INVITRO ANTIBACTERIAL ACTIVITY OF TAMRA BHASMA

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Abstract

Tamra bhasma [copper ash] is used in treating various disorders like *jvara* [fever], *twakvikaras* [skindisorders] etc based on its antipyretic, anti parasitic and antileprotic properties mentioned in our classics. The present study was undertaken to evaluate antibacterial role of *tamra bhasma* on gram positive and gram negative bacteria. **Material and methods**: *Tamra* and other raw drugs such as *Parada* [mercury] and *Gandhaka* [sulphur] were purified and *shudha tamra* was subjected to *marana* [incineration] process according to the procedures mentioned in classics. MIC and MBD of the prepared sample was determined by broth dilution method by following NCCLS guidelines. **Results:** *Tamra bhasma* had an antibacterial effect against both gram positive and gram negative bacteria. Minimum Inhibition Concentration [MIC] and Minimum Bactericidal Dose [MBD] was estimated to be 2.5 mg/ml of nutrient Broth for *E. coli* and between 1.25 mg/ml for *Staphylococcus*. **Conclusion**: The exact therapeutic dose detection needs further detailed analysis.

Key Words: Minimum inhibitory concentration (MIC), Minimum bactericidal dose (MBD), *Tamra Bhasma*, *Jvaraghna*, *Krimighna, Kushtaghna.*

Introduction:

Tamra has been used in preparing various medicines for thousands of years. Our ancient classics mentions the therapeutic use of copper in treating various disorders such as *abhisyanda* [conjunctivitis], *krimiroga* [antihelminthic & antiparasitic], *visuchika* [cholera], various *jvaras* [fever], *tvakvikaras* [skin disorders] etc.

The following Historical review on copper shows it has antibacterial effect

The first medical use of copper found in Papyrus, The Egyptian Medical Text, records use of copper in sterilizing chest wounds.

Acharya susruta mentioned preparation of *Shalaka* [needle] out of *Tamra* in operation of cataract.

In Hippocratic collection copper was recommended in the treatment of leg ulcers associated with varicose veins.

Greeks used dry powder composed of copper oxide and copper sulphate on the wound.

Inorganic copper preparations were found to be effective in treating eczema, impetigo, tubercular infections etc.

Thus based on its use on certain bacterial infections since ancient times and important properties such as *jvaraghna , krimighna* and *kushtaghna* mentioned in our classics present study on *tamra bhasma* was undertaken to prove its efficacy as an antibacterial agent.

AIMS AND OBJECTIVES:

To carry out Purification of the raw drugs – *Parada* [Mercury], *Gandhaka* [Sulphur] and *Tamra* [Copper].

To prepare *Tamra Bhasma* [Copper Ash]

To find the Antibacterial action of the prepared sample and

To determine the MIC & MBD of prepared sample against gram positive bacteria and gram negative bacteria.

MATERIALS AND METHODS:

Raw materials were collected from genuine sources and subjected initially for the purification process.

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***PARADA SHODHANA* [MERCURY PURIFICATION] (1)**

Reference : *Rasendra sara samgraha 1/28*
Method : *Urdhva patana* method
Equipments : *Khalva yantra, Urdhva patana yantra,*
Ingredients : *Ashudha parada* – 500g, *Kumari svarasa* [aloe vera juice]– Q.S, *Haridra* [turmeric] – 500g

Procedure*:* Mercury and Turmeric taken in known quantity was triturated well till the complete mercury is assimilated into Turmeric then aloe vera juice was added and *chakrikas* [small thin plates] were prepared with it. *Chakrikas* are dried and placed into *urdhva patana yantra* [equipment to procure mercury] and subjected for heating for 6 hours. After self cooling mercury was collected and washed thoroughly in hot water for 3-5 times.

