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ANTIMICROBIAL ACTIVITY OF MARIGOLD (*TAGETES MINUTA* L.)

MARIGOLD (*TAGETES MİNUTA* L.)'UN ANTİMİKROBİYAL AKTİVİTESİ

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In this study, ethyl acetate, acetone, chloroform and ethanol extracts of Tagetes minuta L. called Marigold were tested for antimicrobial activity by Disc Diffusion method on the following test microorganisms: Escherichia coli ATCC 11230, Enterobacter aerogenes CCM 2531, Staphylococcus aureus 6538P, Corynebacterium xerosis CCM 2824, Mycobacterium smegmatis RUT, Salmonella thyphimurium, Candida utilis La991, Rhodotorula rubra CCY, Aspergillus oryzae, Aspergillus flavus, Botrytis cinariae, Fusarium oxysporium, Streptomyces murinus ISP 5091 and Nocardia cornea IFO 14403.

According to our findings, they have shown antimicrobial activity against Gram (+) and Gram (-) bacteria, yeasts, filamentous fungi and actinomycetes. Acetone and ethanol extracts of Tagetes minuta L. were found to the particularly effective against M.smegmatis which is acid-fast, as compared to CFR (= Cefadroxil) used as reference.

Bu çalışmada, Tagetes minuta L.'dan etil asetat, aseton, kloroform ve etanol ekstreleri hazırlanarak disk difüzyon yöntemiyle test mikroorganizmalarına karşı bu ekstrelerin antimikrobiyal aktiviteleri denenmiştir. Test mikroorganizmaları olarak Escherichia coli ATCC 11230, Enterobacter aerogenes CCM 2531, Staphylococcus aureus 6538P, Corynebacterium xerosis CCM 2824, Mycobacterium smegmatis RUT, Salmonella thyphimurium, Candida utilis La991, Rhodotorula rubra CCY, Aspergillus oryzae, Aspergillus flavus, Botrytis cinariae, Fusarium oxysporium, Streptomyces murinus ISP 5091 ve Nocardia cornea IFO 14403 kullanılmıştır.

Bulgularımıza göre, Tagetes minuta L. ekstrelerinin bazı Gram (+) ve Gram (-) bakterilere, mayalara, filamentöz funguslara ve actinomycete'lere karşı antimikrobiyal aktivitesi bulunmaktadır ve özellikle asit-fast özellik gösteren M.smegmatis'e karşı aseton ve etanol ekstresi mukayese antibiyotiği olarak seçilen CFR (=Cefadroxil)'den daha etkilidir.

Keywords: *Tagetes minuta L.; Marigold; Antimicrobial activity*

Anahtar kelimeler: *Tagetes minuta L.; Marigold; Antimikrobiyal aktivite*

Introduction

Tagetes minuta L., which is originally distributed around Southern America (Argentina) and Mexico and naturally found in Africa, Europe (France, Italy and old Yugoslavia), Asia and Northern America, is a widespread plant. It has escaped from horticulture and naturalized in some parts of Turkey. It is locally called "Kokar Ot" in Turkey (1).

Tagetes minuta L., grown in India, France, Australia, Argentina, Nigeria and Egypt, has an economical importance as its oil is used in the perfume industry. Under several conditions, the ratio of essential oil obtained from flowers, leaves, and stem shows differences. Mainly, there are tagetone, ocimene, myrcene, linalool, limonene, pinene, carvone, citral, camphene, valeric acid, phenylacetaldehyde in *Tagetes* (2-5).

Tagetes essential oils are used in medicine, in cosmetics industry, and to impart smell and aroma to tobacco and drinks (6,7).

The powerful antihelmintic, antispasmodic, bactericidal, carminative, diaphoretic, emmenagogue, fungicidal and stomachic effects of *Tagetes* have been determined at various studies (2-7).

In this study, using the above facts, we aimed to determine the antimicrobial activity of the extracts of *Tagetes minuta* growing in Turkey against various microorganisms.

Materials and Methods

Materials

Tagetes minuta L. is an erect, globrous, strong smelling annual up to 50-150 cm, with short branches. Leaves 3-15x3-5 cm, pinnatisect; segments 3-7, 2-8x0.2-0.8 cm, linearlanceolate; only the lower leaves opposite. Capitula numerous in dense terminal corymbs. Involucre 8-12x1.5-2 mm, cylindirical of 3-4 yellowish-green bracts. Ligules usually 1-3 mm, obovate-spathulate, yellowish-green. Tubular florets 4-5, 3-4 mm, green. Achenes 4-6x0.5-1 mm, linear, black, with adpressed white hairs. Pappus of 5 scales 0.5-3 mm(8). Wild growing plants were collected at Bursa region:

A2 Bursa : Misi köy, on the river banks, 25.10.1994, H.Malyer, A. Bıçakçı BULU 9285.

