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# AN ANTIBACTERIAL STUDY OF *SWARNAMAKSHIKA BHASMA*

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**ABSTRACT**

*Swarnamakshika* is one among *Maharasas* which has given very much importance in both *Dhatuvada* and *Dehavada*. It is therapeutically used in treating various kinds of diseases like *kushta, krimi, netraroga* etc. It has *krimighna*, *kushtaghna* properties, thus based on above aid facts by our ancient *acharyas* (scholars) the present study was undertaken to prove the efficacy of *Swarnamakshika bhasma* as an antibacterial agent. The *marana* of *shodhita Swarnamakshika* is done in the pharmaceutical study and analytical changes from raw to *shodhita* and *shodhita* to *bhasma* were done and thus prepared *bhasma* was subjected for antibacterial study against gram +ve and gram –ve organisms ie., staphylococcus aureus and E-coli. *Swarnamakshika bhasma* prepared by *putapaka* method passed classical *bhasma pareekshas* and NPST test. *Swarnamakshika* when tested against gram positive and gram negative organism. It effectively inhibits the growth of staphylococcus aureus at 8mg concentration after ½ an hour and E.coli at 4mg concentration after ½ an hour where the MIC range was 1mg.

**Keywords:** MIC – Minimum Inhibitory Concentration, MBD – Minimum Bacterial Dose, *Swarnamakshika Bhasma, Krimighna, Kushtaghna*

## INTRODUCTION

*Rasashastra* is a well established branch of *Ayurveda* serving humanity with its unique heritage of drugs derived from minerals metals and animal origin processed with herbs. The important features of *rasoushadhis* stated as:

*Alpamatropa yogitwat*
*arucheraprasangataha*
*Kshipramaarogya dayitwaat*
*aushadhebhyo radhiko rasaha*[1]

*Bhasmas* are most commonly used in many formulations for treatment purpose. *Swarnamakshika* is one among *Maharasas*, which has given very much importance in both *Dhatuvada* and *Dehavada*. It is a compound of copper, iron and sulphur. It is a good *rasayana*[2] and it can be used in *Swarna abhava*[3], *Swarna Makshika bhasma* is indicated in many diseases such as *prameha, Netraroga, Apasmara, Jwara, krimi*[4] etc. It has also got very good effect over *twak vikaras*.

*Swarnamakshika bhasma* is mentioned in many formulations which were indicated in various kinds of *kushta* and *krimi rogas*[5] like *vicharchika, dushta vrana* etc probably due to its *krimighna* and *kushtaghna* properties it was indicated in these disorders. Thus based on its use on certain infections and its important properties such as *krimighna, kushtaghna karmas*, the present study was undertaken to prove its efficacy over micro organisms.

## AIMS OF OBJECTIVES

1. To carry out *Shodhana* and *Marana* of *Swarnamakshika* as per classics
2. To carry out analysis of *Swarnamakshika bhasma*.

3. To carry out anti-microbial activity of *Swarnamakshika bhasma*

## MATERIALS AND METHODS

**Materials**: Major Raw Material – *Swarnamakshika* which is identified by AAS method.

**Other Raw Materials**

a) *Eranda Taila, Matulunga swarasa* for *shodhana*.

b) *Gandhaka, Matulunga swarasa* for *marana*.

*Yantra*: *Tula yantra, Khalvayantra* & *Putayantra* are used for present study.

*Upayantras*: Iron Pan, Iron Pessel

1. *Shodhana* was done by boiling *Swarnamakshika* powder in *eranda taila* & *Matulunga Swarasa* for 2 hrs.[6]

2. *Marana* of *Sodhita Swarnamakshika*[7] is carried out by adding equal quantity of *Gandhaka* with *Matulunga Swarasa* for 1st *Puta* & adding half the quantity of *Gandhaka* to that of Quantity of *Swarnamakshika Bhasma* obtained after 1st *Puta* and in subsequent *Putas*. *Shodhana* of *Gandhaka* was done by *dalana* method.[8]

***Shodhana* of *Swarnamakshika***

Raw *Swarnamakshika* powdered was taken in an iron vessel equal quantity of *Eranda taila* was added and boiled for 2hrs and it is washed in hot water dried and weighed.

- Raw *Swarnamakshika* was grey in colour with Yellowish Shiny Particles.
- During process of heating thick fumes of Sulphur with suffocating odour was noticed.
- The colour of *Swarnamakshika Choorna* on heating appeared very black.
- After 1 hr, *Swarnamakshika* melted completely and converted into black thick liquid.
- At the end of 2 hrs. – Blackish shiny particles were observed.

*Swarnamakshika* which was obtained after 1st experiment was taken in *Loha Paatra* and boiled with *matulunga Swarasa* for 2 hrs.

