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Antimicrobial screening of *Alchemilla vulgaris* herbs and flowers

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Abstract: *Medical herbs have many bioactive component and they are used in microbial treatment since ancient time. Alchemilla vulgaris is one of them and it is important for folkloric medicine in Turkey. A. vulgaris related antimicrobial research isn't common, therefore herbs and flowers of this medical plant investigation were applied against 17 bacteria and 1 fungi by using disk diffusion method. These microbial strains include Bacillus, Enterobacter, Enterococcus, Escherichia, Klebsiella, Listeria, Pseudomonas, Salmonella, Staphylococcus and Candida geniuses. The results were presented that A. vulgaris ethanol extract has antimicrobial activity against all tested microbial strains.*

Keywords: *Alchemilla vulgaris, medicinal plant, antimicrobial activity, disc diffusion method ethanol extract, bacterial strains, fungal strain.*

I. Introduction

Before microorganism's observations, healing potential of some plants have been known. Since ancient time, human was used plant for infection treatment and some of these plants are still traditionally inclusive as part of the disease therapy [1]. In developing country, traditional herbs have critical significance for disease treatment and they aid to detect novel antibiotics [2]. Therefore, plant biochemical research is needed for continue of human health against pathogen infection. Besides, broad spectra antimicrobial activity related researches are important due to potential of drug development and investigation [3]. In the last three decades, antimicrobial activity related experiment have been applied by using plant extract [4]. This investigation demonstrate that Turkish medical plant related essential oils and extracts have antimicrobial potential [5]. Although the antimicrobial activity of many natural plant species were determined until today, the broad range antimicrobial activity of *A. vulgaris* herbs and flowers haven't been analyzed by disk diffusion method.
The purpose of present research were to detect the antimicrobial activity of *A. vulgaris* herbs and flowers ethanol extract against 17 bacteria and 1 fungus by disc diffusion method.

II. Materials And Methods

Plant sample
Alchemilla genus define about 250 species [6]. *A. vulgaris* samples were used in this study and they were obtained from herbiest outlet.

Extraction procedure
All *A. vulgaris* samples were dried after collection and the samples were ground by a mortar and a pestle. In order to extract active substances, ethanol (Sigma-Aldrich) was chosen as an extraction solvent. Ground samples were shaken in ethanol at 125 rpm for 2 days at room temperature [7]. All the extracts were filtered through Whatman No. 1 filter paper into evaporation flasks. The filtrate was evaporated by a rotary evaporator (HeidolphHei-Vap Value HL/HB-G1) at 45°C [8]. After evaporation the residues were collected and used to prepare 4.3 and 14.3 mg extracts.

Microorganisms
A wide range of Gram positive and Gram negative bacteria and yeast were selected to test the antimicrobial effect of *A. vulgaris*. The pathogenic microorganisms were chosen for the analyses on the basis of their significance because of potential for contamination of food and human infection. Other strains, which are not standard, were isolated from food and identified in Ankara University, Faculty of Science, and Department of Biology. *Bacillus subtilis* DSMZ 1971, *Enterecoccus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* DSMZ 20044, *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* DSMZ 50071, *Pseudomonas fluorescens* P1, *Salmonella enteritidis* ATCC 13075, *Salmonella typhimurium* SL1344, *Candida albicans* DSMZ 1386, *Enterococcus durans*, *Enterecoccus faecium*, *Listeria innocua*, *Klebsiella pneumonia*, *Salmonella infantis* and *Salmonella kentucky* were used in the study.

Preparation of inoculum

All bacterial strains were incubated at 37°C for 24 hours. But since the requirements for *C. albicans* is different, *C. albicans* was inoculated at 27°C for 48 hours. Inoculum were prepared by transferring morphologically similar colonies of each organism into 0.9% sterile saline solution until the visible turbidity was equal to 0.5 McFarland, thus standard inoculum is adjusted to contain approximately 108 cfu/mL for bacteria and 107 cfu/mL for *C. albicans* [7-10].

Disk diffusion method

Disk diffusion test was performed as described previously by Andrews [11]. The culture medium was poured into 120 mm sterile petri dish to give a mean depth of 4.0 mm ± 0.5 mm [12]. 30 µL and 100 µL aliquots of each extract was applied on sterile paper disks of 6 mm diameter end up with sample on each disk. To get rid of any residual solvent which might interfere with the results, disks were left to dry overnight at 30°C in sterile conditions. The surface of the plates was inoculated using previously prepared inoculum containing saline suspension of microorganisms. Inoculated plates were then left to dry for 5 min at room temperature before applying the disks [13, 14]. Disks were firmly applied to the surface of the plate which had an even contact with the agar. Plates were incubated and inhibition zone diameters were expressed in millimeters.

Controls

Empty sterile disks and extraction solvent (ethanol) loaded on sterile disks which were dried at sterile conditions to remove solvent as done in the study were used as negative controls. Also, gentamicin (10 µg) was used as positive control.

Statistics

The statistical analysis was executed using a non-parametric method Kruskal-Wallis which is one-way analysis of variance with $p< 0.05$.

III. Results And Discussion

Antimicrobial activity of *A. vulgaris* ethanol extract was analyzed. In order to load extracts, empty sterile disks were used. These disks were applied on a Mueller Hinton Agar, after they were inoculated with microorganism. Inhibition zone was observed, when the extracts had activity against these microorganisms. The diameter of these zones were measured in millimeters as Table 1.

