



NOTE

Antimicrobial Activity of Various Extracts of *Satureja spicigera*

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The antimicrobial activity of hexane, methanol, butanol and distilled water extracts of *Satureja spicigera* was evaluated. These extracts were tested against gram-negative bacteria *Salmonella typhi* (CCM 5445), gram-positive bacteria *Streptococcus pneumoniae* (ATCC 29212), *Staphylococcus aureus* (ATCC 6538), methicillin resistant *Staphylococcus aureus* MRSA (ATCC 43300), *Bacillus cereus* (ATCC 7064), *Enterococcus faecalis* (ATCC 15753) and yeast *Candida albicans* (ATCC 10231) using the MIC method. Butanol and methanol extracts performed the highest antimicrobial activity (MIC value of 512 µg/mL) whereas hexane extracts performed the lowest (MIC value of > 4096 µg/mL).

Key Words: Solvent effect, Minimum inhibitory concentration, Antimicrobial activity, *Satureja spicigera*.

Satureja spicigera (*S. spicigera*) is a plant, which grows up in Caucasia, Iran and Turkey¹. *Satureja* species have economical values because they have medicinal properties and are also used as spices². Volatile oil types and antioxidant properties of *S. spicigera* have been studied³ and their antibacterial activity was evaluated^{4,5}. To our best of knowledge neither antifungal nor antimicrobial activity of extracts of *S. spicigera* in different solvents nor antimicrobial activity of the *Satureja* types grew up in Turkey have been reported. Therefore, in the present study, we evaluated the antimicrobial activity of *S. spicigera* leaf extracts in different solvents like, butanol, hexane, methanol and distilled water against gram positive bacteria including *Streptococcus pneumoniae* (ATCC 29212), *Staphylococcus aureus* (ATCC 6538), methicillin resistant *Staphylococcus aureus* MRSA (ATCC 43300), *Bacillus cereus* (ATCC 7064), *Enterococcus faecalis* (ATCC 15753) and gram negative bacteria *Salmonella typhi* (CCM 5445) and yeast *Candida albicans* (ATCC 10231) using minimum inhibitory concentration (MIC) method.

Collection of plants and extraction: *S. spicigera* specimens were collected in August 2008 during the flowering time from high plateau of Kumru/ORDU and authenticated by Prof. Hamdi G. Kutbay (Department of Biological Sciences, Faculty of Science, University of Ondokuz Mayıs). Plant materials were extracted using soxhlet device with butanol, methanol, hexane (merck) and distilled water after the plants were dried. Solvents in extracts were removed by Brand lyophilized at -18 °C. The four different extracts obtained were stored at -30 °C prior to antimicrobial activity tests.

Test microorganisms: Antimicrobial activity of *S. spicigera* was examined against gram-negative bacteria species *Salmonella typhi* (CCM 5445), the gram-positive species *Streptococcus pneumoniae* (ATCC 29212), *Staphylococcus aureus* (ATCC 6538), methicillin resistant *Staphylococcus aureus* MRSA (ATCC 43300), *Bacillus cereus* (ATCC 7064), *Enterococcus faecalis* (ATCC 15753) and the yeast species *Candida albicans* (ATCC 10231).

Minimum inhibitory concentration studies: Minimum inhibitory concentration values were determined by using micro-dilution method of CLSI⁶ for antibacterial activity and NCCLS⁷ micro-dilution method for antifungal activity.

Mueller Hinton Broad and RPMI 1640 and 3-(N-morpho) propansulphonic acid (MOPS) were used as medium for bacterias and yeast, respectively. Solutions used in this study were also tested as negative control. Vancomycin, ceftazidime and amphotericin B were used as reference antibiotics.

Minimum inhibitory concentration values that determine antimicrobial activity of *S. spicigera* are given at Table-1. Especially butanol and methanol extracts of *Satureja spicigera* displayed antimicrobial activity against all microorganisms used in this study. The highest antimicrobial activity was observed in the butanol extract against *S. aureus*, *S. pneumoniae* and *S. typhi* and in the methanol extract against *S. aureus* and *S. pneumoniae* at MIC value of 512 µg/mL.