Observations:

Parada	*Before shodhana*	*After shodhana[a]*
Weight	500g	478g
Appearance	Dull shine,	Lustrous, clear and shiny

***GANDHAKA SHODHANA* [PURIFICATION OF SULPHUR] (15)**

Reference: *Rasa ratna samuchaya 3/21-23*
Method: *Bhudhara method*
Equipments: Mud pot, *Sthali yantra*
Ingredients: *Gandhaka* – 500g, *Godugdha* – 2litres

Procedure: Mud pot was filled with *godugdha* and *gandhaka* was placed on cloth tied over its mouth and covered with *sthali yantra. vanyopalas* [cow dung cakes] placed above in sufficient quantity and ignited after self cooling *gandhaka* collected in the milk was collected and washed in hot water.

Observations:

Gandhaka	*Before shodhana*	*After shodhana[b]*
Weight	500g	496g
Appearance	Yellow, Crystalline	Small Globular and lustrous

***TAMRA* GENERAL AND SPECIFIC PURIFICATION: (1-12)**

References: *Rasa ratna samuchaya* 5/29
Equipments: Steel vessel
Ingredients:
samanya shodhana [general purification] - Tamra -1kg, *Tilataila, takra, gomutra, aranala*, *kulatha kwatha*, in sufficient quantity
Vishishta shodhana [specific purification] – *samanya shodhita tamra, nirgundi swarasa* and *saindhava lavana, nimbu rasa* [lemon juice] in quantity sufficient
Procedure: *samanya shodhana* was done by heating and dipping for 7 times in each above said liquid.and *Vishishta shodhana* was done by applying paste of saindhava and *nimbu rasa* on *tamra patra* heating and dipping it in *nirgundi rasa* this process was done for 8 times
Observations: copper general purification and specific purification

TAMRA	**Initial weight**	**Final weight**	**Weight loss**	**Observed changes**
Taila [Oil]	995 g	995g	No loss	Tamra became soft and blackish discoloration
Takra [Buttermilk]	993g	1.025kg	Gain-32g	Tamra had dark grey layers that peels easily
Gomutra [Cow's urine]	1.025kg	1.015kg	Loss-10g	Grayish black ,fragile,
Aranala	1.015kg	950g	Loss-65g	Grayish layers peels easily,
Kulatha [Decoction]	950g	925g	Loss-25g	Tamra became still more

				brittle
Nirgundi Rasa [Juice of Vitex Nirgundo]	500g	575g	Gain-75g	Tamra powdered easily , saltish taste and smell

***KAJJALI PREPARATION*: [E]**

INGREDIENTS: *shudha [*purified] *Parada* – 500g, *shudha gandhaka* - 500g.

Procedure: *mardana of parada* and *gandhaka* was done till the jet black powder was obtained

Observation: triturating done for 62 hours, prepared kajjali was jet black lusterless and soft. Weight – before trituration was 1000g and after trituration – 970 g. So total loss is 30g

TAMRA BHASMA (3) [F]

Reference : *Rasa tarangini 5/51*

Method : Incineration *[marana]*

Equipments: *Khalva yantra* [mortar pestle] , *sharava,vanyopalas* [cowdung cakes]

Ingredients: *Kajjali* – 2pts[300g], *shudha tamra*[150g] – 1pt ,lemon juice-qs

Procedure*: kajjali* and lemon juice paste was applied on copper and subjected to incineration process by heating with 1000 cow dung cakes each time and after 10th *puta* genuine copper ash was obtained which passed all the classical *bhasma pareekshas* [tests for genunity].

