inhibition against Candida guillermondii (1.27-1.80 cm) and Candida albicans (2.2-2.3 cm).

The highest activity with inhibition zones of more than 2 cm was observed against pathogenic gram-negative bacteria Salmonella typhimurium for both dried leaves of old trees (2.03±0.28) and dried leaves of young trees (2.10±0.38).

However, the water extracts exhibited activity against Escherichia coli M 17 with only a 0.8 cm inhibition zone.

Water extracts obtained from both fresh and dried leaves of apricot trees possess profound antimicrobial activity and might have potential use in medicine.

These results confirmed that dried apricot leaves are potentially rich sources of antimicrobial agents as well. However, apricot leaves from young trees (less than 8 years) exhibited higher antimicrobial activity against test-microorganisms compared to leaves of old trees.

Keywords: apricot leaves, antibacterial activity, antifungal activity, agar well diffusion method.

INTRODUCTION

Plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. A rich heritage of knowledge on preventive and curative medicines has been available since ancient scholastic works, including Armenian medival prominent physician Amirdovlat Amaciatsi (15th century) [*Amirdovlat Amasiatsi, 1990*].

The use and search for drugs and dietary supplements derived from plants have accelerated in recent years. Over 50% of all current pharmaceuticals are derived from plants [*Baker J. et al., 1995; Iwu M. et al., 1999; Jacoby G., 1999*].

Apricot leaves were used in traditional medicine for a wide range of various ailments. Particularly, fresh juice of apricot leaves was applied in Chinese traditional medicine with beneficial results in skin diseases such as scabies, eczema, sunburns and itching of the skin due to cold exposure. Apricot leaves

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were also used in folk medicine for clearing teeth, at toothaches, stomatitis and at such diseases as diarrhea, diphtheria, etc. [*Yeung H.-C., 1985; Amirdovlat Amasiatsi, 1990; Chevalier A., 1996; Duke J., Ayensu E, 1998*].

To understand the mechanism of pharmacological action, the antimicrobial activity of water extracts obtaineed fom dried and fresh apricot leaves of young and old trees was investigated using *in vitro* system.

MATERIAL AND METHODS

Plant Material: Leaves of apricot (*Prunus armeniaca*) were collected from the apricot orchards of the native "*Erevani"* cultivar in Kotayk region (Armenia). The material was fixed by boiling water vapors for 10 *min* and dried at room temperature away from the sun light and then ground into powder using an electric blender.

Extraction: The ground leaves (500 *mg*) were extracted with 5 *mL* distilled water for 20 *min*. The obtained mass was centrifuged for 5 *min* at 8000 *rev/min* and the supernatant was used for further analysis. The same conditions were used for getting an extract from dried leaves, and water was added in view of dry weight.

Antibacterial activity of extracts was investigated by agar well diffusion assay (method of wells) [*Zolotarev P., 2006*]. Briefly, hollow cylinders (5.0 *mm* in diameter) were placed on the agar seeded with particular test microorganism. The sample solution was prepared by dissolving in water to attain a concentration of 10 *mg/mL*. Test extract (0.2 *mL*) was introduced in hollow cylinders in Petri dishes and kept to dry off the solvent. The dishes were put in a refrigerator at 10°C for 20-24 *hrs* and then incubated at 37°C for 24 *hrs*. The antimicrobial activities of the extracts were determined by measuring the respective zone of growth inhibition in *cm*.

Water extracts of apricot leaves were tested against 9 gram-positive and 2 gram-negative bacteria (*Staphylococcus aureus* 205, *Staphylococcus citreus, Escherichia coli* M 17, *Salmonella thyphymurium, Brevibacterium flavum* 14067, *Bac. megatherium, Bac. subtilis* 1759, *Bac. subtilis* 205, *Bac. mycoides, Bac. mesentericus)* and fungi *(Candida guillermondii, Candida albicans).*

The microorganisms were obtained as pure culture from the Museum of the Republican Center of Deposition of Microorganisms, as well as from the collection of cultures of microorganisms available at the Faculty of Microbiology and Biotechnology of Plants and Microorganisms of Yerevan State University and Microbiology Department of Yerevan State Medical University.

Antifungal Screening: Antifungal activity of water extract of dried leaves was determined at concentration of 10 *mg/mL* against fungi *Candida guillermondii* and *Candida albicans*.

