

Antibacterial activity of *Thymbra spicata* var. *spicata* against Methicillin-resistant *Staphylococcus aureus*

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Abstract

Aqueous and ethanolic extracts obtained from *Thymbra spicata* L. var. *spicata* L. have been investigated for their ability 35 hospital isolates of Methicillin-resistant *Staphylococcus aureus* (MRSA). Both aqueous and ethanolic extracts of the plant were effective on MRSA. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) values of the ethanolic extract with greatest antibacterial activity were those of *T. spicata* var. *spicata* MIC 3.2-6.3 mg/mL and MBC 6.3-12.5 mg/mL, respectively.

Keywords: antibacterial activity, methicillin-resistant *Staphylococcus aureus*, *Thymbra spicata* var. *spicata*

1. Introduction

The genus *Thymbra* L., which belongs to Lamiaceae family, includes many native plants in Turkey and locally known as Karabaş Kekik, Kara Kekik, Sater or Zater ^[1]. It is traditionally used for flavouring different kinds of food products as herbal tea, and in folk medicine as an antiseptic agent ^[2-3].

T. spicata has a very large distribution area extending to almost all of the Eastern Mediterranean region countries. *T. spicata* var. *spicata* grows on calcareous, hillsides and open sunny places from 121 to 1249 m both under Mediterranean and semi-arid climatic condition of Turkey ^[1].

The antimicrobial properties of *T. spicata* var. *spicata* from Turkey have been reported ^[4-9]. However, the antimicrobial properties of this plant against hospital isolates of Methicillin-resistant *Staphylococcus aureus* (MRSA) have been relatively unexplored until recently.

Staphylococcus aureus is one of the major causes of both community and hospital-acquired infection ^[10-11]. It produces numerous toxins, including super-antigens that cause unique diseases such as toxic shock syndrome and staphylococcal scarlet fever ^[12]. It has become resistant to almost all the currently available antibiotics except teicoplanin and vancomycin ^[13]. The rapid development of resistance to vancomycin, the last resort antibiotics against MRSA, recently has been reported in several countries ^[14-15]. So, the aim of the study was to determine the effects against hospital isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) of the plant *T. spicata* var. *spicata* as wild-growing in Turkey.

2. Materials and Methods

2.1 Plant material

The plant *T. spicata* var. *spicata* was collected in Duzce University campus area, Duzce, Turkey on 2015. The plant was identified by Assoc. Prof. Dr. Ersin Karabacak from Canakkale Onsekiz Mart University, Canakkale, Turkey. A

voucher specimen was deposited in Department of Medical Biology of Duzce University in author's personal collections (voucher number GD 108-2).

2.2 Microorganisms

Thirty-five clinical isolates of MRSA (BD01 – BD035) were kindly provided by Research Hospital of Medical Faculty of Canakkale Onsekiz Mart University, Turkey, from Trakya University, Edirne, Turkey and Duzce University, Duzce, Turkey, respectively.

2.3 Preparation of extract

25 g of air dried plant samples were extracted in 150 mL of 95% ethanol (Merck, Darmstadt, Germany) for 24h by using Soxhlet equipment ^[16]. The extract was filtered with whatman filter paper no 1, and the filtrate was evaporated under vacuum in a rotary evaporator at 55 °C. The extract yield obtained was 8.7%. The dry extract, which was sticky and black, was stored a labeled sterile screw-capped bottles at -20 °C pending use. Prior to testing 2 g was dissolved in 0.4 L of dimethyl sulfoxide (DMSO) (5 mg/mL).

2.4 Screening for antimicrobial activity

The minimum inhibitory concentration (MIC) was determined by microdilution methods ^[17]. The reconstituted extract was serially diluted two-fold in Mueller Hinton Broth (Oxoid, Hampshire, UK) medium. Duplicate tubes of each dilution (ranging from 50.0 – 0.1 mg/mL) were inoculated with 5×10^5 cfu/mL of the best bacterial strain and cultures inoculated at 37 °C for 48h. Minimum inhibitory concentration (MIC) was taken as the highest dilution (least concentration) of extract showing no detectable growth. Minimum bactericidal concentration (MBC) was determined by subculturing the test dilution on the fresh extract-free solid medium and incubating further for 18-24h. The highest dilution that yielded no single bactericidal colony on a solid medium was taken as MBC.

