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Antimicrobial Activity of *Tulipa sintenisii*

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Abstract: In this study, ethanol, water, methanol and acetone extracts of *Tulipa sintenisii* (Baker) were tested for *in vitro* antimicrobial activity using disc diffusion method. All of the extracts exhibited antimicrobial effect on *Staphylococcus auresus* (ATCC 6538), *Escherichia coli* (ATCC 25922) and *Pseudomonas syringae* pv. Tomato (DSM 60407 (DSMZ)). While streptomycin (10 μg) which is used for control generates a zone diameter of approximately 15 mm against test microorganisms, it is ascertained that acetone extract generates an inhibition zone diameter of 10.5 mm against *Staphylococcus auresus*, 12 mm against *Escherichia coli* and an inhibition zone diameter of 14 mm against *Pseudommonas syringae* pv. tomato. As a result of the study, it can be said that the extracts of *Tulipa sintenisii* involves antimicrobial substances against the microorganisms which are used for this study. However, much more complicated researches are needed.

Key words: *Tulipa sintenisii*, antimicrobial activity, *Staphylococcus auresus, Eescherichia coli*, extracts, inhibition zone

INTRODUCTION

Medicinal plants have been used for medical treatment for ages as they contain compounds that have the value of healing (Nostro *et al*., 2000). According to World Health Organization (WHO), >80% of world population in the developed countries is primarily addicted to plants for their basic health needs in medicine Again, according to one of WHO's research based on some editions about medicinal plants and pharmacopoeias of 91 countries, the total number of medicinal plants which are used for healing is about 20 (Dulger *et al*., 1999; Kirbag and Zengin, 2005). Medicinal plants that are important for medical treatment have been being used in public for ages in our country too as it is in all other world countries (Kirbag and Zengin, 2005).

Some of antimicrobial compounds produced by plants are used against human and plant pathogen microorganisms in clinics (Mitscher *et al*., 1987). However, microorganisms put resistence against many antibiotics and as a result, some problems in treatment of infectious diseases emerge (Parekh and Chanda, 2007). For this reason, human pathogen not only causes bacteria to put resistence against existing antibiotics and their undesired side effect to occur but also it causes the investigation of new sort of chemical antibiotics being held and the investigation of how plant extracts and natural products can be the solution of these problems (Rabanal *et al*., 2002). *Tulipa sintenissi* Baker (*T. sintenisii*) which forms the study's material is an endemic plant in the city, Mus and any published record of study about this plant's *in vitro* antimicrobial effect has been found. For this reason in this study it is aimed to investigate *in vitro* antimicrobial activity of *T. Sintenisii* plant against *Staphylococcus auresus* (*S. Auresus*), *Escherichia coli* (*E. Coli*) and *Pseudomonas syringae* pv. tomato (*P. Syringae* pv. tomato) bacteria.

MATERIALS AND METHODS

Plant material: The samples of *T. sintenisii* were picked up from TIGEM (Head office of agricultural management; Mus, Turkey) and were diagnosed by Dr. Nilufer Selcuk (Yuzuncuyil University, Faculty of Science and Arts, Department of Biology, Major Field of Botanic).

Preperation of plant extracts: Plant extracts were prepared with modified version of Ahmad and Beg (1998)'s method. Bulb, leaf, flower and body parts of the plant were used in extraction process. About 16 g of the plant were weighed by a precision weighing machine and it was grinded thoroughly with the help of a muller in 70% of 50 mL

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ethanol, methanol, acetone and sterilized purified water eluents. Subsequent to grind process, the extracts were taken into sterilized glass beakers and each of the samples was homogenized completely in tissue blender for 5 min.

After that process, the beakers were covered by tinfoil and they were wagged with beater for 24 h. After finished the wagging process and the extracts were homogenized completely, they were filtered by using whatman no:1 filter paper. The obtained extracts were percolated through 0.45 μm millipore filter for the aim of sterilization. The filtrats were taken into clean and sterilized coloured glass bottles and kept there in +4°C until they were used again.

Preperation of test organisms: In the study, strains of *S. aureus* (ATCC 6538), *E. coli* (ATCC 25922) and *P. Syringae* pv. tomato (DSM 60407 (DSMZ)) were used as test organisms. Bacteria strains were incubated in Brain Heart Infusion Agar (oxoid) in 37°C for a night and pure colonies were obtained.

Disc diffusion method: Prepered plant extracts were absorbed in the sterilized 0.6 cm diameter antibiotic test discs in a way that each of them contains 40 μL plant extracts and they were kept waiting for an hour in a sterilized area so as to remove the alcoholic eluents totally. It is incubated by planting from pure colonies to Mueller Hinton Broth in 37°C. Suspension which contains 10^8 cfu mL^{-1} bacteria spreaded on Mueller Hinton Agar's (MHA) surface with the help of sterile ecuvion. Then, the discs in which extracts were absorbed were placed on MHL plates. Just after this process, MHA plates were left to incubation for a night in 37°C. At the end of the incubation process, inhibition zone diameters were measured. This procedure was performed for each bacterium strains and extracts at the same time as two serials.

Statistical analyses: SPSS (version 16.0) was used as a statistic packet program for statistical analyses of all parameters and ±SE values were calculated as an avarage standard error.

