Antibacterial Activity of Itrifal Mundi Against *Staphylococcus aureus*

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Abstract

The study of medicinal properties of plant based products is as old as medicine itself. Curative properties of herbal products have been exploited from the ancient past against various diseases. The recent trend of antibacterial research has revealed interest in search of new antibacterial agents of herbal origin that can have acceptability usually being nontoxic and inexpensive. The present study was undertaken to identify the strains of *Staphylococcus aureus* from the pus samples by using PCR technique. 16S rRNA gene was amplified using Universal primers. The amplified product was sequenced and the sequence was confirmed for *Staphylococcus aureus* after BLAST analysis with the NCBI database. Identified microorganisms were checked for their susceptibility to the Unani drug Itrifal Mundi. The microorganisms were found to be sensitive to the drug with the zone diameters ranging from 15mm to 22mm at the concentration of 10mg/ml. The MIC value of the drug was found to be in the range of 0.3125mg/ml to 0.625mg/ml for most of the tested strains of *S.aureus.*

Keywords: Itrifal Mundi, *Staphylococcus aureus,* Antibacterial activity.

Introduction

The microbial pathogens are responsible for more than 40 million human deaths per annum. Control and prophylactic measures for most of the diseases are far from expectation due to non-availability of effective medicines (Sharma, *et al.,* 2006). Antibiotics are being advised for either treatment or prophylaxis of infection. The first and the foremost criteria is the efficient identification and culture of relevant pathogens. The sequence analysis of genes encoding small sub-unit ribosomal RNA (16S rRNA) is currently the most promising approach in identification of organism, which involves the usage of Polymerase Chain Reaction (PCR) technology and BLAST analysis with NCBI database to identify the organism. *Staphylococcus aureus* is widely found in nature and is responsible for superficial, deep pyogenic infection and for toxin mediated illness. The *Staphylococcus aureus* acts as predominant bacteria in causing secondary infection in scabies (Brook, 2002; Walton *et al.,* 2007).

The study was aimed at the identification and isolation (genomic level) of Gram positive *Staphylococcus aureus* (coagulase positive) from the pus samples and their susceptibility pattern to the Unani drug Itrifal Mundi which is being prescribed by the Unani physicians for the treatment of scabies and itching (Anonymous, 2008).

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Materials and Methods

a. Collection of raw drugs

The formulation Itrifal Mundi (Anonymous, 2008) was prepared using twelve ingredients namely Post-e-Halela Zard (*Terminalia chebula* Retz. (Pericarp) DSM64), Halela Siyah (*Terminalia chebula* Retz. (Pericarp) DSM31), Post-e-Halela Kabuli (*Terminalia chebula* Retz. (Pericarp) DSM60), Post-e-Balela (*Terminalia bellirica* Roxb. (Pericarp) DSM56), Aamla Khushk (*Emblica officinalis* Gaertn. (Fruit) DSM07), Tukhm-e-Kishneez (*Coriandrum sativum* Linn. (Fruit) DSM76), Berg-e-Shahtara (*Fumaria parviflora* Lam. (Leaves) DSM11), Asl-us-Soos (*Glycyrrhiza glabra* Linn. (Root) DSM09), Ustukhuddus (*Lavandula stoechas* Linn. (Inflorescence) DSM85), Gul-e-Mundi (*Sphaeranthus indicus* Linn. (Inflorescence) DSM22), Qand Safaid (Sugar) and Raughan-e-Zard (Ghee) at laboratory scale in Drug Standardisation Research Unit (DSRU), Chennai, for the development of Standard Operating Procedures (SOPs) and to evaluate the pharmacopoeial standards. The raw drugs were procured from R.N. RAJAN & CO. local raw drug dealer, Chennai, identified and authenticated using pharmacognostical and physicochemical methods (Kokate *et al.,* 2000).

