Research Article

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SHILA SINDURA: AN ANTIMICROBIAL AGENT

Dasari Srilakshmi[1]*, S. Swetha[2], K.U. Minchitha[2], Maheshwari Kumari Singh[2]

[1]Ayurvedic Physician and Physiotherapist, S.G.S Hospital, Sri Ganapathy Sachchidananda Ashram, Mysore, Karnataka, India

[2]Department of Nanobiosciences, Centre for Emerging Technologies, Jain University, Jakkasandra post, Ramanagaram, Bangalore, India

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***Corresponding author**

Dr. Dasari Srilakshmi B.P.T, B.A.M.S, M.D (Ayu), Ayurvedic Physician and Physiotherapist, S.G.S Hospital, Sri Ganapathy Sachchidananda Ashram, Mysore-570025, India. Email: drdasari24@gmail.com

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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 kushtahara), skin problems associated with itching (kandu), rakta dosha hara (vitiated raktadhatu) and other diseases of infectious origin like fever (jwara, sannipataja jwara), abscess (vidradhi), gonorrhoea (upadamsha), medhya, 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 re-birth) 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, Basavaraajeeyam, Rasayana sara and Siddha bheshaja 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 ingredients[1,3] 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 Khalvayantra (mortar and pestle), pyrometer, multani mitti, cloth, valukayantra, 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)[6] 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 (svangasheetata), shodhita parada was collected from inner surface of upper pot. Sulphur purification (gandhaka shodhana)[5] 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)[5] was done by triturating with juice of *Gingiber officinale* (ardraka swarasa bhavana) for seven times.

Preparation of Shila Sindura

Samaguna kajjali[5] 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)[5].

Equal quantity (45g) of arsenic di sulphide (suddha manashila) was added to samaguna kajjali and triturated for 5-6 hours. Then *Aloe vera* (kumari swarasa bhavana)[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[2,4]. This bottle (kupi) filled with kajjali was placed in iron vessel filled with sand (valukayantra). After the entire apparatus was ready, fire wood was ignited with help of camphor, following gradual heating (kramagni)[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^{o}C – 250^{o}C for mild heat (mrudvagni), raised to 350^{o}C – 600^{o}C for moderate heat (madhyamagni) and intense heat (teevragni) up to 750^{o}C[2,4]. After the stage of fumes and flames, the bottom of the bottle appeared like rising sun i.e. red in colour (udayabhaskara varna). 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, metabolization 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[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[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^{o}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[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 ardraka swarasa bhavana is because of ardraka satva. In kupipakwa process, maximum output of final product is 50-60%[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.

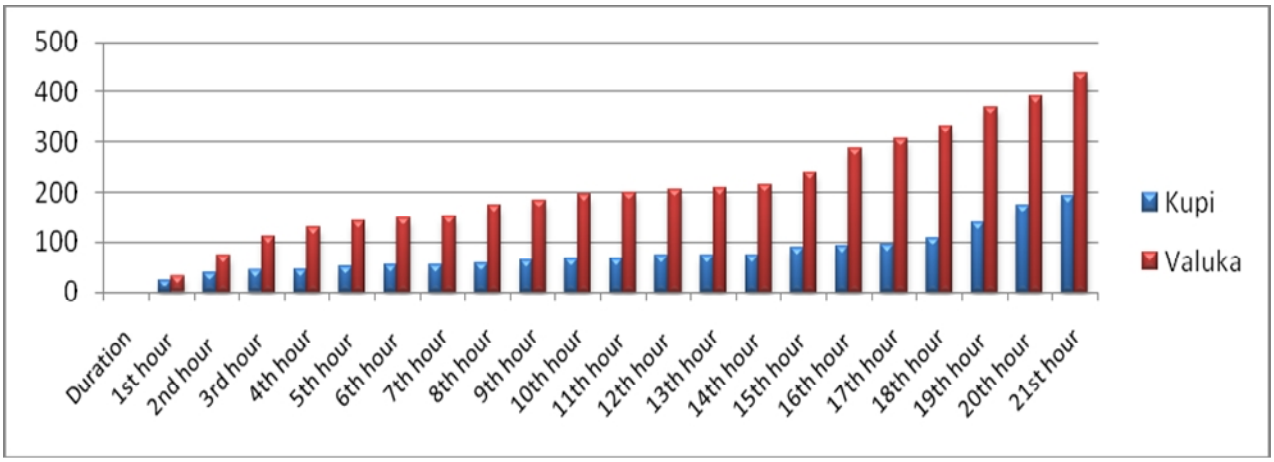

Figure 1: Temperature graph near neck of kupi and valuka during preparation of Shila Sindura

