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Original article

Antibacterial and anticandidal effects of the leaf extracts of *Persea americana* Mill.

H. Halil Biyik, Mediha Onur, Bahadir Torun and Esin Poyrazoglu Coban
Department of Biology, Faculty of Science and Art, Adnan Menderes University, Aydin, Turkey

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Abstract

In this study, the antibacterial and anticandidal effects of the leaf extracts of *Persea americana* Mill. were examined against some pathogenic bacteria and some Candida species. Plant leaves were reduced to powder with liquid nitrogen. n-propanol, methanol, acetone, n-hexane, ethyl acetate, ethanol and boiling water as solvents were used for extraction. The agar well diffusion method is used for the antimicrobial activities of extracts. Test microorganisms were 18 bacteria and 4 yeasts. Also, different antibiotic discs were used for comparison of the zone of inhibition. The extracts of the n-propanol, acetone, and ethanol of *P. americana* leaves showed high activity as regards to other solvents, respectively. The extracts of the n-propanol and acetone of *P. americana* leaves inhibited *S. aureus* ATCC 25923, *P. vulgaris* ATCC 33420 and *B. cereus* ATCC 11778 and the inhibition zones ranged between 13-14 mm. Methanol and ethanol extracts of *P. americana* leaves demonstrated moderate antimicrobial effect against *S. aureus* ATCC 25923, *P. vulgaris* ATCC 33420, *P. aeruginosa* ATCC 35032, *C. albicans* ATCC 10231 and the inhibition zones ranged between 10-11 mm., while they inferred low effect (9 mm) on *S. typhimurium* ATCC 14028. The boiling water extract of *P. americana* leaves showed a high effect against *P. aeruginosa* ATCC 35032 and *C. albicans* ATCC 10231 and the inhibition zones ranged between 12-13 mm., while it indicated low effect (9 mm) on *C. tropicalis*. The ethyl acetate extract of *P. americana* leaves showed moderate effect (10 mm) on *S.epidermidis* ATCC 12228, *C. xerosis* ATCC 373, *P. vulgaris* ATCC 33420, while it demonstrated low effect (9 mm) on *B. cereus* ATCC 11778. However, hexane extract of *P. americana* leaves indicated no effect against tested microorganisms.

Key words: *Persea americana* Mill., antibacterial, anticandidal effect, folkloric medicine

1. Introduction

Since ancient times, many plants have been used for therapeutic purposes. Many researchers have studied the mechanism and benefits of different plants such as antibacterial, antifungal, antioxidant, antitumoral, antiprotozoal (*Akgunlu et al*., 2016; Joshi *et al*., 2017; *Rajesh et al*., 2017).

The avocado (*Persea americana* Mill.) is a tree, thought to have originated in South Central Mexico, belongs to the flowering plant family Lauraceae (Cardosa *et al*., 2016). Avocados are commercially valuable and are cultivated in tropical and Mediterranean climates throughout the world (Ogundare and Oladejo, 2014). They have a green-skinned, fleshy body that may be pear-shaped, egg-shaped, or spherical. Commercially, they ripen after harvesting. Three botanical varieties of avocado adapted to different climate conditions, have traditionally been recognized as *P. americana var. drymifolia* (Mexican), *P. americana var. guatemalensis* L. Wms. (Guatemalan) and *P. americana var. americana* Mill. (West Indian). Most commercial avocado cultivars are inter racial hybrids developed from chance seedlings. Thus, the most important cultivars in subtropical climates such as 'Hass', 'Bacon', and 'Fuerte', are Guatemalan-Mexican hybrids with different degrees of hybridization (Newett *et al*., 2002).

Avocado (*P. americana*) is known as a fruit that contains carbohydrates, protein, fibers, and micronutrient necessary for humans including vitamins, minerals and polyphenols. Like other fruit, the peel of the fruit is generally discarded, while in a study reported that the peel of avocado has a high antioxidant content (Rotta *et al.*, 2016).