*DSM – Drug Standardisation Museum

b. Collection of microorganism

The pus samples were collected from Excellent Laboratory Chennai - 99 and Diagnostic Centre, Chennai-17. The samples were processed to phenotypic identification of various strains of *Staphylococcus aureus* using the existing conventional microscopical and biochemical tests (Mackie & McCartney, 1996). The eight *Staphylococcus aureus* isolates showing positive for coagulase test were isolated in pure form and were stored in the nutrient agar slants for further studies. The molecular level identification was carried out using FD1 and RP2 primers targeting 16S rRNA gene.

c. Molecular identification of micro-organism

i) Extraction of DNA

A loopful of isolated *Staphylococcus aureus* strains were suspended in 250 - 400 ml of Luria Bertani broth and their genomic DNA were extracted according to the standard protocol (Sambrook *et al.,* 1989). The DNA obtained was stored at -20°C until further use.

ii) Polymerase chain reaction (PCR) for amplification and Sequencing

The PCR was carried out in a Gradient Thermal Cycler (AG22331, Eppendorff, Germany) with the profile of initial denaturation at 95°C for 5 min, followed by 30 cycles at 95°C for 1 min, 59°C for 1 min annealing and 72°C for 1 min extension and final extension of 72°C for 7min. The commercial primers and the PCR reagents were used. The FD1 and RP2 universal primers coding for 16S rRNA gene were used for the analysis. The obtained 1460 bp of PCR product were purified and commercially sequenced (Banglore Genie). The sequences were further BLAST analysed using NCBI Database for molecular level identification.

d. Antibacterial activity

The in-vitro antibacterial activity of the drug Itrifal Mundi was performed using the cup plate method (Rai *et al*., 2011). The required number of Muller Hinton agar plates were prepared and swabbed with the molecular level identified *Staphylococcus aureus* isolates after confirmation using NCBI database. The plates were allowed to stand for few minutes. Wells of 10mm diameter were made using the agar gel borer and 100µl drug solution of 10mg/ml dissolved in the solvent DMSO was added into the wells. Commercially available drug norfloxacin (10mcg/disc) was used as positive control. Plain disc with100µl loaded solvent DMSO was placed as the vehicle control and the plates were incubated at 37°C for 24 hours.

e. Determination of Minimum Inhibitory Concentration (MIC)

MIC, the lowest concentration of the drug required to inhibit the microorganism was also determined by the agar diffusion method (Anonymous, 1982). Petridishes containing 20ml of Muller Hinton agar media were prepared and swabbed with different strains of *Staphylococcus aureus* isolates. The plates were allowed to stand for few minutes. Required numbers of 10mm diameter wells were made using the agar gel borer and 100µl of increasing concentration of the drug 0.3125mg/ml, 0.625mg/ml, 1.25mg/ml, 2.5mg/ml and 5mg/ml DMSO were added. The plates were incubated at 37°C for 24 hours. The lowest concentration of the drug that completely inhibits the growth was determined after overnight incubation at 37°C.

Results and Discussion

The Polymerase Chain Reaction amplified 1460bp, 16S rRNA gene from the various isolates of *Staphylococcus aureus* are shown in Fig. 1. The drug Itrifal

Mundi shows significant activity on all the tested strains of *Staphylococcus aureus* at the concentration of 10mg/ml. The drug has exhibited various degrees of inhibition against the tested bacterial strains with zone diameter ranging from 15mm to 22mm. The MIC values of three tested strains are found in the range of 0.3125mg/ml to 0.625mg/ml Fig. 2 (A, B & C). No inhibition was found below the concentration of 0.3125mg/ml in any of the tested strains\

