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SCREENING OF *IN VITRO* ANTIMICROBIAL ACTIVITY OF *Sedum hispanicum* ETHANOL EXTRACT AND DETERMINATION OF ITS BIOCHEMICAL COMPOSITION

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ABSTRACT

Although medical sciences are progressing tremendously, the diseases caused by viruses, fungi, and bacteria are still an important issue for public health. For this reason, continuous research to determine new antimicrobial compounds is needed. This study investigated the *in vitro* antimicrobial activity and biochemical composition of *Sedum hispanicum* ethanol extract for the first time in the literature. The *in vitro* antimicrobial activity of ethanol extract obtained from *Sedum hispanicum* by disk diffusion method against seventeen bacteria strains and one fungi strain, and biochemical composition of the ethanol extract was determined by GC-MS. The extract showed antimicrobial activity against all bacteria strains at almost all doses (50, 100, and 200 µL) at different rates, except *Pseudomonas fluorescence* and *Candida albicans*. Some compounds found in the extract are not matching with the libraries. As a reason for that, this medicinal plant is proposed to contain some unknown molecules, which should be identified. Our results indicate that research of compounds, particularly unknown ones in the ethanol extract and their antimicrobial activities should be examined further.

KEYWORDS:
Antimicrobial activity, disk diffusion method, *Sedum hispanicum*, biochemical composition, GC-MS

INTRODUCTION

Although the first written documents about medicinal plants belong to the Chinese, Indian and Near East civilizations 5000 years ago, undoubtedly, treatment with plants is about as old as human beings [1]. Ancient Indians (3000s BC) combined more than 8,000 herbal medicines in a source called Ayurveda [2]. Chinese emperor Shen Nung (2735 BC) wrote the first pharmacopeia (Pun Tsao) containing 365 herbal medicines [2,3]. According to the tablet records (2000s BC) of the Mesopotamian civilizations (Sumerian, Akad, and Assyrians), it is understood that they benefited from about 250 medicinal plants [4]. Many medicinal plants such as grapes, onion, garlic, beans, barley, cedar, and crocus are mentioned in the Hearst and Ebers papyrus (1500s BC) written in Egypt. More than 200 medicinal plants are mentioned in about 150 books written by Hippocrates (460-377 BC), who is accepted as the father of medicine [3,4]. Theophrastus (370-287 BC), known as the father of botany, was the first to report the effects of a fern. Pontus king Mithridates VI (132-63 BC) was interested in poisonous plants growing in Trabzon and Rize mountains [5] and found the antidote "Mithridaticum" containing 48 drugs. In his book "De Materia Medica" written by Pedanius Dioscorides (40-90 AD), the most important medical botanist in the history of Rome, who mentioned more than 600 plants of medical importance [4]. Galenus (129-216 AD), who was born in Pergamon (Bergama), also mentioned about 500 drug compositions [3]. Abu Hanifa Dinawari (820-895 AD) has written books on the classification of plants, ways to utilize them, and substances that increase sexual potency. Abu Rayhan Al-Biruni (973-1051 AD) described 200 herbal drugs. Ibn Sina (980-1037 AD), the great Turkish scientist knew in the world as Avicenna, wrote 785 herbal, animal, and mineral drug preparations and how they were used [4].

Today, plants are an important pharmaceutical raw material worldwide. 25% of the drugs prescribed in the USA contain substances obtained from plants [1]. According to the estimates of the WHO, approximately 80% of the total population of developing countries meets their primary health needs from traditional medicines. A large part of this is plant extracts [6]. 735 from about 6000 ready drugs currently used in Germany are of herbal origin. 155 from 2500 drugs in Turkey include herbal medicine or extracts [4].

Microbiologically, the emergence of pathogenic microorganisms with multiple drug

resistance, side effects of antibiotics, and viral diseases that do not have reliable treatment options such as AIDS, Hepatitis B, Hepatitis C increase the interest in researching herbal drugs. Medicinal plants, in particular, are seen as the main potential in this regard and are researched for this purpose [7-12].

