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Anti-staphylococcal activity of *Scutellaria albida* subsp. *albida* against methicillin-resistant *Staphylococcus aureus*

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Abstract
The antibacterial activity of ethanol extracts of *Scutellaria albida* L. subsp. *albida* L. (Lamiaceae) was investigated against methicillin-resistant *Staphylococcus aureus* (MRSA). The antibacterial activity was evaluated by agar-well diffusion and microdilution method. The ethanol extract showed antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) strains (MIC < 1024 µg/mL). When compared with methicillin and gentamicin, the ethanol extract was more effective against MRSA, being a promising anti-staphylococcal agent.

Keywords: *Scutellaria albida* subsp. *albida*, anti-staphylococcal activity, methicillin-resistant *Staphylococcus aureus*

Introduction
Scutellaria L., known as skullcaps, is a cosmopolitan genus of Lamiaceae with approximately 350 species [1-2]. It is widely distributed in temperate regions and tropical mountains, including Europe, North America and South Asia [3]. This genus is represented by 34 taxa of which 15 species are endemic to Turkey in the flora of Turkey [4].
Scutellaria species have been used in herb species, fragrances, traditional and folk medicines in different parts of the world for centuries. They are well known many people as powerful medicinal herbs which are wild relaxants that affect the neural and muscular-skeletal systems [5].
The dry root of this genus is one of the most popular and multi-purpose herb used in China and several oriental countries are *Scutellaria baicalensis* Georgi, *S. viscidula* Bge, *S.amoena* C.H., *S. rehderiana* Diels, *S. ikonnikori* Jaz., *S. likiangensis* Diels and *S. hypericifolia*. It has been widely used in treatment of hepatitis, jaundice, tumor, leukemia, arteriosclerosis, diarrhea, and inflammatory diseases [5, 6]. In Turkey, it has been only used as styptic, wound for healing and strengthening in Anatolian folk medicine [7].
S. albida subsp. *albida* is a herbaceous perennial taxon, often somewhat woody at its base. It is distributed from N. Italy to the Balkan peninsula and Crimea [8]. During our routine field excursions, it was found that this plant is used to treat externally boils and abscesses. Therefore, the aim was determined the antibacterial effect of *S. albida* subsp. *albida* extracts against hospital isolates of methicillin-resistant *Staphylococcus aureus* (MRSA).

Materials and Methods
Plant Materials
Leaves of the plant were collected in Golyaka, Duzce – Turkey on May, 2015 and identified by Assoc. Prof. Dr. Ersin Karabacak from Department of Biology, Faculty of Science and Arts, Canakkale Onsekiz Mart University, Canakkale, Turkey. A voucher specimen of the plant (Voucher number GD 108-4) was deposited in Department of Medical Biology of Duzce University in the author's personal collection.

Preparation of extracts
The leaves of the plant were dried in an oven at 40°C (12 h) and powdered. Each dry powdered plant material (20 g) was extracted with 150 mL of 95% ethanol (Merck, Darmstadt, Germany) for 24 h by using Soxhlet equipment [9]. The extract was filtered using Whatman no.1, and the filtrate solvent was evaporated under vacuum using a rotary evaporator at 55°C

(yield: 14.4% for ethanol). The resulting dried extract (in the sticky black substances) amounting to around 2 g was dissolved in 0.1 mL of DMSO (5 mg/mL) (dimethyl sulfoxide) before testing.

Strains

Escherichia coli (ATCC 11230 and ATCC 10536), *Pseudomonas aeruginosa* (ATCC 25619 and ATCC 9027), *Staphylococcus aureus* (ATCC 6538P and ATCC 26923) were used as positive controls. The clinical strains and methicillin resistant (*Staphylococcus aureus* (MRSA) were obtained from research hospital at Duzce University, Duzce, Turkey. All strains were stocked at room temperature on Brain Heart Infusion Agar slants (Oxoid), and prior to assay, the cells were grown overnight at 35-37 °C in Brain Heart Infusion Broth (Oxoid).

