

Antimicrobial Activity of *Arbutus unedo* L.⁺

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Summary : The water, ethanol and n-hexane extracts from *Arbutus unedo* leaves have been evaluated for antimicrobial activities. The antimicrobial activities of the extracts are reported against *Escherichia coli* ATCC 29998, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus epidermidis* ATCC 12228, *Salmonella thyphimurium* CCM 5445, *Enterobacter cloacae* ATCC 13047 and *Enterococcus faecalis* ATCC 29212 as bacteria and *Candida albicans* ATCC 10239 as yeast by using the disc diffusion method. It is observed that ethanol extract showed activity against Gram (+) and Gram (-) bacteria. None of the tested extracts showed antifungal activity against *Candida albicans* ATCC 10239.

Key Words: *Arbutus unedo*, Antimicrobial activity

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Introduction

Arbutus unedo L. (Ericaceae) is a plant widely found in the Mediterranean area^{1,2}. *Arbutus unedo* has edible fruits. Its wood is used for carving spindles, stools and other small articles of furniture². The leaves are used as a diuretic, astringent, anti-diarrhoeic and hemorrhoids. It is used against hypertension in Morocco³⁻⁵.

A study on *Arbutus unedo* L. included isolation and identification of its flavon glycoides (afzelin, juglanin, avicularin, quercitrin, hyperine)⁶. In the other studies, phenol glycosides (Arbutin and met-

Arbutus unedo L.'nin Antimikrobiyal Aktivitesi

Özet : *Arbutus unedo*' nun yapraklarından hazırlanan su, etanol ve n-hegzan ekstralarının antimikrobiyal aktivitesi değerlendirildi. Ekstrelerin antimikrobiyal aktivitesi, bakteri olarak *Escherichia coli* ATCC 29998, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus epidermidis* ATCC 12228, *Salmonella thyphimurium* CCM 5445, *Enterobacter cloacae* ATCC 13047, *Enterococcus faecalis* ATCC 29212 ve maya benzeri fungus olarak *Candida albicans* ATCC 10239'a karşı değerlendirilmiştir. Etanol ekstresinin, Gram (+) ve Gram (-) bakterilere karşı aktif olduğu gözlemlendi. Test edilen ekstraların hiçbiri *Candida albicans* ATCC 10239'a karşı aktivite göstermedi.

Anahtar kelimeler : *Arbutus unedo*, Antimikrobiyal aktivite

ylarbutin), lipids, tannins and vitamin E have been reported⁷⁻¹⁰.

No report concerning the antimicrobial activity of the extracts of this plant was encountered during our literature survey. Therefore, the present study was undertaken to evaluate the antimicrobial activity of *Arbutus unedo* by the disc diffusion method.

Experimental

Plant Material

Dried leaves of *Arbutus unedo* L. (Ericaceae) were collected from Izmir, near the Çiçekliköy in No-

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vember, 1998. The plant was identified by B. KIVÇAK. A voucher specimen is kept in the Herbarium of the Department of Pharmacognosy, University of Ege (1251).

Preparation of Plant Extracts

Air-dried and powdered leaves of *Arbutus unedo* L. (100g) were extracted with *n*-hexane, and ethanol by percolation. The aqueous extract was prepared by infusion. The extracts were evaporated to dryness in vacuo and weighed. All of the extracts were prepared at 100% concentrations (g/ml).

Preliminary chemical tests

Preliminary phytochemical properties of the extract were studied using the following reagents and chemicals^{11,12}: Flavonoids with Mg and HCl, tannins with FeCl₃ solution, phenol glycoides (arbutin and methylarbutin) with FeSO₄ solution, and Vit E with 2,2'-dipyridyl and ferric chloride solutions.

Test Microorganisms

The following Gram (+) and Gram (-) bacteria were used for testing antibacterial activity.

Escherichia coli ATCC 29998, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus epidermidis* ATCC 12228, *Salmonella thyphimurium* CCM 5445, *Enterobacter cloacae* ATCC 13047 and *Enterococcus faecalis* ATCC 29212 were used as bacteria and *Candida albicans* ATCC 10239 as yeastlike fungi.

Lyophilised bacteria and yeast were obtained from the Standard ATCC bacteria strain and Standard ATCC fungus strain collection of Science Faculty of Ege University, Department of Basic and Industrial Microbiology Section.

Media

The solid growth medium used for bacteria was Mueller Hinton Agar (Oxoid) and Sabouraud Dextrose Agar (Difco) for yeast.