Although there are differences between geographic regions in terms of frequency of use of therapy with plants in Turkey, which is still practiced [5,13-16].

The *Sedum* L. genus (Crassulaceae) is represented by about 348 species worldwide and 33 species in Turkey. It is called Damkorugu in Anatolia [17,18]. These species are used both as vegetable and herbal remedies. They are ethnobotanically used to treat many diseases such as athlete's foot, constipation, hemorrhoids, and wounds. They have also been used as a laxative and diuretic [19-21]. *Sedum hispanicum* L., also called Spanish stonecrop, is an Iranian-Turanian element with a widespread distribution in Turkey [17].

In this study, we report the *in vitro* antimicrobial activity of *S. hispanicum* ethanol extract against nine Gram-negative, eight Gram-positive bacteria strains and *C. albicans* with disk diffusion method and biochemical composition of the extract was determined with GC-MS for the first time in the literature.

MATERIALS AND METHODS

Plant sample. *S. hispanicum* was collected from Canakkale (Turkey) and identified by Dr. Bozyel. The plant samples were placed in sample bags and kept in room conditions until use.

Active compound extraction. Dried *S. hispanicum* aerial part samples were ground to obtain a fine powder, to increase the surface area for extraction. The active compounds were extracted by ethanol (Sigma Aldrich) through shaking at room conditions for two days [22]. After filtering through filter paper (Whatman No.1), the ethanol in the extract was evaporated at 45°C under vacuum by using a rotary evaporator (Heidolph Hei-Vap Value HL/HB-G1) [23]. The remnant was weighed and an extract stock was prepared by using a defined volume of ethanol, and 50 µL, 100 µL, and 200 µL of the extract were transferred on sterile empty antibiotic disks to load 1.69, 3.38, and 6.75 mg extracts on disks respectively.

Microorganisms. *B. subtilis* DSMZ 1971, *E. aerogenes* ATCC 13048, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *L. monocytogenes* ATCC 7644, *P. aeruginosa* DSMZ 50071, *P. fluorescence* P1, *S. enteritidis* ATCC 13076, *S. typhimurium* SL 1344, *S. aureus* ATCC 25923, *S. epidermidis* DSMZ 20044, and *C. albicans* DSMZ 1386 are standard strains and *E. durans*, *E. faecium*, *K. pneumoniae*, *L. innocua*, *S. infantis*, and *S. kentucky* are food isolates.

Determination of antimicrobial activity. To determine the antimicrobial activity of *S. hispanicum* ethanol extract, the disk diffusion method previously described in detail by Bozyel et al. [24] was used. The Petri dishes containing disks, on which the extract was loaded, were incubated according to the suitable time-temperature combinations, and the inhibition zones were observed and recorded in millimeters.

Negative control. Empty antibiotic disk and ethanol loaded disk were used as negative controls.

GC-MS Analysis. The biochemical composition of *S. hispanicum* ethanol extract was determined by GC-MS analysis according to the protocol given in previous studies [25].

Statistics. All tests were applied as triplicates. One-way analysis of variance (ANOVA), which is a parametric method was performed (P = 0.05) [26]. Pearson correlation coefficient was determined for any possible correlation between the intensity of antimicrobial activity and concentration. Statistical analysis was performed using R Studio (version 4.0.5) [27].

RESULTS AND DISCUSSION

Antimicrobial activity. Data from the study on inhibition zone diameters are shown in Table 1. According to the results, negative controls show no activity [28]. Additionally, statistical analysis verified that the differences between the results of three replicates of each extract volume were statistically non-significant (p>0.05). Furthermore, obtaining the Pearson correlation coefficient of 0.1774 showed a very weak positive correlation between antimicrobial activity and extracts' volumes.

It is seen in Table 1 that 50 µL ethanol extract of *S. hispanicum* shows antimicrobial activity against all strains, except *P. fluorescens*, *E. faecalis*, and *C. albicans* with inhibition zones between 9 to 11 mm. 100 µL and 200 µL ethanol extract of *S. hispanicum* was presented antimicrobial activity against all microorganism except *P. fluorescens*, and *C. albicans* with inhibition zones ranging between 7 to 13 mm (100 µL), and 8 to 14 mm (200 µL).

