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INVESTIGATION OF THE ANTIMICROBIAL ACTIVITY OF *GAGEA DUBIA*

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ABSTRACT. The main objective of this study is to evaluate the antimicrobial effect of ethanol solution extracts of *G. dubia* A. Terracc. on nineteen gram positive and negative bacteria *(Bacillus subtilis, Enterococcus faecium, Enterococcus faecalis, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis, Salmonella infantis, Salmonella typhimurium, Salmonella kentucky, Salmonella enterica, Pseudomonas aeruginosa, Pseudomonas fluorescens*) and one fungi (*Candida albicans*). Different parts (flowers, leaves, stems, and roots) of *Gagea dubia* samples, were extracted with 80% ethanol solution. These extracts were tested in vitro for their antimicrobial activity against 15 microorganism by disk diffusion (DD) and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests. Ethanol extracts showed antimicrobial activity against some bacteria. According to the results of disk diffusion test *G. dubia* have effect on four bacteria (*B. subtilis, S. aureus, L. innocua* and *E. durans*) and (*C. albicans*) and didn't show effect on other microorganisms. The antibacterial effect observed in 10 µL extract of *G.dubia* against *B. subtilis, S. aureus, L.innocua* with 7,66; 13 and 9 mm zone diameter respectively and 50 µL extract of *G. dubia* against *E.durans* with 11 mm zone diameter. *G. dubia* extract show antimicrobial activity at three concentration (10;50 and 100 µL) against *C. albicans* between 9-15,66 mm zone diameter. MIC result for *G. dubia* determined as 5; 1,25; 10; 5 and 0,15 mg/100 µL against *B. subtilis, S. aureus, L.innocua, E.durans* and *C. albicans* respectively. According to MBC test all MIC value against maicroorganism determined as bacteriostatic.

1. INTRODUCTION

The plants are the main source for drug development for many centuries. Ethno medical plant heavily utilized in the development of pharmacopeias, because it is a major focus in global health care. Medicinal plants contain physiologically active that utilized for the treatment of various ailments in traditional medicine. These plants contain anti-microbial properties [1]. The drug plant usage also originates from ancient times and recorded as practiced by the Native Americans [2]. Moreover, due to the mobility of humankind throughout the world history, specific

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usages and knowledge also spread and improved with the discovery of Europe and America due to the new species at these locations.

Reversely, the knowledge mobility from the America to Europe made difference in terms of improvements too. Some scientific publications about the development of plant usage in America was focused and the facts that the native population of America has founds more than 2500 herbs to use as cures and also consume more than 1500 plants as food. Historically, plants consume provided new drug compounds that help to human health besides well-being. Their role these drug compounds is huge in the progress of new medications. Quinine is important to medicine of herb origin with a past of utilize. This alkaloid found in the bark of Cinchona tree itself. It was usefulness to cure malaria, also to decrease nocturnal leg cramps . Traditional medicine utilized plants for many years because of their antimicrobial properties. Drug value of these because of the chemical they have inside that create physiological effects. These bioactive basics they are alkaloids, which are mainly secondary metabolites [3-8].

The resistance of bacteria to antibiotics can be described in two ways: natural (intrinsic) or acquired. Natural resistance occurred due to the microorganisms naturally do not posses target sites for the drug action and therefore the drug does not affect them or due to the low permeability to those agents because of the differences in the chemical nature of the drug and the microbial membrane structure. The acquired resistance whereby a naturally susceptible microorganism acquires ways of not being affected by the antimicrobial agents due to the presence of an enzyme that lead to inactivates the antimicrobial agent or a mutation in the antimicrobial agent's target, which reduces the binding of the antimicrobial agent or due to active efflux of the antimicrobial agent from the cell [3, 9,10].

Gagea dubia A. Terracc. is a Mediterranean type of herbs in the lily family. It grows and originates in countries such as Morocco, Sardinia, and Turkey. *Gagea dubia* is a bulb-forming plant and it has yellow flowers.

Ali-Shtayeh et al. [11] investigate antimicrobial activities of 56 Palestinian medicinal plants including *Gagea chlorantha* against *Propionibacterium acnes, S. aureus, E. coli, P. aeruginosa, P. vulgaris* and *K. pneumonia* using disc diffusion and broth dilution methods.

The main objective of this study is to evaluate the antimicrobial effect of ethanol solution extracts of *Gagea dubia* on eighteen gram positive and negative bacteria and one fungi (*Candida albicans*).

