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Phytochemical Analysis and Antimicrobial Activity of Different Extracts of Fig Leaves (*Ficus carica* L.) from West Anatolia against some Pathogenic Microoorganisms

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The aim of the study was to investigate phyto chemical compoundsof fig leaves and antimicrobial activity of different extracts. The extract of ethanol, methanol, aqueous fig leaves (*Ficus carica* L.) were screened *in vitro* for their antimicrobial activity against Gram-positive, Gram-negative bacteria and one yeast.. Ethanolic extracts of fig leaves showed various antimicrobial activity (12-28 20μL^{-1} inhibition zone) to the microorganisms tested. The methanolic extracts showed antimicrobial activity (9-15 20μL^{-1} inhibition zone) to the microorganisms tested. The aqueous extracts showed no inhibition zone three out of ten microorganisms. The ethanolic extract displayed the best activity (MIC 6.25 μg/mL) against *Bacillus cereus* CCM 99. Other microorganisms (*Escherichia coli* ATCC 35218, *Salmonella typhimurium* CCM 583, *P. aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633) were showed between MIC 6.25-200 μg/mL. The methanolic and aqueous extracts were between MIC 25-400< μg/mL and MIC 200-400< μg/mL, respectively. The GC/MS analyses allowed 11 compounds to be determined; the main constituents of the fig leaves extract were etanol (94.36%), 6-methylthiol(1)benzothienoquinoline (0.94%) and methyl benzoylformate (0.84%).

Key words: *Ficus carica* L., Leaf extract, Antimicrobial activity, Phytochemical composition, GC/MS.

Ficus constituted one of the largest genera of medicinal plant with about 750 species of woody plants, trees, and shrumbs primarily occuring in subtropical and tropical regions with through out the world. The genus is remarkable for the large variation in the habits of its species[1]. *Ficus carica* is commonly referred as ''Fig'' various parts of the plant like bark, leaves, tender shoots, fruits, seeds and latex are medicinally important, belongs to the mullberry tree (Moraceae) which is one of the oldest fruits in the world. It has been used as a digestion promoter and a cure for ulcerative inflammation and eruption in Turkey. Its fruit, root and leaves are used in the native system of medicine because of high content of alkoloids, flavonoids, coumarins, saponins, terpenes and phenolic compounds[2-6]. These compunds causes antimicrobial activity. Based on these findings, some researchers have tested antimicrobial activity of fig leaves[7-10].

The present study is the first report on antimicrobial properties of West Anatolian fig leaves against a wide spectrum of antibiotic-resistant bacteria and human pathogenic bacteria. The objectives of this work is to investigate the antimicrobial activities of three different solvent extracts from West Anatolian fig leaves and to determine the chemical compound content to find

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out the relationship between antimicrobial activity and the compound content. Therefore, we have tested antimicrobial effect against some microorganisms and the antimicrobial activity was measured by using disk diffusion method and minimal inhibitory concentration (MIC).

MATERIALS AND METHODS

Collection of plant material

Fig (*Ficus carica* L.) leaves were obtained from Cine region and were collected in September West Anatolian of Turkey (N:37-32' 30.1'', E:28° 08' 35.6 altitude: 520m).

Preparation of leaf extracts

They were sun dried to a constant weight and milled to a fined powder using a porcelain muller. The powdered leaf (25 g) was soaked in 150 ml of distilled deionizer water to prepare the aqueous extract and in 150 ml of absolute ethanol (96º, Fluka Chemical) and in 150 ml of absolute methanol (97º, Fluka Chemical) to prepare the ethanolic and methanolic extracts. The suspension was stirred at 200 rpm at room temperature for 4 days after which it was filtered with the aid of a Whatman No 1 filter paper. The residue was re-extracted with 150 ml of the solvent as described. The combined extract were then evaporated to dryness at 40 ºC, re-dissolved in the corresponding solvent to obtained extracts (400 mg/ml) and stored at 4 ºC prior to use[11].

Microorganisms and media

The bacteria (*Staphylococcus aureus* ATCC 6538/P, *Bacillus cereus* CCM 99, *Bacillus subtilis* ATCC 6633, *Streptococcus faecalis* ATCC 8043, *Klebsiella pneumoniae* CCM 2318, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* CCM 583, *Aeromonas hydrophila* ATCC 19570, *Escherichia coli* ATCC 35218) and *Candida albicans* ATCC 10239 were obtained the stock culture collection of the Basic and Industrial Microbiology Section of Ege University, Izmir, Turkey. Cultures of these bacteria were grown in brain heart infusion broth (Merck) at 37 ºC for 24 h and *C. albicans* was incubated in sabouraud dextrose broth (Merck) at 30 ºC for 48 h.

Screening of antimicrobial activity of extracts

The antimicrobial activity of *Ficus carica* leaf extracts against the selected microorganisms was evaluated by disc diffusion method[11].