Extracts from the epicarp of the immature avocado fruit have been indicated to have both antifungal and antibacterial properties (Chia and Dykes, 2010). The seed of the immature fruit was also found to have antibacterial and antioxidant properties (Antasionasti *et al.*, 2017). The antifungal properties of the immature avocado were established to be due to the idioblast oil cells, which are made up of alkaloids, sesquiterpene hydroperoxides, other terpenes, persin, and a group of 2-alkylfurans (Rodriguez-Carpena *et al*., 2011; Vinha *et al.*, 2013).

In this study, the antibacterial and anticandidal effects of the leaf extracts of *P. americana* were examined against some pathogen bacteria and some candida species.

Author for correspondence: Dr. H. Halil Biyik
Professor, Department of Biology, Faculty of Science and Art, Adnan Menderes University, Aydin, Turkey
E-mail: hbiyik@adu.edu.tr
Tel.: +90-2562182000

2. Material and Methods

2.1 Plant materials

P. americana was bought from a local market in Aydin province in Turkey and leaves of the plant were used for the study.

2.2 Preparation of plant extracts

Leaves of the plant sample were washed with distilled water and reduced to powder with liquid nitrogen. Ten gram of this material was added to separately in 100 ml of n-propanol, methanol, acetone, n-hexane, ethyl acetate, ethanol, and boiled water. Then, the mixtures were agitated for a period of 72 h. They were filtered with Whatman No. 389 filter paper. Under aseptic conditions, the extracts were filtered through 0.45 µ-pore size diameter filters and stored at 4°C (Coban *et al.,* 2017a).

2.3 Microorganisms and condition for cultivation

The eighteen bacteria and four yeasts species were: *Escherichia coli* ATCC 35218, *Stapylococcus aureus* ATCC 25923, *Stapylococcus epidermidis* ATCC 12228, *Enterobacter aerogenes* ATCC 13048, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 13882, *Mycobacterium smegmatis* ATCC 607, *Corynebacterium xerosis* ATCC 373, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 27336, *Serratia marcescens* ATCC 13880, *Proteus vulgaris* ATCC 33420, *Listeria monocytogenes* ATCC 19112, *Pseudomonas aeruginosa* ATCC 35032, *Streptococcus mutans, Micrococcus luteus* ATCC 9341, *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, *Candida utilis* ATCC 9950, *Candida tropicalis, and Candida glabrata*.

The bacteria and yeasts were cultured in Tryptic Soy Agar (Merck) at 30-37°C, Malt Extract Agar (Merck) at 27-30°C for 24 h, respectively (Coban *et al.,* 2017b).

2.4 Antimicrobial assays

2.4.1 Disc diffusion method

Screening for antimicrobial activities was carried out by the agar well diffusion method against test microorganisms (Collins *et al.,* 1995; CLSI, 2015). The inoculum size of each group of bacteria and yeast were prepared by using a No. 0.5 McFarland tube to give a concentration of 1×10^8 bacteria and 1×10^6 yeasts per milliliter. In order to test the antimicrobial activity of plants, 20 ml of Mueller Hinton Agar (MHA) were poured in petridishes and kept to solidify at room temperature. Then, it was inoculated with strains of bacteria and yeasts by taking 0.1 ml from cell culture media. After, a hole of 6 mm in diameter and depth were made on top with a sterile stick and was filled with 50 µl of plant extracts. Then, bacterial cultures were incubated at 30-37°C and yeast cultures were incubated at 27-30°C for 18-24 h. At the end of incubation time, the diameters of the inhibition zones formed on the MHA were evaluated in millimeters. Discs of chloramphenicol (C30), gentamycin (CN10), tetracycline (TE30), erythromycin (E15), ampicillin (AM10), and nystatin (NS100) were used as positive controls (Coban *et al*., 2017c).

2.4.2 Minimum inhibitory concentration (MIC)/minimum bactericidal concentration (MIC/MBC)

Minimum inhibitory concentration (MIC) was carried out according to the reported method (Jones *et al*., 1985; CLSI, 2009). The used bacteria were inoculated in tryptic soy broth (TSB) and brain heart infusion broth (BHIB) and incubated at 30-37°C for 24 h while the yeasts were inoculated in malt extract broth (MEB) and incubated at 27°C for 24 h. The inoculums were adjusted according to 0.5 McFarland standard tubes. Firstly, 100 µl of mueller hinton broth (MHB) was placed in each well, after the extracts were added into the first well. Two-fold serial dilutions of the compounds were carried out to determine the MIC, within the concentration range 256 to 0.25 μgml^{-1}. Bacteria cultures were grown at 30-37°C (18-20 h) and the final inoculum was approximately 10^6 $cfuml^{-1}$ while yeasts cultures were grown at 27°C (18-20 h) and the final inoculum was approximately 10^5 $cfuml^{-1}$.

