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Determination of the chemical composition and antimicrobial activity of *Frankenia hirsuta*

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Abstract

Frankenia hirsuta is widely located in Turkey, but the antimicrobial potential and biochemical composition analysis of it weren't determined yet. By using the disk diffusion method, the susceptibility of 17 bacteria and 1 fungi were analyzed, which included *Bacillus, Candida, Enterobacter, Enterococcus, Escherichia, Klebsiella, Listeria, Pseudomonas, Salmonella* and *Staphylococcus* genera. 0.8, 1.5 and 3.1 mg of samples were prepared by using absolute ethanol. The bioactive composition of the plant extract was determined by gas chromatography-mass spectroscopy, and National Institute of Standards and Technology library was used for the mass spectra analysis. The results showed that *F. hirsuta* had antimicrobial activity against all of the studied micro-organisms except *E. aerogenes* and *E. coli*. Several active metabolites were identified, but some composition of this sample didn't match with the library. These results are the first report for the antimicrobial potential and biochemical composition of *F. hirsuta*.

Introduction

Foodborne pathogens cause critical problems all over the world. They don't just cause dangerous illnesses, but cause food spoilages, which is main problem in the food production and storage. Food source microbiological quality is an important topic for human health and the main reason for food contamination is foodborne pathogens. There are 15 major foodborne pathogens, which cause annually 76 million illness and 5,000 death in US. The most common are *Listeria monocytogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella* and *Candida* spp., which exist in various types of foods (Mead et al., 1999). According to US report in 2010, only *E. coli* and *Salmonella* spp. cause 1.4 million foodborne illnesses and responsible for a $2.7 billion loss (USDA, 2010).

Discovery of new drugs/agents has critical importance, thus the investigation of antimicrobial potentials of aromatic plants and the analysis of their biochemical composition are required. They have the potential to synthesize different antimicrobial compounds, therefore scientific research became a popular trend to determine their unknown biochemical composition and their activity (Daglia, 2011).

Frankenia genus are salt tolerant aromatic plants and related research is limited (Hamzaoglu and Akson, 2009). *Frankenia hirsuta* belongs to Frankeniaceae family and it is widely distributed in Turkey (Vural et al., 2014). Its antiphage (Delitheos et al, 1997) and antischistosomal (Yousif et al., 2007) activities were identified before, however antimicrobial activity and its biochemical composition weren't analyzed yet.

The main purpose of this study was to investigate antimicrobial activity of *F. hirsuta* against 18 microorganisms and determining its biochemical composition.

Materials and Methods

Plant samples

F. hirsuta is an aromatic halophytic plant in Turkey. Samples collected from salty soil ground of Aksaray.

Extraction procedure

F. hirsuta samples were dried under shade and they were ground into fine powder by a grinder. These samples were shaken in absolute ethanol (Sigma-Aldrich) at 125 rpm for 2 days at room temperature (Canli et al., 2016a). After that, all of them were filtrated through Whatman No. 1 filter paper into evaporation flasks. Filtrates were evaporated by a rotary evaporator (Buchi R3) at 45°C (Altuner et al., 2012). Finally, remnants were collected and they are used to prepare as 0.8, 1.5 and 3.1 mg/mL stocks.

Microorganisms

A broad range of Gram positive bacteria, Gram negative bacteria and yeast were chosen to analyze the antimicrobial effect of *F. hirsuta*. For this reason, 17 bacteria and 1 fungi species were used and these microorganisms were sustained on nutrient agar (BD Difco, USA). There were 11 bacteria and 1 fungus. Five Gram positive bacteria were *Bacillus subtilis* DSMZ 1971, *Enterecoccus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* DSMZ 20044. The Gram negative bacteria were *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* DSMZ 50071, *Pseudomonas fluorescens* P1, *Salmonella enteritidis* ATCC 13075 and *Salmonella typhimurium* SL1344. The fungus was *Candida albicans* DSMZ 1386. Besides, there were 6 non-standard bacteria, which were isolated from the food at Ankara University, Department of Biology, Microbiology Laboratory. Three of them are Gram positive bacteria, which were *Enterococcus durans, Enterecoccus faecium* and *Listeria innocua*. The others are Gram negative bacteria, which were *Klebsiella pneumoniae, Salmonella infantis* and *Salmonella kentucky*.

Preparation of inocula

All bacterial strain were incubated at 37°C for 24 hours, however *Candida albicans* DSMZ 1386 was incubated at 27°C for 48 hours (Canli et al., 2017a). Each bacteria and yeast were inoculated into 0.9% sterile saline solution and adjusted to 0.5 McFarland standard, in order to standardize the inocula to contain about 10^8 cfu/mL for bacteria and 10^7 cfu/mL for *Candida albicans* (Canli et al., 2015).

