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Research article

Anti-Microbial Activity of Talakeshwara Ras

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Abstract

Rasa Shastra, one of the Pharmaco-therapeutic branches of Ayurveda where metals, minerals, poisonous plants and animal products are used after proper processing for internal administration. Talakeshwara Ras is one of Khalvi rasayanas where Emblica officinalis (Dhatri) and minerals Arsenic tri sulphide (Haratala) & Borax (Tankana) are the ingredients. It is indicated for Sarva Kushta at one Masha (1 gm) dose. Anti –Microbial activity of Talakeshwara Ras was done with an intention to evaluate its efficacy against gram positive and gram negative bacilli. So an honest attempt has been made to put forth the "Anti – Microbial activity of Talakeshwara Ras" which had its anti microbial activity against Staphylococcus aureus and Pseudomonas aeruginosa.

Keywords: Talakeshwara Ras, Anti-Microbial Activity.

Introduction:

Rasa Shastra is one of the Pharmaco-therapeutic branches of Ayurveda where processing of minerals, some poisonous plants and animals materials for therapeutic benefit and metallic pharmaceutical preparations.

Talakeshwara Ras is one of Herbo-mineral preparation, where in Dhatri (*Emblica officinalis* L.), Suddha Haratala (Arsenic trisulphide) & Suddha Tankana (Borax) are processed in Dhatri svarasa. It is indicated for all skin disorders (Sarva Kushta hara) and other diseases of infectious origin. So an anti-microbial study of it was undertaken to study its effects on the two clinical types of the bacteria i.e., gram positive and gram negative bacilli.

Talakeshwara ras is said to be prepared in 78 different methods as mentioned in the various texts of Ayurveda. For convenience the preparation mentioned in the text 'Rasayoga Sagara' (1) was taken for the study as the ingredients mentioned in it were easily available and the preparation is easier to prepare. Even thoughthere is a slight variation in the preparation methods in different texts, the important compounds are the same and the indications are also similar. but most of them were indicated in Kusta apart from other indications.

Talakeshwara rasa: (2)

Ingredients

Equal parts of Dhatri (25gm), Suddha Haratala (25gm) and Suddha Tankana (25gm) are taken and triturated (Bhavana) with sufficient quantity of the Dhatri svarasa. (1-8,11)

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Method of Preparation: (Plate 1)

Dried fruit of Emblica officinalis (Dhatri phala) are made to fine powder and 25 gms of fine powder was taken.

Arsenic trisulphide was purified (Haratala sodhana) by boiling (Swedana) it in the juice of *Benincasa hispida* Thunb. (Kushmanda swarasa) for 3 hours. This processed Arsenic trisulphide (sodhita Haratala) was pounded and sieved to collect 25 gms of fine powder.

Borax (Tankana) was purified by heating it till the moisture content in it was lost. After purification 25 gms of Tankana was taken.

Sufficient amount of fruit juice of Emblica officinalis (Dhatri Ras) was taken and triturated till subhavitha lakshanas. All the above mentioned ingredients were mixed well by triturating for 6 hrs. Then the pills (Vatis) were prepared and dried.

Indications: Sarva Kushta hara & Deepana, Pachana.

Anti-Microbial Study: (9-10)

The bacilli are grossly divided as gram positive and gram negative. So to test the anti microbial activity, a gram positive and a gram negative bacilli are selected for the study. ***Staphylococcus aureus*** (facultatively anaerobic, Gram positive coccus) & ***Pseudomonas aeruginosa*** (gram negative) which are the most common causes of the diseases in the human beings were selected for the test.

Staphylococcus aureus is the most common cause of "Staph Infections". It is frequently part of the skin flora in the nose and on skin. Staphylococcus aureus can cause a range of illnesses from skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome and abscesses to life threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteraemia and sepsis. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections, often causing post surgical wound infections.

Pseudomonas aeruginosa is a Gram-negative, aerobic, rod-shaped bacterium with unipolar motility. It is the most common cause of infections of burn injuries and of the external ear (otitis externa) and is the most frequent colonizer of medical devices (e.g., catheters).