Table 1. Disk diffusion test results for A. vulgaris and gentamicin (10 µg) (Inhibition zones in mm).

	30µL	100µL	Gentamicin
E. faecium	21	25	28
L. monocytogenes ATCC 7644	16	23	28
B. subtilis DSMZ 1971	19	22	30
C. albicans DSMZ 1386	18	22	-
S. epidermidis DSMZ 20044	17	21	25
P. aeruginosa DSMZ 50071	16	20	15
S. aureus ATCC 25923	15	17	24
S. kentucky	14	17	13
S. typhimurium SL 1344	14	14	23
K. pneumonia	12	14	22
*P. fluorescens*P1	10	13	12
E. faecalis ATCC 29212	9	13	13
S. infantis	9	13	24
L. innocula	9	12	16
E. durans	8	11	14
S. enteritidis ATCC 13075	7	10	24
E. aerogenes ATCC 13048	9	9	23
E. coli ATCC 25922	-	9	20
"-": No inhibition			

No activity for empty sterile disks and ethanol loaded on disks evaporated before application. Also, positive control was applied with gentamicin (10 µg). It is aminoglycoside antibiotic and protein synthesis is inhibited by binding 30S subunit of bacterial ribosome [15]. It has bactericidal activity against most gram negative and some gram positive, however restricted information is existing for gram positive bacteria [16]. Our analyses demonstrate that, this antibiotic has antibacterial activity against all tested bacteria and there aren't critical differences between these types. In previous in vivo and in vitro experiment demonstrate that gentamicin has candidacidal activity, however our disc diffusion result show that 10 µg gentamicin hasn't candidacidan

activity against *C. albicans* DSMZ 1386 [17, 18].*C. albicans*could beenreached resistance against gentamicin after thirty five years.

In Djipa et al. [19] experiment, *A. vulgaris* herbsaqueous extract antibacterial activity was investigated against 19 bacterial strains, which are only 13 of them different species, with MIC. According to their result, all tested bacterial strains less sensitive (MIC ≥ 1000mg/ml) except *S. huminis* 214 (750 mg/ml), *S. huminis* 218 (125 mg/ml) and *S. warneri* 215 (125 mg/ml).This result demonstrate that most of the tested microorganisms have low level antimicrobial activity.

In Keskin et al. [20] study, *A. vulgaris* leaves ethanol extract antimicrobial activity was determined against 10 bacteria and 1 fungus with disc diffusion method at 4 mg. According to their result, *K. rhizophila*(14 mm), *S. aureus* (12 mm), *E. faecalis* (12 mm), *P. vulgaris* (10 mm) and *C. albicans* (10 mm) have sensitivity against this plant extract. However, there aren't inhibition zone at *E. coli*, *B. cereus*, *B. subtilis*, *S. typhimurium*, *E. cloaceae* and *E. aerogenes*. This result demonstrate that *A. vulgaris* leaves have only moderate antimicrobial activity against half of the tested microorganisms. In our study, *A. vulgaris* herbs and flowers ethanol extract antimicrobial activity was determined against 17 bacteria and 1 fungi with disc diffusion method at 4.3 mg and 14.3 mg. According to our result, *A. vulgaris* has high antimicrobial activity against *E. faecium*(25 mm), *L. monocytogenes*(23 mm), *B. subtilis*(22 mm), *C. albicans* (22 mm), *S. epidermidis* (21 mm), *P. aeruginosa* (20 mm), *S. kentucky* (17 mm), *S. aureus* (17 mm) at 14.3 mg. Besides, this extract has moderate antimicrobial activity against *K. pneumonia* (14 mm), *S. typhimurium* (14mm), *E. faecalis*(13 mm), *S. infantis*(13 mm), *P. fluorescens*(13 mm), *L. innocua*(12 mm), *E. durans*(11 mm) and *S. enteritidis*(10 mm) at 14.3 mg. It has also low antimicrobial activity against *E. aerogenes* (9 mm) and *E. coli* (9 mm) at 14.3 mg. This result show that *A. vulgaris* herbs and flowers ethanol extract have antimicrobial activity against all tested strain at 14.3 mg. Eight of them have high susceptible; seven of them have moderate susceptible and only two of them have low susceptible.

When compare to gram negative and gram positive bacteria, gram negative bacteria have more resistance than gram positive bacteria against bioactive component [21]. Therefore, much greater activity was obtained against gram positive bacteria in related research. Our experiment has similar result and top of the highest activity was observed against gram positive bacteria. Concentration or dilution of bioactive component is essential, so 4.3 mg ethanol extract was used for comparison. Also, 14.3 mg extract was loaded on disc, so among increasing activity change was determined. According to Table 1, high amount of extract was achieved crucial antimicrobial activity. When our result compare with Keskin et al. [20] study result, our analyses antimicrobial activity level is much higher than their analyses result at about 4 mg plant ethanol sample. This differences can been obtained from its flowers antimicrobial activity. Therefore, *A. vulgaris* flowers ethanol extract analyses is needed for reaching exact consequence.

IV. Conclusion

Medicinal plants, which are used as antimicrobial agents, researches are required for discovery of novel drug development, therefore standard method application is critical. The present paper deals with the screening of *A. vulgaris* flowers and herbs. According to result, this plant activity was determined and they have antimicrobial activity against all tested microbial strain. Further researches are required in order to analysis the mechanism of action, interactions with antibiotics or other medicinal plants compounds, in order to determination of their chemical pharmacokinetic profile.

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