**Observations**

- Before boiling with *matulunga Swarasa* the colour of *Swarnamakshika* after washed with hot water was grayish black in colour.
- The colour of *matulunga swarasa* changed from milky white to golden yellow colour.
- After ½ hr. *Swarnamakshika* melted and whole mixture turned into black thick liquid.
- After 2 hrs the *Shodhana Swarnamakshika* obtained was blackish brown in colour.

Table 1: Showing the *Shodhana* of *Swarnamakshika*

| *Paachana* Done with | Wt. of Raw *Swarna Makshika* | *Shodhana Dravya* & Q.S. Taken | Wt. of *Swarna Makshika* after *Paachana* | Wt Gain. | Colour | Odour, Touch | Temp. & Duration of Heat |
|---|---|---|---|---|---|---|---|
| *Eranda Taila* | 130 g | Eranda Taila 130 ml | 150 g | 20g | Blackish Shiny | Characte -ristic Powdery | 230-520$^0$C 2hrs |
| *Matulunga Swarasa* | 150 g | *Matulunga Swarasa* 500 ml | 160 g | 10 g | Blackish Brown | Characte -ristic Powdery | 230-520$^0$C 2hrs |

***Marana* of *Shodhita Swarnamakshika***

Procedure:

- *Shodhita Swarnamakshika* was pounded well till it became fine powder in a *Khalwa*. *Shodhita Gandhaka* is also powdered and taken equal to the quantity of *Swarnamakshika*. Both are taken in a *Khalva Yantra* and triturated till they become homogenous. To this mixture *Matulunga Swarasa* was added triturated well till it becomes semisolid consistency. The paste were made into shape of pellets and kept for drying.
- In 1st *Sharava* the *Swarnamakshika Chakrikas* were arranged separately leaving little space to avoid over lapping. Over this *Sharava*, another *sharava* was placed invertedly, without leaving any space at the junction. *Sandhi bandhana* was done with the help of a *cora* cloth smeared with *multani mitti*. Two layers of *multani mitti* smeared cloth were applied & it is dried after application of each layer. Completely dried *Samputa* was subjected to *varahaputa*. *Vanopalas* used – 500 in number. 350 *Vanapolas* were arranged and filled in the pit. Then the *sharava samputa* which are ready was placed in pit upon the cakes evenly. The rest 150 *Vanopalas* were arranged upon the *samputa*. And fire was lit.
- The burning continues for 8 hrs and *samputa* was left for *Swanga Sheeta* over night. On next day the *samputa* was removed out of the pit and opened continuously. This whole procedure was repeated for 9 *putas* till it passes all *bhasma pareekshas*. Temperature was recorded at each *puta* with the help of pyrometer.

Table 2: Observation of Pharmaceutical Study of *Swarnamakshika Bhasma*

| *Puta* | Sw. M. in g | *Gandhaka* in g | Wt.of *Chakrikas* before | *Vanopalas* Total No. | *Vanopalas* above | *Vanopalas* below | Wt. after *Puta* in g |
|---|---|---|---|---|---|---|---|
| 1 | 150 | 150 | 303 | 500 | 350 | 150 | 145 |
| 2 | 145 | 72 | 219 | 500 | 350 | 150 | 138 |
| 3 | 138 | 70 | 209 | 500 | 250 | 250 | 135 |
| 4 | 135 | 65 | 203 | 500 | 250 | 250 | 132 |
| 5 | 132 | 65 | 198 | 450 | 225 | 225 | 130 |
| 6 | 130 | 35 | 165 | 450 | 225 | 225 | 125 |
| 7 | 125 | 30 | 158 | 400 | 200 | 200 | 123 |
| 8 | 123 | 15 | 140 | 400 | 200 | 200 | 120 |
| 9 | 120 | 15 | 138 | 350 | 175 | 175 | 118 |

Table 3: Showing the organoleptic characters of *Swarnamakshika Bhasma*

| No. of the *Puta* | Organoleptic Characters of S.M. *Bhasma* | | | Odour | Appearance |
|---|---|---|---|---|---|
| | Colour | Touch | Taste *Gatarasatva* (Metallic Taste) | | |
| 1 | Gray | hard | Metallic | Metallic | Powdery Shiny |
| 2 | Blackish Brown | Soft | Metallic | Metallic | Powdery Shiny |
| 3 | Reddish Black | Soft | Metallic, Sour | Metallic | Powdery Shiny |
| 4 | Reddish | Soft | Metallic | Metallic | Powdery |
| 5 | Muddy Red | Soft | Metallic | Characteristic | Fine Powder |
| 6 | Muddy Red | Soft | Metallic | Characteristic | Fine Powder |
| 7 | Red | Soft | Tasteless | Characteristic | Fine Powder |
| 8 | Dard Red | Soft | Tasteless | Characteristic | Fine Powder |
| 9 | Dark Red | Soft | Tasteless | Odourless | Fine Powder |

## ANALYTICAL STUDY

Classical parameters and Modern parameters were carried out for *Swarnamakshika Bhasma*.