The lowest concentration of antimicrobial agent that resulted in complete inhibition of the microorganisms was represented as MIC (μgml^{-1}). MBC was known as the concentration of plant extract that did not demonstrate any bacterial growth on the inoculated agar plates. As positive controls, Streptomycin (I.E. Ulagay) for bacteria and Nystatin (NS100, Oxoid) for yeast were used in the dilution method. In each case, the test was performed in triplicate and the results were expressed as means.

2.5 Statistical analysis

Mean values and standard deviation calculations were made by SPSS v22 (Statistical Package for Social Sciences).

3. Results and Discussion

3.1 Antimicrobial activity

The antimicrobial activity of n-propanol, methanol, acetone, n-hexane, ethyl acetate, ethanol and boiled water of *P. americana* plant was investigated and the results were given in Tables 1 and 3.However, it showed low effect (9-11 mm) against *E. coli* ATCC 35218, *E. aerogenes* ATCC 13048, *S. typhimirium* ATCC 14028, *K. pneumoniae* ATCC 13882, *C. xerosis* ATCC 373, *E. faecalis* ATCC 29212, *L. monocytogenes* ATCC 19112, *P. aeruginosa* ATCC 35032, *M. luteus* ATCC 9341, *B. subtilis* ATCC 6633 and *C. utilis* ATCC 9950. Acetone extract showed the best effective (12-14 mm) against *S. aureus* ATCC 25923, *P. vulgaris* ATCC 33420, *L. monocytogenes* ATCC 19112, *P. aeruginosa* ATCC 35032, *M. luteus* ATCC 9341, *B. cereus* ATCC 11778 and *C. utilis* ATCC 9950. But, the acetone extract indicated no effect against *E. aerogenes* ATCC 13048, *S. typhimirium* ATCC 14028, *K. pneumoniae* ATCC 13882, *S. pneumoniae* ATCC 27336, *S. marcescens* ATCC 13880 while had low effect (9-11 mm) against *E. coli* ATCC 35218, *S. epidermidis* ATCC 12228, *M. smegmatis* ATCC 607, *C. xerosis* ATCC 373, *E. faecalis* ATCC 29212, *S. mutans*, *B. subtilis* ATCC 6633, *C. albicans* ATCC 10231, *C. tropicalis* and *C. glabrata*. Ethanol extract had high effect (13-14 mm) against *Listeria monocytogenes* ATCC 19112, *Micrococcus luteus* ATCC 9341, *Bacillus cereus* ATCC 11778. However, it showed low effect (9-11 mm) against *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *E. aerogenes* ATCC 13048, *S. typhimirium* ATCC 14028, *P. vulgaris* ATCC 33420, *P. aeruginosa* ATCC 35032, *B. subtilis* ATCC 6633 and *C. albicans* ATCC 10231 while had no effect against *E. coli* ATCC 35218, *K. pneumoniae* ATCC 13882, *M. smegmatis* ATCC 607, *C. xerosis* ATCC 373, *E. faecalis* ATCC 29212, *S. pneumoniae* ATCC 27336, *S. marcescens* ATCC 13880, *C. utilis* ATCC 9950, *C. tropicalis* and *C. glabrata*. Boiled water extract showed the only effect against *P. aeruginosa* ATCC 35032, *C. albicans* ATCC 10231 and *C. tropicalis* while had no effect other microorganisms.

