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Research Article

ANTIMICROBIAL STUDY OF *Shorea Robusta* (SHALA) W.S.R. ABOUT KRIMIGHNA PROPERTY IN KSHARA SUTRA & OTHER MEDICINAL PREPARATION

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ABSTRACT

Ayurveda is a complete self being system, which dominantly stresses on living a good and healthy life style that does not have any imbalance in the harmony and system of the body.

Since the time immemorial the society relay on plants not only for dietic purpose but also for medicinal purpose. Also in the present scenario human being relay in 75% of herbal remedies. Lots of description regarding plants available from the Vedic eras to Nighantu period.

As single plants having ability to cure multiple diseases that mentioned in our classcal Ayurvedic texts now need to be scientifically authentication in order to make the society believe that what had ever been written by our Acharyas are right.

To establish the same above said by taking a scientific study of *Shorea robusta* in order to establish its antimicrobial activity with the clue that mentioned in different classical texts.

Out of three solvent used for preparation of resin (Methanol, Ethanol and Toluene), methanol extraction showed highest response in term of sensitivity (high zone inhibition), while the least sensitivity was observed with toluene extract.

Keywords: 'Aasavyonivriksha', 'Vedanasthapana Mahakashya'and in 'Kashaya skandha'.

Introduction and Need of study

The Ayurvedic perspective, an individual who is balanced and healthy has a strong immune system and, therefore, it will be difficult for microbial infection to take hold. Balance in Ayurveda is equivalent to health, which is equivalent to a strong and well-functioning immune system capable of defending against microbial infection. The Ayurvedic approach is to treat the whole person, including application of correct diet, lifestyle recommendation, and herbal supplements. When a person develops an infection, the design of an Ayurvedic herbal formula reflects the holistic approach. Based on traditional use, herbs are selected and combined for their ability to inhibit microbial overgrowth in various parts of the body and support those organ systems responsible for detoxification and immune function.

It shows the immense need of an alternate antibiotic of natural plant product to avoid or alleviate the resistance & adverse effect of modern antibiotics against human body. It stresses that the herbal drug are safer in present scenario.

To establish the same above said by taking a scientific study of *Shorea robusta* in order to establish its antimicrobial activity with the clue that mentioned in different classical texts as follows--

शालस्तु सर्जकाश्योश्वकर्णकाः शस्यशम्बरः । अष्वकर्णः कषायः स्याद् ब्रणस्वेदकफक्रिमीन ।।

ब्रघ्नविद्रधिबाधिर्ययोनिकर्णगदान हरेत् । (भा.प्र. वटादिवर्ग)

AIMS AND OBJECTIVES OF THE STUDY

To evaluate the Antimicrobial activity by culture and sensitivity test.

Necessity at finding safer microbicide and need for preventing environment degradation.

To assess the drug on Ayurvedic parameter as described in Dravya Guna (Namarupa Vigyana).

Drug Review

Shorea robusta order Dipterocarpaceae is a large, deciduous tree up to 50 m tall.

As the drug having Kashaya rasa, Ruksha guna, Sheeta virya, Katu vipaka and it pacifies Pitta and Kapha, so prevent the formation and growth of Krimis.

In Charaka Samhita the Shala has been described in 'Vedanasthapana Mahakashya'and in 'Kashaya skandha'also mentioned in 'Aasavyonivriksha'.

In Sushruta Samhita the Shala has been described in Eladi Gana, Shalsaradi Gana, Rodhradi Gana and in Shiro Virechana Dravya.

Shala is still not well known for its antimicrobial properties as mentioned in classics. Extraction of drug in different solvent in different concentration or direct administration of drug proved antibacterial action. To find out the action of Shala resin on different microbial strain with the help of modern parameter, the present work has been taken.

Anti-microbial Study

To evaluate the comparative Anti-microbial activity of the test drug samples prepared, this study was performed. Since, no documented study is found mentioned in Ayurveda regarding antibacterial activity, basic microbiological techniques mentioned for evaluating antibacterial activity in the modern medicine were followed. For this study, five common pathogenic strains of bacteria were procured from IMTECH, Chandigarh, already mentioned in Anti-microbial study earlier.

Three different sample of resin extract will be taken with each sample having 3 diff. concentrations (higher, medium, and lower). Then each sample will be taken into study on five different bacteria for evaluating the efficacy.

