Evaluation of an antibacterial efficacy of Denibadi Yoga against Staphylococcus aureus

Sampath, D.L I [1], Peiris, K.P.P [2], Samaranayake, G.V.P. [3]

[1]BAMS undergraduate[2] Professor in Shalakya, Dept. of Shalya – Shalakya,
[3]Lecturer (Probationary), Dept. of Ayurveda Basic Principles, Gampaha Wickramarachchi University of Indigenous Medicine, Sri Lanka

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Abstract: Staphylococcus aureus a gram-positive coccus which leads to produce several diseases in Urdvajatrugata region (parts above the clavicles). But in present era gradually organisms get resisted to the antibiotics is the main potential hazard to treat the infectious disease conditions. An increasing incidence of multidrug resistance bacteria has triggered immense interest in the search for new drugs or alternatives. The purpose of this study is to investigate and identify antibacterial efficacy of two different dosage forms of Denibadi kashaya and Denibadi panta, against Staphylococcus aureus. Denibadi yoga is prescribed as one of the anupana of Sitharama vati (traditional indigenous preparation). It is widely used in Shalakya related disease conditions which deals with treating the diseases of nose, eyes, ears, oral cavity and head region. The antibacterial efficacy of Denibadi yoga is not scientifically evaluated. Therefore, this study was designed to evaluate the antibacterial efficacy of Denibadi yoga in two different dosage forms against staphylococcus aureus. The two dosage forms of Denibadi yoga were prepared according to the standard preprational procedures described in Ayurveda authentic texts. The inoculum spreads over the agar plates uniformly to obtain microbial growth. The prepared 8mm wells were poured with Denibadi yoga (Denibadi kashaya [D1] and Denibadi phanta [D2]) as research drug, Amoxicillin as the positive control [P] and distilled water as the negative control [N]. All four samples were triplicated, and plates were incubated at 37℃ for 24 hours. The antibacterial efficacy against Staphylococcus aureus was determined by using Mean Inhibitory Zone Diameter (IZD). Data revealed that two dosage forms of Denibadi yoga has an antibacterial efficacy against Staphylococcus aureus, but kashaya form is more efficacious than phanta form was evident.

It can be concluded that while compared with positive control kashaya form of Denibadi yoga has more than 50% efficacy against Staphylococcus aureus.

Keywords: Denibadi kashaya, Staphylococcus aureus, Denibadi phanta

Introduction

As soon as man reached the stage of reasoning, plants were discovered to be useful as medicines. In many countries, they are also used as micro nutrient powders to treat various ailments. In addition, these formulations have been reported to possess antimicrobial properties when consider as single drug. In this study, comparative testing for the antibacterial effect of Denibadi yoga in two different dosage forms (Kashaya and Phanta) against the bacteria Staphylococcus aureus was designed to investigate scientifically.

Staphylococcus aureus leads to cause many Shalakya (parts above the clavicles) related diseases in ear, nose and oral cavity. Therefore, it is important to find a suitable remedy to treat Staphylococcus aureus [1]. Denibadi Yoga is one of the effective drug using in Shalakya Roga. It is widely prescribing in two different dosage forms as Kashaya or Phanta. Plant origin antibacterial has an enormous therapeutic potential in the treatment of infectious diseases and simultaneously mitigating most side effects that are often associated with synthetic antimicrobials [2]. Increase in incidence of multidrug resistance bacteria has triggered immense interest in search of new drugs or alternatives from natural sources which include plants against the pathogens such as methicillin resistant Staphylococcus aureus, Escherichia coli, Klebsiella spp and Pseudomonas aeruginosa & Candida albicans [3].

Research methodology

Drug preparation: All the raw ingredients were identified and authenticated by the Department of Dravyaguna, Faculty of Indigenous Medicine, GWUIM, Sri Lanka.

Table - 1 Ingredients of Denibadi Yoga

Ingredient	Botanical Name	Part used
Nimbha	Azadirachta indica	Bark
Bhu Nimbha	Munronia pinnata	Whole plant
Vruhathi	Solanum melongena	Root
Kantakari	Solanum virginianum	Fruit
Shonaka	Oroxylum Indicum	Bark
Pata	Cissampelos pareira	Whole plant
Varuna	Crateva adansoni	Bark
Shunti	Zingiber officinale	Rhizome

Preparation of Kashaya and Phanta

Both dosage forms were prepared according to the authentic text Sharangadhara Samhita[4]

Preparation of Nutrient Broth

The nutrient broth was prepared by dissolving 13 g in 1000 ml. The medium dispensed as desired and sterilized by autoclaving at 15 lbs pressure 121ºC for 15 minutes and kept the broth to cool at room temperature. Staphylococcus aureus bacteria (ATCC 25923) inoculated into the broth to grow and incubated at 37ºC for 18 hours. [5]

Preparation of Inoculum

Dilution series prepared by using the broth. Staphylococcus aureus (ATCC 25923) bacteria spread by using a sterilized cotton swab through the Mueller Hinton Agar (MHA) plates.