In this study, the following microorganisms were used : *Escherichia coli* ATCC 11230, *Enterobacter aerogenes* CCM 2531, *Staphylococcus aureus* 6538P, *Corynebacterium xerosis* CCM 2824, *Mycobacterium smegmatis* RUT, *Salmonella thyphimurium*, *Candida utilis* La991, *Rhodotorula rubra* CCY, *Aspergillus oryzae, Aspergillus flavus, Botrytis cinariae, Fusarium oxysporium, Streptomyces murinus* ISP 5091 and *Nocardia cornea* IFO 14403. Test microorganisms were obtained from culture collection of Ege University, Faculty of Science, Basic and Industrial Microbiology Department.

Method

Preparation and Antimicrobial activity of Extracts

The plant material was ground to fine powder. 15 g of this material was subjected to Soxhlet extraction for 12h each using 150 ml of the following solvents ethyl acetate, acetone, chloroform and ethanol. The extracts were kept at +4°C (9-10).

In vitro antimicrobial studies were carried out by the Agar-Disc Diffusion method against test microorganisms. As a consequence of Mueller Hinton Agar (OXOID) was used as the most suitable medium for Antimicrobial activity studies. The sterilized medium at 45-50°C was poured into petri dishes. Agar depth was 4 mm. For 90 mm diameter plates 25 ml medium was used According to this method, Ethanol, Ethyl acetate, Acetone and Chloroform extacts were impregnated as four discs in ranging concentrations from 50 µl. Then all discs were dried in 50°C and placed into the bacteria and yeasts petri dishes. Each disc was 6 mm diameter. For each experiment a fifth disc which contained only solvent was used as control disc. As reference, antibiotic CFR (=Cefadroxil) was used. Experiments were repeated three times and results were expressed as average values.

Bacteria and yeast cultures were suspended in 4-5 ml Brain Heart Infusion Broth (OXOID). Bacteria were incubated in 37°C for 2-5 hrs. Yeast cultures were incubated in 30°C for 5-7 hrs. When a visible turbidity was obtained at the end of this time, the turbidity of bacterial suspension was adjusted against Macfarland Standart Tube with physiologic serum and inoculation was performed. Prepared bacterial suspension was mixed with a sterile applicator and excess fluid of applicator was removed by rotating the applicator to one side of the tube. Streak the entire Mueller Hinton Agar surface in 3 different directions by rotating the plate 60° angles after each streaking. Yeast cultures were inoculated into Mueller-Hinton Agar (10^2 cfu/ml). All petri dishes after inoculation were allowed to dry for 15-20 minutes in room temperature. For bacteria (in 35°C) and yeasts (in 30°C), inhibition zone diameters were measured after 24-48 hours using Agar-Disc Diffusion method (11, 12).

Spore suspension of filamentous fungi and actinomycetes were cultured on Sabouraud's Dextrose agar (10^5 cfu/ml) by plate dilution techniques using Thoma and Howard slides (13-15). It was observed that Agar-Disc diffusion method was generally not suitable for filamentous fungi and actinomycetes. Therefore this method was used after modification. In this experiment, the solutions (from 10 to 200 µg/ml) were added into the medium after autoclaving. Erythromycine (15 µg/ml) was used as a comparison antibiotic against filamentous fungi. The antibiotic was added into the medium. The evaluation of filamentous fungi and actinomycetes was carried out by means of reproduction on the medium and reduction of the colony numbers at the end of the seven days (16).

Results and Discussion

The zone diameters of the plates after incubation are given in Table 1.

According to our findings; All the extracts of *Tagetes minuta* have been found to be ineffective against *Escherichia coli*, as Gram (-) bacteria. Ethanol and ethyl acetate extracts have shown antimicrobial activity against *Enterobacter aerogenes* and the activity of ethanol was higher than that of CFR used comparison antibiotic. Against *S. aureus* and *Salmonella thyphimurium*, the extracts of *Tagetes minuta* have been determined to be less effective than CFR. The activity of acetone and ethanol extracts were more intense than the comparison antibiotic against *Corynebacterium xerosis*. Nevertheless, it has been determined that the antimicrobial activity of the comparison antibiotic was less against acid-fast *Mycobacterium smegmatis*.

