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ROLE OF SHODHANA ON ANTIMICROBIAL ACTIVITY OF TUTTHA

Research Article

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Abstract

Tuttha is one among the *Maharasas*, well known and frequently used mineral in *Rasaaushadis*. *Ayurveda* classical texts indicate to use in skin diseases, ulcer, sinus, worm infection, vitiligo, obesity, pain, asthma, hyper acidity, hemorrhoids, diseases of eyes and as *Krimighna* etc. But advocates only after specific procedures called *Shodhana* (purification procedures) before making any pharmaceutical form since crude *Tuttha* causes vomiting and giddiness etc. The present antimicrobial study was carried out on two *Tuttha* named crude *Tuttha* and *Shodhita Tuttha* before and after *Shodhana.* Antibacterial activity on *Escherichia coli, Bacillus subtilis, Salmonella typhi, Staphylococcus aureus* bacterias and antifungal activity on fungi *Candida albicans* and *Trichophyton rubrum* were carried out by agar disk diffusion method. The *shodhana* process results that *Shodhita Tuttha* was found to be more effective compared to crude, as the diameter of the zone of inhibition was higher, even at low concentration of 1mg. The present study shows the importance of *Shodhana* process by enhancing the antimicroabial activity.

Keywords: Tuttha, Agar disk diffusion method, Antifungal, Antibacterial, Ayurveda

Introduction

Tuttha is a mineral and chemically copper sulphate(1), that has been indicated in various diseases from skin diseases(2) to eye diseases(3) etc. Reviews of classical texts of Ayurveda suggest to use more in the form of external use than internal use. Caraka samhita is the texts of general medicines suggest to use mainly in external dosage form. Later by other masters of Ayurveda suggested use as orally. Tuttha is mentioned by Ayurvedic classic as *Katu* (pungent), *Kshara* (alkali), *Kashaya* (astringent), *Laghu* (light to digest), *Vamaka* (emetic), *Lekhana* (scraping), *Bhedana* (penetrating), *Usna* (hot) in potency, *Cakshushya* (good for eyes), pacifies *Kaphapitta* (4) and *Krimighna* (antimicrobial).(5)

The Ayurvedic Pharmacopoeia of India, suggests *Tuttha* shall not be used in formualtions without subjecting it to *Shodhana*.(6) The crude of *Tuttha* causes vomiting and giddiness etc.(7) Rasashastra advocates only after specific procedures called *shodhana* (purification procedures) by different medias like triturated with lemon juice(8)/*rakta varga(9)* [*Rakta Varga* consists of following *Kusumbha (Carthemus tincterius), Khadira (Acacia catechu), Laksa (Laccifera lacca), Manjishta (Rubia cardifolia), Rakta candana (Pteroceupus santalinus) Aksi,*

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Bandhujiva (Pentapetis phoenicea) Karpugandhini and Honey] and then *sneha varga*(10) [*Sneha Varga* consists of Ghee, (Seasame) oil, Animal fat & fat from the bone marrow cavity]/ boiled in cows', goats', buffalows' urine(11) , which have their own significance in detoxifying and increasing the therapeutic potential of minerals. Since a comparative microbial activity was not available, this study was conducted to know the role of *Shodhana* on their antimicrobial activity.

Materials and methods

Tuttha was collected from local market and matched with *grahya lakshanas* (qualities as described in the classics), viz., S*nigdha* (unctuous), *Guru* (heavy), *Mahaujjala* (very bright blue in color) were observed and selected the sample for the study. The qualitative estimation of copper and sulpur was done by A.A.S. (Atomic absorption spectroscopy)(12) revealed that crude Tuttha contains copper - 23.78 %, Sulphur - 11.98%, Iron - 0.33% and undetectable sodium.

Tuttha was subjected *Shodhana* as per classical text mentioned in the text Rasa Tarangini.(13) 750gms of Tuttha was taken in a mortar and pestle and 350ml of Lemon juice (*Citrus acida Roxb.*) was poured to make it sufficiently wet. Then with pestle trituration was done with pressure for 6 hours; then allowed the paste to dry and then collected. There was a loss of 10gms of Tuttha after purification. After purification of Tuttha with Lemon juice, quantitative chemical analysis done by A.A.S. (Atomic absorption spectroscopy), the copper content was increased to 26.74%, iron content was increased to 0.76% and sulphur content was decreased to 8.46%.
The crude and *Shodhita* sample of Tuttha were subjected to anitmicrobial study by agar disk diffusion method.