Minimum inhibitory concentration values of methanol extracts of *S. spicigera*'s were found as 512 for *S. aureus* and *S. pneumoniae*, 1024 for MRSA and *S. typhi*, 2048 for *B. cereus*,

TABLE-1
MINIMUM INHIBITORY CONCENTRATION ($\mu\text{g/mL}$) VALUES OF *Satureja spicigera*

	<i>S. pneumoniae</i>	<i>B. cereus</i>	<i>E. faecalis</i>	<i>S. aureus</i>	<i>S. typhi</i>	MRSA	<i>C. albicans</i>
Butanol extract	512	1024	1024	512	512	1024	1024
Methanol extract	512	2048	2048	512	1024	1024	2048
Hexane extract	> 4096	> 4096	> 4096	> 4096	> 4096	> 4096	2048
Water extract	4096	4096	> 4096	2048	4096	1024	> 4096
Butanol	2048	2048	2048	2048	2048	2048	2048
Hexane	2048	> 4096	> 4096	> 4096	> 4096	> 4096	> 4096
Methanol	2048	2048	4096	4096	2048	> 4096	> 4096
Ceftazidime	–	–	–	–	1	–	–
Vancomycin	0.125	0.5	1	0.5	–	0.125	–
Amphotericin B	–	–	–	–	–	–	0.5

C. albicans and *E. faecalis*. Minimum inhibitory concentration values of hexane extracts of *S. spicigera* were higher than 4096 for all microorganisms. Minimum inhibitory concentration values of butanol extracts of *S. spicigera* were 512 for *S. aureus*, *S. pneumoniae* and *S. typhi*, 1024 for *B. cereus*, *C. albicans*, *E. faecalis* and MRSA. Minimum inhibitory concentration values of water extracts of *S. spicigera* were 1024 for MRSA, 2048 for *S. aureus*, 4096 for *S. pneumoniae*, *B. cereus* and *S. typhi*, higher than 4096 for *C. albicans* and *E. faecalis*.

The maximal MIC values for bacterial and yeast strains, which were sensitive to the butanol and methanol extracts of *S. spicigera*, were in the range of 512-2048 $\mu\text{g/mL}$. MIC value of the *C. albicans* sensitive to the hexane extract was 2048 $\mu\text{g/mL}$ (Table-1). Based on these results, it is possible to conclude that butanol and methanol extracts had stronger and broader spectrum of antimicrobial activity as compared to hexane extract. This observation was confirmed in a previous study, which reported that in comparison to hexane, methanol was a better solvent for extraction of antimicrobial substances from the medicinal⁸.

While *S. aureus* and *S. pneumoniae* showed the highest sensitivity to butanol and methanol extracts, *S. typhi* showed the highest sensitivity to only butanol extract. On the other hand, MRSA showed the same sensitivity to butanol and methanol extracts. *B. cereus*, *C. albicans* and *E. faecalis* displayed lower sensitivity than the other microorganisms. *C. albicans*, which was used for determination of antifungal activity, performed the highest activity in butanol extract and the lowest activity at hexane extracts (Table-1).

In research done by Tumen and Baser⁹, main components of *S. spicigera*'s volatile oils were found as thymol (19.6-34.9 %), *p*-cymene (9.1-34.1 %), carvacrol (1.9-26.1 %), methyl carvacrol (5.7-21.2 %) and γ -terpinene (3.4-14.7 %). In this study, high antimicrobial activity was observed for extracts of high polar solvents as methanol and butanol. This situation was probably due to the good extraction of polar oil carvacrol, thymol and other volatile oils, which are known to have antimicrobial activity. As it could be seen in Table-1, antimicrobial activity of an apolar solvent like hexane was lower than polar solvents.

Minimum inhibitory concentration values for gram-positive bacteria were reported at Table-1 and it can be inferred that butanol and methanol extracts had performed higher antibacterial

activity than others. The highest antibacterial activity of butanol extracts and lowest activity at hexane extracts was observed for *S. typhi*, a gram-negative bacteria species. Congruent with the previous reports, it is found that the strength and spectrum of activity varied between *Satureja* species; gram-positive bacteria were generally more sensitive to the effects of the oils compare to gram-negative bacteria¹⁰.

The reference antibiotics, vancomycin, ceftazidime and amphotericin B, had MIC values in a trustable range. We did not observe any effect of solvents used for negative control on the MIC values of the extracts.

Conclusion

In this research, antimicrobial activities of *S. spicigera* extracts dissolved in different solvents were investigated. The highest MIC values of antibacterial and antifungal activities were observed by butanol and methanol extracts. The lowest antimicrobial activity was observed by hexane extract. According to results of this study, the methanolic and butanoic extracts of *S. spicigera* may be recommended as a new potential source of natural antimicrobial agent for human health. By this research information related *S. spicigera* is added to studies done for determination of antimicrobial activities of *Satureja* species.

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