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Investigation of antimicrobial activities of some sideritis species

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ABSTRACT

This research has been conducted on tea grown in the western Mediterranean region and also on the consumption of *Sideritis stricta* and *Sideritis condensate* species, in order to investigate their antimicrobial activity against 6 pathogenic bacteria. The plants were collected and dried at room temperature in the flowering stage. The essential oil of the plants was obtained with a Clevenger apparatus by the hydrodistillation method. Antibacterial activities of the extracts were determined with the disc diffusion method. In this method, MIC of the extracts which showed antibacterial activity were determined with the microdilution method. *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enteretidis* RSK 95046, *Klebsiella pneumoniae* ATCC 700613 were used as test bacteria. The results were evaluated according to the Clinical and Laboratory Standards Institute (CLSI) criteria. Ampicillin and penicillin were used as the positive control antibiotics. It was determined that hot water extracts of both plants did not have antibacterial activity. The results of the statistical analysis showed that there was a significant difference (P<0.05) between the antimicrobial activities of the essential oil of *Sideritis stricta* and *Sideritis condensate*. It was found that there was no significant difference between the antimicrobial activity of *Sideritis stricta* and ampicillin used as a control antibiotic. On the other hand, the antimicrobial activity of *Sideritis condensata* was lower when compared to the control antibiotic.

1. Introduction

Plants have always been the nutriments and the first medicines of people up to the present day. Humans discovered whether plants were poisonous or curative by using them in different ways. Thus, they succeeded in obtaining their active ingredients. Plants have been used for treatment for thousands of years (Çopuroğlu 2013).

It is estimated that there are approximately 750000-1000000 plant species in the world. However, it's been claimed that only 500000 of them have been identified (Baytop 1999). According to the ''Flora of Türkiye and East Aegean Islands'', there are 1251 genus and more than 12000 species and subspecies belonging to 174 families in Türkiye. This means that Türkiye's flora is substantial (Davis et al. 1988; Güner et al. 2000). 234 of these taxons have foreign origins and cultivated plants. The rest of them are plants naturally found in this region (Ekim et al. 1989).

The history of medicinal plants is as old as the history of humanity. Sumerians and Assyrians in B.C 5000-3000 were using medicinal plant, with as many as. 250 different plants being utilized. Greeks, Egyptians, and Hittites were also using medicinal plants. According to the data of the World Health Organization (WHO), there are about 20000 medicinal plants in the world. Since some of them are only being used locally, they cannot be listed completely. In recent years, there is a growing interest in using natural treatment methods; this has brought medicinal plants back onto the agenda. Today, approximately 70% of medicinal plants are being gathered from the natural environment and the rest of them are being cultivated (Baytop 1999).

In recent years, there has been a growth in the number of treatments with medicinal plants, and they are being used for many different types of illnesses (Sağlıkoğlu 2004). Consequently, the number of studies on medicinal plants has also increased.

It is known that, the extract obtained via different methods and volatile oils of aromatic and medicinal plants have some antimicrobial effects (Dorman and Deans 2000). If a plant is rich in alkaloids, volatile oils, glycosides, flavonoids, phenols, coloring agents, tannins and resins named as secondary compounds, it takes place in the group of aromatic and medicinal plants (Baydar 2005). The volatile oils or extracts of these plants have antimicrobial effects (Akgül 1993; Dorman and Deans 2000; Rauha at al. 2000; Marino et al. 2011; Proestos 2006). These antimicrobial effects arise from volatile oils and phenolic compounds with 150-600 IU molecular weight. The compounds, such as basic phenols, phenolic acids, quinones, flavonoids, tannins, coumarins, alkaloids, glycosides, lectins, polypeptides, volatile oils and terpenoids have biological activity and are found in plants. It was determined by some research that these compounds were effective on some pathogenic bacteria such as

Staphylococcus aureus, E. coli, Enterococcus faecalis, (E.faecalis) Pseudomonas aeruginosa, Salmonella enteretidis, Klebsiella pneumoniae (Uğur et al. 2005; Kılıç 2006).

