Original Article

Antimicrobial Activity and Chemical Composition Screening of *Epilobium montanum* Root

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ABSTRACT

Medical herbs have many bioactive component and they are used in microbial treatment since ancient time. The resistance of pathogen to antibiotic is became a critical problem, so novel antimicrobial agent related research is required. *Epilobium montanum* related antimicrobial research doesn't exist, therefore root of this medicinal plant investigation was applied against 17 bacteria and 1 fungi by using disk diffusion method. These microbial species include *Bacillus, Enterobacter, Enterococcus, Escherichia, Klebsiella, Listeria, Pseudomonas, Salmonella, Staphylococcus* and *Candida genera*. Besides, chemical composition of this sample was determined by Gas Chromatography-Mass Spectroscopy. The results were presented that *E. montanum* has antimicrobial activity against all tested microbial species except *E. coli*. Seventeen major chemical components were determined, but some composition of this sample is not match with library. For this reason, this medicinal plant contain unknown molecules and this molecules should be analysed by NMR spectra for 3D structure determination and identification.

Keywords: *Epilobium montanum*, Medicinal Plant, Antimicrobial Activity, Chemical Composition, Disk Diffusion Method, GC-MS.

INTRODUCTION

A tremendous progress in medicine has been observed especially at the last decades. But unfortunately microorganisms, including viruses, are still on the agenda of the scientist, because diseases caused by them are still threatening the public health especially in the developing countries, despite the remarkable progression in the medicine.[1-3] The growing number of new resistant pathogens direct the researches to discover new antimicrobial agents inevitably. Plants are accepted to be good candidates of being sources for new drug hits.

Epilobium montanum, which is one of the willow herb species and commonly known as broad-leaved willow herb, is distributed in North, South and Central Anatolian region between 780 m - 2300 m elevations. It is mostly found on the riversides in forests.[4] It is known to be used as an ethno medicine especially against kidney, urinary tract and prostate diseases in Austria, but it also have some common uses around the world against urinary system and prostate problems.[5-6] There are several researches about the anticancer properties of *E. montanum*.[5] But the research about its antimicrobial activity is very limited according to the current literature.

The main purpose of this study was to investigate antimicrobial activity of *E. montanum* against 17 bacteria and 1 fungi and to determine its biochemical composition to contribute the literature with this information.

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MATERIALS AND METHODS

Plant samples preparation

E. montanum is medicinal plant was obtained from local market. 2.75 mg and 9.17 mg *E. montanum* samples were prepared with ethanol extraction process as mentioned in previous study.[7]

Antimicrobial activity test

17 bacteria and 1 fungi was used and sustained on Nutrient Agar (BD Difco, USA). Microorganism inoculum process was applied as done in previous study.[8] The antimicrobial activity of *E. montanum* was performed by disk diffusion test.[9]

Gas chromatography-mass spectrophotometry method (GC-MS)

For the identification of chemical components, each sample was analyzed by Shimadzu GCMS QP 2010 ULTRA equipped with RTX-5MS capillary column (30m * 0.25 mm; coating thickness 0.25 µm). Analytical conditions were an injector temperature of 250 ºC; carrier gas Helium at 1.78 mL/min; injection mode: split, split ratio 10:1; volume injected: 1 µL of sample in ethanol extract and oven temperature programmed from 40 ºC to 290 ºC at 4 ºC/min, pressure: 100 kPa, total flow: 13.7 mL/min. The MS scan conditions were an interface temperature of 250 ºC, and an ion source temperature of 200 ºC. Identification of the components was conducted by matching the retention times against Wiley Data Library and crosscheck was applied with previously published data.[10] The chemical components found to be higher than 1% were accepted as the major components and the list of these components and information regarding them are given in Table 2.

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.[11] In order to put forward any correlation between concentration and antimicrobial activity Pearson correlation coefficient was calculated.[12] All statistical analysis were conducted by using R Studio, version 3.3.2.[13]

RESULTS AND DISCUSSIONS

The diameter of these zones were measured in millimeters and given as mean values with standard errors in Table 1. No activities were observed for empty sterile disks and ethanol loaded disks, which are negative controls. Furthermore, statistical analysis proved that

Table 1: Disk diffusion test result for *Epilobium montanum* (Inhibition zones in mm).