Table 1: Antimicrobial activities of the extracts of *P. americana*

Test microorganisms	Inhibition zones (mm)												
	Leaf extracts							References					
	1	2	3	4	5	6	7	C30	CN10	TE30	E15	AMP 10	NS 100
Escherichia coli ATCC 35218	10	-	9	-	-	-	-	24	21	15	11	-	NT
Stapylococcus aureus ATCC 25923	13	10	14	-	-	11	-	23	20	22	23	20	NT
Stapylococcus epidermidis ATCC 12228	14	-	10	-	10	9	-	22	17	19	11	17	NT
Enterobacter aerogenes ATCC 13048	11	-	-	-	-	9	-	19	20	14	-	-	NT
Salmonella typhimirium ATCC 14028	10	9	-	-	-	9	-	17	16	15	8	8	NT
Klebsiella pneumoniae ATCC 13882	9	-	-	-	-	-	-	21	19	20	14	-	NT
Mycobacterium smegmatis ATCC 607	14	-	9	-	-	-	-	23	18	26	25	19	NT
Corynebacterium xerosis ATCC 373	10	11	10	-	10	-	-	20	17	25	26	27	NT
Enterococcus faecalis ATCC 29212	11	-	11	-	-	-	-	16	11	19	-	14	NT
Streptococcus pneumoniae ATCC 27336	-	-	-	-	-	-	-	24	20	25	15	14	NT
Serratia marcescens ATCC 13880	-	-	-	-	-	-	-	23	19	13	-	19	NT
Proteus vulgaris ATCC 33420	14	10	13	-	10	10	-	17	24	17	20	-	NT
Listeria monocytogenes ATCC 19112	11	-	12	-	-	14	-	19	14	12	-	12	NT
Pseudomonas aeruginosa ATCC 35032	10	11	12	-	-	10	12	22	20	20	21	-	NT
*Streptococcus mutans***	14	-	9	-	-	-	-	28	22	19	-	-	NT
Micrococcus luteus ATCC 9341	10	-	12	-	-	13	-	25	15	26	30	28	NT
Bacillus cereus ATCC 11778	13	13	14	-	9	13	-	-	23	24	25	26	NT
Bacillus subtilis ATCC 6633	10	-	10	-	-	10	-	22	20	12	25	-	NT
Candida albicans ATCC 10231	-	11	10	-	-	10	13	NT	NT	NT	NT	NT	22
Candida utilis ATCC 9950	10	12	12	-	-	-	-	NT	NT	NT	NT	NT	21
*Candida tropicalis**	-	-	9	-	-	-	9	NT	NT	NT	NT	NT	20
*Candida glabrata**	12	-	9	-	-	-	-	NT	NT	NT	NT	NT	21

1. n-Propanol, 2. Methanol, 3. Acetone, 4. n-Hexane, 5. Ethyl acetate, 6. Ethanol, 7. Boiling water. C30: Chloramphenicol (30 mg Oxoid), CN10: Gentamycin (10 mg Oxoid), TE30: Tetracycline (30 mg Oxoid), E15: Erythromycin (15 mg Oxoid), AMP10: Ampicillin (10 mg Oxoid), NS: Nystatin (100 mg Oxoid)
(-): Zone did not occur. NT: Not tested,
(*): Special gift from Faculty of Medicine, Adnan Menderes University, Aydin, Turkey
(**): Special gift from Department of Microbiology, Ege University, Aydin, Turkey

Table 2: Mean values of each extract with standard deviation

Solvents	Mean	Std. Dev.
n-propanol	9,5	4,96
Methanol	3,95	5,4
Acetone	8,41	4,92
n-hexane	0	0
Ethyl acetate	1,73	3,85
Ethanol	5,36	5,63
Boiling Water	1,55	4,03

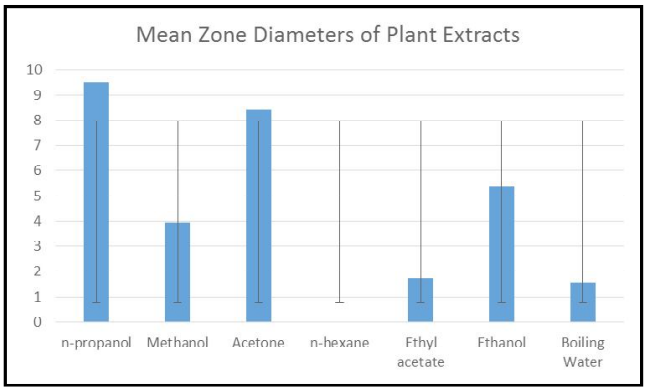

Figure 1: Zone range of each extract with standard deviation.