Statistical analysis: All analyses were carried out in triplicate and data were reported as Means±SD (P=0.05). Results were processed using Excel Anova.

RESULTS

Data pertaining to antimicrobial potential of plant water extracts are presented in Tables 1 and 2 and Fig. respectively.

Screening for antibacterial activity using agar well diffusion assay showed that from the investigated test microorganisms gram-positive bacteria *Staphylococcus aureus* 205, *Staphylococcus citreus, Bac. subtilis* 1759, *Bac. subtilis* 205, *Bac. mycoides, Brevibacterium flavum 14067, Bac. mesentericus, Bac. megatherium* and gram-negative bacteria *Escherichia coli* M 17 and *Salmonella typhimurium* appeared to be sensitive to water extracts obtained from leaves.

The results revealed that both fresh and dried apricot leaves are potentially rich sources of antimicrobial agents. However, apricot leaves from young

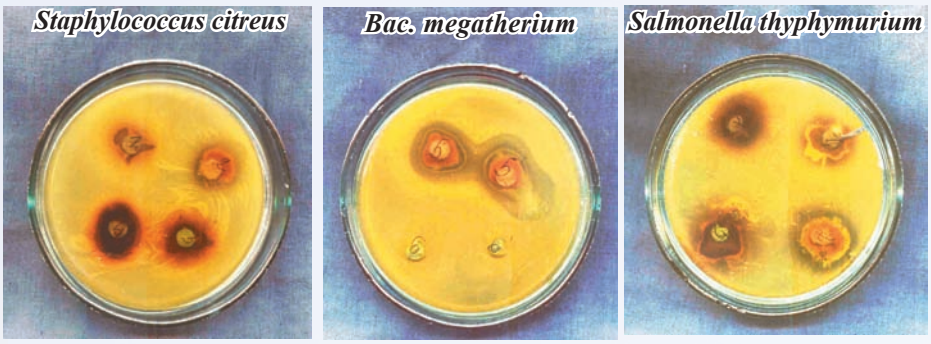

Figure. *Growth Inhibition of test organisms*

trees exhibited a broad-spectrum antimicrobial activity compared with leaves of old trees (Table 1). Fresh leaf extract from young trees (YFL) showed antibacterial activity against all of the tested microorganisms, while *Staphylococcus citreus* had not been inhibited by fresh leaf extract of old trees (OFL). Likewise, 3 of the test microorganisms (*Brevibacterium flavum* 14067*, Bac. mycoides, Escherichia coli* M 17*)* were not susceptable to water extract obtained from dried leaves of old trees (ODL), while dried leaves of young trees (YDL) were found to be inactive against *Bac. mycoides* and *Bac. subtilis* 205.

The microorganism *E. coli*, which is already known to be multi-resistant to drugs, was also resistant to water extracts obtained from old trees (both fresh and dried leaves).

The antimicrobial potency of extracts obtained from dried apricot leaves of young and old trees was quantitatively assessed for each susceptible microbe. The growth of *Salmonella typhimurium, Staphylococcus citreus*, *Bac. subtilis* 1759, *Bac. Megatherium, Staphylococcus aureus* 205 was strongly inhibited. Specifically, these extracts produced inhibition zones of 1.1-2.73 *cm* (Table 2).

The highest activity with inhibition zones of more than 2 *cm* was observed against pathogenic gram-negative bacteria *Salmonella typhimurium* for both ODL (2.03±0.28) and YDL (2.10±0.38).

However, water extracts exhibited activity against *Escherichia coli* M 17 with an inhibition zone of only 0.8 *cm*.

The water extract of apricot leaves showed high antifungal activity against tested fungi *Candida guillermondii* (1.3-1.8 *cm*) and *Candida albicans* (2.2-2.3 *cm*). Again, apricot leaves from young trees exhibited higher antifungal activity in comparison with leaves of old trees.

Table 1.