5 Diff. Bacteria
 3 Diff. Concentration
 45 observations will be made
 3 Diff. Sample

Three Different Samples

- Toluene Extract of resin
- Methanol extracts of resin
- Ethanol extracts of resin

Three different concentrations

Crude extracts a. 50mg b. 75 mg c.100 mg

Five Different bacteria

For the present study out of a vast group of Micro organisms only followings strains of bacteria were selected and procured from MTCC, IMTECH, Chandigarh and the study was carried out at Birla Institute of Scientific Research, Jaipur.

	MTCC NO.
Staphylococcus aureus	3160
Streptococcus pyogenes	1928
Pseudomonas aeruginosa	424
Escherichia coli	901
Salmonella typhi	733
5 control Group	
	Streptococcus pyogenes Pseudomonas aeruginosa Escherichia coli Salmonella typhi

Total observation = 5x3x3 + 5 control group

= 45 + 5 = 50 observation was made.

MATERIALS AND METHODS

Apparatus Required

Measuring cylinder Conical flask (1lt) Conical flask (500 ml) Sterile Petri plates pH meter Auto clave Analytical balance ILV chamber

Chemical required

1N HCl, 1N NaOH, NaCl

Ingredients

Composition of growth medium 3 (for bacteria Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa & Salmonella typhi)

Beef extract	=	1.0gm
Yeast extract	=	2.0gm
Peptone	=	5.0gm
NaCl	=	5.0gm
Agar powder	=	15gm
Distilled water	=	1lt

Composition of growth medium for Bacteria $\it Streptococcus pyogens$

Nutrient Broth	=	2.6gm	
Agar powder	=	4.0gm	
Distilled water	=	200ml	
Blood		=	10ml

Evaluation of antibacterial activity

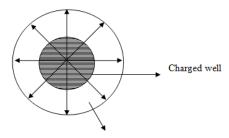
Well method was adopted for assessment of antimicrobial activity of the drug obtained from *Shorea robusta*.

Well method

In this method media plates were placed inside the U.V chamber, then the plates were divided into 5 sectors for 3 samples with the help of a marker pen. Before this the floor of the chamber was cleaned by the help of absolute alcohol and tissue paper. A spreading loop, borer of 8mm diameter was dipped in a beaker containing absolute alcohol. Micro pipette and sterilized pipette tips were also placed inside the chamber. Then one sector was picked up for each bacteria & in each plate $100\mu l$ of concerned bacterial culture was poured with the help of micro pipette and spread all over the plate uniformly by means of a sterilized spreader . This process was adopted for each sector. Then a well was made at the center of each plate by the help of a sterile cork borer having diameter 8mm. Each time the borer as well as the spreader was dipped in the alcohol and placed over the flame in order to prevent the cross contamination of bacteria.

Measurement of inhibition zone

The zone of inhibition of bacterial growth around the well measured in mm. with the help of a scale. The readings were taken at 4 different planes as shown in figure below:



Inhibition zone (Boundary)

Then the mean was calculated of the four readings taken.

Observations and Results

The antibacterial activity of different extracts of *Shorea robusta* in different solvents as mentioned earlier were evaluated against a number of pathogenic bacterial stains and zone of inhibition was observed. This was based upon the scale developed by Arora D.S et al (1997). The zone of inhibition and result of drug sensitivity was described below.

Table 1: It shows-- Relation between zone of Inhibition and drug sensitivity

S.No.	Zone of Inhibition (m.m.)	Drug Sensitivity
1.	N.I. (below 6)	Insensitive
2.	6 < 9	Less sensitive
3.	9 < 12	Moderate sensitive
4.	> 12	Highly sensitive

Table 2: It shows-- Zone of inhibition of methanol extract of resin of *Shorea robusta*

Resin extract	M.T.C.C Bacteria code				
Weight	1928	3160	901	424	733
50mg	8,9,7,8	9,8,8,9	10,10,11,11	N.I.	8,9,7,8
	M=8	M=8.5	M=10.5		M=8
75mg	10,11,10,9	11,10,10,11	11,11,12,11	N.I.	10,11,10,9
	M=10	M=10.5	M=11.25		M=10
100mg	11,12,13,12	12,13,11,12	12,13,12,12	N.I.	10,12,13,12
	M=12.75	M=12	M=12.25		M=12.75

Results

Methenol extract taken for five microbial strains -

On increasing concentration *Streptococcus pyogen* showed slight high sensitivity (>12mm) inhibition Zone.

On increasing concentration *Staphylococcus aureus* showed moderate sensitivity (<12mm) inhibition Zone.

On increasing concentration *Escherichia coli* showed moderate sensitivity (<12mm) inhibition Zone.

Pseudomonas aeruginosa showed no inhibition Zone.

On increasing concentration $Salmonella\ typhi$ showed slight high sensitivity (>12mm) inhibition Zone.