		
Purified Parada (Mercury)		Purified Gandhaka (Sulphur)
		
Copper		Purified Copper

		
Kajjali		Copper after 10th Puta

Observations:

Puta	First	Second	Third	fourth	Fifth	Sixth	seventh	eight	ninth	Tenth
Kajjali taken	300g	250g	240g	240g	230g	226g	210g	196g	170gg	220g
Tamra taken	150g	125g	120g	119g	115g	113	105g	98g	85g	110g
Weight before puta	450g	375g	360g	360g	350g	115g	107g	102g	175g	220g
Weight after puta	145g	120g	119g	115g	113g	105g	98g	97g	107g	108g
Vanyopalas	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Observation	blackish green	Blackish with green and brown tinge	Brownish black and soft than before	Shine reduced comparatively Chakrikas soft and easily breakable	Black and easily powdered	Blackish brown	Brownish on powdering	Black colored chakrikas	Brown tinge	Blackish brown
Bhasma pareeksha Test for genuinity of bhasma	-	-	-	*Varitaratva –ve Rekhapoornatva +ve*	*Varitaratva—ve*	*Varitaratva-80% passed*	*Varitaratva-95% passed Curd test passed*	*Unama positive Curd test +ve*	*Apunarbhasva passed Niruth passed*	*All the tests passed*

ANTIBACTERIAL STUDY

After the sample was prepared the antibacterial study was conducted in Department of Microbiology, JSS Medical College Mysore**.**

Methodology:

Preparatory procedures:

A] Four different methods were adapted to make *tamra bhasma* suitable for this study.

1. Sample I – Preparation of stock solution by adding 1g of *tamra bhasma* sample in 10 ml of distilled water
2. Sample II - Preparation of *tamra bhasma* paste by triturating well.
3. Sample III - Pure filtrate was obtained from the original stock solution
4. Sample IV - Solution obtained by diluting pure Filtrate with equal amount of distilled water

B] Control groups:

Positive control group: In which bacterial suspension was maintained to check the

Viability of organisms

Negative control group: Was only distilled water for validation of the test was used.

C] Gentamycin in different solution with particular dose was used for comparison at the same time.

D] A suspension was prepared with standard strains of *E. coli* and *Staphylococcus*.

METHOD ADOPTED:

Invitro Study by Broth Dilution Method (16)

The Antibacterial action of the prepared *tamra bhasma* was tested against both gram positive cocci and gram negative bacilli. MIC & MBD of the sample was determined by following NCCLS guidelines.

In the present study ATCC strains of gram positive bacteria (*Staphylococcus* Aureus) and gram negative Bacilli (E coli) was selected as these are the common agents of infections including Nosocomial infections.

- Sample I [G] -*Tamra bhasma* was made into fine powder and stock solution was prepared by adding 10ml of distilled water to 1g of sample.

  From this stock solution different dilutions of *bhasma* in decreasing concentration was prepared ranging from 0.1g to 0.0125g /ml of nutrient broth in this way two sets of test tubes was prepared and named as set A and set B

To each of these set of test tubes 1 loop full of each bacterial suspension [staph and *E. coli*] matching to 0.5 mcfarland turbidity standard was added. Set A test tubes were inoculated with *E. coli* and set B was inoculated with *Staphylococcus*. All the test tubes were then incubated at 37 c for 18-24 hrs.

Sample II [F]- *Tamrabhasma* paste was prepared by triturating well with known quantity of distilled water and dilutions were prepared with decreased concentrations of sample. Two such sets of test tubes were sample concentration ranging from 5mg to 0.15mg/ml of nutrient broth were made and named as set a and set b. these sets were also inoculated with bacterial suspensions in the same manner and incubated at 37c for 18-24 hours.

Similarly the III sample - pure filtrate and the IV sample – dilute solution of filtrate were prepared and 1ml of each sample was taken with one ml of nutrient broth in different test tubes and each of these were inoculated with bacterial suspensions and incubated for 18 hrs.

Negative and positive control groups and gentamycin dilutions were also prepared at the same time.

After 18 hrs of incubation test tubes were taken out and wet mount preparation was done for each of the dilutions and examined microscopically for viability of the bacteria. At the same time from each test tubes and also from the control group one loop full of material were aseptically inoculated on a blood agar plate. And all the blood agar plates were incubated at 37c for 18 hours.