Antimicrobial Assay

The solid medium diffusion technique using agar wells was used for screening the extracts for antimicrobial activity. 100 µL of the bacterial suspension (approximately 10^5 CFU/mL) was uniformly spread on sterile Brain Heart Infusion Agar petri dishes, and 50 µL of extract (10 mg/mL) were added inside agar wells of 6 mm in diameter. These plates were incubated at 25-37 °C for 24 h. The data of antibacterial activity were used only when the growth inhibition zone had a diameter ≥10 mm. MICs were determined by micro dilution method by adding 100 µL of each strain suspensed in Brain Heart Infusion Broth (final concentration 10^5 CFU/mL) to a 96 well microtiter plate with wells containing 100 µL of two-fold serial dilutions of extracts [10]. The final concentrations of extract were 512 to 8 µg/mL. MIC was defined as the lowest concentration required for growth inhibition. The minimal bactericidal concentration (MBC) was determined by inoculating Brain Heart Infusion Agar (Oxoid) plates with sample from non-growth wells [11, 12].

The MRSA strains 009-016 and 036 were assayed with methicillin and gentamicin (Sigma Co.) at final concentrations of 1024 to 1 µg/m L. All plates were incubated aerobically for 24h at 35-37 °C. MBC was defined as the lowest concentration showing no growth. All antimicrobial assays were performed twice and the results were expressed as the average of two repetitions. The solutions of the antibiotics were prepared using the recommendations of Clinical and Laboratory Standard Institute - CLSI [13].

Results and Discussion

Table 1 shows the inhibition zones generated by the extracts obtained from *S. albida* subsp. *albida* assayed against clinical isolates of *S. aureus* and bacteria used as positive controls.

The extracts obtained from *S. albida* subsp. *albida* have shown a strong antibacterial activity against used as positive controls with inhibition zones of 11.8-15.6 mm. Especially, *E. coli* ATCC 10536 is the most sensitive bacterium to the extracts (15.6 mm). The lowest effect has shown against *P. aeruginosa* ATCC 25619 (11.8 mm). Notably, the extracts inhibited the growth of all MRSA strains with zones of 10.8-18.8 mm. Five strains showed inhibition zones with diameter ≥ 17.0 mm such as 005, 009, 014, 016 and 036. The smallest inhibition zones were found with the MRSA strain 032 (10.8 mm), while the largest one was found with the MRSA strain 036 (18.8 mm).

Table 2 shows the anti-staphylococcal effect of the extract compared to the aminoglycoside Gentamicin and the β-lactam Methicillin. MIC and MBC values for the extracts were ≥1024/≥1024 µg/mL, were 1024/≥1024 µg/mL, 512/1024 µg/mL, 512/1024 µg/mL µg/mL and 512/1024 µg/mL µg/mL for the *S. aureus* strains 005, 009, 014, 016 and 099, respectively. The extracts were 1-2 times more effective in inhibiting *S. aureus* growth than these drugs against strain 014,016 and 036, but not against strain 005. The extracts against strain 009 were a little more effective than those of drugs.

The reports on the essential oil composition of *Scutellaria* L. species showed β-caryophyllene (27.75%), germacrene D (19.0%) and γ-amorphene (6.5%) as the major constituents from *S. scandens* [14]; germacrene D (39.7%) and β-caryophellene (15.0%) from *S. pinatifida* ssp. *alpine* [15]; linalool (52.63%) and trans-nerolidol (9.03%) from *S. albida* ssp. *albida* [16] and hexahydro farnesyl acetone (11.0%), 1,7,11,15,tetramethyl-2-hexadecen-1-ol (7.8%), menthol (7.7%) and 1-octen-3-ol (7.1%) as the principal constituents of the essential oil from *S. barbata* [17].

There are many investigations on antibacterial activity of *Scutellaria* species against *S. aureus*. For example, antibacterial properties have been demonstrated in extracts of *S. barbata*, but they were specific to *S. aureus*. Two ethanol-soluble extracts with NMR peaks corresponding to luteolin and apigenin were examined [17]. The apigenin analogue had MIC's for MRSA strains that were lower than those of the antibiotics; methicillin and oxacillin. Also, there seemed to be no significant activity against then other bacterial strains tested, suggesting that the flavonoids in *S. barbata* extracts may be toxic only to *Staphylococcus* [18].