N.I. = No inhibition, M = Mean

Zone of Inhibition - I (Media-Blood Agar)



Figure 1: Group – I :: Control S. pyogens



Figure 2: Group - II :: MeOH Extract (Resin)
S. pyogens

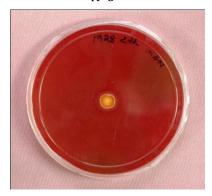


Figure 3: Group – III :: EtOH Extract (Resin) S. pyogens

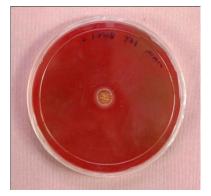


Figure 4: Group – IV :: Toluene Extract (Resin)
S. pyogens

Zone of Inhibition - III (Media-Nutrient Agar)

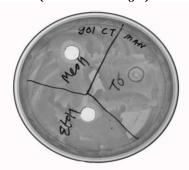


Figure 5: Group – I :: Control E. coli



Figure 6: Group – II :: MeOH Extract (Resin) *E. coli*



Figure 7: Group - III :: EtOH Extract (Resin) *E. coli*

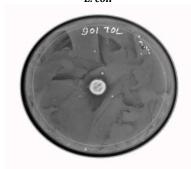


Figure 8: Group – IV :: Toluene Extract (Resin) E. coli

Table 3: It shows-- Zone of inhibition of Ethanol extract of resin of Shorea robusta

Resin extract		M.T.C.C Bacteria code			
Weight	1928	3160	901	424	733
50mg	5,6,5,6	9,8,9,8	9,8,7,8	N.I.	7,8,7,8
	M = 5.5	M=8.5	M=8		M=7.5
75mg	7,8,7,8	10,11,11,10	10,9,8,9	N.I.	9,10,8,9
	M = 7.5	M=10.5	M=9		M=9
100mg	9,9,8,9	13,15,13,15	11,10,12,11	N.I.	13,11,12,12
_	M=8.75	M=14	M=11		M=12

RESULTS

Streptococcus pyogen showed mild sensitivity (<9mm) inhibition Zone

On increasing concentration *Staphylococcus aureus* showed slight high sensitivity (>12mm) inhibition Zone.

On increasing concentration *Escherichia coli* showed moderate sensitivity (<12mm) inhibition Zone.

Pseudomonas aeruginosa showed no inhibition Zone.

On increasing concentration *Salmonella typhi* showed moderate sensitivity (<12mm) inhibition Zone.

Table 4: It shows-- Zone of inhibition of Toluene extract of resin of Shorea robusta

Resin extract	M.T.C.C Bacteria code				
Weight	1928	3160	901	424	733
50mg	6,7,6,7	7,6,6,7	8,7,8,7	N.I.	N.I.
	M = 6.5	M=6.25	M = 7.5		
75mg	7,8,7,8	8,7,9.8	9,8,9,8	N.I.	N.I.
	M = 7.5	M=8	M=8.5		
100mg	9,8,9,8	8,9,9,8	10,11,10,11	N.I.	N.I.
-	M = 8.5	M=8.5	M=10.5		

Results

Streptococcus pyogen showed mild sensitivity (<9mm) inhibition Zone

 $\it Staphylococcus\ aureus\ showed\ mild\ sensitivity\ (<9mm)\ inhibition\ Zone$

On increasing concentration $\it Escherichia~coli~showed~moderate~sensitivity~(<12mm)~inhibition~Zone.$

Pseudomonas aeruginosa showed no inhibition Zone.

Salmonella typhi showed no inhibition Zone.

CONCLUSIONS

". The following conclusion was drawn from this research work:-

After analysis of different Ayurvedic text about Shala, the word Shala, Sarja, Aswakarna and Ajkarna may be considered as different plants.

As the drug having Kashaya rasa, Ruksha guna, Sheeta virya, Katu vipaka and it pacifies Pitta and Kapha, so prevent the formation and growth of Krimis.

'Krimi' includes all the micro-organism as well as macro-organisms (worms) like pathogenic & nonpathogenic organisms covering a wide range of infections & infestations.

Different extraction and different concentration of drug sample shows varying zone of inhibition on different bacteria.

Methanol extract of resin shows at increasing concentration, slightly high sensitivity on *Streptococcus pyogenes and Salmonella typhi*

Ethanol extract of resin shows at increasing concentration, slight high sensitivity on *Staphylococcus aureus*.

Toluene extract of resin shows at increasing concentration, moderate sensitivity on *Escherichia coli*.

No zone of inhibition was observed in any control plates.

Shala can be used in kshara sutra preparation also.

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