According to Table 1, the extracts of the n-propanol, acetone, and ethanol of *P. americana* was found to be high effective against tested microorganisms from high to low, respectively. Methanol and ethyl acetate displayed moderate effect while n-hexane showed no effect on tested microorganisms. n-propanol extract was found to be most effective (13-17 mm) against *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *P. vulgaris* ATCC 33420, *S. mutans*, *B. cereus* ATCC 11778, *M. smegmatis* ATCC 607 and *C. glabrata* while it showed no effect against *S. marcescens* ATCC 13880, *S. pneumoniae* ATCC 27336, *C. albicans* ATCC 10231 and *C. tropicalis* (Figures 2 a, b).

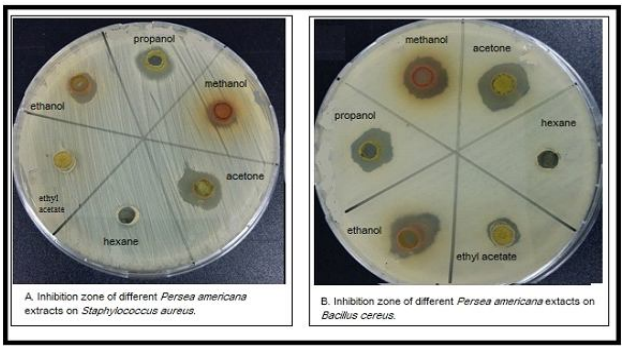

Figure 2. Antimicrobial effects of *P. americana* extracts. **a.** *Staphylococcus aureus* ATCC 25923, **b.** *Bacillus cereus* ATCC 11778. 1. n-Propanol, 2. Methanol, 3. Acetone, 4. n-Hexane, 5. Ethyl Acetate, 6. Ethanol, 7. Boiling water.

Dennis *et al.* (2017) evaluated that the antibacterial effect of ethanol extract of the avocado seed of *P. americana* as an alternative root canal irrigants against *Porphyromonas gingivalis*. They showed that ethanol extract of the avocado seed has an antibacterial effect against *Porphyromonas gingivalis* with MIC of 50% and MBC of 60%. Lubis *et al.* (2017) researched the antimicrobial activity of ethanolic extract of *P. americana* peel against *Staphylococcus aureus, Proteus vulgaris, Enteroccus faecalis, Escherichia coli* and *Pseudomonas aeruginosa*. The extract of plant was effective against Gram-positive and negative bacteria between inhibition zone at 100% and 0,4%, respectively. Cardoso *et al.* (2016) investigated antibacterial activity against *Streptococcus agalactiae* of ethanol and dichloromethane extracts obtained from avocado seeds, The ethanol extract showed antimicrobial activity only for some isolates of *S. agalactiae* of human origin. The dichloromethane extract showed activity against all isolates of *S. agalactiae* of human and fish origins.

Boadi *et al.* (2015) obtained methanol, ethyl acetate, chloroform and petroleum ether extracts from leaves of *P. americana* and tested against some microorganisms. They showed that the methanolic extract had effect the most potent antimicrobial activities. Ogundare and Oladejo (2014) tested the antibacterial activities of the methanolic leaf and bark extract of *P. americana* against some Gram-positive and negative bacteria. The results showed the highest zone of inhibition of 6.0 mm and 12.0 mm was observed on *Staphylococcus aureus* for the leaf and bark extract, respectively, while the least zone of inhibition was observed on *Klebsiella pneumoniae* (2.0 mm) for leaf extract and *Proteus mirabilis* (3.0 mm) for bark extract. Rodríguez-Carpena *et al.* (2011) analyzed the antibacterial activities of ethyl acetate, acetone, and methanol extracts of the peel, pulp, and seed obtained from two avocado, *P. americana* varieties. The avocado extracts had moderate antimicrobial effects against Gram-positive bacteria. Chia and Dykes (2010) provided ethanol and distilled water extracts from epicarp and seed of *P. americana* and evaluated against Gram-positive and negative bacteria. The results showed that the ethanol extracts had effect against Gram-positive and negative bacteria except *Escherichia coli* while the water extracts had only effect against *Listeria monocytogenes* and *Staphylococcus epidermidis*. Gomez-Flores *et al.* (2008) researched the antimicrobial activity of methanol extract of *P. americana* (Lauraceae) (Avocado) leaves against *Mycobacterium tuberculosis*. They found that methanol extract of plant leaves had high effect antibacterial activity against *Mycobacterium tuberculosis*.