Antimicrobial Activity of Fresh and Dried Apricot Leaf Extracts of Old and Young Trees against Some Microorganisms

Test organism	Presence of the growing absence zone of test-organisms			
	Fresh Leaves		Dried Leaves	
	Old trees	Young trees	Old trees	Young trees
Gram positive Bacteria				
Staphylococcus aureus 205	+	+	+	+
Staphylococcus citreus	-	+	+	+
Bac. megatherium	+	+	+	+
Brevibacterium flavum 14067	+	+	-	+
Bac. subtilis 1759	+	+	+	+
Bac. subtilis 205	+	+	+	-
Bac. mesentericus	+	+	+	+
Bac. mycoides	+	+	-	-
Gram negative Bacteria				
Escherichia coli M 17	-	+	-	+
Salmonella thyphymurium	+	+	+	+

Notes: (+) susceptibility (inhibition zone 0.7 *cm*);
(-) absence of susceptibility.

Table 2.

Antimicrobial Activity of Dried Apricot Leaf Extracts from Old and Young Trees

Test organism	Diameter of Zone Inhibition, cm (P=0,05, n=8)	
	Old	Young
Bacteria		
Staphylococcus aureus 205	1,10±0,25	1,53±0,63
Staphylococcus citreus	1,73±0,25	2,73±0,63
Bac. megatherium	1,30±0,38	1,70±0,38
Bac. subtilis 1759	1,93±0,29	1,83±0,14
Escherichia coli M 17	0	0,80±00
Salmonella thyphymurium	2,03±0,28	2,10±0,38
Fungi		
Candida guillermondii	1,27±0,14	1,80±0,25
Candida albicans	2,2±0,30	2,3±0,25

DISCUSSION

Interest in plants with antimicrobial properties has revived as a result of current problems such as resistance, associated with the use of antibiotics [*Cohen M., 1992; Gislene G. et al., 2000*]. The primary benefits of using plant-derived medicines are that they are relatively safer than synthetic drugs [*Athnasiadou S. et al., 2001*]. Over the past several years, intensive efforts have been made to discover clinically useful antimicrobial drugs, which have been reviewed by many researchers [*Iwu M. et al., 1999; Mali R. et al., 2007; Khadatkar S. et al., 2008; Paramesha M. et al., 2009*].

The current study revealed that water extracts of apricot leaves possess potent antibacterial activity at concentration of 10 *mg/mL*. The activity could be attributed to the presence of phytoconstituents such as flavonoids, triterpenoids, alkaloids, steroids, phenolic compounds, and tannins. These metabolites are reported to have many biological actions, including antimicrobial and antioxidant [*Medicinal plants, 1989; Rasadah M., Houghton P., 1998; Jacoby G., 1999; Kahkonen M. et al., 1999; Akiyama H. et al., 2001; Wallace R., 2004; Min B. et al., 2008; Akroum S. et al., 2010*].

Our previous chemical analysis carried out with extracts of apricot leaves showed that many secondary metabolites of various classes occur in water and ethanolic extracts (tannins, flavonoids, and catechines), and the water extracts exhibit antioxidant activity [*Hanisyan R. et al., 2010*].

It is considered that enzyme inhibition by phenolic compounds is the mechanicm for microorganism inhibition [*Scalbert A,. 1991*]. This is possibly done by the oxidized compounds through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins [*Cowan M., 1999; McGaw L. et al., 2002*].

Hence, the difference between in antimicrobial activities of tested extracts could be explained by the amount of antimicrobial substances present in each form and could be attributed to their chemical constituents. Drying the leaves could have concentrated the antimicrobial compounds per amount of the extract as opposed to the antimicrobial compounds present in the same amount of the fresh leaf. On the other hand, drying can affect negatively on the quality of some useful compounds of secondary metabolism in leaves. Identification of these compounds could be a subject of future studies.

The results also showed that water extracts of dried apricot leaves was mainly inactive against *Bacillus* family microorganisms (*Bac. subtilis* 205; *Bac. mycoides*). Such results were not totally unexpected, since these bacteria form resting spores and are more resistant to environmental conditions and

physical factors including water vapour treatment, drying, ultraviolet radiation and oxidizing agents than any other tested bacteria [*Borozdina I., 2011*].

CONCLUSION

Thus, data obtained by us specify antimicrobial action of water extracts of apricot in relation to various gram-positive and gram-negative bacteria and fungi.

The present study revealed that the water extracts obtained from both fresh and dried leaves of apricot trees possess profound antimicrobial activity and might have potential use in medicine.

Furthermore, these results confirmed that dried apricot leaves are potentially rich sources of antimicrobial agents. However, apricot leaves from young trees (less than 8 years) exhibited higher antimicrobial activity against test microorganisms compared with leaves from old trees.

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