Today medicinal and aromatic plants, which have some positive influences have gained more importance. It is thought that the *Sideritis* species, consumed as a kind of tea, can be used as an antimicrobial. In this study, the antimicrobial formatting of *Sideritis stricta* (*S. stricta)* and *Sideritis condensate* (*S. condensate)*'s extracts and volatile oils were analyzed comparatively. The antimicrobial characteristics of these two species were identified and evaluated statistically.

2. Material and Methods

S. stricta and *S. condensata* species grow in the West Mediterranean Region and are also consumed as tea. In this research, the antimicrobial activities of these species against some pathogenic bacteria were analyzed. Plants were gathered from the Bey mountains, Antalya and kept under room temperatures until analysis. For this analysis, *Staphylococcus aureus* ATCC 29213, *E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enteretidis* RSK 95046, *Klebsiella pneumoniae* ATCC 700613 were taken from the Microbiology Laboratory of Akdeniz University Hospital, Antalya.

Volatile oils of *S. stricta* and *S. condensata* were obtained via the hydrodistillation method with a Clevenger apparatus. While obtaining volatile oils, 20 grams of each plant were weighed separately and 300 ml of pure water was added. Then, it was distilled in the Clevenger apparatus for 5 hours until 0.5% yield was achieved (TSI 1991).

In order to obtain hot water extracts of *S. stricta* and *S. condensata*, 0.5 g of each plant was mixed with 100 ml of pure water and shaken. In water bath extracts were obtained in 5 min, 15 min, 25 min at 80°C and 150 rpm rotation. Each extract was filtered by Whatman No:42 filter papers. After this, they were kept under 4°C until further analysis (Zhu at al. 2004).

For antibacterial activity the agar well diffusion and disc diffusion methods were utilized. The bacteria for the disc diffusion method were incubated in a nutrient liquid medium at 37°C for 24 hours. The density of each bacteria, which were revitalized, in sterile physiological serum was adjusted for 0.5 Mc Farland ($1x10^8$ cfu ml^{-1}). Sterile swabs were submerged into the prepared suspensions. By pressing to the wall of the tube, excess liquid from the top was removed. Muller Hilton Agar (MHA, Merck 103872) filled pre-prepared Petri dishes were inoculated by the streak plate technique and left for 2 hours at 4°C. The Petri dishes were left for incubation for 24 hours at 37°C. In this study, ampicillin, penicillin discs were used for positive control. At the end of the incubation, the diameters of the zones were measured by caliper and evaluated (Ezoubeiri et al. 2005; Uğur et al. 2005).

After inoculation to MHA with the streak plate method, wells with 8mm diameter were dug and 50 µl volume extracts were transferred into the wells. The Petri dishes were left for incubation at 37°C for 24 hours (Ezoubeiri et al. 2005; Uğur et al. 2005).

Minimum inhibition concentrations were determined via the Broth microdilution method for the quantitative determination of antimicrobial activities of all the extracts and volatile oils showing inhibition effect on microorganisms. For this analysis 96 well microplates were used. Except for the first well, all other wells were filled with 50 µl Muller Hinton Broth (MHB, Merck 110293). The first and second well of the first row were filled with 50 µl plant extracts, which has a final density of 128 µg ml^{-1}. Starting from the second well, double dilution occurred by transferring 50 µl volume from well to well. The last well medium was chosen as the control well; therefore, from well before the last well 50 µl volume was removed outside and the last well was left empty. The standard antibiotic was diluted to the second raw wells in the same way. As mentioned above, bacteria suspensions which were adjusted Mc Farland 0.5 were added in 50 µl volume except the last well. Population growth was evaluated due to blur of the wells. The last well without blur was determined as value. Ampicillin, and penicillin antibiotics were used for positive control. Results were evaluated according to zone diameters (Andrews 2001; Bilgehan 2002; Toroğlu and Çenet 2006).