Disc Diffusion Method

The disc diffusion method, known as the Kirby Bauer method, was used to determine antimicrobial activities¹³⁻¹⁵.

24 hour cultures containing 10⁸ cfu/ml of microorganisms were used and diluted with sterile distilled water to obtain equivalent to 0.5 Mc Farland's standards of turbidity. 24 hour cultures of the yeast were prepared in Sabouraud Dextrose Broth to obtain 10⁷ cfu/ml.

40 µl of reconstituted crude extracts were absorbed on to sterile 6 mm discs (Oxoid Antibacterial Susceptibility Blank Tests Disc) under aseptic conditions to obtain 30 µg extract/disc and dried at 50 °C. Dried discs were transferred on to plates containing test organisms with sterile forceps. Control disc contained 40 µl of sterile 10% aqueous DMSO. Agar plates containing bacteria were incubated at 37 °C for 24 h and those containing yeast at 27 °C for 48 h. The standard antibacterial agent Ceftazidime (30 µg/disc) was used as a positive control for bacteria and the standard antifungal agent Nystatin (25 µg /disc) was used as the positive control for yeast.

All experiments were done in triplicate.

Results and discussion

Result of antimicrobial activity screening tests were given in Table 1.

If disc diffusion diameter is the same, then it is possible to say that microorganism is resistant, but if the inhibition zone is slightly larger than that of the disc diameter, then the organism is less sensitive. Otherwise, when the inhibition zone of the extracts examined on the organism is two or more times larger, the extract will be fully effective¹⁶.

The ethanol extract of *Arbutus unedo* leaves was found to be more active against five bacteria than the other extracts. This extract exhibited higher antibacterial activity against *E. coli* ATCC 25922 than Ceftazidime. Also it has been found to be as active as Ceftazidime against *Staphylococcus aureus* ATCC

Table 1. Antimicrobial activity of *Arbutus unedo* L.

Extracts/Drugs	Growth inhibition zones of microorganisms (mm)							
	<i>E. coli</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. thyphimurium</i>	<i>E. cloacae</i>	<i>E. faecalis</i>	<i>C. albicans</i>
	ATCC 29998	ATCC 25922	ATCC 6538P	ATCC 12228	CCM 5445	ATCC 13047	ATCC 29212	ATCC 10239
<i>n</i> -Hexane	8	16	12	9	-	12	8	-
Ethanol	12	19	18	11	8	13	8	-
Water	8	8	-	-	8	-	-	-
Ceftazidime	17	17	19	19	9	15	12	-
Nystatin	-	-	-	-	-	-	-	18
DMSO	-	-	-	-	-	-	-	-

6538P. Ethanol extract showed slight activity against *Escherichia coli* ATCC 29998, *Staphylococcus epidermidis* ATCC 12228 and *Enterobacter cloacae* ATCC 13047. It is observed that this extract had lower activity against *Salmonella thyphimurium* CCM 5445 and *Enterococcus faecalis* ATCC 29212.

The *n*-hexane extract of *A. unedo* has been found to be as active as the Ceftazidime against *Escherichia coli* ATCC 25922. However, this extract showed lower activity against *Enterobacter cloacae* ATCC 13047 and *Staphylococcus aureus* ATCC 6538P than ethanol extract and Ceftazidime. This extract had lower activity against *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 29998 and *Enterococcus faecalis* ATCC 29212 but had no effect on the growth of *Salmonella thyphimurium* CCM 5445.

The water extract of *Arbutus unedo* (infusion) was found to have lower activity against *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 29998 and *Salmonella thyphimurium* CCM 5445 but had no effect on the growth of the tested microorganisms.

No inhibition zones are seen against the yeast (*Candida albicans* ATCC 10239) investigated in all of the plant extracts.

DMSO also had no effect on the growth of any of the eight microorganisms.

On phytochemical screening^{11,12}, all extracts gave positive tests for flavonol glycosides, *n*-hexane and

water extracts for phenol glycosides, EtOH and water extracts for tannins but only the *n*-hexane extract was positive for Vitamine E. So flavonol glycosides might have been the active principles responsible for the observed antimicrobial activity. On the other hand, there are some reports concerning the antimicrobial effects of flavonoids^{17,18}, so the possibility of flavonoids as responsible compounds for the antimicrobial effect of *A. unedo* increases, and leaves an open door for pharmacological investigations on the potential antimicrobial activity of this compounds in the ethanol and *n*-hexane extracts.

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