Original Article

Antimicrobial Activities and Some Flavonoids in Extracts of Some Medicinal Plants

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ABSTRACT

Origanum majorana, Melissa officinalis, Anthemis cotula and *Avena sativa* were extracted by using 65% ethanol to isolate their active constituents. The antimicrobial activities of extracts were investigated against 15 microorganisms by using the disk diffusion method, MIC (Minimum Inhibitory Concentration), MBC (Minimum Bactericidal Concentration) and MFC (Minimal Fungicidal Concentration) tests. Furthermore, the presence of some flavonoids were analyzed by using HPLC. It was determined flavonoids in the extracts of *O. majorana*, *M. officinalis*, *A. cotula* and *A. sativa*. As a results it was observed that *O. majorana* was active against *Staphylococcus aureus*, *Enterococcus faecium, Enterococcus faecalis, Salmonella typhimurium* and *Pseudomonas aeruginosa*, where *M. officinalis* was active against *Enterococcus faecium* and *Enterococcus faecalis*. On the other hand, both *A. cotula* and *A. sativa* were observed to be active against *Enterococcus faecium*. The extracts of plant samples showed antibacterial activity against tested microorganisms at different levels.

Keywords: *Origanum majorana, Melissa officinalis, Anthemis cotula, Avena sativa*, Antimicrobial activity, Flavonoid.

INTRODUCTION

For a long period of time, plants have been a valuable source of natural products for maintaining human health. Nowadays, the use of phytochemicals for pharmaceutical purpose has gradually increased in many countries. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use medicinal plants.[1]

The use of crude extracts of plants parts and phytochemicals, of known antimicrobial properties, can be of great significance in the therapeutic treatments. In recent years, a number of studies have been conducted in various countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to the secondary metabolites synthesized by the plants.[2,3] These products are known by their active substances like, phenolic compounds which are part of the essential oils, as well as in tanning.[4] The screening of plant products for antimicrobial activity have shown that the higher plants represent a potential source of novel antibiotic prototypes. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases.[5] This has forced scientist to search for

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new antimicrobial substances from various sources like the medicinal plants. Plant produces a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However, very little information is available on such activity of medicinal plants and out of the 400.000 plant species on earth, only a small number has been systematically investigated for their antimicrobial activities.[6]

MATERIALS AND METHODS

Plant Samples

Origanum majorana, Melissa officinalis, Anthemis cotula and *Avena sativa* were purchased from Özşen Lokman Hekim Company located in Ankara/Turkey, Gimat at 2016.

Extraction method

The plants were washed with water and air dried under shade. The dried plant sample were ground in a mixer. 10-30 g ground samples were extracted with 250 mL of ethanol (65%) in a Soxhlet apparatus by continuous heat extraction for 24 h. Extracts were prepared according to previous studies and stored at 4°C for further studies.[7]

Determination of Antimicrobial Activities

Preparation of Extract Stock

Extract stocks to test the antimicrobial activity were prepared by dissolving 1 mg of extract in each 3 mL of ethanol for disk diffusion test, where the solvent was distilled water for MIC tests. The extract stock prepared for MIC test was sterilized through 0.45 µm filter (Millipore).

Strains

Bacillus subtilis DSMZ 1971, *Candida albicans* DSMZ 1386, *Enterobacter aerogenes* ATCC 13048, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium*, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* DSMZ 50071, *Pseudomonas fluorescens* P1, *Salmonella enteritidis* ATCC 13075, *Salmonella infantis*, *Salmonella kentucky*, *Salmonella typhimurium* SL1344, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* DSMZ 20044 were used in the study.

Preparation of Inocula

All strains were incubated according to their requirements as it was previously mentioned.[7]

The inoculate was prepared according to previous studies and adjusted.[8,9] Thus standard inoculate contained adjusted number of microorganisms.[10,11]

Disk diffusion test

The disk diffusion test was applied as it was mentioned in the previous studies.[12,13] 20, 50 and 100 µL of extracts were loaded on sterile disks as mentioned before.[14] The inoculation process was described as in the previous studies.[13] Inhibition zones were defined in mm.[15]

Minimum Inhibitory Concentration (MIC) Test

A broth micro-dilution MIC test was applied as mentioned before.[16] Two-fold dilutions of the extracts were prepared ranging from 33 mg/mL to 6.50 µg/mL.

Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) Test

The wells, where no visual growth were observed in MIC test were used for further analysis called MBC and MFC tests, which were conducted according to previous studies.[17]

Controls

Empty disks and sterilized broth medium were used as negative controls, where broth medium inoculated with each microorganism was used as positive control of microorganisms.[18,19]

Statistics

All tests were done in triplicates. Statistical analysis was conducted as mentioned in previous studies.[20] $p < 0.05$ was considered as statistically significant.

HPLC Analysis

HPLC analysis were carried out by using an Agilent Eclipse XDB C18 5 µm with 4.6 x 250 mm column and studied at the column temperature of 30 °C. Flavonoids measurements were determined at 280 nm after 20 µL injection volume. Standard solvents were prepared in the ethanol-distilled water (65-35) mixture. For HPLC analysis, mobile phase-A containing water-10% formic acid (95-5), mobile phase-B containing acetonitrile-10% formic acid (5-95) were used. Mobil phases were flowed for during 39 min.

RESULT AND DISCUSSION

Table 1: HPLC analyses results of flavonoids (µg/g plant)

	Catechin (µg/g)	Epicatechin (µg/g)	Rutin (µg/g)	Naringin (µg/g)	Myricetin (µg/g)	Luteolin (µg/g)	Naringenin (µg/g)	Apigenin (µg/g)
O. majorana	17.23	-	18.34	0.79	34.22	33.55	0.70	2.08
M. officinalis	-	-	19.06	10.50	22.85	64.17	961.75	-
A. cotula	-	0.81	4.51	1.41	40.32	1.42	0.48	0.21
A. sativa	-	-	-	-	1.47	0.13	0.36	0.58

Table 2: Disk Diffusion Test (mm) and MIC (*µg*/mL) and MBC/MFC (mg/mL) results

	O. majorana			M. officinalis			A. cotula			A. sativa		
	20 µL	50 µL	100 µL	20 µL	50 µL	100 µL	20 µL	50 µL	100 µL	20 µL	50 µL	100 µL
C. albicans	-	-	-	-	-	8	-	-	-	-	-	-
E. faecalis	-	9	11	-	10	13	-	-	-	-	-	-
E. faecium	-	7	9	-	9	8	-	7	9	-	8	7
P. aeruginosa	-	-	8	-	-	-	-	-	-	-	-	-
S. typhimurium	-	8	10	-	-	-	-	-	-	-	-	-
S. aureus	-	9	10	-	-	-	-	-	-	-	-	-
No activity:	B. subtilis, E. aerogenes, E. coli, K. pneumonia, P. fluorescence, S. enteritidis, S. infantis, S. kentucky, S. epidermidis											

	O. majorana		M. officinalis		A. cotula		A. sativa	
	MIC	MBC MFC	MIC	MBC MFC	MIC	MBC MFC	MIC	MBC MFC
C. albicans	-	-	>3330	-	-	-	-	-
E. faecalis	26.02	>3330	6.50	>3330	-	-	-	-
E. faecium	26.02	>3330	26.02	>3330	26.02	>3330	52.03	>3330
P. aeruginosa	26.02	>3330	-	-	-	-	-	-
S. typhimurium	13.01	>3330	-	-	-	-	-	-
S. aureus	416.25	>3330	-	-	-	-	-	-

The HPLC results of the extracts are presented in **Table 1**, and the antimicrobial activity test results are presented in **Table 2**.