A 20 ml of the molten medium was seeded with 0.2 ml of broth cultures of the test organisms in sterile petri dishes. The petri dishes were rotated slowly to ensure a uniform distribution of microorganisms. The Mueller Hinton Agar (Merck) was left to solidify in the dish for bacterial strains, the sabouraud dextrose agar (Merck) for *C. albicans*. 20 µl of each extract (400 mg of extract/ml) were inoculated into the 6.0 mm diameter sterile discs with the aid of a sterile pipettes. The discs were take placed onto the dishes. The plates were allowed to stand for 30 min at room temperature to allow for proper diffusion of the extract to take place. The bacteria were then incubated at 37 ºC for 24 h and *C. albicans* was incubated at 25 ºC for 48h. At the end of the incubation period, inhibition zones formed on the medium were measured in mm. In addition, commercial antibiotic discs such as tobramycin (10 µg/disc) (Oxoid), ampicillin (10 µg/disc) (Oxoid) and nystatin (30 µg/disc) (Oxoid) were used as positive control and water, ethanol, methanol were used as negative control to determine the sensitivity of the tested strains. Whole studies were performed in three times and the results were expressed as average values.

Determination of minimal inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined for five of test microorganisms that determined the best inhibition on. The microwell dilution assay with slight modification was performed by using the CLSI standards[12,13]. A sterile 96 microtiter well plate was labeled. A volume of 100 µL of extract solution was pipetted into the first row of the plate. To all other wells 50 µL of double strength mueller hinton broth or potato dextrose broth was added. Serial dilutions were performed using a micropipette (A1-A10). Tips were discarded after use such that each well had 50 µL of the test material in serially descending concentrations. Then, 50 µL of broth containing bacterial suspension (5×10^6 cfu/mL) or *C. albicans* (5×10^5 cfu/mL) was added to each well. Each column of wells contained a single antimicrobial extract in progressive dilutions and was inoculated with a single microorganism. This analysis was performed at final concentrations of each extract (400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 mg of extract/ml).

Each plate had a set of both a growth

(A11) and sterility control (A12). Plates were sealed with clean film to ensure that microorganisms did not become dehydrated. The plates were prepared and placed in an incubator set at 37°C for 18-24 h and at 25ºC for 48 h, respectively for bacteria and *C. albicans*. After incubation, added 10 µL of 0.2% 2,3-5 Triphenyl tetrazolium chloride (TTC) solution to each well of microtitre plate. The plates containing TTC were incubated one h at 37°C for reaction. The color change was then assessed visually. Any color changes from purple to pink, which showed the growth of organism. MIC concentration does not exhibit reduction of TTC into formazan so the MIC in mg/ml was defined as that the lowest inhibitory concentration of each extract contained in the microtiter well in which the absence of visual color change (colorless) first observed. The average of five values was calculated and that was the MIC for the test extract and microorganism.

GC/MS analysis

The steam-distilled components were analysed by GC/MS. A HP 6890 gas chromatograph equipped with a HP-PTV and a 0.32mX0.60m HP-Innowax capillary column (0.5µm coating) was employed for the GC analysis. GC/MS analysis was performed on a HP-5973 mass selective detector coupled with a 6890 gas chromatograph, equipped with a HP 6890 gas chromatograph, equipped with HP-1capillary column. The column temperature was programmed from an initial temperature of 60 ºC to a final temperature of 250 ºC at 15 ºC/min. The carrier gas was helium (14.1mL/min). Identification of the individual components was performed by comparison of mass spectra with literature data and by a comparison of their retention time (Rt) relative to a $C_{8-}C_{32}$ *n*-alkanes mixture[14]. A computerized search was carried out using the Wiley 7n.l GC/MS library and ARGEFAR GC/MS library created with authentic samples.

RESULTS AND DISCUSSION

In the study, ethanol and methanol were analysed as negative control and they did not show inhibition against tested microorganisms. Ethanolic extracts of fig leaves showed various antimicrobial activity (12-28 20µL^{-1} inhibition zone) to the microorganisms tested. The methanolic extracts showed antimicrobial activity (9-15 20µL^{-1} inhibition zone) to the microorganisms tested. The aqueous extracts showed no inhibition zone three out of ten microorganisms. The ethanolic extracts was found to be the most effective antimicrobial agent as compared to the methanolic and aqueous extract. Aref *et al.,* (2010) showed that ethyl acetate and chloroform were the best solvents for many constituent extraction of latex antimicrobial substances compared to other solvents such as water, hexane, methanol, ethanol[15].