Methods adopted:

1. Minimum Inhibitory Concentration (MIC) method
2. Diffusion method

Bacterial Strains and Culture Conditions for both Methods:

An ATCC 25922 *Staphylococcus aureus culture* & ATCC 27853 *Pseudomonas aeruginosa culture* was obtained from St. John's medical college, Bangalore, India. The obtained cultures were maintained on nutrient agar slants and the stock cultures were transferred at monthly intervals. Nutrient broth was prepared and sterilized; a loop full of Staphylococcus aureus & Pseudomonas aeruginosa culture was inoculated and incubated at 37^{o}C for 24 hours.

After 24 hours of incubation the final OD (optical density) of the culture broth was determined. The final OD was found to be 0.60 for both the culture broths. The above prepared culture broths were used for the minimum inhibitory concentration assay.

Antimicrobial Agent (medicine):

The sample was rough in texture and was spherical shaped and weighed 0.33gm. The sample was finely grounded and then used for the experiment.

A series of 600mg, 300mg, 150 mg, 75mg, 37.5mg, 18.75 mg, 9.375mg, 4.687 mg, 0mg (control) was prepared by suspending the sample (medicine) in 1ml of appropriate diluent for Minimum Inhibition Concentration. 600mg and 300 mg concentration of the sample (medicine)

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was used for the experiment for Diffusion Method.

Procedure of MIC:

A pure culture of a single micro-organism is grown in Mueller-Hinton broth, or other broth as appropriate. The culture is standardized using standard microbiological techniques to have a concentration of very near 1 million cells per millilitre. The more standard the microbial culture, the more reproducible the test results. The antimicrobial agent is diluted a number of times, 1:1, through a sterile diluents (Mueller-Hinton broth).

After the antimicrobial agent has been diluted, a volume of the standardized inoculums equal to the volume of the diluted antimicrobial agent is added to each dilution vessel, bringing the microbial concentration to approximately 500,000 cells per millilitre.

The inoculated, serially diluted antimicrobial agent is incubated at an appropriate temperature for the test organism for a pre-set period, usually 18 hours. The more standard the incubation period, the more reproducible are the test results.

After incubation, the series of dilution vessels is observed for microbial growth, usually indicated by turbidity and/or a pellet of microorganisms in the bottom of the vessel. The last tube in the dilution series that does not demonstrate growth corresponds with the minimum inhibitory concentration (MIC) of the antimicrobial agent.

Procedure of Diffusion Method:

Mueller-Hinton medium was prepared, sterilized and poured into the sterile petri plates and was allowed to solidify.

Above mentioned cultures were uniformly spread on to the plates containing the media using cotton swabs.

With the help of cork borer small wells were made in the above mentioned plates and the 600mg, 300mg samples were poured into the well and labeled appropriately.

Later the plates were incubated at 37^0C for 24 hours. After 24 hours of incubation the plates were checked for the formation of inhibition zone.

Result:(Plate 2)

Minimum Inhibition Concentration – MIC

Dosage	Staphylococcus aureus	Pseudomonas aeruginosa
600 mg	0.11	0.17
300 mg	0.13	0.19
150 mg	0.15	0.20
75 mg	0.19	0.23
37.5 mg	0.20	0.27
18.75 mg	0.22	0.28
9.375 mg	0.25	0.39
4.687 mg	0.26	0.40

Diffusion method:

Zones of Inhibition were observed for both Staphylococcus aureus and Pseudomonas aeruginosa at the concentrations of 600 and 300mg respectively.

Diffusion Method	Staphylococcus aureus	Pseudomonas aeruginosa
300mg	2.8 cm	2.5 cm
600mg	3.3 cm	2.9 cm

Discussion:

Talakeshwara Ras is one of Khalvi Rasayana. It has Arsenic Tri Sulphide (shuddha Haratala) as one of the ingredients which is least toxic among the arsenic compounds used in Ayurveda.

Among the available literatures, 78 references were available in Rasa yoga sagara. The manufacturing procedure adopted was easy and had fewer ingredients. So evaluating its anti–microbial efficacy through invitro method was conducted. So that Talakeshwara Ras could be one of the drugs which is cost effective and is therapeutically effective in common practise. So, the anti-microbial efficacy was evaluated, as said in classics with modern perspective.