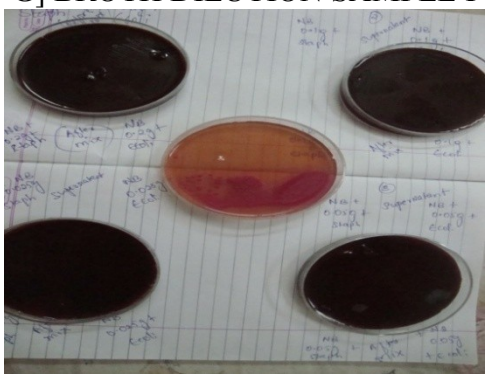
On the third day blood agar plates were examined for presence or absence of bacterial growth [colony formation] which shows no action or effective action of the samples respectively.

OBSERVATIONS: After 18 hours of incubation of blood agar plates.

Blood agar plates	*E. coli* (Gram -ve bacilli)	*STAPHYLOCOCCUS* (Gram +ve cocci)
Positive control group	Growth present	Growth present
Negative control group	Growth absent	Growth absent
Gentamycin dilution test tubes	Growth absent	Growth absent
Sample I [0.1g – 0.0125g]	Growth absent [I]	Growth absent [I]
Sample II [5mg – 0.15mg]	Growth absent in 5mg 2.5mg [J]	Growth absent in 5mg,2.5mg,1.25mg [K]
Sample III [pure filtrate of sample]	Growth absent [L]	Growth absent [L]
Sample IV [dilute filtrate solution]	Growth absent	Growth absent

G] BROTH DILUTION SAMPLE I

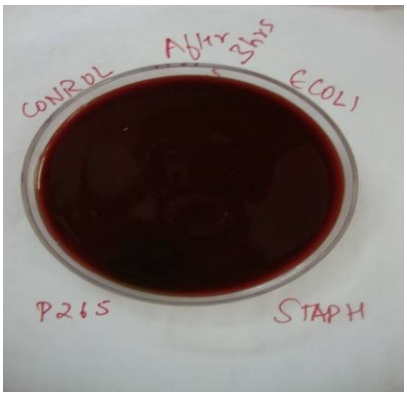
H] BROTH DILUTION SAMPLE II



[I]

[J]

[K]



[L]

RESULTS:

- The pure bacterial suspension or the positive control group showed bacterial growth.
- The negative control group containing only distilled water showed no bacterial growth.
- The gentamycin showed MBD [minimum bactericidal dose] at 0.01mg/ml concentration
- The *tamra bhasma* solution inhibited bacterial growth at 2.5mg/ml concentration for *E. coli* and growth of *Staphylococcus* was inhibited at 1.25mg/ml concentration.
- The *tamra bhasma* filtrate inhibited the bacterial growth in equal concentration even after dilution.

DISCUSSION:

- Antibacterial action of *Tamra bhasma i.e kushtaghna, krimighna, jvaraghna* properties as said in ancient literature is established
- *Tamra bhasma* was used in treating *rakta vikaras, parinama shula* and *yakrit vikaras* .
- Previous studies on *tamra bhasma* have proved its hepato protective, antioxidant and antiulcerogenic effect.
- *Tamra bhasma* given in appropriate doses would have very minimal or no side effects.
- In the present study its anti bacterial action on Ecoli and Staphylococcus was made.
- By the Observations of the results it is proved that *tamra bhasnma* has antibacterial action and has bactericidal effect on both the bacteria.

CONCLUSION:

- *Tamra bhasma* is mentioned in treatment of *tvak roga, rakta vikaras* and *yakrit vikaras.*
- Previous studies on *tamra* have shown that *tamra* is hepatoprotective in nature and when prepared classically its toxic effects can be nullified.
- Its internal administration with an appropriate dose it may have less or no side effects.
- Here the studies show that *tamra bhasma* [4 samples] has a good amount of antibacterial effect.
- Further research is required to find its exact therapeutic dose and with clinical studies on infectious disorders it may be used as an ideal medicine in certain bacterial infections.

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