Table 1. Antimicrobial activity of figs (*Ficus carica* L.) leaves by the disc diffusion method

Microorganisms		*Ficus carica* L.			Standard antibiotics		
		Ethanol extract	Methanol extract	Aqueous extract	TOB	AMP	NYS
E. coli ATCC 35218	G(-)	21	10	11	10	12	NT
S. aureus ATCC 6538/P	G(+)	16	10	9	13	15	NT
A. hydrophila ATCC 19570	G(-)	15	9	8	11	10	NT
S. typhimurium CCM 583	G(-)	20	10	0	10	12	NT
P. aeruginosa ATCC 27853	G(-)	19	11	0	11	9	NT
K. pneumoniae CCM 2318	G(-)	12	10	9	11	9	NT
B. subtilis ATCC 6633	G(+)	28	15	14	17	10	NT
B. cereus CCM 99	G(+)	25	13	11	18	12	NT
S. faecalis ATCC 8043	G(+)	18	10	8	9	14	NT
C. albicans ATCC 10239	Y	12	10	0	NT	NT	20

Results (mean of three replicates) indicate zone of inhibition in mm and include filter paper disc diameter (6 mm), TOB: Tobramycin (10 µg/disc), AMP: Ampicillin (10 mg/disc), NYS: Nystatin (30 µg/disc), G: Gram reaction, Y: Yeast, NT: Not tested.

Aref *et al.,* (2010) reported that in vitro antimicrobial proprieties against five bacteria species and seven strains of fungi.The antimicrobial activity of the extracts was evaluated and based respectively on the inhibition zone using the disc– diffusion assay, minimal inhibition concentration (MIC) for bacterial testing and the method by calculating inhibition percentage (I%) for fungi inhibiting activities.The methanolic extract had no effect against bacteria except for *Proteus mirabilis* while the ethyl acetate extract had inhibition effect on the multiplication of five bacteria species (*Enterococcus faecalis, Citrobacter freundei, Pseudomonas aeruginosa, Escherchia coli* and *Proteus mirabilis*). For the opportunist pathogenic yeasts, ethyacetate and chlorophormic fractions showed a very strong inhibition (100%); methanolic fraction had a total inhibition against *Candida albicans* (100%) at a concentration of 500µg/ml and a negative effect against *Cryptococcus neoformans*. *Microsporum canis* was strongly inhibited with methanolic extract (75%) and totally with ethyl acetate extract at a concentration of 750µg/ml. Hexanoic extract showed medium results[15]. In a similarly, Aref *et al*., (2011) showed that the ethyl acetate extracts had inhibition effect on the growth of five bacterial species: *E. faecalis*, *C. freundii*, *P. aeruginosa*, *E.coli* and *P. mirabilis*. The inhibition values on these microorganisms were sensitive to ethylacetate extracts in the range of 8 to 16 mm, while hexanoic and chloroformic extracts were active against these six tested bacteria at a sensitive range of 8 to 15 and 8 to 14 mm, respectively. Methanolic extracts had no effect against the previous bacteria except for *P. mirabilis* with inhibition diameter of 14 mm. *P. mirabilis* was the most sensitive germ at a range of 0.33 to 0.041 mg/ml. The Hexanoic extracts were the only fractions active against *P. aeruginosa* which was the most resistant germ at MIC of 5.00 mg/ml. These extracts exhibited the most important activity against *P. mirabilis* and *S. auerus*. *E. coli* was also inhibited by all fractions at concentrations of 5 to 0.66 mg/ml[16].

When it was compared to MIC value of the ethanolic, methanolic and aqueous extracts,

Table 2. The MIC values (µg/ml) of *Ficus carica* against microorganisms tested in the microwell dilution assay

Microorganisms		Concentration range (400-0.39 mg of extract/ml) *Ficus carica* L.			Concentration range (400-0.39 µg/ml) Standard antibiotics	
		Ethanol extract	Methanol extract	Aqueous extract	GN	ERY
E.coli ATCC 35218	G(-)	25	400<	400<	0.78	0.39
S. typhimurium CCM 583	G(-)	50	200	400<	3.12	0.78
P. aeruginosa ATCC 27853	G(-)	200	400	400<	1.56	0.39
B. subtilis ATCC 6633	G(+)	6.25	50	200	0.39	0.78
B. cereus CCM 99	G(+)	3.12	25	400	0.39	0.39

GN: Gentamycin, ERY: Erythromycin, G: Gram reaction

Table 3. Volatile components of the ethanol extracts of fig leaves extracts (GC-MS analysis)

Component[a]	Area(%)	Rt[b]
Acetaldehyde	0.43	3.79
Ethyl Acetate	0.22	4.79
Etanol	94.36	5.14
1,1,3,3 Tetramethyl-, 1,3-diethoxydisiloxane	0.5	6.63
Octamethylcyclotetrasiloxane	0.48	5.85
Chloroform	0.71	6.95
Hexamethylcyclotrisiloxane	0.21	13.56
Pentadecane	0.03	14.54
Methyl benzoylformate	0.84	16.27
Decamethyltetrasiloxane	0.12	19.17
6-methylthiol (1) benzothieno-quinoline	0.94	19.57
Unidentified	1.60	19.68
Total	100	-

