TO STUDY THE ANTIBIOTIC SUSCEPTIBILITY OF THE ISOLATED STRAINS OF
STAPHYLOCOCCUS AUREUS AND COMPARATIVE ANALYSIS
WITH NATURAL HERBS

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ABSTRACT
Extracts of three medicinal plants Neem (Azadirachta indica) bark, Tulsi (Ocimum sanctum) leaf, Turmeric (Curcuma longa) and honey were studied for their antimicrobial activity against S. aureus strains, isolated from air and soil of the MGM hospital area, Aurangabad. The isolated strains of S. aureus showed resistance to Methicillin, Oxacillin, Vancomycin and sensitivity to Gentamycin. The antimicrobial activity of antibiotics was determined by using both agar well diffusion method and disc diffusion method. The plant extracts and honey showed various levels of antimicrobial activity against resistant S. aureus under different concentrations. Pure honey showed good activity against most of the isolated S. aureus; whereas other three showed moderate activity against most of the isolated strain of S. aureus. The present study thus suggests the use of these medicinal plants in the treatment of various diseases caused by drug resistant S. aureus strains.

KEY WORDS: antibiotics, disc and agar well diffusion methods, neem extract-bark and leaf, natural honey, S. aureus, tulsi extract, turmeric extract,

INTRODUCTION
Staphylococcus aureus is a facultative anaerobic Gram-positive cocal bacterium. It is frequently found as part of the normal skin flora on the skin and nasal passages. It is estimated that 20% of the human population are long-term carriers of S.aureus. It can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), postsurgical wound infections. Methicillin-resistant S.aureus, is one of a number of greatly-feared strains of S. aureus which have become resistant to most antibiotics. Often found associated with institutions such as hospitals.

Beta-lactam antibiotics like Methicillin, Oxacillin, Aminoglycoside antibiotics like Gentamycin and glycopeptide antibiotics like Vancomycin are used in the prophylaxis and treatment of infections caused by Gram-positive bacteria (Kulkarni et al., 1979). Methicillin acts by inhibiting the synthesis of bacterial cell walls, Oxacillin is a penicillinase-resistant β-lactam. It is similar to Methicillin, and has replaced Methicillin in clinical use. However, resistant strains called Oxacillin-resistant Staphylococcus aureus (MRSA/ORSA) are highly prevalent. The original indication for Vancomycin was for the treatment of penicillin resistant Staphylococcus aureus. Vancomycin never became the first-line treatment for Staphylococcus aureus as it possesses poor oral bioavailability; it must be given intravenously for most infections, found to be toxic to the ears and to the kidneys; Natural herbs like Turmeric, Tulsi, treeslike Neem – bark and leaves and natural Honey are known from long times for their medicinal properties (Yagoub, S. et al, 2006). Turmeric’s active ingredient is curcumin with a distinctly earthy flavor and a mustardy smell, known for its antibiotic activity in rural India since ages (Chattopadhyay and Bhattacharyya, 2007; Chopra et al., 1992).

Products made from Neem trees have been used in India for over two millennia for their medicinal properties: neem products are believed to be antihelminthic, antidiabetic, antibacterial, antifungal, antiviral,(Ram Kumar et al., 2010). It is considered a major component in Ayurvedic and Unani medicine for skin disease. (Nayak Aarati et al., 2010). Tulsi is widely known across South Asia as a medicinal plant, commonly used in Ayurveda. It has anti-oxidant properties with main chemical constituents like oleanolic acid, rosmarinic acid, eugenol, carvacrol, linalool, β-caryophyllene, β-elemene (c.11.0%), β-caryophyllene (about 8%), and germacrene D (about 2%) (Rubina Lawrence et al., 2012). Antibacterial properties of honey are the result of the low water activity causing osmosis, chelation of
free iron, high acidity, and the antibacterial activity of methylglyoxal, effective in killing drug-resistant biofilms (Shahedur Rahman et al., 2011).

MATERIALS AND METHODS

Collection of *S. aureus*: Isolation of *S. aureus* from the soil of GMCH, AURANGABAD and from the air of MGM, AURANGABAD by serial dilution and spread-plate technique on nutrient agar plates initially and later on MSA agar (*Mannitol Salt Agar*).

Identification of *S. aureus*: Identification of *S. aureus* was done by Gram’s staining and by observing typical yellowish shining colonies on the media. It is later confirmed by biochemical and enzymatic tests.