Analyses were carried out with 2 parallels and 2 repetitions. Results were evaluated with variance analysis and different mean values were evaluated with Duncan's multiple range test (Düzgündeş et al. 1987).

3. Results and Discussion

The analysis of variance results in Table 1 display the antimicrobial activities of volatile oils which were obtained from *S. stricta* and *S. condensata*.

The results of Duncan's multiple range test for antimicrobial effects of volatile oils from *S. stricta* and *S. condensata* are given in Table 2.

It was detected that there is a significant difference between the antimicrobial effects of these two species. Results show that the antimicrobial effect of *S. stricta* is not significantly different from the control antibiotic's average effect but *S. condensata* has a lower effect than the control antibiotic.

Karanika et al. (2001) determined that extracts of *Sideritis montana, Origanum dictamnus, Mentha piperita, Rosmarinus officinalis* and *Origanum marjorana* species, which belong to the *Lamiaceae* family, were effective on *Yarrowia lipolytica* yeast. It was believed that the difference between this research was due to the microorganism and plant species.

Uğur et al. (2005), and Kılıç (2006) have conducted similar research. Their results demonstrate that volatile oils of both plants are effective on the growth of all microorganisms. In comparison with other research, it has been thought that the differences originated from the use of different plant species and compounds. In addition, regional and seasonal differences were relevant in this situation (Toroğlu and Çenet 2006).

Table 1. Variance analysis results of antimicrobial effects of volatile oils obtained from two different *Sideritis* species and ampicillin

Variation Sources	S.D.	K.O.	F
Antimicrobial Effect	2	85.18586	66.28**

**Significant at $P<0.01$ level.

Table 2. Duncan's multiple range test results of antimicrobial effects of volatile oils obtained from *S. stricta* and *S. condensata* plants (mm)

Antimibrobial Effect	N	Diameter of zone (mm)
S. condensata	4	$14.52^{a} \pm 0.55$
Ampicillin	4	$22.78^{b} \pm 0.23$
S.stricta	4	$22.23^{b} \pm 0.78$

The difference between values that have different letters and at the same column ($P<0.05$).

MIC values of *S. sitricta* and *S. condensata* on *E. faecalis* bacteria were determined by the microdilution method and result MIC value of plant was found 4 µg ml^{-1}. For positive control, penicillin and ampicillin were used (Table 3).

In this study, the antimicrobial effects of *S. sitricta* ve *S. condensata* species on 6 pathogenic bacteria *(Staphylococcus aureus* ATCC 29213, *E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enteretidis* RSK 95046, *Klebsiella pneumoniae* ATCC 700613) were found.

Results show that, volatile oils are effective on *E. faecalis* ATCC 29212. Hot water extracts of plants did not have any antimicrobial effect on the test bacteria (Table 4).

According to the findings, plant species are very effective in terms of antimicrobial effects *(P*<0.01). It was observed that the antimicrobial activities of the examined bacteria *S. stricta* and *S. condensata* are significantly different ((*P*<0.05). *S. condensata* species has a lower antimicrobial effect than the control antibiotic's antimicrobial activity. It was determined that *S. stricta*'s antimicrobial effect was not different from the control antibiotic.

Table 3. Results of *S.sitricta'*s MIC analysis on *E. faecalis*

E. faecalis	MIC Values (µg ml^{-1})											
	128	64	32	16	8	4	2	1	0.5	0.13	0.06	0.03
S.stricta	-	-	-	-	-	-	+	+	+	+	+	+
Ampicillin	-	-	-	-	-	-	+	+	+	+	+	+
Penicillin	-	-	-	-	-	-	+	+	+	+	+	+

Table 4. Results of *S.condensata'*s MIC analysis on *E. faecalis*

E. faecalis	MIC Values (µg ml^{-1})											
	128	64	32	16	8	4	2	1	0.5	0.13	0.06	0.03
S.condensata	-	-	-	-	-	-	+	+	+	+	+	+
Ampicillin	-	-	-	-	-	-	+	+	+	+	+	+
Penicillin	-	-	-	-	-	-	+	+	+	+	+	+

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