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Antimicrobial and Cytotoxic Activities of the Extracts Obtained from the Flowers of *Alcea Rosea* L.

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Introduction

Alcea rosea L. (Malvaceae), populary known as Holyhock, is especially grown in gardens and parks in the Southern Europe and Turkey [1]. The flowers of *A. rosea*, sold in the Indian market under the trade name Gulkhairo, are well known for their expectorant, cooling and diuretic properties, and are used in many indigenous cough mixtures in India [2]. Its flowers have also widely varied applications in Turkish folk medicine[1]. The flowers of *A. rosea* are reported to contain mucilaginous polysaccharides, antocyanins and flavonoids [3-5].

No report about the cytotoxicity and antimirobial activity of the extracts of this plant was encountered during literature survey. Therefore, the aim of this study was to investigate the possible antimicrobial activity of *Alcea rosea* extracts against ten bacterial species and *C. albicans* and to evaluate the cytotoxic activity against Brine shrimp.

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Material and Methods

Plant Material

The flowers of *A. rosea* (blue variety of hollyhock, Malvaceae) were collected from the Botanical Garden of the Forest Nursery in Karşıyaka (Izmir), Turkey. A voucher specimen was deposited in the herbarium of the Department of Pharmacognosy, University of Ege (No:1261).

Preparation of Plant Extracts

Air dried and powdered flowers of *A. rosea* L. (20 g) were extracted with *n*-hexane, ethanol, methanol, ethyl acetate and water (infusion) at room temperature; the extracts were evaporated to dryness in vacuo and weighed.

Cytotoxic Studies

Cytotoxicity was studied by Brine shrimp (*Artemia salina*) lethality bioassay [6]. Cytotoxic activity of all extracts were compared with umbelliferone and colchicine as the active cytotoxic substances [7,8].

Materials

Brine shrimp was obtained from San Fransico Bay Brand Inc. Newark, CA94560 USA. Sea salt (Sigma-9883) was used in activity tests.The small tank was purchased from Otsuka Pharmaceutical Co.Ltd., (Tokyo, Japan).

Method

Cytotoxicity was evaluated by the brine shrimp lethality bioassay [6]. The sea salt (3.8g) was dissolved in 100 ml water and filtrated. Brine shrimp (Artemia salina) eggs were placed in to the sea water and allowed to incubate for 48h at 28°C in a small tank. Each extracts were tested at 1000, 100 and 10 ppm. 20 mg plant extract was dissolved in 2 ml of chloroform to prepare a stock solution of 10 mg/ml. From the stock solution, 500, 50 and 5 ml was transferred to different vials and allowed to evaporate. After evaporation, 5 ml of sea salt solution was

added to each vial to prepare concentrations corresponding to 1000, 100 and 10 ppm. Each concentration was prepared in triplicate. Also, a vial including chloroform (500ml) was prepared for control. After incubation, 10 brine shrimp larvae (nauplii) were introduced into vials containing graded concentrations (ranging from 10 to 1000 ppm) of the test extracts. After 24h, the number of surviving shrimps at each concentration of the extracts was counted and data was analysed with Finney Computer programme (Cambridge University, England) to determine the LC_{50} at 95 % confidence interval.

Antimicrobial Studies

The disc diffusion method, known as the Kirby Bauer method, was used to determine the antimicrobial activities [9-11].

24 Hour cultures containing 10^8cfu/ml of microorganisms were used and diluted with sterile distilled water to obtain equivalent to 0.5 Mc Farland's standards of turbudity. 24 hour cultures of the yeast were prepared in Saboraud Dextrose Broth to obtain 107 cfu/ml. 40 ml of reconstituted crude extracts were absorbed on to the sterile 6 mm discs (Oxoid Antibacterial Suspectibility Blank Tests Disc) under aseptic conditions to obtain 30 mg extract/disc and dried at 50^0C.The dried discs were transferred on to the plates containing test organisms with sterile forceps. The control disc contained 40 ml of sterile 10 % aqueous DMSO. The agar plates containing bacteria were incubated at 37^oC for 24h and those containing yeast at 27^0C for 48h. The standard antibacterial agent Ceftazidime (30 mg/disc) was used as a positive control for bacteria and the standard antifungal agent Nystatine (25 mg /disc) was used as the positive control for yeast. All experiments were done in triplicate.

Test Microorganisms

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

Escherichia coli ATCC 29998, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC11230, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Salmonella thyphimurium* CCM 5445, *Enterobacter cloacae* ATCC 13047,

Enterococcus faecalis ATCC 29212, *Pseudomonas aeriginosa* ATCC 27853 were used as bacteria and *Candida albicans* ATCC 10239 as yeast like fungi.

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

Media

The solid growth medium used for bacteria was Mueller Hinton Agar (Oxoid, USA) and for yeastlike fungi was Saboraud Dextrose Agar (Difco, England).

Results and Discussion

The antimicrobial activities of *Alcea rosea* are presented in Table I. The tested plant extracts were found to posses an activity against tested microorganisms except for *E. Coli* 25922, *E. Cloaceae* 13047, *E faecalis* 29212 and *C. Albicans* 10239. There was no significant activity difference between extracts. But all of the extracts were found to be slightly active against tested microorganisms than Ceftazidime.