Antibiotic susceptibility tests

The activity of 4 antibiotics Gentamicin (10 mcg/disc), Oxacillin (5mcg/disc), Vancomycin (30mcg/disc), Methicillin (5mcg/disc) was studied on the isolated strain. These 4 antibiotics were collected from lab of MGM’s Institute of Biosciences and Technology. Nutrient Agar plates were prepared and bacterial suspensions were spread on plates and labeled for different antibiotics by disc diffusion and agar well diffusion. (Aljabri 2005; Holt et al., 1986)

Preparation of natural antibiotic extract from herbs:

**Turmeric aqueous extract:** The aqueous extract were prepared by adding 25gm of turmeric powder in sterilized distilled water to make 100 ml of aqueous extract. Mixture was to be kept undisturbed at room temperature for 24 hrs in sterile flask covered with aluminum foil. After 24 hrs the extract was filtered by Whatman filter paper No.1 and evaporated in water bath until 25 ml extract was left. (Firdaus Jahan et al, 2011; Ramkumar and Jain, 2010)

**Neem aqueous extract:** Neem aqueous extract was prepared by mixing 15gm of dry powder of neem leaves with 100ml sterile distilled water, the extract was then filtered through muslin cloth and finally through Whatman filter paper No.1 (Parekh and Chanda, 2007; Prashant et al, 2007).

**Neem bark methanolic extract:** 20 gm of neem bark powder was mixed with 100ml of methanol and this mixture was kept for distillation process. After the distillation we get methanolic extract of neem bark. (Nayak Aarati et al, 2011; Parekh and Chanda 2007; Galang et al., 1994) The extract can be stored at 4ºC use for future use.

**Tulsi acetone extract of by distillation method:** 1.7gm of air dried tulsi leaves crushed into 32ml of acetone and we kept for distillation process. After the distillation we got the acetone extract of tulsi. The extract was stored at 4c for further use. (Parekh and Chanda, 2007; Chatopadhyaya and Bhattacharya, 2007).

**Honey:** Natural raw honey was taken and warmed at 28°C and dilutions were made with sterile distilled water as follows – 100%, 75%, 50%, 25%, 12.5% and 6.25% (Shahedur Rahman, 2011; Bansal et al., 2005; Agbaje et al., 2006).

Natural Extracts susceptibility tests

**By Disc diffusion method:** Nutrient agar medium was used to test the antibacterial activity. Discs of 10mm were punched out from the blotting filter paper and sterilized in autoclave. Discs are soaked and impregnated with 100ug of respective solvent extracts ml(dry form) were placed on the surface of the petri plates seeded with 0.1 ml of microbial suspension and incubated at 37 c for 24hrs.

**By using agar well diffusion method:** Antimicrobial activity was checked by agar well diffuson method also. Nutrient agar medium was poured into sterile petri plates and allow solidifying. 100ul of respective extracts were placed into wells punched into the medium in the petri plates seeded with 0.1ml of microbial suspension. After allowing the extract to diffuse into agar, the plates were incubated at 37c for 24 h.
Measurement of zone of inhibition (ZIH): The zones of inhibition of the tested microorganisms by the extracts were measured and compared with those observed with standard antibiotic discs. All the tests, both standard antibiotics and natural herbs were performed in duplicates and average observations are estimated.

RESULTS AND DISCUSSION
The antibacterial activity by the commonly used antibiotics was checked by disc diffusion method (Figure 1, 2, 3 and 4) and the results are represented in table 1 to 5.

Table-1 Zone of Inhibition of standard Antibiotics

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Standard antibiotics</th>
<th>Zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methicillin (10mcg/disc)</td>
<td>No Zone</td>
</tr>
<tr>
<td>2</td>
<td>Oxacillin (5mcg/disc)</td>
<td>No Zone</td>
</tr>
<tr>
<td>3</td>
<td>Gentamicin (10mcg/disc)</td>
<td>1.9 cm Zone</td>
</tr>
<tr>
<td>4</td>
<td>Vancomycin (30mcg/disc)</td>
<td>No Zone</td>
</tr>
</tbody>
</table>

Fig-1 methicillin activity  
Fig-2 oxacillin activity  
Fig-3 vancomycin activity  
Fig-4 gentamycin activity

Antibacterial activity of Turmeric aqueous extract by disc diffusion is shown in Figure 5 and table -2. Turmeric aqueous extract did not control Staphylococcus as expected. Turmeric oil needs to be extracted and tested further.

Table2. Zone of inhibition by aqueous extract of turmeric

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Concentration aqueous extract of turmeric</th>
<th>Zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disc diffusion</td>
<td>Agar well diffusion</td>
</tr>
<tr>
<td>1</td>
<td>5%</td>
<td>No Zone</td>
</tr>
<tr>
<td>3</td>
<td>15%</td>
<td>No Zone</td>
</tr>
<tr>
<td>5</td>
<td>25%</td>
<td>Mild Zone</td>
</tr>
</tbody>
</table>

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Figure 5. Turmeric extract plate

Antibacterial activity of neem bark methanolic extract by agar well diffusion method is shown in Figure 6 and 7 and table-3. Neem bark methanolic extracts showed a better zone of clearance than aqueous extracts in all the treatments, at higher concentrations.

