SHILA SINDURA: AN ANTIMICROBIAL AGENT

Dasari Srilakshmi1,2, S. Swetha2, K.U. Minchitha2, Maheshwari Kumari Singh3

1Ayurvedic Physician and Physiotherapist, S.G.S Hospital, Sri Ganapathy Sachehidananda Ashram, Mysore, Karnataka, India
2Department of Nanobiosciences, Centre for Emerging Technologies, Jain University, Jakkasandra post, Ramanagaram, Bangalore, India

ABSTRACT

Kupipakwa rasayana is a unique and highly evolved pharmaceutical preparation of the four murchita parada yogas. Shila Sindura is sagandha (with sulphur), sagni (processing with heat), kantastha (near the neck of the bottle) murchita parada yoga, which has mercury (parada), sulphur (gandhaka) and arsenic di sulphide (manashila) as ingredients. It is indicated in all types of skin disorders (sarva kushtah), skin problems associated with itching (kandu), rakta dosha hara (vitiated raktadhatu) and other diseases of infectious origin like fever (jwara), abscess (vidradhi), gonorrhoea (upadamsa), mediha, rasayana and hridya at a dose of 125-250 mg (1-2 ratti). Antimicrobial activity of Shila Sindura was conducted against gram positive, gram negative bacteria and fungus to evaluate its efficacy as broad spectrum antibiotic. So an attempt had been made to put forth “Shila Sindura: An Antimicrobial Agent”. Shila Sindura has an effective antimicrobial activity against Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus mutans and Candida albicans in both Gradient plate technique and Kirby bauer method.

Keywords: Shila Sindura, Kupipakwa rasayana, Antimicrobial agent.

INTRODUCTION

The main aim of Rasa shastra is to attain jivanmukti (liberation from the cycle of rebirth) by means of dehavedha i.e. healthy physique with rasoushadhis along with lohavedha (converting lower metals to higher metals). The preparation of Sindura kalpa can be traced back to 12th century A.D of Rasapraksha sudhakara by the name udayabhaskara ras, but drug Shila Sindura has been introduced in the early years of 20th century as indicated by the books Rasamrutam, Rasendra sambhava, Basavarajeevam, Rasayana sara and Siddha bhesjha manimala. Shila Sindura is one of the mineral preparations by kupipakwa method. It was prepared as per the reference of the text ‘Rasamrutam’ which has equal quantity of mercury (parada), sulphur (gandhaka) and arsenic di sulphide (manashila) as ingredients. Antimicrobial activity was evaluated by two different methods i.e. by Gradient plate technique and Kirby bauer method against seven clinical isolates such as Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus mutans, Acinetobacter baumannii, Klebsiella pneumoniae and Candida albicans.

MATERIALS AND METHODS

The materials used as ingredients13 were

- Parada – Mercury
- Gandhaka – Sulphur
- Manashila – Arsenic di sulphide
1 part (45g) each of above ingredients
- Kumari rasa – Aloe vera pulp – Quantity sufficient

Other materials required for preparation of Shila Sindura were Khalbayantra (mortar and pestle), pyrometer, multani mitti, cloth, valukanya, karpura (camphor), match box, fire wood, shalaka (thin iron rod), torch, copper coin.

Materials required for antimicrobial study were

(a) Inoculation loop, media, cotton swab, sterile discs, Shila Sindura.
(b) Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus mutans, Acinetobacter baumannii, Klebsiella pneumoniae and Candida albicans.

Method of preparation of antimicrobial agent Shodhana of ingredients

Purification of mercury (parada shodhana)2 was done by triturating with powder of Curcuma longa (haridra churna) and Aloe vera pulp juice (kumari swarasa). This paste was made into small flat cake like pieces (chakrikas) dried in shade and subjected to sublimation (urdhwapatana). On self-cooling (svangashetata), shodhita parada was collected from inner surface of upper pot. Sulphur purification (gandhaka shodhana)2 was done with cow’s milk (godugdha) by bhudhara puta method. Later shodhita gandhaka was washed with warm water and collected. Purification of arsenic di sulphide (manashila shodhana)2 was done by triturating with juice of Gingiber officinale (ardraka swarasa bhavana) for seven times.