Al-Qudah et al. [29] showed that *Sedum microcarpum* caused 21 mm of inhibition zones for 1000 µg/µL aqueous methanol extract against *S. epidermidis* ATCC 12228 and 19 mm of inhibition zones for 1000 µg/µL aqueous methanol extract against *E. faecalis* ATCC 29212. Bensouici et al. [30] presented that 128 µg/mL of chloroform extract of *Sedum caeruleum* showed an inhibition zone of 13

mm against *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 700603 and *S. aureus* ATCC 43300, and 12 mm against *E. coli* ATCC 25922.

E. faecium is a gram-positive bacterium [31] and was thought to be a normal member of the gastrointestinal microflora of humans and animals, but it also turned out to be a pathogenic bacterium that causes diseases such as neonatal meningitis or endocarditis [32]. Also, an inhibition zone over 10 mm, 12 mm, and 12 mm for *E. faecium* against Ampicillin, Norfloxacin, and Vancomycin respectively are accepted to be susceptible [33]. Our extract presented a 13 mm inhibition zone for 200 µL (6.25 mg) extract, which clearly shows that the *E. faecium* strain used in our study is susceptible against 200 µL of *S. hispanicum* ethanol extract.

L. monocytogenes is a gram-positive facultative anaerobic bacterium that can survive in the presence or absence of oxygen. This pathogenic bacteria species causes listeriosis. It can grow and multiply within its host cells and is one of the most dangerous foodborne pathogens. 20-30% of food-borne listeriosis can be fatal in individuals at high risk [34,35]. Besides, an inhibition zone over 13 mm for *L. monocytogenes* against Benzylpenicillin is accepted to be susceptible [33]. 200 µL (6.25 mg) of our extract presented a 13 mm inhibition zone against *L. monocytogenes*, which also shows that *L. monocytogenes* ATCC 7644 strain used in our study can be accepted to be susceptible against 200 µL of *S. hispanicum* ethanol extract.

Biochemical composition of ethanol extract. The major components of *S. hispanicum* ethanol extract that are higher than 1% and their composition percentages are given in Table 2, according to the data obtained from the GC-MS analysis. In Figure 1, the chromatogram of *S. hispanicum* ethanol extract obtained from the GC-MS analysis is given.

According to Table 2, .beta.-Amyrin (36.57%), Germanicol (20.05%), Stigmast-5-en-3-ol, (3.beta.)- (5.54%), Lup-20(29)-en-3-ol, acetate, (3.beta.)- (3.90%) are major components in the biochemical composition of *S. hispanicum* ethanol extract.

TABLE 1
Antimicrobial activity results for *S. hispanicum* (the diameters are in mm)

Microorganisms	50 µL*	100 µL*	200 µL*
B. subtilis DSMZ 1971	9.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00
E. aerogenes ATCC 13048	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00
E. faecalis ATCC 29212	-	7.00 ± 0.00	8.00 ± 0.00
E. coli ATCC 25922	10.00 ± 0.00	11.00 ± 0.58	12.00 ± 0.00
L. monocytogenes ATCC 7644	9.00 ± 0.00	11.00 ± 0.00	13.00 ± 0.00
P. aeruginosa DSMZ 50071	11.00 ± 0.00	12.00 ± 0.00	13.00 ± 0.00
P. fluorescens P1	-	-	-
S. enteritidis ATCC 13076	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00
S. typhimurium SL1344	10.00 ± 0.00	12.00 ± 0.00	12.00 ± 0.00
S. aureus ATCC 25923	11.00 ± 0.58	13.00 ± 0.00	14.00 ± 0.00
S. epidermidis DSMZ 20044	11.00 ± 0.00	12.00 ± 0.00	13.00 ± 0.00
E. durans	9.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.58
E. faecium	10.00 ± 0.00	11.00 ± 0.00	13.00 ± 0.58
K. pneumoniae	9.00 ± 0.00	9.00 ± 0.00	10.00 ± 0.00
L. innocua	9.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00
S. infantis	10.00 ± 0.00	11.00 ± 0.00	11.00 ± 0.00
S. kentucky	9.00 ± 0.00	9.00 ± 0.00	10.00 ± 0.00
C. albicans DSMZ 1386	-	-	-