[a] Components listed in order of elution from a HP-1capillary column
[b] Retention time (as min).

the ethanolic extract displayed the best activity (MIC 3.12 µg/ml) against *B. cereus* CCM 99. Other microorganisms (*E.coli* ATCC 35218, *S. typhimurium* CCM 583, *P. aeruginosa* ATCC 27853, *B. subtilis* ATCC 6633) were showed between MIC 6.25-200 µg/ml. The methanolic and aqueous extracts were between MIC 25-400< µg/ml and MIC 200-400< µg/ml, respectively. The results of MIC showed that ethanolic extracts were the best activity compared to methanol and aqueous like disc diffusion method. Methanol is a toxic solvent, so it must not be used in food systems.

Lee & Cha (2010) reported that the antimicrobial activity of methanol (MeOH) extract of fig leaves against methicillin resistant – *Staphylococcus aureus* (MRSA) isolated in clinic. The synergistic effect of MeOH extract with oxacillin or ampicillin was shown as reduced ≥4-8 fold in most of tested MRSA, producing a synergistic effect as defined by (the fractional inhibitory concentration index) FICI ≤0.375-0.5. Furthermore, combination of the MeOH extract with oxacillin or ampicillin showed a more rapid decrease in MBC (minimum bactericidal concentration) than MeOH extract alone[7]. The MeoH extract (MICs, 2.5-to 20 mg/mL; MBCs,5 to 20 mg/mL) was demonstrated as antibacterial activityin isolates MRSA 1-20. In a similar study, Jeong *et al* (2009) showed that the antibacterial activity of the MeOH extract of *F.carica* leaves showed strong activities aganist *Streptococcus gordonii*, *Streptococcus anginosus, Prevotella intermedia, Aggregatibacter actinomycetem-comitans* and *Prevotella gingivalis* (MIC, 0.15 to 0.625 mg/mL, MBC 0.313 to 0.625 mg/mL)[8]. And also, Sharma and Sharma (2010) reported that the methanolic extract of *Ficus carica* possessed significant antibacterial and antifungal activity when compared with the other extracts (petroleum ether, chloroform, ethyl ether, ethanol) and standard drugs. In contrast to the other studies, we suggested that the ethanolic extract was more efficient than other extracts[17]. The main disadvantage of the results of *in vitro* studies that is difficult to compare each other because of the different test methods, different methods of extraction, test assays, and variation in chemical phytoconstituents in plants due to different agroclimatic conditions and plant phenotype[18]. The antibacterial activities were wide variations according to the species, subspecies or variety and essential oils of some plants belonging to the same taxa but collected from different localities showed different levels of antimicrobial activities[19,20].

Al-Sabawi (2010) tested ethanolic extract of leaves (EEL) at different concentrations and latex (LX) of *Ficus carica* against *Enterococcus faecalis*, and to evaluate the most effective concentration of EEL and LX against *Enterococcus faecalis* in dentinal tubules when used as intracanal medicament[21]. The different concentrations of EEL (5%, 2.5%,1.25%, 0.6%, 0.3%, 0.1%) and LX against *Enterococcus faecalis* was evaluated by broth microdilution method using spectrophotometer. According to this study, 5% EEL had best effect than other concentrations but its effect less than LX but significantly not different.. Al-Sabawi (2010) concluded that the EEL at 5% and LX had sufficient antibacterial effect against *Enterococcus faecalis* in the infected dentinal tubules when they are used as intracanal medicaments[21].

It is not suprising that that there are differences in the antimicrobial effect of extract, due to soluble differences among the antimicrobial compounds.These kinds of differences in susceptibility among the microorganisms against antimicrobial substances in plant extracts may be explained by the differences in cell wall composition.

The ethanol extracts of fig leaves were also evaluated for their chemical composition by GC/MS in this study. The GC/MS analyses allowed 11 compounds to be determined; the main constituents of the fig leaves extract were etanol (94.36%), and 6-methylthiol(1) benzothi-enoquinoline (0.94%) and methyl benzoylformate (94.36%), and 6-methylthiol(1) (0.84%) (Table 3). Quinolone derivatives useful as an antimicrobial agents. It contains quinoline alkaloids.

Conclusion, on the basis of the present investigations, it can be highlighted that ethanolic extract of fig leaves show promising antibacterial properties and could be exploited in herbal preparations against bacterial infections at least external uses.Antimicrobial activity based on quinoline alkaloids.The effect of this plant on more pathogenic organisms and toxicological investigations and further purification however, needs to be carried out.

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