2. Material And Methods

2.1 Plant Samples and Extraction

Plant samples collected from naturel lands (surround of Kastamonu Culture village, April 2018) and were washed with distilled water. Than samples dried in a sun-free environment for several days. Dried plants are made into fine powder by crush machine. 50 grams of powder plant sample is weighed and placed in a conical bottle. 300 mL of a 60% ethanol-water mixture was added as a solvent and the samples were placed in a shaker for better extraction (100 rpm for 3 days). At the end of three days, the samples from the shaker were filtered with the aid of filter paper. To gate dry plant extract ethanol evaporated with help of Rotary evaporator and water wit help of Lyophilizator. 1 gr Dry plant extract solved in 10 ml ethanol to prepare stock extract for disk diffusion test and 1 gram dry extract solved in strile distilled water than filtered with help of sterile syringe filter to make sterile for prepare stock extract of Minimum Inhibition Concentration (MIC) test [12, 13].

2.2 Test Microorganisms

In this thesis study the antimicrobial activity of the extracts were tested against 18 Microorganisms (*Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecium, Enterococcus faecalis, Listeria monocytogenes, Listeria innocua, Enterococcus durans, Bacillus subtilis, Salmonella typhimurium, Salmonella kentucky, Salmonella infantis, Salmonella enteritidis, Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa, Pseudomonas fluorescens, Klebsiella pneumoniae*) and one fungi (*Candida albicans*).

2.3. Preparation of Inocula

For each microorganism, which will be used in the study an inoculum was prepared. To prepare the inoculum morphologically similar colonies of the microorganism were transferred in 0,9% sterile NaCl solution to adjust the turbidity against 0,5 standard McFarland solution [7].

2.4. Loading Extracts to Empty Disks

Different volumes (10 µL, 50 µL and 100 µL) of stocks extract, which were prepared previously loaded on empty sterile antibiotic disks in aseptic conditions and the ethanol was removed by leaving disks for 24 h at 40 ºC, to prevent any interaction with microorganisms [12].

2.5 Disc Diffusion Method (DD)

In disk diffusion test, inocula of eigteenth microorganisms and disks loaded with extracts were used. The surface of Mueller Hinton Agar (MHA) were inoculated by the inoculum using a sterile cotton swab, and 4 disks (one empty, one 10 µL extract containing, one 50 µL extract containing and one100 µL extract containing disks) were applied to the surface of MHA. The plates were incubated at 37 ± 1 ºC for 24 h for bacteria and 27 ± 1 ºC for 48 h for fungi . After incubation the diameters of inhibition zones were measured by a ruler and the inhibition zones were defined in millimetres [14].

2.6. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of any anti-infective compound is accepted as the lowest power of the concentration that inhibits visual growth. The MIC values were determined by over-culturing an identified amount of microorganism after applying diluted amount of extracts [15].

2.6. Determination of Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

The Minimum Bactericidal Concentration (MBC) determination were performed by sub-culturing suspensions from non-turbid MIC test well to agar medium. The MBC values were defined as the lowest concentration of extract inhibiting bacterial growth.

2.7. Controls

Ten different standard antibiotic discs (streptomycin, gentamicin, meropenem, vancomycin, ampicillin, and gentamicin) were used to test the efficacy of plant extracts for positive controls when empty discs were used for negative controls. In

the MIC test, the well containing the culture medium and microorganism was used as a positive control, whereas the well containing only culture medium was used as a negative control.

3. Results and discussions

The results of the experiments carried out in this study are given Table1, Table 2 and Table 3. All results given in this section, if any, are the mean values of the results of three parallel tests with standard deviation.

TABLE 1. Disk diffusion (DD) results of *Gagea dubia* extract (Zone diameter in mm).

	G. dubia extract concentration (µL)		
Microorganisms	10	50	100
B. subtilis	7,66	0,00	0,00
S. aureus	13	0,00	0,00
L. innocua	9,00	0,00	0,00
E. durans	0,00	11,00	0,00
C. albicans	9,66	9,00	15,66

TABLE 2. Minimum inhibition concentration (MIC) test and Minimum bactericidal/fungicidal (MBC/MFC) test results of *Gagea dubia* extract (mg/100 µL).

