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THE CYTOTOXICITY AND THE ANTIMICROBIAL EFFECTS OF SOME TURKISH PLANTS

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SUMMARY

In this research, the cytotoxic effects of the aerial parts of *Hypericum triquetrifolium* (Guttiferae), *Ononis natrix* subsp. *natrix* (Leguminosae), *Fumaria officinalis* (Papaveraceae) and the fruits of *Capparis spinosa* (Capparidaceae) have been investigated by the Brine Shrimp Method (*Artemia salina*). Then the antimicrobial activity of the mentioned drugs from the antibacterial and antifungal point of view have been investigated by the agar well diffusion and tube dilution methods.

Keywords: *Hypericum triquetrifolium*, *Ononis natrix* subsp. *natrix*, *Fumaria officinalis*, *Capparis spinosa*, Brine Shrimp Method (*Artemia salina*), Cytotoxicity, Antimicrobial.

ÖZET

Bu çalışmada *Hypericum triquetrifolium* (Guttiferae), *Ononis natrix* subsp. *natrix* (Leguminosae), *Fumaria officinalis* (Papaveraceae) bitkilerinin toprak üstü kısımlarıyla, *Capparis spinosa* (Capparidaceae) bitkisinin meyvalarının sitotoksik

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aktiviteleri Brine Shrimp (*Artemia salina*) yöntemi ile araştırılmıştır. Daha sonra bu bitkilerden hazırlanan ekstrelerin antimikrobiyal etkileri, antibakteriyel ve antifungal aktiviteleri yönünden agar oluk difüzyon ve tüp dilüsyon yöntemleri ile incelenmiştir.

Anahtar Kelimeler: *Hypericum triquetrifolium*, *Ononis natrix* subsp. *natrix*, *Fumaria officinalis*, *Capparis spinosa*, Brine Shrimp (*Artemia salina*), Sitotoksisite, Antimikrobiyal.

INTRODUCTION

The aerial parts of *Hypericum triquetrifolium* (Guttiferae), *Ononis natrix* subsp. *natrix* (Leguminosae), and the fruits of *Capparis spinosa* (Capparidaceae) were collected from Üzümlü, Fethiye. The plants were identified by Prof.Dr.Ertan Tuzlacı and voucher specimens are kept in the Herbarium of the University of Marmara, Faculty of Pharmacy. (MARE 6442, 7242, 7244 respectively). The aerial parts of *Fumaria officinalis* (Papaveraceae) were obtained from herb dealers. *Hypericum* species (Guttiferae),are known for their antispasmotic, antidiarrheic and antiseptic effects in folk medicine. These species contain hypericine, tannins, volatile oil and flavone derivatives. *Ononis* species (Leguminosae) are also used in folk medicine. The most known one is *Ononis spinosa* which is known as "kayışkıran" in Turkey. It is used for its diuretic and antiseptic effects. The fruits of *Capparis spinosa* (Capparidaceae) contain flavone derivatives. They are used for their diuretic, antidiarrheic and tonic effects. The buds of the plant are used to make pickles. *Fumaria officinalis* (Papaveraceae) is a plant which is known for its alkaloidal content and used as diuretic, sedative and hypotensive agent.

In our study, in addition to all of the activities of these plants mentioned above, we wanted to investigate their cytotoxic and antimicrobial activities.

RESULTS AND DISCUSSION

According to the Brine Shrimp *(Artemia salina)* method all of the extracts are found to be inactive. Results are shown in Table-1.

Table 1: LC_{50} values by the Brine Shrimp (*Artemia salina*) method.

Name of the plant	Type of extract	Brine Shrimp LC_{50} (ppm)
Hypericum triquetrifolium	% 60 ethanol	>1000
Ononis natrix subsp. *natrix*	PE:E:EtOH	>1000
Capparis spinosa	Methanol	>1000
Fumaria officinalis	Tertiary alkaloid	>1000
Fumaria officinalis	Quarternary alkaloid	>1000

LC_{50} values of the plants are more than 1000, so that they are inactive.

According to the antibacterial and antifungal research, while the extract prepared from *H. triquetrifolium* showed some activity which was not considered to be significant, extracts prepared from *O. natrix* subsp. *natrix* (extract no. 2), *C. spinosa* (extract no. 3), *F. officinalis* which contains tertiary alkaloids (extract no. 4) and *F. officinalis* which contains quaternary alkaloids (extract no. 5) were found to be inactive.

