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In vitro Antimicrobial Activity Screening of *Galanthus trojanus* A.P.Davis & Özhatay

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Abstract*: In recent years, researchers tend to search for biologically active compounds against pathogenic microorganisms isolated from plant species due to the resistance of microorganisms to antibiotics. In this study in vitro antimicrobial activity of ethanol extract of endemic species Galanthus trojanus was investigated against Bacillus subtilis DSMZ 1971, Enterococcus faecalis ATCC 29212, Listeria monocytogenes ATCC 7644, Staphylococcus aureus ATCC 25923, Staphylococcus epidermis DSMZ 20044, Staphylococcus hominis ATCC 27844, Staphylococcus warneri ATCC 836, Bacillus cereus RSKK 863, Enterococcus durans, Enterococcus faecium, Listeria innocua, Staphylococcus aureus, Staphylococcus mutans, Staphylococcus hominis, Staphylococcus haemolyticus, Staphylococcus lugdunensis, Staphylococcus aureus, Enterococcus faecalis, Streptococcus pneumonia MDR, Staphylococcus aureus MRSA, Staphylococcus aureus MRSA+MDR, Enterobacter aerogenes ATCC 13048, Escherichia coli ATCC 25922, Pseudomonas aeruginosa DSMZ 50071, Pseudomonas fluorescens P1 , Salmonella enteritidis ATCC 13076, Salmonella typhimurium SL 1344, Shigella flexneri RSKK 184, Acinetobacter baumannii CECT 9111, Klebsiella pneumoniae, Salmonella infantis, Salmonella kentucky, Escherichia coli, Shigella boydi, Acinetobacter baumannii, Shigella flexneri, Klebsiella pneumoniae, Escherichia coli MDR, Klebsiella pneumoniae MDR, Acinetobacter baumannii MDR, Enterobacter aerogenes MDR, Serratia odorifera MDR, Proteus vulgaris MDR, Providencia rustigianii MDR, Achromobacter sp. MDR, Candida tropicalis, Candida glabrata, and Candida albicans DSMZ 1386 by using the disk diffusion method. It is observed that ethanol extract of endemic Galanthus trojanus has no antimicrobial activity against some microorganisms tested.*

Keywords: *Galanthus trojanus*; Endemic species; Ethanol extract; Disk diffusion method; Antimicrobial activity

1. INTRODUCTION

Using plants against diseases is assumed to be as old as human history. Today there is an increasing interest by the scientists to define the secrets of these traditional herbal medicines [1-3] The search for new antimicrobial agents has increased mainly because of the increase in infections particularly in developing countries with medically indigent populations and more so because of extensive bacterial resistance to current antimicrobial agents [4].

World Health Organization (WHO) has predicted increasing antimicrobial resistance as a major threat to public health for the 21st century. To prevent the spreading of antibiotic-resistant infections, scientists have been conducting intensive researches to determine new antimicrobial agents. One way to prevent antibiotic resistance of microorganisms is by using new compounds that are not based on existing antimicrobial agents. [5,6].

The purpose of the present research was to detect the antimicrobial activity of *Galanthus trojanus* ethanol extract against 48 microorganisms by the disk diffusion method.

2. MATERIALS AND METHODS

Plant Sample: The endemic plant sample used in this study was obtained from the Dokuz Eylül University Fauna and Flora Research and Application Center Laboratory.

Extraction: *G. trojanus* samples were dried after collection and the samples were ground by a mortar and a pestle. To extract active substances, ethanol (Sigma-Aldrich) was chosen as an extraction solvent. Ground samples were shaken in ethanol at 160 rpm for 2 days at room temperature [7-9]. 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 41°C [10]. After evaporation, the residues were collected and used to prepare 2,643 g extracts.

Microorganisms: A wide range of microorganisms was selected to test the antimicrobial effect of *G. trojanus*. These strains are *Bacillus subtilis* DSMZ 1971, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermis* DSMZ 20044, *Staphylococcus hominis* ATCC 27844, *Staphylococcus warneri* ATCC 836, *Bacillus cereus* RSKK 863, *Enterococcus durans*, *Enterococcus faecium*, *Listeria innocua*, *Staphylococcus aureus*, *Staphylococcus mutans*, *Staphylococcus hominis*, *Staphylococcus haemolyticus*, *Staphylococcus lugdunensis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus pneumonia* MDR, *Staphylococcus aureus* MRSA, *Staphylococcus aureus* MRSA+MDR, *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* DSMZ 50071, *Pseudomonas fluorescens* P1, *Salmonella enteritidis* ATCC 13076, *Salmonella typhimurium* SL 1344, *Shigella flexneri* RSKK 184, *Acinetobacter baumannii* CECT

9111, *Klebsiella pneumoniae*, *Salmonella infantis*, *Salmonella kentucky*, *Escherichia coli*, *Shigella boydi*, *Acinetobacter baumannii*, *Shigella flexneri*, *Klebsiella pneumoniae*, *Escherichia coli* MDR, *Klebsiella pneumoniae* MDR, *Acinetobacter baumannii* MDR, *Enterobacter aerogenes* MDR, *Serratia odorifera* MDR, *Proteus vulgaris* MDR, *Providencia rustigianii* MDR, *Achromobacter* sp. MDR, *Candida tropicalis*, *Candida glabrata*, and *Candida albicans* DSMZ 1386. Namely antimicrobial activities were investigated on 17 standards, 7 food isolates, 13 clinical isolates, and 11 multi-drug resistance (MDR) strains.

