

Antimicrobial Efficacy of Selected Ayurveda Formula against Laboratory Specimen of *Staphylococcus aureus*

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Abstract: Selected formula consisted of three ingredients including dried leaves of *Azadirachta indica*, dried seeds of *Sesamum indicum* and Bees' honey which is clinically use for open wounds. The current study was designed as a microbiological assay and the key objective was to evaluate the antibacterial efficacy of TNK against laboratory specimen of *Staphylococcus aureus* (ATCC25923). The Anti-Bacterial Sensitivity Test was conducted according to the Kirby Bauer method using Agar Well Diffusion method by comparing the effect of Amoxicillin as the positive controller and distilled water as the negative controller in triplicates. The testing drug was assessed as D1 and D2 in 1:2 concentration ratios respectively. Results of the study were obtained through the diameter measurement of inhibitory zone and assessed using one - sample T – test. D1 depicted p value as 0.024 and T – test was 4.44. D2 depicted p value as 0.100 and T- test was 1.89. Comparing to the positive control drug, the hypothesis was generated as $H_0: \mu \leq 19$ mm and $H_1: \mu > 19$ mm. Referring to hypothesis, H_0 of D1 was rejected and H_0 of D2 was not rejected. The significant level was considered less than 5%. D2 concentration of TNK was significant against laboratory specimen of *Staphylococcus aureus*. The study suggests that the TNK is consisted with an extrinsic effective antibacterial application for infected wounds which were caused by *Staphylococcus aureus*. Further clinical study on human subjects will verify the efficacy of TNK in clinical manifestations.

Key Words: Antibacterial Activity, *Staphylococcus aureus*, TNK, Kirby Bauer method

I. INTRODUCTION

Herbs are the key ingredients used in Ayurveda medication. Various parts such as barks, flowers, roots etc. are manually processed in different methods to discover their optimal potential efficacy. Among herbs, *Azadirachta indica* and *Sesamum indicum* are two herbs widely used in Ayurveda pharmacological practice. The current study discusses on antibacterial efficacy of *Thilanimbadi Kalka* (TNK) against laboratory specimen of *Staphylococcus aureus*. The study focusses on wound healing effect due to *Staphylococcul* infections.

Ayurveda authentic text 'Susruta Samhita' and Kaiyadewa Nighantu highlights that *Azadirachta indica* is consisting with *Vruna Shodhana* (wound cleansing) and *Krimighna* (destroying worms / antibacterial) properties. [1-2] Another authentic text ' Bhava Prakash' mentions that leaves of *Azadirachta indica* is effective in cleansing maggot infested wounds. [3] Several recent studies have already proven that *Azadirachta indica* is consisting with antibacterial effects against *Staphylococcus aureus*. [4-5]

Effective antibacterial properties are available in *Sesamum indicum* plant, specially for common skin pathogens such as *Staphylococcus* and *Streptococcus* infections. [6] Ancient Ayurveda literature elaborates positive evidences on antimicrobial effect of *Sesamum indicum* plant including wound cleansing and wound healing effect. [7, 8, 9]

Bees' honey clears wound infection to facilitate healing of deep surgical wounds with infection. [10-11] Antimicrobial effect of bees' honey against *Staphylococcus aureus* is considerably high. [12]

Azadirachta indica, *Sesamum indicum* and Bees' honey individually contain antimicrobial properties against wounds infected with *Staphylococcus aureus*. *Thilanimbadi Kalka* (TNK) is prepared using *Azadirachta indica*, *Sesamum indicum* and Bees' honey. Therefore, the current study was conducted to evaluate the antibacterial activity of *Thilanimbadi Kalka* (TNK) against laboratory specimen of *Staphylococcus aureus*.

II. METHODOLOGY

Collection of Plant Materials

Fresh leaves of *Azadirachta indica* and Seeds of *Sesamum indicum* were collected at Thambagalle area of Nikadalupotha, Kurunegala District in Sri Lanka. Bees' honey was collected at Galgamuwa of Kurunegala District in Sri Lanka.