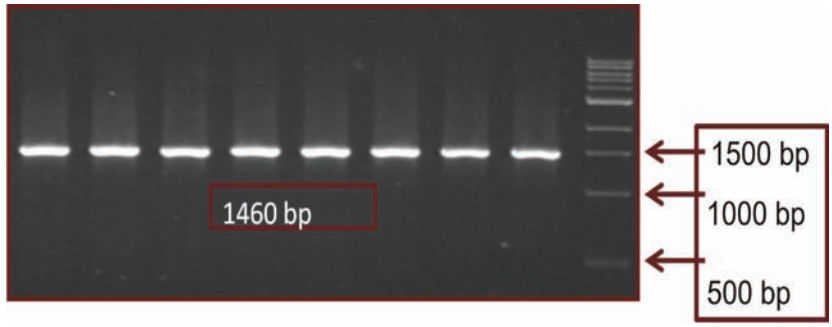

Fig. 1: PCR amplified 16S rRNA gene of Staphylococcus aureus Isolates

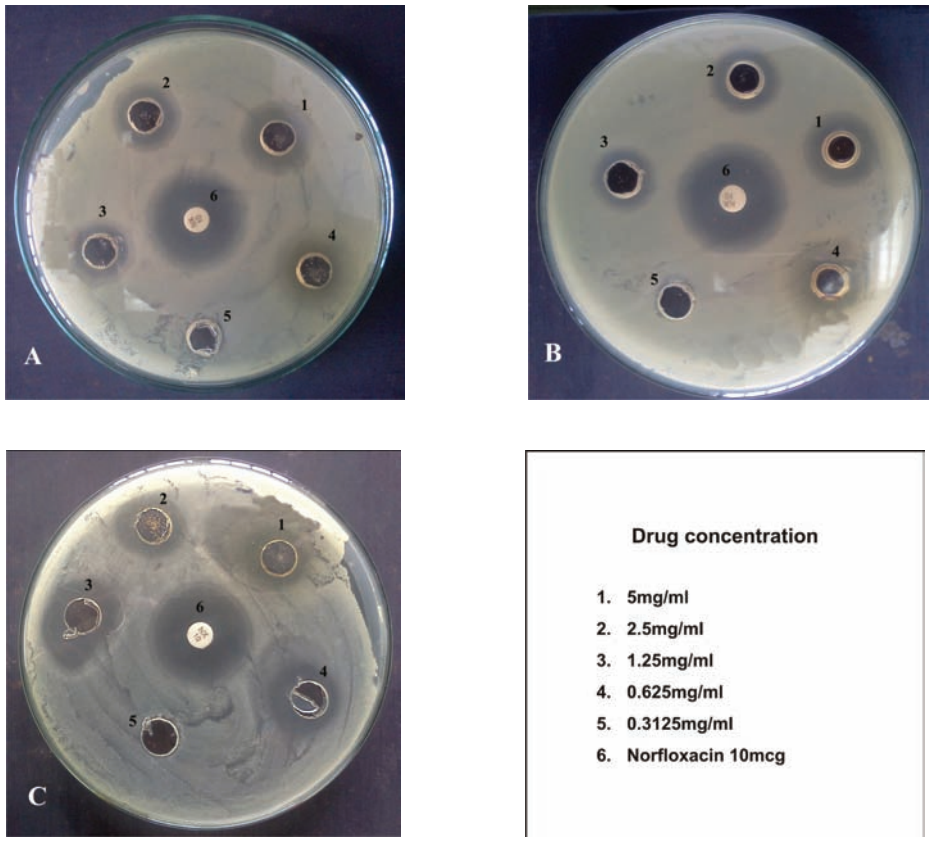

Fig. 2: Antimicrobial activity of Itrifal Mundi against different strains of Staphylococcus aureus

Conclusion

The study reveals the antibacterial activity of Itrifal Mundi against the pathogen *Staphylococcus aureus* responsible for skin infection. The use of PCR based technology has enabled easier and accurate identification of *Staphylococcus aureus* upto species level.

Acknowledgement

The authors are highly thankful to the Director General, Central Council for Unani Medicine, New Delhi for encouragement and providing necessary facilities. The authors also express their sincere thanks to SDDL, Vaccine Research Center - Viral Vaccines, TANUVAS, Madhavaram, Chennai, for allowing to use of PCR facility.

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