Table 3. Zone of inhibition by neem bark methanolic extract

<table>
<thead>
<tr>
<th>sr.no</th>
<th>Concentration of neem bark methanolic extract</th>
<th>Zone of inhibition</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4%</td>
<td>0.9cm</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>8%</td>
<td>1.0cm</td>
<td>1.3cm</td>
</tr>
<tr>
<td>3</td>
<td>12%</td>
<td>1.2cm</td>
<td>1.5cm</td>
</tr>
<tr>
<td>4</td>
<td>16%</td>
<td>1.4cm</td>
<td>1.5cm</td>
</tr>
<tr>
<td>5</td>
<td>20%</td>
<td>1.6cm</td>
<td>1.7cm</td>
</tr>
</tbody>
</table>

Figure 6. Neem methanolic extract-20%

Figure 7. Neem methanolic extract-12%

Antibacterial activity of tulsi acetonic extract by agar well diffusion method are shown in Figure 8 and table-4. Tulsi acetonic extract showed promising zone at higher concentrations than in diluted treatments.

Table 4. Zone of inhibition by tulsi acetonic extract

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Concentration of tulsi acetonic extract</th>
<th>Zone of inhibition</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4%</td>
<td>1.0</td>
<td>1.1cm</td>
</tr>
<tr>
<td>2</td>
<td>8%</td>
<td>1.0cm</td>
<td>1.1cm</td>
</tr>
<tr>
<td>3</td>
<td>12%</td>
<td>1.1cm</td>
<td>1.3cm</td>
</tr>
</tbody>
</table>
Figure 8. Tulsi extract 12%

Antibacterial activity of honey by agar well diffusion method is shown in Figure 9 and table 5. Honey had a positive effect at higher concentrations than in diluted treatments.

Table-5. Zone of inhibition by Honey.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Concentration of honey</th>
<th>Zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>12.5%</td>
<td>No Zone</td>
</tr>
<tr>
<td>3</td>
<td>25%</td>
<td>No Zone</td>
</tr>
<tr>
<td>4</td>
<td>50%</td>
<td>1.1cm</td>
</tr>
<tr>
<td>5</td>
<td>75%</td>
<td>1.3cm</td>
</tr>
<tr>
<td>6</td>
<td>100%</td>
<td>1.8cm</td>
</tr>
</tbody>
</table>

Figure 9. Honey -75% & 100%

Our results showed that the test organism i.e MRSA exhibited varying degrees of resistance to standard antibiotics, used in this study. However, of all the natural herbs taken up for this study namely turmeric (Curcuma longa), neem (Azadirachta indica), tulsi (Ocimum tenuiflorum), and honey the isolated microbes showed strong susceptibility to honey. Honey appears to be effective in killing drug resistant biofilms which are implicated in chronic rhinosinusitis. It has been shown that natural unheated honey has some broad-spectrum antibacterial activities when tested against pathogenic bacteria, oral bacteria as well as food spoilage bacteria. The potency of honey at 100% concentration was found to be higher than all other concentrations tested. However, no effect was observed at concentration of 6.25% v/v honey. The different types of concentration of raw honey was made as 100%, 75%, 50%, 25% and 12.5%. The zone of inhibition was observed in three concentrations i.e. 100%, 75% and 50%. The aqueous extracts of turmeric are less promising in killing the bacterium which is already resistant to potent chemical compositions, suggesting that either the suitable variety of turmeric needs to be identified or the extraction of chemical principle needs to be standardized. For tulsi, the extraction procedure was done in acetone etc. Neem bark methanolic extracts showed varying degree of anti-bacterial activity, which suggests that extraction procedures can be standardized to show efficient activity. The present study thus suggests the use of these medicinal plants in the treatment of various diseases caused by drug resistant S. aureus strains. For thousands of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs. The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief. It is estimated that local
communities have used about 10% of all flowering plants on Earth to treat various infections, although only 1% have gained recognition by modern scientists. With the present analysis we conclude that S. aureus strain is sensitive to honey and honey can be used as alternative therapy. More investigation is needed to explore the possible benefits of the use of honey among therapies in the treatment of bacterial infections.

ACKNOWLEDGEMENT
We would like to thank our Director and HOD. Dr. Sanjay N. Harke, who constantly supported us to carry out this project. We also thank Mr. Ashok Shinde and Mr. Devendra Deshmukh, colleagues at MGM’s Institute of Biosciences and Technology, for their valuable guidance and help.

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