Culture and Maintenance of Microorganisms

Pure cultures of *Staphylococcus aureus* (ATCC 25923) were obtained from the Medical Technology Division of Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya, Sri Lanka. The pure bacterial cultures were maintained on nutrient agar medium. Each bacterial was further maintained by sub culturing regularly on the same medium and stored at 4°C before use in experiments. Kirby's Single disc antibiotic sensitivity testing of Staphylococci was used in the current study. [13]

Preparation of Testing Drug Extract

The testing drug was prepared according to the 'Kalka Paribhasha' of Sharangadhara Samhita, 'Fresh raw materials should be obtained in double quantity. Liquids should be obtained in double quantity. Drug should be grounded well in soft paste with no fibrous particles left in it'. [14] Ingredients were authenticated by Department of Dravyaguna Vijnana, Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya, Sri Lanka.

Fresh leaves of *Azadirachta indica* and Seeds of *Sesamum indicum* were collected from source plant were washed for 2-3 times with tap water and finally with distilled water, followed by ethanol wash and then allowed to dry at 30 °C and 50 °C for overnight respectively. Herbal ingredients were grounded well into soft paste while adding Bees' honey till no fibrous particle felt in it.

Sample D₁ which finely grounded paste, sample D₂ which squeezed extract of paste were tested for Antibacterial Sensitivity Test.

Microbiological screening

Antimicrobial activities of different extracts were evaluated by the Agar Well Diffusion method [15] modified by Olurinola, 1996 [16] and Minimum Inhibitory Concentration (MIC) [17]

Media Preparation and Its Sterilization

For agar well diffusion method (Murray et al., 1995 later modified by Olurinola, 1996) antimicrobial susceptibility was tested on solid (Agar) media in petri plates. For bacterial assay nutrient agar (NA) (38.16 gm/L) was used for developing surface colony

growth. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined by serial micro dilution assay.

The suspension culture, for bacterial cells growth was done by preparing 2% Nutrient Broth (w/v) taken for evaluation. All the media prepared was then sterilized by autoclaving the media at (121°C) for 15 min.

Agar Well Diffusion Method

Agar well-diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with 18 hour old - broth culture of respective bacteria and fungi. Wells (8mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. About 50 µl of different concentrations of study drug solvent extracts were added sterile syringe into the wells and allowed to diffuse at room temperature for 2hrs.

Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37°C for 24 h for bacterial pathogens. The diameter of the inhibition zone (mm) was measured and the activity index was also calculated. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

Preparation of Inoculum

The antibacterial assay was carried out by micro-dilution method in order to determine the antibacterial activity of compounds tested against the pathogenic bacteria. The bacterial suspensions were adjusted with peptone water to a concentration of 1.0×10^7 CFU/ml. The inocula were prepared and stored at 4 °C until use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculum. All experiments were performed in duplicate and repeated three times. According to the turbidity standard method (0.5 McFarland standard), (-1) Dilution series was selected and put in to the Muller-Hinton agar plates.

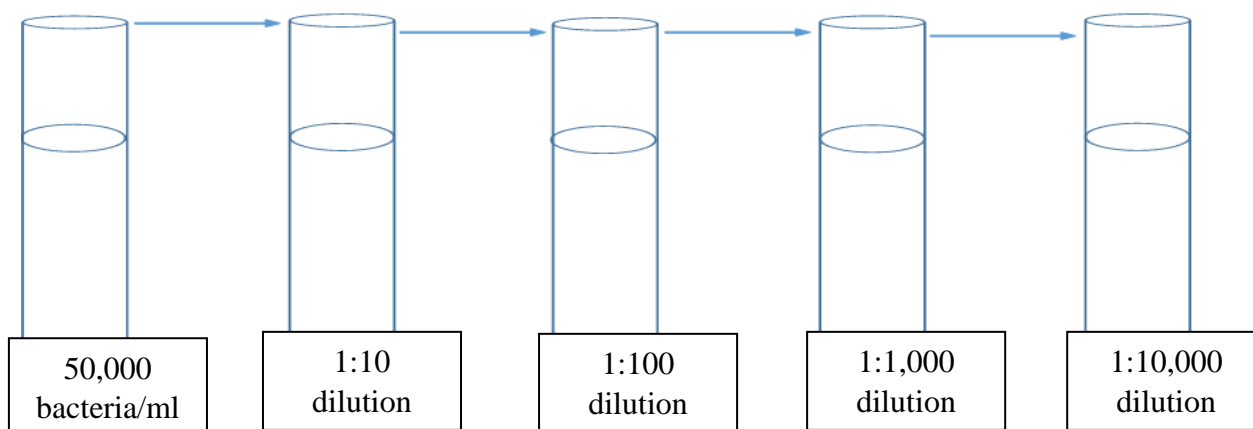


Figure 01: Dilution Series

Determination of MIC

The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs.