Microorganisms	MIC	MBC/MFC	
		Bc/Fc	Bs/Fs
B. subtilis	5	-	5
S. aureus	1,25	-	1,25
L. innocua	10	-	10
E. durans	5	-	5
C. albicans	0,156	-	0,156

Bc/Fc; Bacterisidal/Fungicidal, Bs/Fs; Bacteriostatik/Fungistatic

Results showed that *G. dubia* extract show antimicrobial activity against *B. subtilis, S. aureus, L. innocua, E. durans* and *C. albicans* but no activity was found against *E. aerogenes, E. coli, E. faecium, P. aeruginosa*, *S. enteritidis, K. pneumonia, P. fluorescens, S. typhimurium, S. epidermidis, S. epidermidis* and *S. kentucky*.

G. dubia extract exhibite antimicrobial activity at 10 µL volume against *B. subtilis, S. aureus* and *L. innocua* with 7,66; 13 and 9,00 mm respectively while 50 and 100

µL volume of extract doesn't show any activity against the same bacteria. Extract exhibit antimicrobial activity against *E. durans* only at 50 with 11 mm zone diameter. Extract show antifungal activity against *C. albicans* at three volumes between 9-15,66 mm zon diameter. Ali-Shtayeh et al. [11] observed antimicrobial activity of *Gagea chlorantha* extract against *P. acnes, S. aureus, E. coli, P. aeruginosa, P. vulgaris* and *K. pneumonia* with 10; 4; 22; 10; 10 and 4 mm zon diameter respectively.

MIC value of G. dubia extract determined as 5; 1,25; 10; 5; 0,156 mg/100 µL for *B. subtilis, S. aureus, L. innocua, E. durans* and *C. albicans* respectively and this value also determined as bacteriostatic and fungistatic.

TABLE 3. Positive control antibiotics used in Disk diffusion test (Zone diameter in mm).

Microorganisms	**L 2**	**OFX5**	**MEM 10**	**TE 30**	**CZ 30**	**VA 30**	**AM 10**	**K 30**	**CN 10**	**S 10**	**S 300**	**NA 30**	**SH 100**	**SXT 25**	**N 30**	**CIP 5**	**AMC 30**	**C 30**
E.aerogenes	-	27	28	18	13	-	10	24	25	-	23	24	30	28	20	32	10	30
S.infantis	-	24	37	9	15	-	20	-	20	10	-	-	12	-	10	30	21	30
L.monocytogenes	-	19	25	-	18	-	22	-	20	11	-	-	12	25	10	25	25	27
K.pneumoniae	-	30	30	17	-	-	-	25	24	20	25	24	18	-	20	35	11	30
P.fluorescens	-	23	25	18	-	-	-	-	20	13	16	-	17	-	12	33	-	-
P.aeruginosa	9	19	14	20	-	21	30	12	15	-	-	-	20	25	12	24	30	23
S. kentucky	-	32	33	15	-	-	25	23	14	11	-	23	-	26	21	32	26	30
E.faecalis	-	20	21	10	-	22	30	18	15	-	20	-	19	28	17	23	30	25
L.innocua	-	17	26	22	-	20	28	25	25	26	35	17	21	26	12	22	30	24
S. enteritidis	-	32	32	20	19	-	23	22	21	20	15	25	24	25	18	30	25	28
E.durans	-	17	27	21	12	-	-	25	20	22	22	24	24	26	18	21	10	30
S.typhimurium	-	32	32	15	15	-	25	26	27	-	-	25	33	23	22	35	30	33
E. faecium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S.aereus	25	28	35	25	-	21	40	24	25	20	17	-	21	28	22	30	37	25
S.epidermidis	-	30	32	15	10	-	-	25	24	18	24	28	30	30	21	37	10	33
B.subtilis	16	28	40	33	9	24	46	28	30	20	35	23	30	35	34	35	10	33
E.coli	-	-	36	-	-	-	-	20	25	21	23	-	30	16	22	-	18	25
S. marcescens	-	38	38	18	24	-	-	30	27	25	25	39	33	30	22	43	10	32

(-) no effect, Lincomycin: L2, Ofloxacin: OFX 5, Meropenem: MEM 10, Tetracycline: TE 30, Ceftazidime: CAZ 30, Vancomycin: VA 30, Ampıcillin: AM10 Kanomycin: K 30, Gentamicin: CN 10, Streptomycin: S10, Compound Sulphonamides: S 3 300, Nalidixic acid: NA 30, Spectonomycine: SH 100 Sulphamethoxazole trimethaprim: SXT 25, Chloramphenicol: C 30, Neomycin: N 30, Cıprofloxacin: CIP 5, Amoxycillin clavulanic acid: AMC30.

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