Preparation of media plate

Dissolved 38 g of Mueller Hinton Agar (MHA) medium in 1000 ml of distilled water and boiled in 1 minute until dissolved the medium completely. The medium autoclaved at 15 lbs pressure at 121ºC for 15 minutes and allowed to cooled it until 45ºC. The autoclaved medium mixed well and took 100 ml to pour into sterilized Petri plates (25-30 ml /plate). Allowed to solidify at room temperature [6].

Screening for antibacterial activity

Inoculum spread over the nutrient agar plate by using a sterile cotton swab in order to obtain uniform microbial growth. Three wells bored with the help of cork borer. Denibadi kashaya [D1] and Denibadi phanta [D2] as research drug and Amoxicillin [P] as the positive control and distilled water [N] as the negative control poured into the wells. The plates incubated at 37ºC for 24 hours. All the samples were triplicated and mean Inhibitory Zone Diameter (IZD) was measured by using the normal ruler.

Data Collection

The antibacterial activity was evaluated by measuring Mean Inhibitory Zone Diameter (IZD) of all triplicated samples.

Results & Discussion

Table - 2 Zone Diameters of the samples (mm)

Petri dish	A	B	C	Maximum	Minimum	Mean
Amoxicillin[P]	20 mm	19 mm	22 mm	22 mm	19 mm	20.33 mm
Distilled Water [N]	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm
Kashaya [D1]	12 mm	10 mm	11 mm	12 mm	9 mm	11 mm
Phanta [D2]	9 mm	9 mm	10 mm	10 mm	9 mm	9.33 mm

Fig 1 – Results of antibacterial test of Kashaya and Phanta

Fig 2 – Results of antibacterial test of the Kashaya and Phanta

Data Analysis

Table 3 – Statistics of Paired samples

	Sample plates (IZD)	Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Kashaya [D1]	11.00	3	1.000	.577
	Amoxicillin [P]	20.33	3	1.528	.882
Pair 2	Phanta [D2]	9.33	3	.577	.333
	Amoxicillin	20.33	3	1.528	.882
Pair 3	Amoxicillin	20.33	3	1.528	.882
	Distilled Water [N]	.00	3	.000	.000
Pair 4	Kashaya	11.00	3	1.000	.577
	Phanta	9.33	3	.577	.333

Table 4 - Paired Samples Correlations

	Sample - IZD	N	Correlation	Sig.
Pair 1	Kashaya vs Amoxicillin	3	.327	.788
Pair 2	Phanta vs Amoxicillin	3	.945	.212
Pair 3	Amoxicllin vs Distilled water	3	-	-
Pair 4	Kashaya vs Phanta	3	.000	1.000

Discussion

Medicinal plants are used for treatments of infectious diseases which are caused by different pathogenic microorganisms. Among these microorganisms, bacteria are the most and very hazardous microorganisms in present era[7]. As the result of those characteristics, treatment options should be changed to overcome the threats. Infectious diseases are one of the major problems in developing as well as developed countries. Traditional medicinal plants were widely used to treat the microbial diseases due to their rich source of antimicrobial activity [8] and less cost effective.

Groups of phytochemical compounds commonly associated with combating microbial resistance and having antimicrobial activity in medicinal plants are flavonoids, alkaloids, tannins, triterpenoids, essential oils, saponins, glycosides, and phenols [9]. Even though it is difficult to judge the mechanism of actions of the bioactivity. It is possible to say that the plant has antibacterial activity [10].

Currently, many of the pathogenic bacteria have become multidrug resistant to commonly used antibiotics causing different diseases [11]. Because of this, searching of new drug is important in the recent times. Therefore, this study has been carried out. The results have been revealed that Denibadi kashaya and Denibadi phanta has antibacterial efficacy. By measuring the zone diameter of test samples, it is clear that Denibadi kashaya and Denibadi Phanta have less antibacterial efficacy while compared to positive control, Amoxicillin. But Denibadi Kashaya has greater inhibitory zone diameter than Denibadi Phanta,

It can be concluded that while compared with positive control kashaya form of Denibadi yoga has more than 50% efficacy against Staphylococcus aureus.

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