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Antimicrobial and Cytotoxic Activities of *Ceratonia siliqua* L. Extracts

Bijen KIVÇAK, Tuba MERT
Ege University, Faculty of Pharmacy, Department of Pharmacognosy, Bornova 35100 İzmir - TURKEY
H. Tansel ÖZTÜRK
Ege University, Faculty of Pharmacy, Department of Science, Basic and Industrial Microbiology, Bornova 35100 İzmir - TURKEY

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Abstract: The antimicrobial and cytotoxic activities of *n*-hexane, methanol, ethanol, ethyl acetate and water extracts of *Ceratonia siliqua* L. leaves were evaluated in this study. The antimicrobial activities of the extracts were reported against *Escherichia coli* ATCC 29998, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 11230, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus aureus* ATCC6538P, *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 yeast-like fungi by the disc diffusion method.

The cytotoxic activity of the extracts was evaluated by the brine shrimp lethality bioassay. The ethanol, methanol and water extracts showed cytotoxic activity against brine shrimp.

Key Words: *Ceratonia siliqua* L., Leguminosae, cytotoxic activity, antimicrobial activity

Ceratonia siliqua L. Ekstrelerinin Antimikrobiyal ve Sitotoksik Aktiviteleri

Özet: Bu çalışmada, Cerotonia siliqua L.'nin *n*-hegzane, metanol, etanol, etil asetat and su ekstrelerinin, antimikrobiyal ve sitotoksik aktiviteleri değerlendirildi. Ekstrelerin antimikrobiyal aktiviteleri, bakteri olarak *Escherichia coli* ATCC 29998, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 11230, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus aureus* ATCC6538P, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Salmonella thyphimurium* CCM 5445, *Enterobacter cloacae* ATCC 13047, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853 ve mantar olarak *Candida albicans* ATCC 10239'a karşı disk difüzyon metodu ile tayin edildi.

Ekstrelerin sitotoksik aktiviteleri brine shrimp yöntemiyle değerlendirildi. Etanol, metanol ve su ekstreleri brine shrimp'e karşı sitotoksik aktivite gösterdi.

Anahtar Sözcükler: *Ceratonia siliqua* L., Leguminosae, sitotoksik aktivite, antimikrobiyal aktivite

Introduction

Ceratonia siliqua L. (Leguminosae), commonly known as carob, is distrubuted in the Mediterranean region (1). It is found to contain protein, fat, carbohydrates, polyphenols and tannins (2).

Its bark and leaves are used in Turkish folk medicine as an antidiarrheal and diuretic (1,3). The fruits of this plant are traditionally used as an antitussive and against warts (4,5). Its hormone-like effects have been reported (6).

The objective of the present study was to assess the antimicrobial and cytotoxic properties of *C. siliqua* extracts, the activities of which, to the best of our knowledge, have not been previously evaluated.

Materials and Methods

Plant material

The leaves of *Ceratonia siliqua* L. were collected from Çeşme-Ardıç (İzmir) in July 2001. A voucher specimen was deposited in the herbarium of the Department of Pharmacognosy, Ege University (1273).

Preparation of plant extracts

Air-dried and powdered leaves of *Ceratonia siliqua* 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 the brine shrimp (*Artemia salina*) assay (7). The cytotoxic activity of all extracts was compared with umbelliferone and colchicine as the active cytotoxic substances (8,9).

Materials

Brine shrimp was obtained from San Fransisco Bay Brand Inc. Newark, CA 94560 USA. Sea salt (Sigma-9883) was used in activity tests. A small tank was purchased from Otsuka Pharmaceutical Co. Ltd., (Tokyo, Japan).

Methods

Cytotoxicity was evaluated by the brine shrimp lethality bioassay (7). Sea salt (3.8 g) was dissolved in 100 ml water and filtered. Brine shrimp (*Artemia salina*) eggs were placed into the water and left to incubate for 48 h at 28 ºC in a small tank. Each extract was tested at 1000, 100 and 10 ppm. Then 20 mg of plant extract was dissolved in 2 ml of chloroform (20 mg/2 ml). From this solution 500, 50 or 5 µl was transferred to vials corresponding to 1000, 100 or 10 ppm, respectively. Vials including chloroform and extraction solvents (500 µl) were prepared as controls. 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 24 h, the number of surviving shrimps at each concentration of the extracts were counted and data analyzed with the Finney computer program to determine the LC_{50} at a 95% confidence interval.

Antimicrobial studies

The disc diffusion method, known as the Kirby-Bauer method, was used to determine the antimicrobial activities (10-12).