The extarcts of *Tagetes minuta* have shown antiyeast activity against the yeasts. Ethyl acetate and ethanol extracts were effective against *Candida utilis*, all the extracts except for that of chloroform were found to be effective against *Rhodotorula rubra*.

The table 2 shows that the colony numbers of filamentous fungi and actinomycetes were reduced between 99.95 and 99.98% for the concentrations of 10 µg/ml and 50 µg/ml of the related compounds after the incubation whilst the concentrations 100 µg/ml and 200 µg/ml of these compounds inhibited filamentous fungi and actinomycetes growth completely. All results showed that colony numbers were reduced because of the activity of the compounds contained in the extracts.

Table 1. Antimicrobial activity of the extracts of Marigold (Tagetes minuta L.) on some bacteria and yeasts.

Tested Microorganisms	Zones of Inhibition (mm)			Comparison antibiotic	
	Ethyl Acetate	Acetone	Chloroform	Ethanol	CFR
Escherichia coli ATCC 11230	-	-	-	-	13.0
Enterobacter aerogenes CCM 2531	12.0	-	-	15.0	14.0
Staphylococcus aureus 6538P	-	14.0	10.0	-	24.0
Corynebacterium xerosis	-	13.0	-	14.0	12.0
Mycobacterium smegmatis RUT	-	19.0	-	20.0	17.0
Salmonella thyphimurium	-	-	-	12.0	18.0
Candida utilis La991	14.0	-	-	16.0	NT
Rhodotorula rubra CCY	14.0	14.0	-	13.0	NT

(-) : No Inhibition Zone, (NT) : Not Tested

Table 2. Antimicrobial activity of the extracts of Marigold (Tagetes minuta L.) on some filamentous fungi and actinomycetes.

		The colony numbers after incubation Compounds*				
Tested Organisms	Concentrations (µg/ml)	Ethyl Acetate	Acetone	Chloroform	Ethanol	Erythromycin (15 µg/ml)
Aspergillus Oryzae	10 50 100 200	43 30 - -	27 17 - -	46 32 - -	15 10 - -	15
Aspergillus flavus	10 50 100 200	52 41 - -	36 27 - -	33 28 - -	24 19 - -	18
Botrytis cineriae	10 50 100 200	48 35 - -	47 44 - -	39 35 - -	35 24 - -	15
Fusarium oxysporium	10 50 100 200	36 30 - -	44 36 - -	38 32 - -	33 25 - -	16
Streptomyces murinus ISP 5091	10 50 100 200	42 40 - -	35 25 - -	32 30 - -	30 28 - -	NT
Nocarida cornea IFO 14403	10 50 100 200	34 30 - -	18 15 - -	40 38 - -	22 17 - -	NT

* : Datas are the average of n=3 experiments
- : No growth NT: Not Tested

Inhibition zone diameters around control disc were measured as between 0-1 mm.

According to a previous study, the essential oil obtained from the flowers of *Tagetes minuta* showed antimicrobial activity against Gram (+) and Gram (-) bacteria and fungi using Agar-Diffusion and Atmospheric Diffusion methods (4). The essential oil was found to be particularly effective against *Mycobacterium pelligrino, Corynebacterium xerosis, Xanthomonas versicolor* and *Candida albicans*. In another study, the leaf oil of *Tagetes minuta* and *T. filifolia* showed antifungal activities against *filamentous fungi* such as *Sclerotium cepivorum, Colletotrichium coccodes* and *Alternaria solani, T. minuta* and *T. filifolia* exhibited the strongest fungitoxicity by completely inhibiting the mycelial growth of the test pathogen (17). Our findings were partly parallel to those reported in the above study. Since the secondary metabolites show difference on the parts of stem, flowers and leaves under different conditions (2-4), it can be said that the effect of *Tagates minuta* extracts on the microorganisms used is expected to vary according to the antimicrobial properties of the materials contained in these extracts.

As the result, *Tagetes minuta* has antimicrobial activity against some Gram (+) and Gram (-) bacteria, yeasts, filamentous fungi and actinomycetes. All extracts showed more antifungal activities than antibacterial activities. For antimicrobial activity, ethanol extract was the most effective. In addition, *Mycobacterium smegmetis* was found to be the most sensitive bacteria against the extracts of *Tagetes minuta.*

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