RESULTS AND DISCUSSION

The results of inhibition zone and diameters which were obtained from this study that investigates the antimicrobial activity of *T. Sintenisii*'s ethanol, water, methanol and acetone extracts on *S. aureus*, *E. coli* and *P. Syringae* pv. tomato has been shown in Table 1. It is observed that streptomycin (10 μg) which is used for control, generates zone diameter of approximately 15 mm. It is also ascertained that the ethanol extract obtained from the plant creates proximately 8 mm zone diameter on *E. coli*, 7 mm zone on *S. aureus* and 10 mm zone diameter on *P. Syringae* pv. tomato. It is designated that water extracts generate 7.5 mm zone diameter against *S. aureus*, 11.5 mm against *E. coli* and again 11.5 mm zone diameter against *P. Syringae* pv. Tomato. It is also determined that methanol extract creates inhibition zone diameter of approximately 7-8 mm on each of the three bacteria while acetone extract's effect creates 12 mm zone diameter on *E. coli* which is much more close to the inhibition zone created by streptomycin than the other extracts, 10.5 mm zone on *S. aureus* and 14 mm zone diameter on *P. Syringae* pv. tomato.

In this study, the antimicrobial effect of *T. Sintenisii*'s different extracts on *S. aureus* Gram (positive) and *E. coli* Gram (negative) bacteria which are human pathogens and *P. Syrringae* pv. tomato Gram (negative) bacteria which is plant pathogen was searched.

The results in this study are discussed over the studies of the other species which belong to *Tulipa* genus because any kind of study about antimicrobial activity of this plant could not be found throughout the litterateur review. In the study that Bazzaz and Haririzadeh investigate the antimicrobial activities of the extracts, which were obtained with methanol from the plants grown up in Iran, on *Bacillus subtilis* (ATCC6633), *Candida albicans* (ATCC10231), *Escherischia coli* (ATCC10536), *Klebsiella pneumoniae* (ATCC10031), *Morganella morganii* (PTCC1078), *Pseudomonas aeruginosa* (ATCC4027), *Salmonella typhi* (PTCC1185) and *Staphylococcus aureus* (ATCC29737), it is observed that the methanol extracts which belong to *Tulipa micheliana*'s whole parts create antimicrobial effect which does not make sense against *Staphylococcus aureus* and antimicrobial effect which makes sense against *Bacillus subtilis*, *Escherischia coli Morganella morganii*, *Pseudomonas aeruginosa* and *Salmonella typhi* while it does not create any antimicrobial activity against *Candida albicans* and *Klebsiella*.

Again at the same study, it is reported that the methanol extracts obtained from *Tulipa montana's*

Table 1: Inhibition zone diameters of *T. sintenisii*

	Inhibition zone diameters (mm)				
Test organisms	St	1	2	3	4
S. aureus (ATCC 6538)	15±1.0	7±0.0	7.5±0.5	7±0.0	10.5±0.5
E. coli (ATCC 25932)	15±0.0	8±1.0	11.5±0.5	8±1.0	12.0±1.0
P. syrringae pv. tomato (DSM 60407)	15±0.0	10±1.0	10.5±0.5	8±1.0	14.0±1.0

St: Streptomycin (10 μg), 1: Ethanol extract, 2: Water extract, 3: Methanol extract, 4: Acetone extract

whole plant parts and antennas has an antimicrobial effect which makes sense on *Bacillus subtilis*, *Escherischia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus* while it does not have such kind of an effect on *Candida albicans* and *Morganella* morganii (Bazzaz and Haririzadeh, 2003). In the study, it is detected that methanol extract obtained from *T. sintenisii*'s whole parts generates inhibition zone diameter of approximately 7-8 mm on the three bacteria used in the study.

The creation of zone on *Tulipa tarda* and *Tulips wilsoniana* species is not witnessed in the study which investigates antimicrobial activities of *Tulipa* species from extracts obtained by Shoji *et al*. (2005) using gel filtration and high-performance liquid chromatography on *Esherischia coli* (IFO3972), *Salmonella enteritidis* (IFO3313), *Pseudomonas aeruginosa* (IFO13275), *Staphylococcus aureus* (IFO13276), *Bacillus subtilis* (IFO3007) and *Candida albicans* (IFO1594) while it is witnessed that it creates inhibition zone diameter of 13.5 mm on *Tulipa batalinii*, 13.0 on *Tulipa chrysanta*, 16.0 on *Tulipa clusiana*, 14.3 on *Tulipa hageri*, 12.0 on *Tulipa kolpakowskiana*, 19.3 on *Tulipa linifolia*, 17.3 on *Tulipa maximowizii*, 13.8 on *Tulipa orpanidea*, 15.0 on *Tulipa pulchella*, 13.5 on *Tulipa praestans*, 16.5 on *Tulipa sosnowskyi*, 10.3 on *Tulipa urumiensis* and 16.8 mm *Tulipa vvedenskyi*. Again at the same study, it is ascertained placing the anthers which belong to *Tulipa* species on plates directly, creates that inhibition zone (Shoji *et al*., 2005). It is indicated in the study, which is handled by Shigetomi *et al*. (2010) and done with using especially 6-Tuliposide B which is a secondary metabolite formed in tulip anthers that this metabolite has a strong antibacterial activity. In addition, Fujimura *et al*. (2004) purified two candidate antimicrobial peptides as Tu-AP 1 and Tu-AMP 2 in bulbs which belong to *Tulipa gesneriana* (Fujimura *et al*., 2004).

CONCLUSION

It is identified that water, ethanol, methanol and acetone extracts of *Tulipa sintenisii* have antimicrobial effect against all test organisms researchers used in the study. Especially acetone extract produced almost the same result of inhibition zone diameter which is generated by streptomycin that we have used for the aim of control against *P. syringae* pv. tomato. As a result of the study, *in vitro* antimicrobial activity of *T. sintenisii* extracts against microorganisms has been exerted for the first time and it is detected that it is a necessity to explore the active ingredients which have antimicrobial feature with more complex studies of searching the effects of them on lots of microorganisms to rendering them be useful in industry and medicine.

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