The drugs *Emblica officinalis* (Dhatri) eleminates all excessive 3 doshas (Tridosha hara), *Especially Pitta hara i.e. Rakta dosha hara*. Purified Borax (Suddha Tankana) is Teekshna and exudative action (Saaraka). Indicated for mucolytic (kapha visleshana), cough (kasa) & respiratory disorders (swasa hara) and heals all kinds of ulcerative conditions (vividha vrana nashana). (Apamarga mula) is Kapha dosha hara, reduces itchy conditions (Kandugna), skin problems (Kustagna) and lekhana.

Arsenic tri sulphide (Haratala) has Sleshma, Rakta dosha hara properties, along with indicated in toxic and skin disorders (*Visha hara and Kushta hara).* So due to these properties the drug Talakeshwara Ras might be effective in all skin disorders (Sarva Kushta hara). As skin disorders (Twak vikaras) are mainly due Rakta dhatu vitiation. Above mentioned drugs have their activity as Rakta dosha hara and Kushta hara (eliminates skin diseases).

Both the bacilli i.e Staphylococcus aureus and Pseudomonas aeruginosa are normal skin flora which are harmless with intact skin, but causes skin disorders in cases of skin lesion. The bacilli grow in suitable conditions and these conditions were provided and checked for anti microbial activity with anti microbial agent i.e Talakeshwara Ras.

The dose of Talakeshwara Ras was taken with upper limit of 600mg as 1 gm was said to be the maximum therapeutic dose as per Ayurvedic texts.

The dosage was reduced to half the previous dose to form the successive dose. Thus doses of 600 mg, 300 mg, 150 mg so on till minimum of 4.687 mg was taken for testing the efficacy of drug and 0 mg as control dose.

In Minimum inhibition concentration (MIC) method, the efficacy of the drug was calculated with Optical density (OD). The OD increases with the turbidity i.e growth of organisms. The decrease in the value of OD is suggestive of decrease in the growth of organisms or more efficacy of the drug.

In MIC method, growth of both the bacilli was decreased with successive increase in the concentration of Talakeshwara Ras.

In diffusion method the zone of inhibition was observed to be effective at 300 mg and this zone diameter was increased with 600 mg, i.e the dose was effective at 300 mg.

In both the methods Talakeshwara Ras was effective on both the bacilli, but more effective on Staphylococcus aureus when compared to Pseudomonas aeruginosa in respective same doses.

Conclusion:

Talakeshwara Ras, the name might suggest its ingredient Haratala.

The anti-bacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* as per MIC was effective with decreased OD & in Diffusion method was effective at 300 mg & with increased Zone of Inhibition at 600 mg.

So it may be prescribed in skin disorders like furuncles, folliculitis, carbuncles, scalded skin syndrome and abscesses at a dose of 300mg to 600mg of dose. Thus the awareness of our Acharyas in selecting drugs in formulations as per

indications and dose fixing as Talakeshwara Ras as Kushta hara and 1Masha dose was understood with modern perspective also along with aptavachana.

Thus the classical reference of Talakeshwara Ras as Kushta hara was proved effective in both gram positive and gram negative bacilli.

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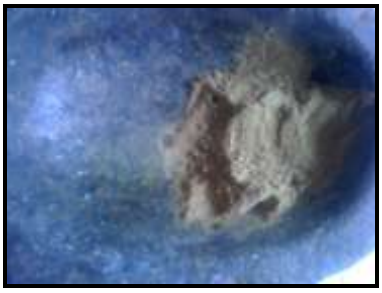
Plate 1: Preparation of Talakeshwara Ras


1) Dried fruit of Emblica officinalis

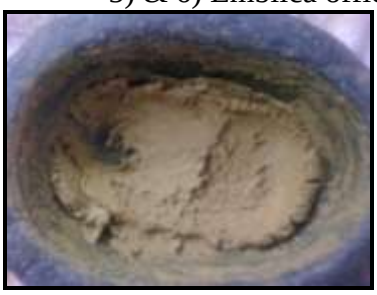

2)Arsenic tri sulphide


3) with Borax


4) Mixing


5) & 6) Emblica officinalis fruit juice for triturating


7) Sufficient quantity of juice


8) Proper consistency for pills


9) Talakeshwara Ras

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Plate 2: Anti-Microbial Activity

Before & after incubation with Staphylococcus aureus

Before & after incubation with Pseudomonas aeruginosa