"*": The data is given as the average values of three repetitions with standard errors; "-": No inhibition

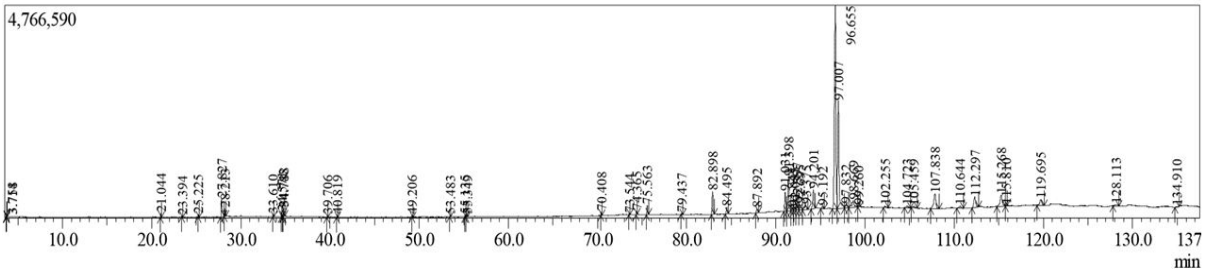

FIGURE 1
GC-MS chromatogram of *S. hispanicum* ethanol extract

TABLE 2
The major components of *S. hispanicum* ethanol extract

No	Retention Time	Components	Formula	Molecular Weight (g/mol)	Area (%)
1	27.827	1,2,3-Benzenetriol	$C_6H_6O_3$	126.110	2.08
2	82.898	Tetrapentacontane	$C_{54}H_{110}$	759.451	2.95
3	91.031	Tetrapentacontane	$C_{54}H_{110}$	759.451	2.60
4	91.398	Stigmast-5-en-3-ol, (3.beta.)-	$C_{29}H_{50}O$	414.707	5.54
5	92.397	.alpha.-Amyrin	$C_{30}H_{50}O$	426.717	1.21
6	92.695	.alpha.-selinene	$C_{15}H_{24}$	204.351	1.05
7	93.375	Lup-20(29)-en-3-ol, acetate, (3.beta.)-	$C_{32}H_{52}O_2$	468.754	1.13
8	94.201	Lupeol	$C_{30}H_{50}O$	426.717	3.20
9	96.655	.beta.-Amyrin	$C_{30}H_{50}O$	426.717	36.57
10	97.007	Germanicol	$C_{30}H_{50}O$	426.717	20.05
11	98.669	Lup-20(29)-en-3-ol, acetate, (3.beta.)-	$C_{32}H_{52}O_2$	468.754	2.11
12	107.838	Lup-20(29)-en-3-ol, acetate, (3.beta.)-	$C_{32}H_{52}O_2$	468.754	3.90
13	112.297	17.beta.-Acetoxy-4-hydroxy-4-propyl-5-androsten-3-one	$C_{24}H_{36}O_4$	388.540	2.47
14	115.268	Lup-20(29)-en-3-ol, acetate, (3.beta.)-	$C_{32}H_{52}O_2$	468.754	3.23
15	119.695	UNKNOWN	-	-	1.45

"-": No information

CONCLUSION

S. hispanicum has antimicrobial activity against a large range of tested strains. Some compounds found in the extract are not matching with the library. As a reason for that, this medicinal plant is proposed to contain some unknown molecules and they should be identified and their 3D structure should also be determined. The unknown compound, which consists of 1.45%, should be analyzed in detail. Also, the mode of action(s) of the extract should be determined in further studies.

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