Antimicrobial study

Organisms

Antibacterial study on gram-positive organisms used in the study were *Staphylococcus aureus, Bacillus subtilis* and gram-negative organism were *Salmonella typhi* and *Escherichia coli*. The fungi *Candida albicans* and *Trichophyton rubrum* were used in the study.

Preparation of test/stock solution

Suspension of crude and *Shodhita Tuttha* were prepared by dissolution of test sample - at 1mg/ml, the bhasma was vortexed in distilled water and loaded as such taking into consideration the particulate nature of the salt and its usage.

Preparation of agar plates

The Petri dishes which measured around 32 cm diameter and 2 cm thickness were selected after sterilizing by dry heat in an oven. Base layer was obtained by pouring around 20-30 ml of Muller Hinton Agar solution to obtain a thickness of 4 mm. It was then kept for solidification. The overnight grown subculture from the mother culture (procured from T- Stanes laboratory, Coimbatore) was taken in definite volumes of peptone water and incubated at 37°C at least for 2-4 hours prior to plating. After incubation with the help of cotton swab, the organisms were streaked on Petri dish containing base layer medium.(14) Following this, the cylinders are placed from a height of approximately 1/8 inch onto the agar surface as an alternate to well as per diffusion method. The cylinders on each plate are filled with the test sample. After the cylinders are filled, the plates are carefully placed in the 37°C in incubator where they remain for 15 to 16 hours. The diameter of each circle of inhibition is measured to the nearest 0.25 mm.

Readings the test samples are averaged, unless it is obvious that a cylinder has been jarred or that it leaks badly.(Zones around such cylinders are

often abnormally large or not perfectly circular.) (15)

The antimicrobial activity is expressed as zone diameter in millimeters, which is measured by a scale.

Results

The drug concentration in each microliter of test solution was as 1mg, 5mg, 10mg and 20 mg. The zone of inhibition for *Tuttha* samples were measured by a scale and the measurements are tabulated. [Table No. 1]

The zone of inhibition images are shown in plates from image number 1 to 10. [Image No. 1 to 12]

Discussion

Copper sulaphate exothermically dissolves in water to give the aquo complex [Cu(H2O)6]2+, which has octahedral molecular geometry and is paramagnetic. It is uses as a herbicide, fungicide and pesticide. During the process of *Shodhana,* copper sulaphate triturated with lemon juice it converts to copper citrate. The bright blue colour in crude form converts to a green or bluish green crystalline powder after purification. *Tuttha* sample was studied on *Escherichia coli, Bacillus subtilis, Salmonella typhi Staphylococcus aureus, Escherichia coli, Candida albicans* and *Trichophyton rubrum*.

The samples were assessed by agar method, by comparing zone of inhibition between test samples. In low concentraion, it was found that *Shodhita Tuttha* found is a better antifungal than antibacterial agent. *Shodhita Tuttha* was found to be more effective compared to crude, as the diameter of the zone of inhibition was higher, even at low concentration of 1mg. It is bactericide to *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi* and *fungicide* to *Trichophyton rubrum* and *Candida albicans.* Crude *Tuttha* was alone bactericidal to *Salmonella typhi.*

In high concentration, it was found that both samples have comaparable zone of inhibition for both antifungal than antibacterial activity. Both samples have shown better showed better antibacterial result against *Bacillus subtilis* compared to other organisms and showed better antifungal result in *Trichophyton rubrum* against *Candida albicans.*

The comparative zone of inhibition towards microbial organisms in lowest and highest concentration of Crude and *Shodhita Tuttha* are show below in graphs number 1 and 2. [Graph No. - 1 and 2]

Conclusion

The cultures were sensitive to the test samples above 1mg, hence the concentration was increased to 5mg, 10mg, 20mg. No zone of inhibition was observed at concentration below 1 mg (i.e. @ 100µg, 500µg). *Shodhita Tuttha* was found to be more effective compared to crude, as the diameter of the zone of inhibition was higher, even at low concentration of 1 mg. The present study shows that *Shodhana* procedure enhanced the antimicrobial activity in *Shodhita Tuttha* compared to crude *Tuttha*.