Antimicrobial activity test

The antimicrobial activity *F. hirsuta* of ethanol extract was performed by disk diffusion test, as described by Altuner et al. (2013). Firstly, Mueller Hinton agar (BD Difco, USA) was poured into 90 mm sterile petri dish in order to reach a meant depth of 4.0 ± 0.5 mm. 20, 40 and 80 µL of extracts were loaded on 6 mm oxoid antimicrobial susceptibility test disks. Disks were left to dry overnight at 30°C in sterile conditions in order to prevent any remaining of solvent, which may interfere with the results. After that, prepared microorganisms, which were inoculated into the saline solution were streaked on the surface of the petri dishes. These plates were left to dry for 5 min at room temperature in aseptic conditions. Next, disks were tightly applied to the surface of plates. Finally, these plates were incubated and the inhibition zone diameters were observed (Canli et al., 2016b; 2016c).

Gas chromatography-mass spectrophotometry method (GC-MS)

For the identification of chemical components, each sample was analyzed by Agilent GC 6890N-Agilent MS 5973 equipped with HP5-MS capillary column (30 m* 0.25 mm; coating thickness 0.25 µm). Analytical conditions were an injector temperature of 350°C; carrier gas helium at 1 mL/min; injection mode: split, split ratio 10:1; volume injected: 1 µL of sample in ethanol extract and oven temperature programed from 40 to 350°C at 4°C/min, pressure: 48.2 kPa, split flow: 9.9 mL/min. The MS scan conditions were a transfer line temperature of 280°C, an interface temperature of 280°C, and an ion source temperature of 230°C. Identification of the components was conducted by matching the retention times against National Institute of Standards and Technology (NIST Mass Spectrometry DATA CENTER) data library and crosscheck was applied with previously published data (Canli et al., 2017b).

Controls

Empty sterile disks and extraction solvent (ethanol) were used as negative controls.

Statistics

The statistical analysis was executed using a parametric method, the one-way analysis of variance (ANOVA), with a significance level of 0.05 (Chambers and Hastie, 1992). In order to put forward any correlation between the concentration and antimicrobial activity Pearson correlation coefficient was calculated (Becker et al., 1992). All statistical analysis were conducted by using R Studio, version 3.3.2 (Team, 2016).

Table I

Disk diffusion test result for *F. hirsuta*

	Inhibition zone (mm)		
	20 µL	40 µL	80 µL
B. subtilis	8 ± 0.0	9 ± 1	10 ± 0.0
C. albicans	-	8 ± 0.0	12 ± 0.0
E. aerogenes	-	-	-
E. coli	-	-	-
E. durans	-	9 ± 1	10 ± 0.0
E. faecalis	-	8 ± 0.0	10 ± 0.0
E. faecium	8 ± 0.0	14 ± 1	16 ± 0.0
K. pneumoniae	-	-	7 ± 0.0
L. innocula	-	7 ± 0.0	8 ± 0.0
L. monocyto-genes	-	8 ± 1	10 ± 0.0
P. aeruginosa	8 ± 0.0	8 ± 0.0	9 ± 1
P. fluorescens	8 ± 1	9 ± 0.0	11 ± 0.0
S. aureus	8 ± 0.0	10 ± 0.0	12 ± 0.0
S. enteritidis	8 ± 0.0	19 ± 1	14 ± 0.0
S. epidermidis	11 ± 0.0	14 ± 0.0	16 ± 0.0
S. infantis	-	8 ± 0.0	10 ± 0.0
S. kentucky	10 ± 0.0	12 ± 0.0	14 ± 0.0
S. typhimurium	9 ± 0.0	10 ± 0.0	12 ± 0.0

"-": No inhibition

Results

Antimicrobial activity of the *F. hirsuta* ethanol extracts were analyzed. In order to load extracts, the empty sterile disks were used. Then these disks were applied on a Mueller Hinton agar (culture medium), which was inoculated with the micro-organisms. Inhibition zone was observed, when the extracts had activity against these micro-organisms. The diameter of these zones were measured as diameters in mm as given in Table I. No activities were observed for empty sterile disks and ethanol loaded disks, which are negative controls. Furthermore, the statistical analysis proved that there were no significant difference between the activities of three parallels of each extract volumes, which were 20, 40 and 80 µL, with p values of 0.9973, 0.9706 and 0.9972 respectively. On the other hand, the difference between the activities of three extract volumes were observed to be statistically significant with a p value of 0.0017. In addition, a weak positive correlation was observed between increasing the extract volume tested and the activity observed, where the Pearson correlation coefficient was 0.4273.