EXPERIMENTAL

Preparation of extracts:

- *Hypericum triquetrifolium*: The aerial parts of the plant (650 g) were powdered and macerated in 60% ethanol for 48 hrs. Then the macerate was evaporated.

-*Ononis natrix* subsp. *natrix*: The aerial parts of the plant (400 g) were powdered and macerated in PE:E:EtOH (1:1:1) for 48 hrs. and evaporated. The crude extract was dissolved in methanol and kept in the freezer for 48 hrs. The precipitated part was filtered. The residue was used for this investigation.

-*Capparis spinosa* : The air dried (450 g) and powdered fruits of the plant were macerated in methanol and evaporated.

-*Fumaria officinalis* : The aerial parts of the plant (250 g) were powdered and macerated in ethanol for 48 hrs. The extract was evaporated and dissolved in 3% HCl. Then the solution was extracted with diethylether. The acidic part of the solution was basified with 10% NH_3 and extracted with $CHCl_3$. The extract obtained from the chloroform fraction contained the tertiary alkaloids. The aqueous part was acidified with 3% HCl and the saturated solution of KI in water was added in the ratio of 4%. The solution was extracted with $CHCl_3$ again. The extract obtained from the chloroform fraction contained the quaternary alkaloids.

Cytotoxic activity determination

The Brine Shrimp (*Artemia salina*) method:

1. 3,8 g sea salt is dissolved in 100 ml of water.
2. Sea water is put into the tank and shrimp eggs are added to the tank.
3. The shrimp are kept for two days to hatch and mature.

4. 20 mg of sample is weighed and dissolved in 2 ml of solvent. Vials for testing are prepared in 1000, 100 and 10 µg /ml concentrations and 3 vials are prepared for each concentration.
5. 2 days later when shrimp larvae are ready, 5 ml of sea water is added to each vial and 10 shrimp per vial are counted.
6. 24 hrs. later the number of survivors are counted and recorded.
7. The data is analysed with a computer to determine LC_{50} values and 95% confidence intervals.

Antimicrobial activity determination:

Agar well diffusion and tube dilution method were used in the antimicrobial activity tests. Mueller Hinton Agar, Mueller Hinton Broth for the bacteria, Sabouraud Dextrose Agar, Sabouraud Dextrose Broth and RPMI 1640 for the yeasts are used as media. The bacteria and the yeasts used in this research are shown in Table-2.

Table 2: Used microorganisms.

Bacteria	**Yeasts**
Staphylococcus epidermidis ATCC12228	*Candida albicans* ATCC 10231
Escherichia coli ATCC 11229	*Candida tropicalis* KUEN 1021
Staphylococcus aureus 6538 P	*Candida kefyr* KUEN 1012
Pseudomonas aeruginosa ATCC 1539	*Candida krusei* ATCC 6258
Proteus mirabilis ATCC 14153	*Candida guilliermondii* KUEN 998
Klebsiella pneumoniae ATCC 4352	*Candida glabrata* ATCC 90030

Agar well diffusion method: Mueller Hinton Agar (MHA), Mueller Hinton Broth (MHB) for the bacteria, Sabouraud Dextrose Agar (SDA), Sabouraud Dextrose Broth (SDB) for the yeasts are used as media. 1 ml of the dissolved *H. triquetrifolium* extract and 2 ml of the dissolved *O. natrix* subsp. *natrix, C. spinosa, F. officinalis* tertiary alkaloid and *F. officinalis* quaternary alkaloid extracts were diluted with distilled water in the ratio of 1/10. The prepared stock suspensions of the extracts were as follows, Extract no. 1 (16.49 mg/ml), Extract no. 2 (67.42mg/ml), Extract no. 3 (332.5 mg/ml), Extract no. 4 (23.5 mg/ml) and Extract no. 5 (50.67 mg/ml). Microorganism suspensions were prepared equal to the turbulence of Mc Farland 0.5 standart and diluted in the ratio of 1/100 and they were cultivated on the agar medium and its surface was set to dry. After that, 7 wells, each in 6 mm in diameter, were cut in the agar plate and 0.1 ml from each stock suspension of the extracts were placed in the wells. Thus the final concentrations of the extract suspensions were decreased in the ratio of 1/10. The solvent used for the extraction which has been diluted in the ratio of

1/10 and meropenem (50μg/ml) and fluconazole (100 μg/ml) for extract no.1 and meropenem (100μg/ml) and fluconazole (1000 μg/ml) for the other extracts which were tested in the same manner as controls. Then the petri dishes were kept in 37°C incubators for one night and the inhibition of the growth zones around the wells were evaluated subsequently. Occured zones were calculated in mm. The solvents were found to have no antibacterial and antifungal effect.