Preparation of Inocula: All bacterial strains were incubated at 37°C for 24 hours. But *Candida* strains were inoculated at 27°C for 48 hours. The inoculum was 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 10^8 cfu/mL for bacteria and 10^7 cfu/mL for *Candida* strains [11,12].

Disk Diffusion Test: Disk diffusion test was performed as described previously by Andrews [13]. The culture medium was poured into a 120 mm sterile petri dish to give a mean depth of 4.0 mm ± 0.5 mm [14]. 50 µL, 100 µL, and 200 µL aliquots of each extract were applied on sterile paper disks of 6 mm diameter end up with a 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 a saline suspension of microorganisms. Inoculated plates were then left to dry for 5 min at room temperature before applying the disks [15,16]. 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.

Statistics: All extracts were tested in triplicate and MacAnova (version 5.05) was used for statistical analysis of the data. P values of < 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

The main aim of this study was to identify the antimicrobial activity of ethanol extract of *G. trojanus*. To do this, a disk diffusion test was performed in the study. In this test, extracts were loaded on empty sterile disks and these disks were then applied on a culture medium inoculated with microorganisms. If the extracts were shown activity against these microorganisms, they have caused an inhibition zone. The diameters of the inhibition zones recorded in millimeters are given in Table 1. No activity was observed for the negative controls; extraction solvent and empty sterile disks.

Table 1. Disk diffusion test results for *G. trojanus* (Inhibition zones in mm)

Microorganism	50 µL	100 µL	200 µL
B. subtilis DSMZ 1917	-	-	-
C. albicans DSMZ 1386	-	-	-
E. aerogenes ATCC 13048	-	-	-
E. feacalis ATCC 29212	-	-	-
E. coli ATCC 25922	-	-	-
L. monocytogenes ATCC 7644	-	-	-
P. aeruginosa DSMZ 50071	7	8	8
P. fluorescens P1	-	-	-
S. enteritidis ATCC 13076	-	-	-
S. typhimurium SL 1344	-	-	-
S. aureus ATCC 25923	-	-	-
S. epidermidis DSMZ 20044	-	-	-
S. hominis ATCC 27844	-	-	-
S. warneri ATCC 27836	-	-	-
B. cereus RSKK 863	-	-	-
S. flexneri RSKK 184	-	-	-
A. baumannii CECT 9111	-	-	-
E. durans	-	-	-

E. faecium	-	7	10
K. pneumoniae	-	-	-
L. innocua	-	-	-
S. infantis	-	-	-
S. kentucky	-	-	-
E. coli	-	-	-
S. aureus	7	8	8
S. mutans	-	-	-
S. hominis	-	-	-
S. haemolyticus	-	-	-
S. lugdunensis	-	-	-
S. boydi	-	-	-
A. baumannii	-	-	-
S. flexneri	-	-	-
S. aureus	7	7	7
E. faecalis	-	-	-
K. pneumoniae	-	-	-
C. tropicalis	-	-	-
C. glabrata	-	-	-
E. coli	-	-	-
K. pneumoniae	-	8	8
A. baumannii	-	-	-
E. aerogenes	-	-	-
S. odorifera	-	-	-
P. vulgaris	-	-	-
S. .pneumoniae	-	-	-
S. aureus MRSA	13	14	13
S. aureus MRSA+MDR	-	-	7
P. rustigianii	-	-	-
Achromobacter sp.	-	-	-

"-": No inhibition

In our study, antimicrobial activity of *G. trojanus* was determined against 48 microorganisms with disc diffusion method. According to our result *Galanthus trojanus* has antimicrobial activity against *P. aeruginosa* DSMZ 50071 (8 mm), *E. faecium* (10 mm), *S. aureus* (8 mm), *K. pneumoniae* (8 mm), *S. aureus* MRSA (14 mm), and *S. aureus* MRSA+MDR (7 mm). However, there is no antimicrobial activity determined except for these bacteria strains.

In Keskin et al. [17] 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. cloacae*, and *E. aerogenes*. This result demonstrates that *A. vulgaris* leaves have only moderate antimicrobial activity against half of the tested microorganisms. In our study for *G. trojanus* ethanol extract, antimicrobial activity was determined against 45 bacteria and 3 fungi with disk diffusion method at 2,643 g. According to our result, low antimicrobial activity was observed.

Conter et al. [18] reported that *L. monocytogenes* strains are susceptible to the antibiotics commonly used in human listeriosis treatment, but *L. monocytogenes* is slowly becoming antibiotic-resistant and perpetual surveillance of emerging antimicrobial resistance of this pathogen is critical to ensure effective treatment of human listeriosis. From this point of view, having antibacterial activity *against L. monocytogenes* may be very important. However, no

antibiotic resistance was detected for this bacterium in this study.

According to the literature review, adequate antimicrobial studies have not been conducted for this plant.

4. CONCLUSION

As a result, it can be concluded that there is a clear antimicrobial activity of *G. trojanus* against some of the strains tested. The results of our study present that *G. trojanus* could have possible medicinal uses especially against *P. aeruginosa* DSMZ 50071 *E. faecium*, *S. aureus*, *K. pneumoniae*, and *S. aureus* MRSA. But further researches are needed to be conducted to analyze the active substances and their activity mechanisms in detail.

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