Determination of MBC

The MBCs were determined by serial sub-cultivation of 2 µl into plates containing 100 µl of broth per well and further incubation for 72 hours.

The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum and compared with the standards Amoxicillin (Table 01) for Bacteria (Hi-media) as the positive control.

All experiments were performed in duplicates (D₁ & D₂) and repeated three times.

Table 01: Standard Amoxicillin Sensitivity Chart

Antibiotic (Antimicrobial agent)	Disc code	Resistance (µ)	Intermediate	Susceptible
		<or=mm	mm	=or>mm
Amoxicillin (Other)	AMC	<13	14-17	>18
Amoxicillin (Staph)	AMC	19		20

(Fall. 2011 – Jackie Reynolds, Richland College, BIOL 2421)

Results were compared with the standard Amoxicillin sensitivity chart and drug resistance were taken as µ ≤19 vs µ > 19.

iii. RESULTS

Hypothetical assessment elaborates H₀ µ ≤19 vs µ > 19

Table 02: One Sample T Test (Test of µ ≤19 vs µ > 19)

Variable	Sample size	Mean	Standard deviation	SE Mean	95% lower bound	T value	P value
D ₁	3	24.33	2.08	1.2	20.82	4.44	0.024
D ₂	3	20.667	1.528	0.082	18.091	1.89	0.100

Significant level was considered less than or equal 5%.

Table 03: Hypothetical Assessment

Sample	T - Test	P - value	Conclusion
D ₁	4.44	0.024	H ₀ is rejected
D ₂	1.89	0.100	H ₀ is not rejected

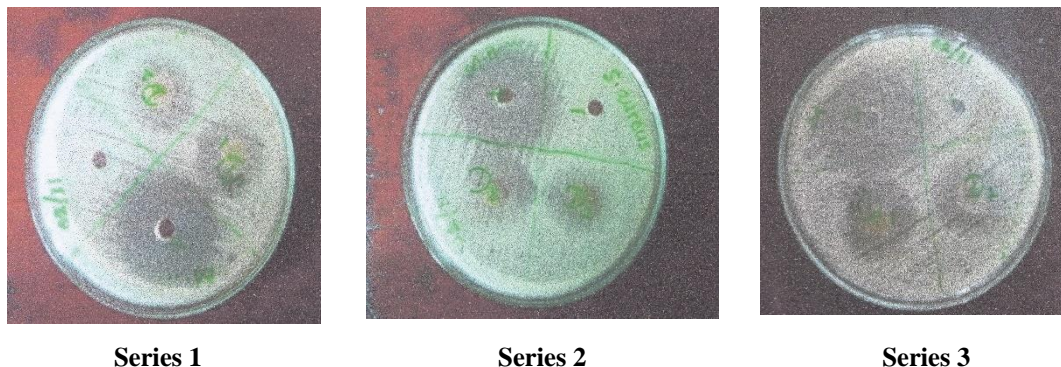


Figure 02: Inhibitory Zone Diameters

IV. DISCUSSION

Considering adverse effects and eco-friendly usage, search for antimicrobials from natural sources has achieved a time-honored attention and efforts have been put in to identify compounds that can act as suitable antimicrobials agent to replace antibiotics carrying adverse effects. [18, 19] Plants derived phytochemicals serve as a prototype to develop more effective medicines in controlling microbial infections. Among Ayurveda medicines, TNK is introduced as a wound disinfectant medicine in Ayurveda authentic text Susruta Samhita.

The current study was subjected to incipient screening for antibacterial activity against laboratory specimen of *Staphylococcus aureus*. The ABST elaborated mean inhibitory zone diameter of D₁ = 24 mm and D₂ = 21 mm. among both samples, finely grounded sample depicted most antibacterial sensitivity.

V. CONCLUSION

The present investigation elaborates that TNK contains potential antimicrobial components that may be of great use for the development of pharmaceutical industries as a therapy against infected wounds with the purpose of wound cleansing.

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