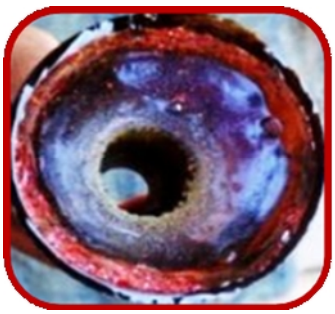

Figure 2: Kantastha Shila Sindura

Figure 3: Final Product – Shila Sindura

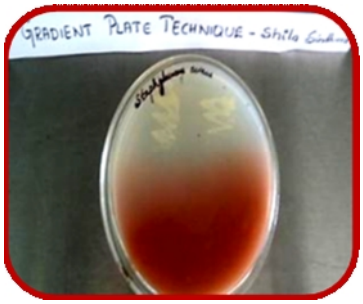

Figure 4: Gradient Plate technique – *Staphylococcus aureus*

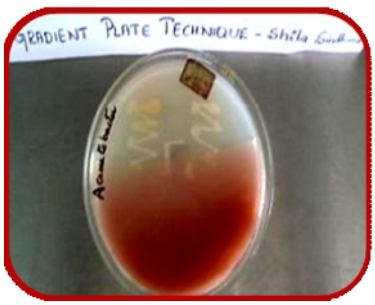

Figure 5: Gradient Plate technique – *Acinetobacter baumannii*

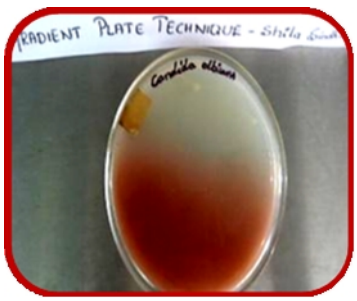

Figure 6: Gradient Plate technique – *Candida albicans*

Figure 7: Gradient Plate technique – *Escherichia coli*

Figure 8: Kirby Bauer method – *Escherichia coli*

Figure 9: Kirby Bauer method – *Pseudomonas aeruginosa*

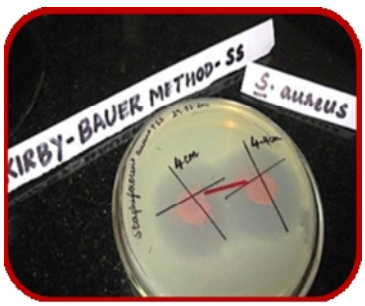

Figure 10: Kirby Bauer method – *Staphylococcus aureus*

Table 1: Pharmaceutical work

SN	Drug Used for Process	Duration of Process	Quantity before Process	Quantity after Process	Loss/ Gain	Percentage of Yield
1.	Parada Shodhana	6 Hrs	250g	238g	12g	95.2%
2.	Gandhaka Shodhana	--	250g	246g	04g	98.4%
3.	Manashila Shodhana	3 Hrs (each time)	250g	273g	23g	109.2%
4.	Samaguna Kajjali	36 Hrs	260g	257g	03g	98.8%
5.	Kajjali (Haratala Yukta)	06 Hrs	375g	373g	02g	99.4%
6.	Shila Sindura	22:45 Hrs of heating and 24 Hrs of Self-cooling	135g	76g	59g	54.28%

Table 2: Activity of Antimicrobial agent in both the methods

S.N	Bacteria	Dosage	Activity by Gradient plate method	Zone of inhibition for Shila Sindura
1.	*Escherichia coli*	100 mg	+	1.7 cm
2.	*Pseudomonas aeruginosa*	100 mg	+	1.9 cm
3.	*Streptococcus mutans*	100 mg	+	2.4 cm
4.	*Candida albicans*	100 mg	+	3.9 cm
5.	*Staphylococcus aureus*	100 mg	+	4.2 cm
6.	*Klebsiella pneumoniae*	100 mg	--	--
7.	*Acinetobacter baumannii*	100 mg	--	--

DISCUSSION

Kajjali is a khalvi rasayana (one of murchita parada yogas) intended to remove the chapalatva 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, subtleness 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[1,5] acts as rogaghna, rasayana, yogavahi. Gandhaka[5] has rasayana, kushtaghna, kandughna properties. It is indicated in twak and raktagata vikara. Manashila[5] has rasayana, lekhana, kasahara, shwasahara, kandughna, panduhara, varnya, vishaghna, kapha vata nashaka properties. Kumari[6,7] also helps in treating twak, rakta and yakrit vikara. Shila Sindura[1,3,4] is effective in conditions like skin disorders (kushta nashana sreshta), 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), gonorrhoea (upadamsha), 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.

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[10]. *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 ($Hg+S+As_2S_2$) 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

1. Vaidya Yadavji Trikamji, Rasamrutam, Translation by Damodar Joshi, 1st edition 1998, Chaukamba Sanskrit Bhavan, Varanasi; pp 117.
2. Shri Harisharanananda Ji, Kupipakwa rasa nirmana vijnana, 1st edition 2000, Chaukamba Sanskrit Series, Varanasi; pp 337-340.
3. Vaidya Pandit Hariprapanna Sharma, Rasayoga sagara Volume-I, 2nd edition 1983, Krishna Das Academy, Varanasi, pp 438-440.
4. Vasudev Mulashankar Dwivedi, Parada vignaneeyam, 2nd edition 1997, Chaukamba Sanskrit sansthan, Varanasi, pp 223-224.
5. Vagbhata Acharya, Rasa Ratna Samucchaya, Translation by Dr. Indra Deva Tripathi, edition 2000, Chaukamba Sanskrit Sansthan, Delhi, pp 28, 35, 87, 88.
6. Sri Bhatta Gopala Krishna, Rasendra Sara Sangraha, Translation by Dr. Indradev Tripathi, Jai Krishnadas Ayurveda Series, 3rd Edition 2003, Chaukamba Orientalia, Varanasi, pp 9.
7. P.V. Sharma, Dravyaguna vignan Volume-II, 5th edition 1999, Chaukamba Samskrita Samsthana, Varanasi, pp 162-163; 332-333; 446-448.
8. Jacquelyn G Black, Microbiology principles and explorations, 7th edition, John Wiley and Sons, INC. 2008, USA, Chapter-13, pp376-390.
9. Lansing M Prescott, John P Harley and Donald A Klein, Microbiology, 5th edition 2002, Tata Mc Kathleen Park Talaro and Barry Chess, Foundations in Microbiology, 8th edition 2008, Mc Graw-Hill companies, New York, Chapter-35, pp805-825.
10. Kathleen Park Talaro and Barry Chess, Foundations in Microbiology, 8th edition 2008, Mc Graw-Hill companies, New York, Chapter-12, pp337-385.

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