4. Conclusion

The extracts of the n-propanol, acetone, and ethanol of *P. americana* leaves showed high effect comparing to methanol, hexane, ethyl acetate and boiled water. The leaf extracts obtained in n-propanol, acetone and ethanol were found to have secondary metabolites like alkaloids, tannins, and flavonoids effective against microorganisms. The difference in the efficiency of the extracts can be explained by the difference in polarity of solvents. During the extraction process, polarity influences solubility of the main active substance, leading to difference in their chemical composition and consequently, in their biological activity The yield of extract and concentration of the extract might also be the reason for difference in .activity of the extract against a particular organism (Idris *et al.*, 2009).

Table 3: Antimicrobial activities of the extracts of *P. americana* (MIC/MBC, µg mL^{-1})

Test Microorganisms	1	2	3	5	6	7	Str	NS 100
Escherichia coli ATCC 35218	128/256	-	256/-	-	-	-	64/128	NT
Stapylococcus aureus ATCC 25923	64/128	128/256	64/128	-	128/256	-	32/64	NT
Stapylococcus epidermidis ATCC 12228	64/128	-	128/256	128/256	256/-	-	32/64	NT
Enterobacter aerogenes ATCC 13048	128/256	-	-	-	256/-	-	32/64	NT
Salmonella typhimirium ATCC 14028	128/256	256/-	-	-	256/-	-	64/128	NT
Klebsiella pneumoniae ATCC 13882	256/-	-	-	-	-	-	64/128	NT
Mycobacterium smegmatis ATCC 607	64/128	-	256/-	-	-	-	128/256	NT
Corynebacterium xerosis ATCC 373	128/256	128/256	128/256	-	128/256	-	64/128	NT
Enterococcus faecalis ATCC 29212	128/256	-	128/256	-	-	-	64/128	NT
Streptococcus pneumoniae ATCC 27336	-	-	-	-	-	-	128/256	NT
Serratia marcescens ATCC 13880	-	-	-	-	-	-	64/128	NT
Proteus vulgaris ATCC 33420	64/128	128/256	64/128	-	128/256	128/256	64/128	NT
Listeria monocytogenes ATCC 19112	128/256	-	64/128	-	-	64/128	32/64	NT
Pseudomonas aeruginosa ATCC 35032	128/256	128/256	64/128	-	128/256	64/128	64/128	NT
*Streptococcus mutans***	64/128	-	256/-	-	-	-	-	-
Micrococcus luteus ATCC 9341	128/256	-	64/128	-		64/128	32/64	NT
Bacillus cereus ATCC 11778	64/128	64/128	64/128	-	256/-	64/128	64/128	NT
Bacillus subtilis ATCC 6633	128/256	-	128/256	-	-	128/256	64/128	NT
Candida albicans ATCC 10231	-	128/256	128/256	-	128/256	64/128	NT	64/128
Candida utilis ATCC 9950	128/256	64/128	64/128	-	-		NT	64/128
*Candida tropicalis**	-	-	256/-	-	-	256/-	NT	64/128
*Candida glabrata**	64/128	-	256/128	-	-	-	NT	64/128

1. n-Propanol, 2. Methanol, 3. Acetone, 5. Ethyl Acetate, 6. Ethanol, 7. Boiling Water.
Hexane extract did not show antibacterial activity.
Str = Streptomycin, NS100 = Nystatin.
(–) = No effect.
(*) From Faculty of Medicine, Adnan Menderes University, Aydin, Turkey
(**): Special gift from Department of Microbiology, Ege University, Aydin, Turkey

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Conflict of interest

We declare that we have no conflict of interest.

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