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ANTI-METHICILLIN-RESISTANT *S. aureus* ACTIVITY OF FRUITING BODY AND MYCELIAL CULTURE EXTRACTS OF *Xylaria longipes* NITSCHKE (ASCOMYCOTA)

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Resistance to penicillin by *Staphylococcus aureus* gave rise to methicillin-resistant *S. aureus* (MRSA) and the emergence of vancomycin-resistant *S. aureus* (VRSA) that was reported later. Finding an alternative antimicrobial in the treatment of Staphylococcal infections is the need of the hour. In this context, secondary metabolites of both the fruiting body and mycelia of wood-rotting fungi *Xylaria longipes* were evaluated for anti-MRSA activity. Thin layer chromatographic (TLC) separation and bioautography of the acetone extract revealed a strong anti-MRSA activity at $R_f = 0.69 \pm 0.28$. The bioactive anti-MRSA compound was partially characterized by Fourier transform infrared (FTIR) spectroscopy and liquid chromatography—tandem mass spectrometry (LC-MS/MS). The analysis suggested anti-MRSA activity could be due to integric acid, eremoxylarin C, or a related compound.

Keywords: antimicrobials; drug resistance; *Staphylococcus aureus;* wood-rotting fungi; *Xylaria longipes.*

1. INTRODUCTION

Staphylococcus aureus is an important human pathogen and the most common cause of nosocomial infections among human beings. Penicillin was an effective antibiotic in the treatment of *S. aureus* infections, however, most of *S. aureus* strains have developed resistance to penicillin. To counter this problem, methicillin was introduced but, after several years of its usage, methicillin-resistant *S. aureus* (MRSA) strains emerged. Currently, MRSA is resistant to most of the penicillin-like antibiotics called beta-lactams, which include amoxicillin, oxacillin, dicloxacillin, carbapenems, and others. Vancomycin, a glycopeptide antibiotic was the only effective alternative available in the management of multi-drug resistant MRSA [1]. However, the emergence of vancomycin-resistant *S. aureus* (VRSA) was reported later [2]. The resistance of *S. aureus* to various antibiotics and the increase in life-threatening infections caused by *S. aureus* in hospitalized and non-hospitalized patients is a great health care concern among the physicians and public health authorities. This necessitates the search for an alternative, potent antibiotic in the management of infections caused by *S. aureus*.

Macromycetes (macrofungi/mushrooms) are the groups of fungi with macroscopic 'fruiting bodies' belonging to the division Basidiomycetes and a few Ascomycetes. Macromycetes also include the lignicolous or wood-rotting (White-Rot) fungi which decompose the wood using both enzymatic and non-enzymatic reactions [3]. Macrofungi contain a variety of pharmacologically active compounds with antimicrobial, antiparasitic, antioxidative, antidiabetic, antineoplastic, antiulcer and hepatoprotective activities. The antimicrobial activities of these fungi against both Gram-positive and Gram-negative bacteria are well reported in the literature [4, 5]. In spite of their potential medicinal properties [6, 7], only 10% of mushrooms were described and even less have been tested for therapeutic values [8, 9].

Xylariaceae is a family of ascomycetes fungi that cause pseudo white rot/soft-rot type II of the wood [10]. Apart

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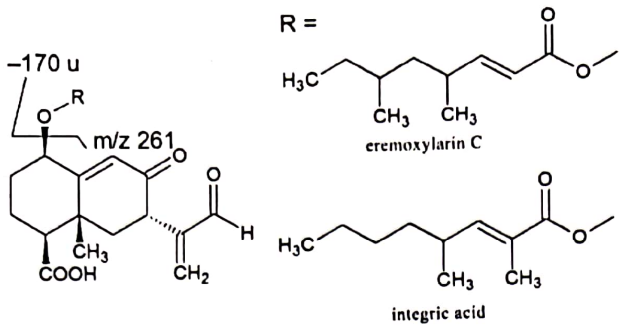

Fig. 5. Formulas of two suggested candidates for identity of the anti-MRSA compounds.

such as sesquiterpenoids, diterpenoids, diterpene glycosides, triterpene glycosides, steroids, N-containing compounds, pyrone derivatives, and polyketides [39]. Among various bioactivities, the antimicrobial activity of *Xylaria* spp. against human pathogens including *S. aureus* [29, 40 – 43] was demonstrated in laboratory experiments. Different species of *Xylaria* possessing antimicrobial activity include *X. polymorpha* [40, 41, 44], *X. curta, Xylaria* sp. Strain R005 and Strain R006 [29, 34, 41 – 42], and *X. feejeensis* 2FB-PPM08M [45].

Some of secondary metabolites are often similar within a group of organisms demonstrating similar bioactivities. Likewise, the compound identified in the present study and the similar compounds isolated from different species of the order Xylariales [46], also shows other biological activities [33, 34] including antibacterial properties [45, 46]. This sharing of secondary metabolites among various species of the same/different genus is due to the similar biosynthetic pathways resulting from evolutionary links originating from a common ancestor. However, there are possibilities of obtaining a molecule with superior biological activities due to structural variations in the molecules, because structural changes significantly influence the biological activities [47]. For example, three eremophilane sesquiterpenes, i.e., eremoxylarin A, B [48], and C [49] were isolated from various species of *Xylaria* with different biological activities. In the present study, *X. longipes* demonstrated anti-MRSA activity with antimicrobial potential not well reported in the literature (except Schneider, et al. [50] who revealed the presence of an 'antifungal' compound xylaramide). Similarly, this study carries importance in signifying the occurrence of an antibacterial (anti-MRSA) component in *X. longipes*, which further adds up to the knowledge of secondary metabolites of *Xylaria* species.

Thus, the present study demonstrated the antibacterial activity of *X. longipes* due to integric acid/eremoxylarin C or a related compound against MRSA. To the best of our knowledge, this is the first report on anti-MRSA activity of *X. longipes*. Further purification and structural characterization of the anti-MRSA compound and evaluation of its cytotoxicity are necessary to qualify the anti-MRSA compound for clinical applications.

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