**Classical parameters:**

- *Bhasma Varna* : The colour of prepared *Bhasma* obtained was observed
- *Nischandratva*: Luster less ie., no shining particles were seen when *bhasma* was observed in the bright sunlight.
- *Gatarasatva* : On *Rasanendriya pareeksha bhasma* should not possess any metallic taste is called *Gatarasatva*
- *Rekhapurnata*: When fine powder of *Swarnamakshika Bhasma* was rubbed between the thumb and index finger the fine particles entered in to the furrows of the fingers. This indicated fineness of *bhasma*.
- *Varitaratva*: When *Swarnamakshika bhasma* was sprinkled on water taken in a beaker containing water, *bhasma* floated on water.
- *Apunarbhava*: *Apunarbhava bhasma* is that, from which the original metal cannot be re-obtained even after intense heating in the fire, after mixing the *bhasma* with *Guda, Gunja, Madhu, Ghrita* & *Tankana*.

Table 4: Showing physical properties of *Swarnamakshika Bhasma*

| No of Puta | Colour | Touch | Taste | Odour | Appea-rance | *Rekha Purnata* | *Varitara tva* | *Apunar Bhava* | *Dadhi* test |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Grey | Soft | Metallic | Metallic | Powdery Shiny | - | - | - | - |
| 2 | Blackish Brown | Soft | Metallic | Metallic | Powdery Shiny | + | - | - | - |
| 3 | Reddish Black | Soft | Metallic Sour | Metallic | Powdery Shiny | + | - | - | - |
| 4 | Reddish | Soft | Metallic | Metallic | Powder | ++ | - | - | - |
| 5 | Muddy Red | Soft | Metallic | Characteristic | Powder | ++ | Partially Positive | - | - |
| 6 | Muddy Red | Soft | Metallic | Characteristic | Fine Powder | +++ | Partially Positive | - | - |
| 7 | Red | Soft | Tasteless | Characteristic | Fine Powder | +++ | Partially Positive | - | - |
| 8 | Dard Red | Soft | Tasteless | Characteristic | Fine Powder | +++ | Positive | - | - |
| 9 | Dark Red | Soft | Tasteless | Odourless | Fine Powder | +++ | Positive | + | + |

Table 5: Showing the Elemental Analysis

| Elements | Raw S M | *Shodhita* S M | S M *Bhasma* |
|---|---|---|---|
| Cu | 29.68% | 26.17% | 5.12% |
| Fe | 25.20% | 18.12% | 38.0% |
| S | 14.48% | 4.71% | 1.72% |

Table 6: Showing the Analytical Study of the *Swarnamakshika Bhasma*

| Sl. No. | Parameters | Results |
|---|---|---|
| 1 | Loss on drying | 1.18% |
| 2 | Total ash | 94.55% |
| 3 | Acid insoluble ash | 61.33% |
| 4 | Water soluble ash | 6.89% |
| 5 | pH value | 5.66% |
| 6 | Particle size | 1.2 microns |
| 7 | $Fe_2O_3$ | 20.10% |
| 8 | CuO | 6.40% |

**Nambhuri phased spot test**

As *Swarnamakshika* $CuFeS_2$ contains both Cu & Fe both *Tamra* group and *Lauha* group method (both) were tested in order to identify the presence of Cu and Fe.

**Result**

- Deep blue wide with a central bleached white shows the presence of Fe in *Swarnamikshika bhasma*.
- Chocolate coloured central spot with blue periphery shows the presence of Cu in *Swarnamakshika bhasma*.

**Anti Bacterial Study of *Swarnamakshika Bhasma***

Antibacterial potency was tested against gram positive cocci (staphylococci) and gram negative bacilli (E.Coli) organisms which are the common nasocomial pathogens causing variety of HAI (Hospital acquired Infections).

**Preparation of test compound**

To make the sample suitable for study, semisolid paste and suspension was made ready. Gentamycin solution was used as a control antibiotic. Suspension was prepared out of standard strains of ATCC staphylococcus aureus and ATCC E-Coli under CLSI guidelines.

**Control Groups**

Positive control: In which bacterial suspension was maintained to check the viability of the organism. Negative control – was only distilled water validation of the test was used.

**Broth dilution Method (9)**

- 1ml of suspension of the *bhasma* containing 1mg of *Swarnamakshika bhasma* concentration was taken in a sterile test tube. 0.1ml of bacterial suspension was added. The same procedure was employed using 2mg, 4mg, and 8mg concentrations of *bhasma*. These tubes were incubated for 24hrs at $37^0$C. Next day tubes were observed for turbidity.