Phytochemicals of *Scutellaria* are flavones, flavonoids, chrysin, iridoids, neo-clerodanes, scutapins, and isoscutellarein [5]. Flavonoids may be responsible for their antimicrobial activity. Activity od flavonoids is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. Lipophilic flavonoids may be disrupted microbial membranes [19]. It is declared only one study on antimicrobial effect of *S. albida* subsp. *albida* [16]. The oil (essential oil, linalool and trans-nerolidol) of this plant was tested against four bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* BBL 12084, *Escherichia coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853) and two yeast cultures (*Saccharomyces cerevisiae* ATCC 2366 and *S. cerevisiae* ATCC 9763). The oil has shown a moderate activity against the tested microorganisms. However, the oil (essential oil, linalool and trans-nerolidol) has a weak activity against *Staphylococcus aureus* ATCC 25923 with inhibition zones ranged between 9-10 mm. The results obtained from this study indicated that *S. albida* subsp. *albida* possessed significant activity against bacteria. Our findings clearly show that *S. albida* subsp. *albida* has strong effects against MRSA strains. The emergence of MRSA is one of the most serious issues in public health in developed countries. It does not only a high prevalence (<1-80 %), but it has also become resistant to almost the currently available antibiotics except teicoplanin and vancomycin [20]. The rapid development of resistance to vancomycin, the last resort antibiotics against MRSA recently has been reported in several countries [21].

Table 1: Origin, resistance profile of *Staphylococcus aureus* strains and inhibitory activity of *S. albida* subsp. *Albida*

			Inhibition (mm)
Strain	**Origin**	**PRP[a]**	**The plant extract**
E.coli ATCC 11230	-	-	14.2
E.coli ATCC 10536	-	-	15.6
P. aeruginosa ATCC 25619	-	-	11.8
P. aeruginosa ATCC 9027	-	-	12.4
S. aureus ATCC 6538P	-	-	14.8
S. aureus ATCC 25923	-	-	13.6
MRSA 001	Surgical wound	1	14.2
MRSA 002	Surgical wound	2	13.8
MRSA 004	Abscess	3	15.2
MRSA 005	Abscess	3	18.6
MRSA 009	Surgical wound	2	17.2
MRSA 011	Surgical wound	4	14.6
MRSA 014	Abscess	2	18.2
MRSA 015	Windpipe secretion	3	11.2
MRSA 016	Surgical wound	3	17.6
MRSA 032	Abscess	1	10.8
MRSA 033	Abscess	1	13.2
MRSA 036	Surgical wound	2	18.8
MRSA 038	Surgical wound	3	15.2
MRSA 039	Surgical wound	4	15.0

[a]Phenotipic Resistance Profile: PRP; 1: Oxacillin, Gentamicin, Tobramycin, Amikacin, Chloramphenicol, Rifamycin, Novobiocin; 2: Oxacillin, Gentamycin, Tobramycin, Amikacin, Kanamicin, Neomycin, Paromomicin, Butirocin, Sisomicin, Netilmycin; 3. Oxacilin, Penicillin, inductive Erythromycin, Kanamycin, Streptomycin, Gentamicin, Amikacin, Tobramycin, Tetracycline-minocin, 4: Oxacillin, Penicillin, Constitutive Erythromycin, Kanamycin, Streptomycin, Gentamicin, Amikacin, Tobramycin, Tetracycline-minocin

Table 2: Comparative MICs and MBCs of ethanol extracts of *S. albida* subsp. *albida* and antibiotics against MRSA strains isolated from clinics (µg/mL)

Strain	Methicillin MIC/MBC	Gentamycin MIC/MBC	The plant MIC/MBC
MRSA 005	≥1024 / ≥1024	≥1024 / ≥1024	≥1024 / ≥1024
MRSA 009	≥1024 / ≥1024	≥1024 / ≥1024	1024 / ≥1024
MRSA 014	≥1024 / ≥1024	≥1024 / ≥1024	512/1024
MRSA 016	≥1024 / ≥1024	≥1024 / ≥1024	512/1024
MRSA 036	≥1024 / ≥1024	≥1024 / ≥1024	512/1024

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