Preparation of Shila Sindura

Samaguna kajjali3 was prepared by triturating equal quantity by weight of mercury (suddha parada) and sulphur (suddha gandhaka) till signs of complete formation of kajjali (samyak siddha kajjali lakshanas)3.
Equal quantity (45g) of arsenic di sulphide (suddha manashila) was added to samaguna kajjali and triturated for 5-6 hours. Then Aloe vera (kumari swaras bhavana)\textsuperscript{1,3} leaf pulp juice was added and triturated. After drying it was filled in glass bottle (kacha kupi) covered with 7 consecutive layers of cloth smeared with multani mud up to mouth of kupi\textsuperscript{2,4}. This bottle (kupi) filled with kajjali was placed in iron vessel filled with sand (valukyantra). After the entire apparatus was ready, fire wood was ignited with help of camphor, following gradual heating (kramagni)\textsuperscript{2,4} pattern. Pyrometer was used for recording temperature every hour at neck and base of kupi along with valuka near the neck. The temperature near the base of kupi was maintained between 150°C – 250°C for mild heat (mrudvagni), raised to 350°C – 600°C for moderate heat (madhyamagni) and intense heat (teevragni) up to 750°C\textsuperscript{2,4}. After the stage of fumes and flames, the bottom of the bottle appeared like rising sun i.e. red in colour (udayabhaskara vana). After confirming with copper coin test, corking of bottle was done and intense heat (teevragni) was continued for 2 hours. Kupi was left for self-cooling (svangasheetata), approximately equal time taken for heating process, later bottle was removed from valuka yantra. The cloth with multani was scrapped off and bottle was broken 2 inches below the collection of the Shila Sindura. Later the drug was collected by tapping over the outer surface of glass bottle.

Antimicrobial Activity
Antibiotics are useful for the treatment of infectious diseases in situations where the normal host defence cannot destroy pathogens. Antimicrobial agents differ not only in their action and activity but also in their distribution, metabolism and excretion from the body. When immediate antimicrobial therapy is essential there is no time to culture and identify the disease causing agent. So drug (Shila Sindura) specificity was checked with two different methods i.e by Gradient plate technique and Kirby bauer method\textsuperscript{8-10}.

Bacterial Strains and Culture Conditions
All the cultures were obtained from St. John’s Medical College, Bangalore, India. The obtained cultures were maintained on nutrient agar and potato dextrose agar slants and the stock cultures were transferred at monthly intervals.

Antimicrobial Agent
The sample (Shila Sindura) was made to fine powder. It was brick red in colour.

I) Gradient Plate Technique

Principle
Gradient plate technique is used to isolate antibiotic resistant bacterial mutants by exposing an agar plate containing concentration gradient of antibiotic to an inoculation of microbes to be tested\textsuperscript{8,10}.

Procedure
Agar plate was placed on a pencil or other object to tilt one end up, so that plate was at a right angle to the object the plate was sitting on. The tilt of the plate was maintained such that the liquid doesn't reach to the top edge of the angled plate. Molten agar medium was poured into the plate without antibiotic and was allowed to harden. After the hardening of agar (2-5 minutes), the plate was set flat on the desk and medium containing the antibiotic was added. It was allowed to harden for 15 to 20 minutes. Using sterile inoculation loop, microbes were streaked in a zigzag manner over the surface of the medium, taking care not to tear the agar. Later it was incubated for approximately 72 hours. Then the plate was observed for the pattern of microbial growth.

II) Kirby Bauer Method

Principle
Diffusion of the antibiotics from the filter paper soaked in antibiotic solution results in a concentration gradient of drug. Sensitivity is measured as the zone of clearance on the lawn of sensitive bacteria. Effectiveness of antibiotics in this test is based on the size of inhibition. The zone of inhibition also depends on the diffusability of the antibiotic, the size of the inoculum, type of media and other factors.