F. hirsuta showed antimicrobial activity against all of the studied micro-organisms except *E. aerogenes* and *E. coli*. Two of them had high susceptibility (higher than 15 mm). Eleven of them had moderate susceptibility (14 -10 mm) and only three of them had low susceptibility (9-7 mm).

According to Table II, oleic acid (26.3%), γ-sitosterol (12.9%), vitamin E (11.5%), *n*-hexadecanoic acid (9.6%), benzene,1,3,5-trimethyl- (7.0%), 9,12-octadecadienoic acid (Z,Z)- (6.5%) were mainly found in the composition of *F. hirsuta* ethanol extract.

Discussion

F. hirsuta was analyzed against *S. enteritidis*, *S. infantis*, *S. kentucky*, *S. typhimurium*, and a moderate activity was observed. Salmonella genus is a group of Gram negative bacteria and the species belong to this genus are important foodborne pathogens. Gram negative bacteria have more resistance against aromatic plants than Gram positive bacteria (Canli et al, 2016b). *F. hirsuta* has antimicrobial activity against all studied Gram positive bacteria and some Gram negative bacteria has resistance against this aromatic plant extract. This result demonstrate that *F. hirsuta* can be used for large range of microbial infection treatment.

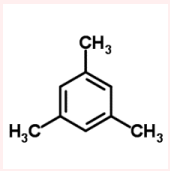
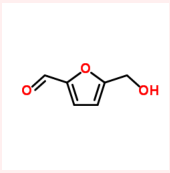
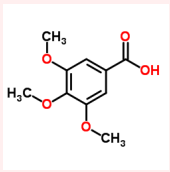
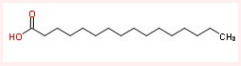
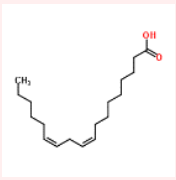
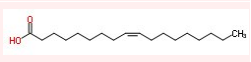
The highest activity was reached against *S. epidermidis* and *E. faecium* (16 mm at 3.1 mg). *Staphylococcus* and *Enterococcus* genera are Gram positive bacteria and these results are consistent with previous studies (Altuner et al., 2012).

Bacterial fatty acid synthesis is controlled by enoyl-acyl carrier protein reductase (FabI) and this enzyme is significant in novel antibacterial agent research (Payne et al., 2001). Oleic, palmitic (*n*-hexadecanoic acid) and linoleic (9,12-octadecadienoic acid (Z,Z)-) acids are known to inhibit FabI activity. Therefore, these unsaturated fatty acids have antibacterial potential (Zheng et al., 2005). According to GC-MS result, *F. hirsuta* ethanol extract include high amount of these molecules and their antibacterial potential can be related to these fatty acids.

Mesitylene (benzene,1,3,5-trimethyl-) is used in synthesis of new benzofuran compounds and it was previously proved that these compounds have antimicrobial activity against *E. coli*, *C. albicans* and *S. aureus* (Kirilmis et al., 2008). Eudesmic acid (benzoic acid, 3,4,5-trimethoxy-) has also antimicrobial activity against *C. albicans* and *S. aureus* (Bisignano et al., 2000). Stearic acid (octadecanoic acid) is an unsaturated fatty acid and some stearic acid analogues has antibacterial activity against *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli* (Jubie et al., 2012). *B. subtilis*, *P. aeruginosa*, *C. albicans* and *S. aureus* growth were inhibited by *F. hirsuta* ethanol extract and the inhibitions of these micro -organisms were important as a reason of being

Table II

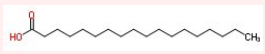
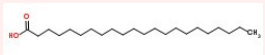
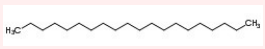
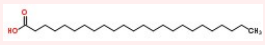
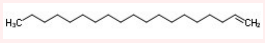
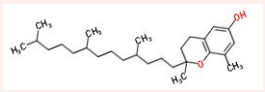
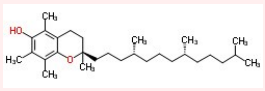
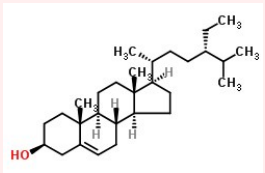
Major chemical components of *F. hirsuta* according to the GC-MS analysis