	30µL	100µL
Bacillus subtilis DSMZ 1971	8,00 ± 0,00	11,00 ± 0,00
Candida albicans DSMZ 1386	11,00 ± 0,71	13,00 ± 0,00
Enterecoccus faecalis ATCC 29212	-	9,00 ± 0,00
Enterecoccus faecium	17,00 ± 0,71	22,00 ± 0,00
Enterobacter aerogenes ATCC 13048	-	8,00 ± 0,00
Enterococcus durans	7,00 ± 0,00	8,00 ± 0,00
Escherichia coli ATCC 25922	-	-
Klebsiella pneumonia	12,00 ± 0,71	14,00 ± 0,71
Listeria innocua	-	8,00 ± 0,00
Listeria monocytogenes ATCC 7644	11,00 ± 0,00	14,00 ± 0,00
Pseudomonas aeruginosa DSMZ 50071	16,00 ± 0,00	21,00 ± 0,71
Pseudomonas fluorescens P1	7,00 ± 0,00	9,00 ± 0,00
Salmonella enteritidis ATCC 13076	11,00 ± 0,00	14,00 ± 0,00
Salmonella infantis	-	8,00 ± 0,00
Salmonella kentucky	13,00 ± 0,00	15,00 ± 0,00
Salmonella typhimurium SL1344	18,00 ± 0,00	21,00 ± 0,00
Staphylococcus aureus ATCC 25923	15,00 ± 0,00	16,00 ± 0,00
Staphylococcus epidermidis DSMZ 20044	14,00 ± 0,00	17,00 ± 0,71

"-":No inhibition

Table 2: The major chemical components of *Epilobium montanum*.

No	Retention Time	Compound name	Formula	Molecular Weight (g/mol)	Area (%)
1	27.875	1,2,3-Benzenetriol	$C_6H_6O_3$	126.110	5.05
2	42.864	Neophytadiene	$C_{20}H_{38}$	278.516	2.65
3	48.139	Palmitic acid	$C_{16}H_{32}O_2$	256.424	9.27
4	53.563	Phytol	$C_{20}H_{40}O$	296.531	2.20
5	54.331	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.445	2.25
6	54.583	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278.430	8.04
7	64.522	Limonen-6-ol, pivalate	$C_{15}H_{24}O_2$	236.350	1.45
8	65.613	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.503	1.11
9	66.190	Unknown	-	-	2.71
10	70.460	1-Heptacosanol	$C_{27}H_{56}O$	396.733	14.97
11	73.788	Squalene	$C_{30}H_{50}$	410.718	1.01
12	91.633	.gamma.-Sitosterol	$C_{29}H_{50}O$	414.707	23.00
13	92.619	.beta.-Amyrin	$C_{30}H_{50}O$	426.717	1.09
14	93.445	Phytol, acetate	$C_{22}H_{42}O_2$	338.568	1.97
15	94.338	METHYL COMMATE C	-	-	1.39
16	96.829	.alpha.-Tocopheryl acetate	$C_{31}H_{52}O_3$	472.743	1.37
17	102.589	Phytol, acetate	$C_{22}H_{42}O_2$	338.568	1.07

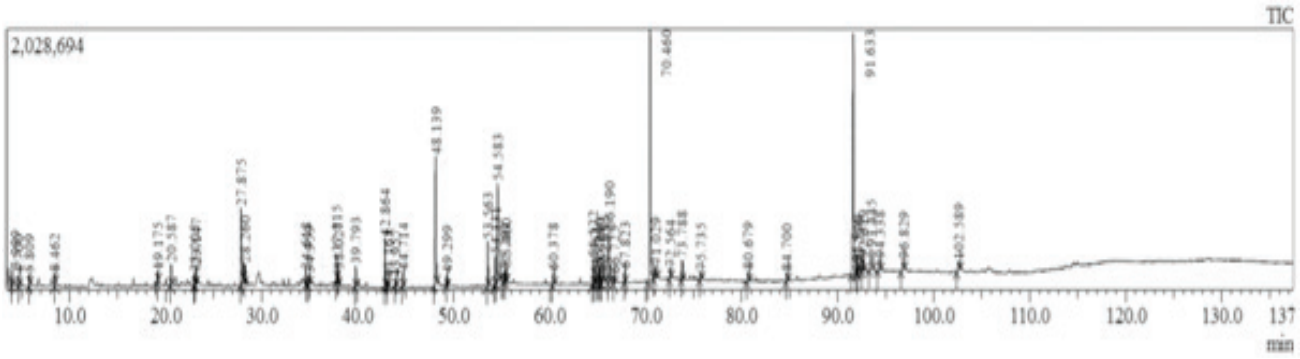

Figure 1: GC-MS chromatogram of *Epilobium montanum*.

there are no significant difference between the activities of three parallels of each extract volumes, which are 30 and 100 µL, with p values of 0.9896 and 0.9983 respectively. On the other hand, the difference between the activities of two different extract volumes are observed to be statistically insignificant with a p value of 0.09235, which can be accepted as significant for p value < 0.1. In addition, a very weak positive correlation was observed between increasing the extract volume tested and the activity observed, where the Pearson correlation coefficient is 0.2847267.