Procedure
Mueller-Hinton medium was prepared, sterilized, poured into the sterile petri plates and was allowed to solidify. Above mentioned cultures were uniformly spread on the plates containing the media using cotton swabs. 100 mg of the medicine was dissolved in 1ml methanol and 2ml water. Sterile discs of himedia were soaked in the suspension of medicine for 5 to 10 minutes and later it was dried. The dried discs were placed on the previously swabbed petri plates. Later the plates were incubated at 37°C for 24 hours. After 24 hours of incubation, the plates were checked for the formation of inhibition zone. The diameter of zone of inhibition was measured\textsuperscript{8,10}.

RESULTS
The weight loss during shodhana of parada and gandhaka is due to removal of impurities and collection of final product. Weight gain of shodhita manashila after 7 ardrika swaras bhavana is because of ardrika satva. In kupipakwa process, maximum output of final product is 50-60%\textsuperscript{2} as a result of loss of free molecules of ingredients during the stage of fumes, especially sulphur, in the form of vapours and burning of sulphur and other remnant organic material, added as bhavana dravya, in the stage of flames following gradual heating pattern, Shila Sindura obtained was 54.28% (Table 1), (Figure 1-3) The results of antimicrobial activity against test organisms are given in Table 2. Gradient plate method on test organisms are in Figure: 4-7 and in Kirby bauer method, activity is measured as zone of inhibition (Figure 8-10). It is very clear that Shila Sindura is effective against 5 microbes i.e. Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus mutans, Klebsiella pneumoniae and Candida albicans. In both the methods Klebsiella pneumoniae and Acinetobacter baumannii are resistant to Shila Sindura.
Figure 1: Temperature graph near neck of kupi and valuka during preparation of Shila Sindura

Figure 2: Kantastha Shila Sindura

Figure 3: Final Product – Shila Sindura

Figure 4: Gradient Plate technique – Staphylococcus aureus

Figure 5: Gradient Plate technique – Acinetobacter baumannii

Figure 6: Gradient Plate technique – Candida albicans

Figure 7: Gradient Plate technique – Escherichia coli

Figure 8: Kirby Bauer method – Escherichia coli

Figure 9: Kirby Bauer method – Pseudomonas aeruginosa

Figure 10: Kirby Bauer method – Staphylococcus aureus

Table 1: Pharmaceutical work

<table>
<thead>
<tr>
<th>SN</th>
<th>Drug Used for Process</th>
<th>Duration of Process</th>
<th>Quantity before Process</th>
<th>Quantity after Process</th>
<th>Loss/Gain</th>
<th>Percentage of Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Parada Shodhana</td>
<td>6 Hrs</td>
<td>250g</td>
<td>238g</td>
<td>12g</td>
<td>95.2%</td>
</tr>
<tr>
<td>2.</td>
<td>Gandhaka Shodhana</td>
<td>--</td>
<td>250g</td>
<td>246g</td>
<td>04g</td>
<td>98.4%</td>
</tr>
<tr>
<td>3.</td>
<td>Manashila Shodhana</td>
<td>3 Hrs (each time)</td>
<td>250g</td>
<td>273g</td>
<td>23g</td>
<td>109.2%</td>
</tr>
<tr>
<td>4.</td>
<td>Samaguna Kajjali</td>
<td>36 Hrs</td>
<td>260g</td>
<td>257g</td>
<td>03g</td>
<td>98.8%</td>
</tr>
<tr>
<td>5.</td>
<td>Kajjali (Haratala Yukta)</td>
<td>06 Hrs</td>
<td>375g</td>
<td>373g</td>
<td>02g</td>
<td>99.4%</td>
</tr>
<tr>
<td>6.</td>
<td>Shila Sindura</td>
<td>22:45 Hrs of heating and 24 Hrs of Self-cooling</td>
<td>135g</td>
<td>76g</td>
<td>59g</td>
<td>54.28%</td>
</tr>
</tbody>
</table>
Kajjali is a khalvi rasayana (one of murchita parada yogas) intended to remove the chapaalata and durgrahatva of parada and potentiating it. Kajjali should pass the tests like rekhapurnata (kajjali fills in the furrows when rubbed between two fingers), slakshnata (fine powder), nischandrata (without any shining particles) and tamra pareeksha (kajjali rubbed over copper foil should not leave any white streak). All these tests signify the fineness, subteness and to strike out the chances of free mercury. Trituration of arsenic di sulphide (manashila) with samaguna kajjali and later with liquid media ensures even mixing and may help in bonding, thus reducing free molecules of mercury, sulphur or arsenic. Amount of kajjali should be 1/3rd of the capacity of kupi, which may facilitate space for the free movement of gases and boiling of kajjali during the process. Valuka yantra was specially designed for uniform, indirect heating through sand (valuka) and sand is inert material, so that it may prevent the sudden rise or fall of temperatures of the kupi and also may render resistance to the apparatus from atmospheric temperature variations. The objective of mrith lepana for kupi is to strengthen kacha kupi (glass bottle) to sustain heat, as it’s more pyro-sensitive. During the process of kupipakwa, parada was steadily heated along with gandhaka and manashila after excessive free sulphur and arsenic escapes from kajjali thus forming very intimate bond between the ingredients as the form, colour and consistency of final product was different from that of kajjali which may help Shila Sindura to exhibit superior qualities compared to other formulations with same ingredients. Parada acts as rogaghana, rasayana, yogavahi. Gandhaka has rasayana, kushtaghna, kundughna properties. It is indicated in twak and raktagata vikara. Manashila has rasayana, lekhana, kasahara, shwasahara, kundughna, panduhara, varnya, vishaghna, kapha vata nashaka properties. Kumarp also helps in treating twak, rakta and yakrit vikara. Shila Sindura is effective in conditions like skin disorders (kusha nasana sreshhta), skin problems associated with itching (kanduhara), sarva rakta dosha hara (vitiated raktadhatu) and other diseases of infectious origin like fever (jwara, sannipataja jwara), abscess (vidradhi), gonorrohoe (upadamsa), medhya, rasayana and hridya due to the properties of ingredients. Antibiotics which are effective against a wide range of gram-positive and gram-negative bacteria are said to be broad spectrum. So Shila Sindura may be grouped under broad spectrum antibiotic to understand the various aspects Acharyas tried to explain with all the indications enumerated of infectious origin, with modern perspective.