**Results**

Turbidity was observed only in positive control groups where as no turbidity was seen in any other series of test tubes. These shows all the samples had effectively inhibited the growth of the bacteria.

Mueller Hinton Agar plates was divided into 4 sections & labeled. With the help of sterilized loop the content from each test tube was taken out & applied over the agar plates. At ½ an hr. 1hr, 2hr, 4hr, 8hr. suspension was sub-cultured first followed by the sub-culturing of the solution after mixing. The similar procedure was done with all the series of test tubes & incubated for 24hrs.

**Results**

- *Swarnamakshika bhasma* inhibited the growth of staphylococcus aureus at a

concentration of 8mg after ½ an hr. the MIC range was 1mg – 8mg.

- *Swarnamakshika bhasma* inhibited the growth of E-coli at a concentration of 4mg after ½ an hr. the MIC range was 1mg – 8mg.

## DISCUSSION

*Swarnamakshika* is as *Rasendra Parna*, used in various mercurial operations. *durmelanasya melanam* - it can bring together the *dhatus* (metals) having antagonistic properties, and it is said as *Rasayanagrya*. *Swarnamakshika* is used in both *Dehavada* and *Lohavada*. This might be the reason that earliest *Rasacharyas* were included *Swarnamakshika* under *Maharasas* group.

*Shodhana* - removes impurities and nullifies the harmful effects , as well as brings about some changes in the qualities of the drug.

The *matulunga swarasa* contains citric acid 7-10% phosphoric acid 4%, sugar 10.9%, vitamin C, vitamin A, citrine, 7.6%, citrol 7.8%, this might have brought some chemical reaction - the little quantity of Hcl which is liberated from *amla dravyas* takes away impurities like carbonates.

At the end of *shodhana* colour of *Swarnamakshika* was brownish black.

In *marana*, *bhavana* with *matulunga swarasa* helps in neutralyzing the harmful effects of *Swarnamakshika*, especially the copper content. Addition of *Gandhaka* helps in easy disintegration of metals and minerals and also addition of some therapeutic qualities also.

Before subjecting *Swarnamakshika* to 1st *puta* - triturated - 2 days along with *marana drayvas* as the particles were hard in nature and not easily powdered. On subjecting to further *putas*, however the trituration became easier. The *rekhapurnata* test was noticed from 3rd *puta* onwards which indicates *sukshmatwa* of *bhasma*. *Varitara* test - 5th *puta* - only partially positive. In 7th and 8th *puta*- achieved *varitara* test. This indicates the lightness of *bhasma*.

Thus the 9th *putita bhasma* gave all positive results for *rekhapurnata*, *varitara*, *apunarbhava*, and *nirutha pareekshas*.

According to classic there was reference of 5 *varaha puta*. But it satisfied only *Rekhapurnata* test but no other test like *varitara*. Therefore, it was subjected for further *putas*. In quantitative analysis (AAS) there is a variation in percentage of all the elements in *shodhita* as well as in *bhasma*.

To test the sample against gram positive and gram negative micro organisms, it's important to see its solubility in solvents. For the study distilled water- 70% of water with either acidic or alkaline pH, and as water is an universal solvent, therefore *bhasma*s was checked for solubility in distilled water in acidic pH of 1.2, in alkaline pH of 7.8. *Swarnamakshika bhasma* was sparingly soluble so suspension was taken for the study.

In serial dilution method the supernatant portion as well as the mixed portion containing nutrient broth showed antibacterial effect, which explains that the active principles present in the *bhasma* was absorbed by the distilled water so probably the same occurs in the body the active principles of the *bhasma* are absorbed by fluids in our body and remaining unabsorbed portion is excreted out.

*Swarnamakshika bhasma* -staph at concentration of 8mg after ½ an hour and E-coli - at the concentration of 4mg after ½ an hour. This shows that the

*Swarnamakshika bhasma* has effectively inhibited the growth of microorganisms. Based on the ancient literature – SM *bhasma* - *vrana ropaka*, *kushtaghna*, *krimighna*, and especially in *twak vikaras* - as its antimicrobial action against both staph and E-coli is proved.

## CONCLUSION

The physical properties like colour and consistency change during process of *shodhana* and *marana*. *Marana* was done ie., *Swarnamakshika* is subjected for many *puta* until *bhasma siddhi lakshanas* found and it took 9 *putas*. The *bhasma* obtained after 9th *puta* fulfilled all the *bhasma lakshanas*. The physical properties like colour, consistency etc. and *bhasma* satisfying all the classical parameters and the expected results obtained through NPST reveals the genuinity of the *bhasma*.

Prepared *bhasma* when tested against staphylococcus aureus and E-coli showed the significant results that SM *bhasma* inhibited the growth of staph at the concentration of 8mg after ½ an hour and inhibited the growth of E-coli at a concentration of 4mg after ½ an hour, where the MIC range was 1mg – 8mg.

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