According to Table 1, *E. montanum* has antimicrobial activity against all tested microbial species except *E. coli*. 15 results were observed to be little effect, where 13 were weak, 5 were moderate and 3 were strong.[14] The frequencies were given in Figure 1. These result are important because of being the first determination of the antimicrobial activity of *E. montanum*.

Gram negative bacteria have more resistance against aromatic plants than gram positive bacteria. The highest activity was reached against *E. faecium* (22 mm), *S. typhimurium* (21 mm), *P. aeruginosa* (21 mm), *S. epidermidis* (17 mm), *S. aureus* (16 mm) and *S. kentucky* (15 mm) at 9.17 mg ethanol samples. *E. faecium*, *S. epidermidis* and *S. aureus* are gram positive bacteria; *S. typhimurium*, *P. aeruginosa* and *S. kentucky* are gram negative bacteria. Therefore, *E. montanum* has approximately the same antimicrobial activity against gram positive and gram negative

bacteria. This result demonstrate that *E. montanum* can be used for large range of microbial infection treatment.

According to the current literature the antimicrobial activity of *E. montanum* wasn't studied in details. Sheikh Akbari Mehr and Malekzadeh studied the antimicrobial activity of some *Epilobium* species against 7 bacteria by using disk diffusion test.[15] There are some differences which could possibly depend on several reasons: (1) the location and collection time of *E. montanum* may change active components, (2) the amount of extracts tested on microorganisms may be different and (3) the type of extraction solvent may change the active compounds extracted from plant samples.

The GC-MS chromatogram is given in Figure 1 and according to Table 2 prepared according to the data in Figure 1; .gamma.-Sitosterol (23.00%), 1-Heptacosanol (14.97%), Palmitic acid (9.27%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (8.04%), 1,2,3-Benzenetriol (5.05%) are mainly found in the composition of *E. montanum*. These results are critical because of being the first identification of the biochemical composition of them by GC-MS.

CONCLUSION

E. montanum has antimicrobial activity against large range of microorganisms, but further researches are required in order to analysis their mechanisms. It contain some fatty acids in relatively high amounts, which are important for foodborne pathogen treatment. Its extract and compound will be used for industrial purposes in order to prevent contamination.

ACKNOWLEDGEMENT

None

CONFLICT OF INTEREST

None

ABBREVIATION USED

GC-MS: gas chromatography-mass spectrophotometry method; NMR: nuclear magnetic resonance; 3D: three dimensional.

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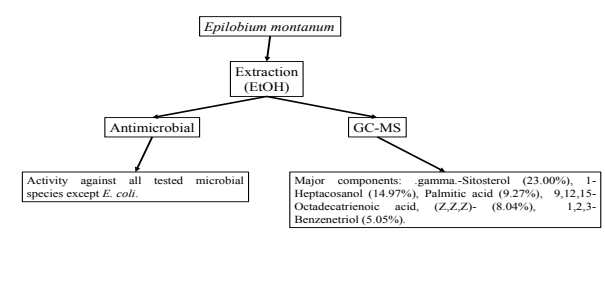
PICTORIAL ABSTRACT



SUMMARY

- The antimicrobial activity of *Epilobium montanum* was analysed against 17 bacteria and 1 fungi by using disk diffusion method.
- The test microorganisms included *Bacillus, Enterobacter, Enterococcus, Escherichia, Klebsiella, Listeria, Pseudomonas, Salmonella, Staphylococcus* and *Candida genera.*
- The chemical composition of this sample was determined by Gas Chromatography-Mass Spectroscopy.
- The extract was observed to be active against all tested microbial species except *E. coli.*
- The major chemical components were observed as gamma.-Sitosterol (23.00%), 1-Heptacosanol (14.97%), Palmitic acid (9.27%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (8.04%), 1,2,3-Benzenetriol (5.05%), but somecomponents of this sample didn't match with the library.

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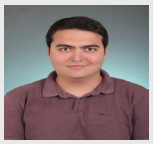

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