**DISCUSSION**

Gradient plate method helps to evaluate the presence or absence of antimicrobial activity. In Kirby bauer method the antibiotic action is evaluated with zone of inhibition. The diameter of zone of inhibition indicates sensitivity of the drug to the test organisms. Acharyas have specified 125-250 mg (1-2 ratti) as maximum treatment dose. So 100mg of Shila Sindura was taken for both the methods on all test organisms, so that the dose is within the limits. Acharyas specified and its effectiveness to species was evaluated. In Gradient plate technique, inhibition to *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus mutans* and *Candida albicans* was observed. This may suggest the wide range of antimicrobial activity of Shila Sindura. The zone of inhibition was in successive order in *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Candida albicans* and *Staphylococcus aureus*. This suggests that *Staphylococcus aureus* is more sensitive at the concentration of 100 mg. *Klebsiella pneumoniae* species often are resistant to many antibiotics, including cephalosporin’s (example: extended spectrum beta-lactamase/ESBL) and aminoglycosides. *Acinetobacter baumannii* is resistant to many classes of antibiotics including penicillin, chloramphenicol and often amino glycosides. Both of them were resistant to Shila Sindura too.

**CONCLUSION**

Murchita parada yogas are unique and highly evolved pharmaceutical preparations with a wide range in therapeutics. Shila Sindura is sagandha, sagni, kantastha kupipakwa rasayana. Kajjali (Hg+S+As) is transformed to Shila Sindura with agni samskara (gradual heating process), thus altering the physico-chemical properties from that of kajjali. Antibacterial activity of Shila Sindura is confirmed against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus mutans* and *Candida albicans* as substantiated from both the methods i.e. Gradient plate technique and Kirby bauer method. *Klebsiella pneumoniae* and *Acinetobacter baumannii* are resistant to Shila Sindura. The present drug was found to be an effective antimicrobial agent against gram positive, gram negative bacteria and fungus which are responsible for various infectious conditions like urinary tract infections, respiratory tract and skin infections. So Shila Sindura may be used as an effective antibiotic in above mentioned conditions as instructed and used by Acharyas.
REFERENCES

Cite this article as:

Source of support: Nil, Conflict of interest: None Declared