No	Retention time	Chemical structure[a]	Compound name	Formula	Molecular Weight (g/mol)	Area (%)	Known activity[b]
1	8.0		Benzene, 1,3,5-trimethyl-	C_9H_{12}	120	7.0	-
2	17.4		2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	1.5	-
3	31.0		Benzoic acid, 3,4,5-trimethoxy-	$C_{10}H_{12}O_5$	212	2.1	-
4	32.0	-	unknown	-	-	2.8	-
5	33.1	-	unknown	-	-	0.7	-
6	40.7		*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	9.6	5-Alpha-reductase-inhibitor, antialopecic, antiandrogenic, antifibrinolytic, anti-oxidant, FLavor FEMA, hypercholesterolemic, lubricant, nematicide, pesticide, propecic, soap
7	46.9		9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	6.5	5-Alpha-reductase-inhibitor, antiacne, antialopecic, antianaphylactic, antiandrogenic, antiarteriosclerotic, antiarthritic, anticoronary, antieczemic, antifibrinolytic, antigranular, antihistaminic, antiinflammatory, antileukotriene-D4, antimenorrhagic, antiMS, antiprostatitic, cancer-preventive, carcinogenic, comedolytic, hepatoprotective, hypocholesterolemic, immunomodulator, insectifuge
8	47.2		Oleic Acid	$C_{18}H_{34}O_2$	282	26.3	5-Alpha-reductase-inhibitor, allergenic, alpha-reductase-inhibitor, anemiagenic, antialopecic, antiandrogenic, antiinflammatory, antileukotriene-D4, cancer, preventive, choleretic, dermatitigenic, FLavor FEMA, hypocholesterolemic, insectifuge, perfumer, propecic

[a]http://www.chemspider.com/; [b]Dr. Duke's Phytochemical and Ethnobotanical Databases; "-"Activity not researched

Table II

Major chemical components of *F. hirsuta* according to the GC-MS analysis (Cont.)

No	Retention time	Chemical structure[a]	Compound name	Formula	Molecular Weight (g/mol)	Area (%)	Known activity[b]
9	47.8		Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.4	-
10	59.4		Docosanoic acid	$C_{22}H_{44}O_2$	340	2.2	Cosmetic
11	62.5		Eicosane	$C_{20}H_{42}$	282	1.7	-
12	64.3		Tetracosanoic acid	$C_{24}H_{48}O_2$	368	2.2	-
13	66.7	-	unknown	-	-	2.2	-
14	67.2		1-Nonadecene	$C_{19}H_{38}$	266	4.1	-
15	72.0		2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]	$C_{27}H_{46}O_2$	402	4.2	Antiatherosclerotic, anticancer, antiCRP, antiinflammatory, anti-oxidant, antiprostaglandin, antitumor, cardioprotective, cyclooxygenase-inhibitor, hypocholesterolemic, natriuretic, NO-inhibitor, PKC-inhibitor
16	73.4		Vitamin E	$C_{29}H_{50}O_2$	430	11.5	Allergenic, antiarteriosclerotic, antiatherosclerotic, antibronchitic, anticariogenic, anticataract, antichorea, anticoronary, antidermatitic, antidiabetic, antiepitheleomic, antileukemic, antileukotriene, antiMS, antimyoclonic, antioxidant, antiparkinsonian, antiproliferant, antitumor (breast), antitumor (colorectal), antitumor (prostate), apoptotic, cancer-preventive, hepatoprotective, hypocholesterolemic, immunostimulant
17	79.7		γ-Sitosterol	$C_{18}H_{34}O_2$	282	12.9	-

[a]http://www.chemspider.com/; [b]Dr. Duke's Phytochemical and Ethnobotanical Databases; "-"Activity not researched

foodborne pathogens.

γ-Sitosterol is also called as clionasterol (triterpenoid). Clionasterol was firstly isolated from *Gracilaria edulis* and it didn't show antimicrobial activity against *A. niger* and *E. coli* (McCabe-Sellers and Beattie, 2004). Many of the antimicrobial activities of γ-sitosterol have not been identified, so large scale micro-organism investigations such as MIC and disk diffusion methods are required.

F. hirsuta extract is observed to contain some fatty acids in relatively high amounts and they are important for foodborne pathogen treatment. Therefore, *F. hirsuta* extract might be used for industrial purposes in order to prevent contamination. On the other hand, the composition of *F. hirsuta* extract revealed that it contains relatively high amounts of some compounds, such as oleic acid and vitamin E, which are used to prepare some food supplements.

Moreover, some compounds were determined in the composition of *F. hirsuta* extract, which is not matching with the present libraries.

Conclusion

F. hirsuta ethanol extract has antimicrobial activity against large range of micro-organisms. These results are important because of being the first determination of the antimicrobial activity and biochemical composition of *F. hirsuta*.

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Conflict of Interest

Authors declare no conflict of interest

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