Cytotoxic activity of *n*-hexane, ethanol, methanol, ethyl acetate and water extracts of the leaves of *A. rosea* L. have been investigated *in vitro* against Brine shrimp (*Artemia salina*). The results were reported in Table II.

The ethyl acetate extract showed cytotoxic activity against Brine shrimp (LC_{50} < 1000). extracts. This extract was even more less active than umbelliferon [7] and colchicine [8].

The tested gram-negative bacteria, with the exception of *Escherichia coli* ATCC 25922 and *Enterobacter cloacae* ATCC 13047, showed sensitivity to the *A. rosea* extracts. The gram- positive bacteria (except for *Enterococcus faecalis* ATCC 29212) were inhibited by all of the extracts. In addition the ethylacetate extract showed cytotoxic activity against Brine shrimp (LC_{50} < 1000).

Finally, the results of the present study support the ethnomedical use of the plant in Turkish folk medicine. Further studies are, therefore, needed to confirm its efficacy and to evaluate its safety.

TABLE I

Antimicrobial activity of Alcea rosea extracts

	Inhibition Zone (mm)*							
Microorganisms	A	B	C	D	E	F	G	H
Escherichia coli ATCC 29998	9	9	9	9	9	15	-	-
Escherichia coli ATCC 25922	-	-	-	-		14	-	-
Escherichia coli ATCC 11230	10	9	10	9	9	18	-	-
Staphylococus aureus ATCC 6538P	8	10	9	7	9	12	-	-
Staphylococcus aureus ATCC 29213	-	9	9	9	9	13	-	-
Staphylococcus epidermidis ATCC 12228	8	10	10	9	8	12	-	-
Salmonella typhimirium CCM 5445	8	8	8	8	7	14	-	-
Enterobacter cloacae ATCC 13047	-	-	-	-	-	13	-	-
Enterococcus faecalis ATCC 29212	-	-	-	-	-	11	-	-
Pseudomonas aeroginosa ATCC 27853	9	9	9	8	8	22	-	-
Candida albicans ATCC 10239	-	-	-	-	-	-	18	-

A : Ethanol extract ; B : n-Hexane extract ; C : Ethyl acetate extract ; D : Methanol extract ; E : Water extract ; F : Ceftazidime ; G : Nystatine ; H : Control (DMSO) ;

*Includes diameter of disc (6 mm).

TABLE II

Cytoxicity assay of *Alcea rosea* L .extracts against *Artemia salina*

PLANT	EXTRACTS	CONCENTRATION(ppm)	LC_{50} (µg/ml)	SD (%) (n=3)	% Capacity
rosea L.	n-hexane	1000:100:10	>1000	0.78	% 2.93
	Ethyl acetate	1000:100:10	545.398	0.54	% 1.41
	Ethanol	1000:100:10	>1000	0.37	% 9.12
	Methanol	1000:100:10	>1000	0.08	% 12.13
Umbelliferon		500:50:5	377.02		
Kolşisin		500:50:5	0.0009		

Summary

The antimicrobial and cytotoxic activities of n-hexane, methanol, ethanol, ethyl acetate and water extracts of *Alcea rosea* L. flowers were investigated. The antimicrobial activities of the extracts were reported against *Escherichia coli* ATCC 29998, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 11230, *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Salmonella thyphimurium* CCM 5445, *Enterobacter cloacae* ATCC 13047, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeroginosa* ATCC 27853 as bacteria and *Candida albicans* ATCC 10239 as yeastlike fungi by disc diffusion method.

These extracts were also tested for cytotoxic activity by brine shrimp assay. Ethyl acetate extract showed cytotoxic activity against brine shrimp.

Key Words: Alcea rosea L., Malvaceae, Cytotoxic activity, Antimicrobial activity.

Özet

Alcea rosea L. çiçeklerinden Hazırlanan Ekstrelerin Antimikrobiyal ve Sitotoksik Aktiviteleri

Bu çalışmada, *Alcea rosea* L.'nin çiceklerinin n-hegzan, metanol, etanol, etil asetat ve su ekstrelerinin, antimikrobiyal ve sitotoksik aktiviteleri değerlendirildi. Ekstrelerin antimikrobiyal aktiviteleri, bakteri olarak *Escherichia coli* ATCC 29998, *Escherichia coli* ATCC 25922, ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Salmonella thyphimurium* CCM 5445, *Enterobacter cloacae* ATCC 13047, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeroginosa* ATCC 27853 ve mantar olarak *Candida albicans* ATCC 10239 **'** a karşı disk difüzyon metodu ile tayin edildi.

Ekstrelerin sitotoksik aktiviteleri Brine shrimp yöntemiyle değerlendirildi. Etil asetat ekstresi Brine shrimp'e karşı sitotoksik aktivite gösterdi.

Anahtar Kelimeler: Alcea rosea L., Malvaceae,, sitotoksik aktivite, antimikrobiyal aktivite.

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