Overnight cultures containing 10^8 cfu/ml of microorganisms were used and diluted with sterile distilled water to obtain turbidity equivalent to a McFarland 0.5 turbidity standard. Overnight cultures of yeast were prepared in Sabouraud Dextrose Broth to obtain 10^7 cfu/ml.

Then 40 µl of reconstituted crude extracts were absorbed onto sterile 6 mm discs (Oxoid Antibacterial Suspectibility Blank Test Discs) under aseptic conditions to obtain 30 µg extract/disc and dried at 50 ºC. Dried discs were transferred onto plates containing test organisms with sterile forceps. The control disc contained 40 ml of sterile 10% aqueous DMSO. Agar plates containing bacteria were incubated at 37 ºC for 24 h and those containing yeast at 27 ºC 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. The antimicrobial activity of each extraction solvent was also tested.

All experiments were done in triplicate.

Test Microorganisms

Escherichia coli ATCC 29998, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC11230, *Salmonella thyphimurium* CCM 5445, *Enterobacter cloacae* ATCC 13047, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus aureus* ATCC 29213 and *Staphylococcus epidermidis* ATCC 12228 were used as bacteria and *Candida albicans* ATCC 10239 as yeast-like fungi for testing antimicrobial activity.

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

Media

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

Results and Discussion

The cytotoxic activity of n-hexane, ethanol, methanol, ethyl acetate and water extracts of leaves of *Ceratonia siliqua* L. was investigated *in vitro* against the brine shrimp (*Artemia salina*). The results are given in Table 1.

The ethanol, methanol, *n*-hexane and water extracts showed cytotoxic activity against the brine shrimp. These extracts were toxic (LC_{50} < 1000) in the brine shrimp bioassay. Ethyl acetate extract showed no cytotoxic activity.

Table 1. Cytoxicity assay of *Ceratonia siliqua* extracts against *Artemia salina*

Extracts (ppm)	Concentration (ppm)	LC_{50}
Methanol	1000:100:10	25.7920
n-Hexane	1000:100:10	356.4297
Ethanol	1000:100:10	269.6858
Ethyl acetate	1000:100:10	>1000
Water	1000:100:10	25.7920
Colchicine (Standard)	500:50:5	0.0009
Umbelliferone (Standard)	500:50:5	377.0223

These extracts were even more active than a cytotoxic substance umbelliferon (8); but all of the tested extracts were less active than colchicine (9).

Results from the antimicrobial screening tests are shown in Table 2. The ethanol extract of *C. siliqua* inhibited the growth of five of the 10 microorganisms but had no effect on the growth of *Escherichia coli* ATCC 29998, *Escherichia coli* ATCC 25922 *Staphylococcus aureus* ATCC 6538P, *Enterococcus faecalis* ATCC 29212 or *Pseudomonas aeruginosa* ATCC 27853. Ethyl acetate and *n*-hexane extracts of *C. siliqua* were more active against *Enterococcus faecalis* ATCC 29212 than Ceftazidime. However, ethanol, methanol and water extracts had no effect on the growth of *Enterococcus faecalis* ATCC 29212. All the extracts (except for ethanol extract) inhibited *Escherichia coli* ATCC 25922. Furthermore, the antibacterial activity of *n*-hexane extract against *Escherichia coli* ATCC 25922 was similar to that of Ceftazidime. On the other hand, the growth of *Escherichia coli* ATCC 11230 was inhibited by the whole extracts used in this study. The growth of *Escherichia coli* ATCC 29998 was only inhibited by methanol extract. The growth of *Staphylococcus aureus* ATCC 29213 and *Staphylococcus epidermidis* ATCC 12228 were inhibited by ethanol, methanol and water extracts. Furthermore, ethanol, methanol and water extracts inhibited the growth of *C. albicans* but n-hexane and ethyl acetate extracts had no effect on the growth of *C. albicans* None the tested extracts showed activity against Pseudomonas aeroginosa ATCC 27853.

However, the standard antibacterial agent, Ceftazidime, inhibited the growth of all of tested microoorganisms but had no effect on the growth of *Candida albicans*. On the other hand, the standard antifungal agent, nystatine, inhibited the growth of *Candida albicans* but had no effect on the growth bacteria. DMSO also did not affect the growth of any tested microorganisms.

Table 2. Antimicrobial activity of Cerotonia siliqua extracts

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

In conclusion, whole extracts, especially *n*-hexane and ethyl acetate extracts, can be used for protection against bacteria and fungus in some cases. On the other hand, the cytotoxic and antimicrobial activity observed on the tested extracts may provide useful data for the utilization of cytotoxic and antimicrobial principles of these extracts.

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