While myricetin, luteolin and naringenin were detected in all extracts, catechin was only determined in extract of *O. majorana*. Epicatechin was only found in extract of *A. cotula*. Rutin and naringenin in other extracts apart from *A. sativa*, apigenin in other extracts apart from *M. officinalis*, were detected. The most abundant flavonoids were observed as myricetin (in extracts of *A. cotula* and *O. majorana*), luteolin and naringenin (in extract of *M. officinalis*) in **Table 1**. And Also, disk diffusion test results given in **Table 2** show that *O. majorana* is active against *E. faecalis*, *E. faecium*, *P. aeruginosa*, *S. typhimurium* and *S. aureus* with inhibition zones between 7-11 mm, where *M. officinalis* is active against *C. albicans*, *E. faecalis* and *E. faecium* with inhibition zones between of 8 - 13 mm. On the other hand, *A. cotula* was observed to be active against only *E. faecium* with inhibition zones between 7-9 mm depending on the amount of extract loaded on disks, where *A. sativa* was observed to be active against *E. faecium* with inhibition zones between 7-8 mm.

To the extracts and microorganism combination which presented antimicrobial activity were chosen for MIC value determination. Results given in **Table 2** show that *O. majorana* is active against *E. faecalis*, *E. faecium*, *P. aeruginosa*, *S. typhimurium* and *S. aureus* with MIC values of 26.02 µg/mL, 26.02 µg/mL, 26.02 µg/mL,

13.01 µg/mL and 416.25 µg/mL respectively, where *M. officinalis* is active against *C. albicans*, *E. faecalis* and *E. faecium* with MIC values of >3330 µg/mL, 6.50 µg/mL and 26.02 µg/mL respectively. On the other hand, *A. cotula* was observed to be active against only *E. faecium* with MIC value of 26.02 µg/mL, where *G. A. sativa* was observed to be active against *E. faecium* with a MIC value of 52.03 µg/mL.

MBC/MFC test showed that all the MIC values observed were bacteriostatic/fungistatic concentrations, which means they only inhibit the reproduction of microorganisms. On the other hand, it wasn't possible to identify the "cidal" concentrations for all plant extracts and microorganism combinations, which presented an activity in MIC test, and were given as MBC/MFC>3330 µg/mL.

CONCLUSION

Catechin and epicatechin are the least common flavonoids in the studied plants, whereas myricetin, luteolin and naringenin were found as the most common flavonoids. However, the plant extracts seem to be rich in the flavonoids studied. The active compounds and their mode of actions especially for *O. majorana* needed to be analyzed in further studies.

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CONFLICT OF INTEREST

None

ABBREVIATION USED

HPLC: High Performance Liquid Chromatography.

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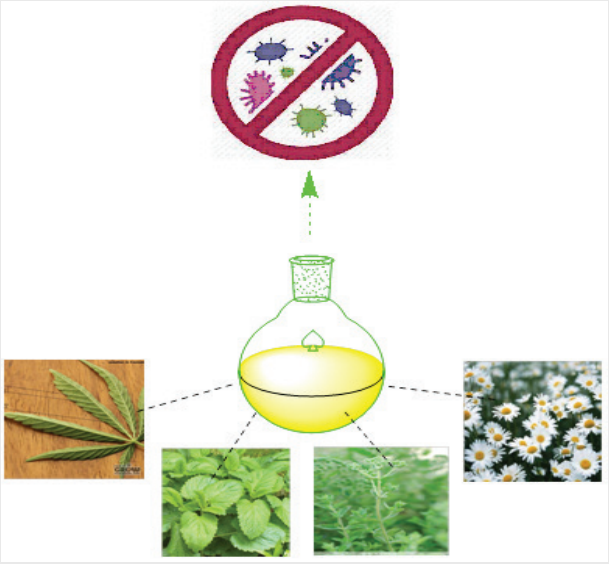
PICTORIAL ABSTRACT



SUMMARY

- *Origanum majorana*, *Melissa officinalis*, *Anthemis cotula* and *Avena sativa* were extracted
- The antimicrobial activities were investigated against 15 microorganisms by using the disk diffusion method, MIC (Minimum Inhibitory Concentration), MBC (Minimum Bactericidal Concentration) and MFC (Minimal Fungicidal Concentration) tests.
- The presence of some flavonoids were analyzed by using HPLC.

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