Tube dilution method: Macrodilution test was used in the evaluation of the Minimal Inhibitory Concentration (MICs) values of extracts that form inhibition zones on the microorganisms. Microorganism suspensions were prepared from the overnight microorganism cultures, equal to the turbulence of 0.5 Mc Farland standart ($1x10^8$ cfu/ml) and the microorganism cultures were diluted in 1/1000 ratio and adjusted approximately $1x10^5$ cfu/ml. Then 1 ml of sterile appropriate medium was distributed to the series of serologic tubes before MIC study. 1 ml was taken from the stock suspension of each extract and put into the 1st tube that contained 1 ml of appropriate broth medium. Then the first solution was mixed and 1 ml of it was transferred to the 2 nd tube, so each time it was diluted twice and this process was repeated for 8 tubes. The microorganism suspensions were added into each diluted tube. The 9th tube, the control tube, only contained the nutrition broth. These tubes were kept in the incubator overnight at 35°C. After incubation, the growth of microorganisms in each tube also was compared with the growth in the control tube (extract free). Meropenem (100μg/ml) for extract no.1 and meropenem (512 μg/ml) for the other extracts were treated in the same manner as the control agent.The lowest concentration that completely inhibited visible growth of the organism as detected by the unaided eye was recorded as the MIC.

For the yeasts, the reference macrodilution method was performed according to the NCCLS guideline (3). Colonies from 24 hr cultures of isolated *Candida* species were suspended in 6 ml of sterile 85 % saline. The final suspension was adjusted to the turbulance of 0.5 Mc Farland standard.

The reference macrodilution method was prepared by an inoculum which was 1:100 diluted with sterile water; this was followed by a 1:20 dilution with RPMI 1640 broth medium (Sigma) buffered to pH 7.0 with 0.165 M morpholinepropane sulfonic acid (MOPS) buffer. The final inoculum was $0.5x10^3$ cfu /ml. 0,9 ml of the adjusted inoculum was added to each tube containing 0.1 ml of the extract in each dilution series and the tubes were incubated at 35°C for 48 hrs. Extract-free tubes acted as growth controls, and yeast-free tubes were accepted as purity controls. Fluconazole (100μg/ml) for extract no.1 and fluconazole (5120 μg/ml) for the other extracts were treated in the same manner as control agent. MICs were determined as the minimum concentration of the extracts that prevented any discernible growth. The results are summarized in Table 3.

Table-3: The Antimicrobial activity of the extracts.

Bacteria	Extract no. 1		Meropenem		Extract no. 2		Extract no. 3		Extract no. 4		Extract no. 5		Meropenem	
	Zone dia. (mm)	MIC (µg/ml)	Zone dia. (mm)	MIC (µg/ml)	Zone dia. (mm)	MIC (µg/ml)	Zone dia. (mm)	MIC (µg/ml)	Zone dia. (mm)	MIC (µg/ml)	Zone dia. (mm)	MIC (µg/ml)	Zone dia. (mm)	MIC (µg/ml)
Staphylococcus epidermidis ATCC12228	15	256	22	6.25	7	>153,776	-		-		4	>28,856	31	0.0625
Eschericia coli ATCC 11229	-		18	1.56	-		-		-		-		21	0.0625
Staphylococcus aureus 6538 P	15	256	22	6.25	4	>153,776	-		5	10.143	5	3607	32	0.0625
Pseudomonas aeruginosa ATCC 1539	-		22	1.56	-		-		-		-		24	0.0625
Proteus mirabilis ATCC 14153	14	256	20	3.13	-		-		-		-		10	0.0625
Klebsiella pneumoniae ATCC 4352	-		17	1.56	-		-		-		-		18	0.0625
Yeast			**Fluconazole**										**Fluconazole**	
Candida albicans ATCC 10231	-		23	6.25	-		-		6	>20,285	14	>28,856	9	10
Candida tropicalis KUEN 1012	-		16	3.13	-		-		-		6	>28,856	9	10
Candida kefyr KUEN 1012	-		20	3.13	-		-		-		15	>28,856	32	1.25
Candida krusei ATCC 6258	-		8	50	-		-		-		6	>28,856	9	10
Candida guilliermondii KUEN 998	-		-		-		-		-		-		-	
Candida glabrata ATCC 90030	-		2.5	>100	-		-		-		5	>28,856	12	5

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