3. Results and Discussion

Significant antibacterial activity, expressed as MICs and MBCs, of crude extracts obtained from *T. spicata* var. *spicata* against the 35 isolates of MRSA are given in Table 1. Ethanolic extracts of the plant were the most active against the MRSA isolates, with MICs and MBCs of 3.2 – 6.3 and 6.3 – 12.5 mg/mL, respectively. Notably, antibacterial assay indicated that the ethanol extracts of the plant used in this study were more efficient than those of the aqueous extracts. In previous study, ethanol was observed as the best solvent for extracting antimicrobial substances [18]. Ethanol extract is also a good solvent extract for obtaining polyphenolic compounds [19]. From the results, ethanol extracts has shown some affinity to extract bioactive compounds. This may be due to extractability of some bioactive compounds in ethanol.

In previous study, it is determined that the compositions of the essential oil from the aerial part of *T. spicata* var. *spicata* from Turkey resulted in the identification of 23 constituents, representing 97.04% of the oil. The major compounds detected in the essential oil were carvacrol (60.39%), γ -terpinene (12.96%), and p-cymene (9.61%) [8]. Other authors, reporting also on the essential oil composition of *T. spicata* from Turkey, found that its main constituents were carvacrol (75.74%), γ -terpinene (9.28%), p-cymene (7.17%), myrcene (1.39%), β -caryophyllene (1.13%) and thymol (0.15%), respectively [20]. The literature indicates that wild populations of *T. spicata* var. *spicata* are almost exclusively represented by the carvacrol containing chemotype [21-23]. In another study, the oxygene-containing phenolic monoterpene carvacrol was found to be the major constituent in the essential oil of *T. spicata*, followed by moderate amounts of the monoterpene hydrocarbons γ -terpinene and p-cymene, which are biosynthetically related [24].

There are many reports on the biological activities of *Thymbra spicata*. In previous study, the *in vitro* efficacy of *T. spicata* essential oil against 21 bacteria and 7 *Candida* species was investigated their antimicrobial activities. The essential oil demonstrated strong antimicrobial effects on a wide spectrum against most microorganisms, particularly the yeast tested [9, 25] and Mueller-Riebau *et al.* [26] have reported that the essential oils of *T. spicata* and its main phenolic constituents, carvacrol and thymol, show a remarkable antifungal activity by inhibition of the mycelial growth of the soil-borne phytopathogenic fungi *Fusarium moniliforme*, *Rhizoctonia solani*, *Sclerotinia sclerotium* and *Phytophthora capsici*, one of the most important plant pathogens of pepper growing in the East Mediterranean region of Turkey. Markovic *et al.* [27] determined that the oil of *T. spicata* and carvacrol showed the highest antimicrobial activity. According to results obtained from this study, ethanolic extracts of *T. spicata* var. *spicata* have antibacterial activity against the MRSA isolates, with MICs and MBCs of 3.2 – 6.3 and 6.3 – 12.5 mg/mL respectively. The findings are parallel to those reported in the mentioned studies. The effects of ethanolic extracts on MRSA were very high. *S. aureus* is a major cause of cutaneous infections, furunculosis, impetigo and abscesses, organ infections, osteomyelitis, endocarditis and arthritis, and toxinoses, such as food poisoning, septic shock, scalded skin syndrome and toxic shock syndrome [28]. According to the latest report from the National Nosocomial Infection Surveillance

System (NNIS), approximately 60% of all *S. aureus* nosocomial infections in intensive care units were methicillin resistant in 2003, representing an 11% increase in resistance compared to the proceeding 5-year period [29].

The results clearly show that *T. spicata* var. *spicata* is interesting source of biologically active compounds that may be useful for therapy against the MRSA infections.

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Table 1. Antibacterial activity of MIC and MBC (mg/mL) of the plant extract on Methicillin-Resistant *Staphylococcus aureus*

Plant tested	Aqueous extract		Ethanolic extract	
	MIC	MBC	MIC	MBC
<i>T. spicata</i> var. <i>spicata</i>	6.3–12.5	12.5–25	3.2–6.3	6.3–12.5

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

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