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

DR. MANOJ ADLAKHA1, DR. AKHLESH KUMAR BHARGAVA2, DR. MITA KOTECHA3

1Asst.prof. P.G.Deptt. of Dravya-Guna,Rajasthan Ayurved University JODHPUR, 2M.D.P.H. Lecturer & H.O.D. Shalya Tantra. Deptt. Govt.Ashastang Ayurved College Indore(M.P.). 3M.D.ph.D. Associate Professor, P.G.Deptt. of Dravya-Guna, National institute of ayurveda, Jaipur. Email: akhlsh.bhargava@yahoo.com

Received: 28 September 2013, Revised and Accepted: 1 October 2013

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 rely on plants not only for dietetic purpose but also for medicinal purpose. Also in the present scenario human being rely 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 classical 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: ‘Asavyonirviksha’, ‘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—

शालशुर आवतारात्त्वभार्यक्रमः। अयोध्य याह्य याय भाष्यदिशेकवलक्ष्येन्।

AIMS AND OBJECTIVES OF THE STUDY

To evaluate the Antimicrobial activity by culture and sensitivity test.

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

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

Drug Review

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

As the drug having Kashaya rasa, Ruksa 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 ‘Asavyonirviksha’.

In Sushrutha Samhita the Shala has been described in Eladi Gana, Shalsaradi Gana, Redhnavi Gana and in Shiro Virechna 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
- 3 Diff. Sample

45 observations will be made
Three Different Samples

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

Three different concentrations

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

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

Total observation = 5x3x3 + 5 control group

= 45 + 5 = 50 observation was made.

MATERIALS AND METHODS

Apparatus Required

Measuring cylinder
Conical flask (1L)
Conical flask (500 ml)
Sterile Petri plates
pH meter
Auto clave
Analytical balance
U.V. chamber

Chemical required

1N HCl, 1N NaOH, NaCl

Ingredients

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

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef extract</td>
<td>1.0gm</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>2.0gm</td>
</tr>
<tr>
<td>Peptone</td>
<td>5.0gm</td>
</tr>
<tr>
<td>NaCl</td>
<td>5.0gm</td>
</tr>
<tr>
<td>Agar powder</td>
<td>15gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1lt</td>
</tr>
</tbody>
</table>

Composition of growth medium for Bacteria Streptococcus pyogenes

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient Broth</td>
<td>2.6gm</td>
</tr>
<tr>
<td>Agar powder</td>
<td>4.0gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>200ml</td>
</tr>
<tr>
<td>Blood</td>
<td>10ml</td>
</tr>
</tbody>
</table>

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, borers 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 100μl 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 borers 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:

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

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Zone of Inhibition (m.m.)</th>
<th>Drug Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>N.I.(below 6)</td>
<td>Insensitive</td>
</tr>
<tr>
<td>2.</td>
<td>6 &lt; 9</td>
<td>Less sensitive</td>
</tr>
<tr>
<td>3.</td>
<td>9 &lt; 12</td>
<td>Moderate sensitive</td>
</tr>
<tr>
<td>4.</td>
<td>&gt; 12</td>
<td>Highly sensitive</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Resin extract</th>
<th>M.T.C.C Bacteria code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>ZONE TABLE</td>
</tr>
<tr>
<td>50mg</td>
<td>1928</td>
</tr>
<tr>
<td>75mg</td>
<td>101110.09</td>
</tr>
<tr>
<td>100mg</td>
<td>111213.12</td>
</tr>
</tbody>
</table>

Results

Methanol 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

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*

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

<table>
<thead>
<tr>
<th>Resin extract</th>
<th>M.T.C.C. Bacteria code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td></td>
</tr>
<tr>
<td>50mg</td>
<td>928 3160 901 424 733</td>
</tr>
<tr>
<td>M=5.5 M=8.5 M=8 M=7.5</td>
<td></td>
</tr>
<tr>
<td>75mg</td>
<td>7.8 7.8 10 11110 10 9.8 9.8 9 10 8.9 9 10 8.9 9</td>
</tr>
<tr>
<td>M=7.5 M=10.5 M=9</td>
<td></td>
</tr>
<tr>
<td>100mg</td>
<td>9.9 9.8 13.15 13.15 11 10 12 11 11 13 11 12 12</td>
</tr>
<tr>
<td>M=8.75 M=14 M=11</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Resin extract</th>
<th>M.T.C.C. Bacteria code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td></td>
</tr>
<tr>
<td>50mg</td>
<td>928 3160 901 424 733</td>
</tr>
<tr>
<td>M=6.5 M=6.25 M=7.5</td>
<td></td>
</tr>
<tr>
<td>75mg</td>
<td>7.8 7.8 8.7 9.8 9.8 9.9 9.8</td>
</tr>
<tr>
<td>M=7.5 M=8 M=8.5</td>
<td></td>
</tr>
<tr>
<td>100mg</td>
<td>9.8 9.8 8.9 9.8 10 11 10 11 11 11 13 11 12 12</td>
</tr>
<tr>
<td>M=8.5 M=8.5 M=10.5</td>
<td></td>
</tr>
</tbody>
</table>

Results

Streptococcus pyogen showed mild sensitivity (<9mm) inhibition Zone.
Staphylococcus aureus showed mild sensitivity (<9mm) inhibition Zone.
On increasing concentration 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.

REFERENCES

2. Sushruta Samhita Comm. Dr. Ambikadatta Shastri Pub.-Chaukhambha Bharti Academy 14th 2003
5. Dhanvantari Nighantu:Editor – Acharya P.V. Sharma Comm. Dr.Guru Prasad Sharma Pub.-Chaukhambha Bharti Academy, Varanasi
8. Adarsha Author- Vaidya Baba Lal reprint 1998 Pub.-Chaukhambha Bharti Academy, Varanasi
10. Aushadham Sangraha Nighantu Author – Chunn Lal Shastri Pub.-Chaukhambha Sanskrit Varanasi
12. Indian Materia Medica KM. Nadkarni Flora of India, Vol 1 & 7
17. Pharmacognosy of Ayurvedic Drugs, Kerala vol.11 Author-Prof. N. Laxmi, A. Jagadamma Pub.- Pharmacognosy Unit, Ay. Research Unit, Poojapura 1996, Thrivunananthpuram-12
18. Material medica of India & their therapeutics
19. Text Book of Medical Laboratory