Acknowledgement

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Table 1: Zone of inhibition measured in mm of Crude Tuttha and Shodhita Tuttha in different concentrations

Sample / Organism	1 mg conc.		5 mg cocnc.		10 mg conc.		20 mg conc.	
	Crude Tuttha	Shodhita Tuttha	Crude Tuttha	Shodhita Tuttha	Crude Tuttha	Shodhita Tuttha	Crude Tuttha	Shodhita Tuttha
E.coli	-	9 mm	15 mm	19 mm	20 mm	25 mm	24 mm	26 mm
B. subtilis	-	5 mm	29 mm	28 mm	29 mm	30 mm	28 mm	30 mm
S.typhi	6 mm	7 mm	15 mm	14 mm	19 mm	18 mm	24 mm	24 mm
S.aureus	-	5 mm	19 mm	16 mm	24 mm	22 mm	28 mm	24 mm
C.albicans	-	12 mm	16 mm	14 mm	19 mm	20 mm	24 mm	26 mm
T. rubrum	-	10 mm	19 mm	12 mm	21 mm	20 mm	24 mm	20 mm

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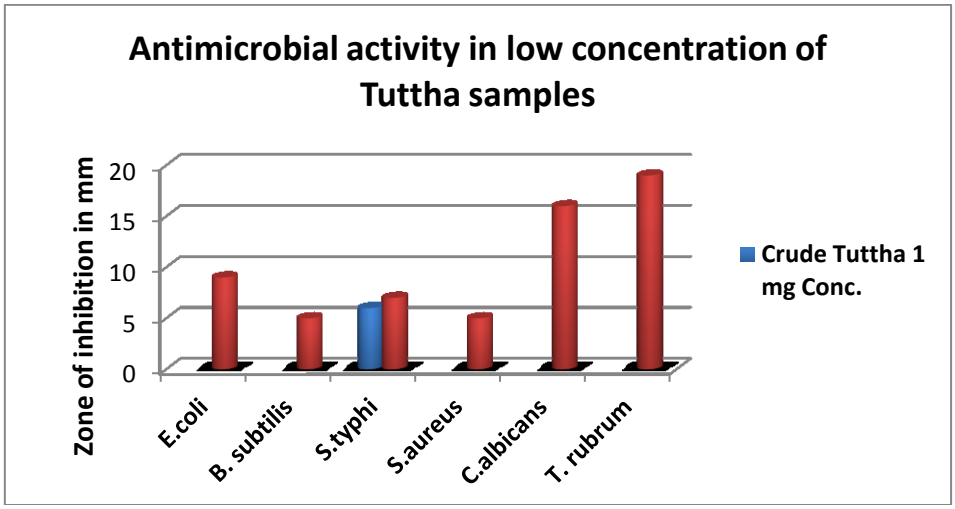
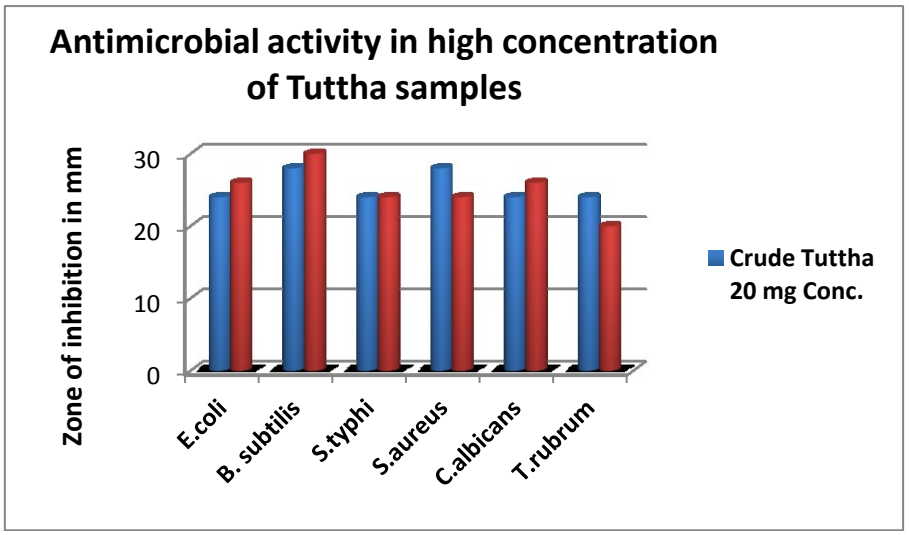
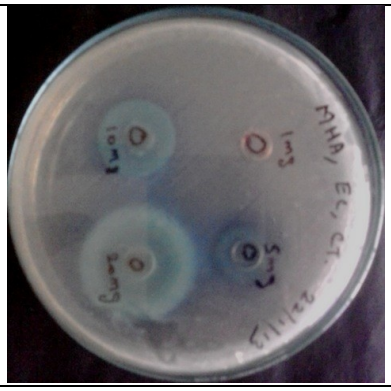
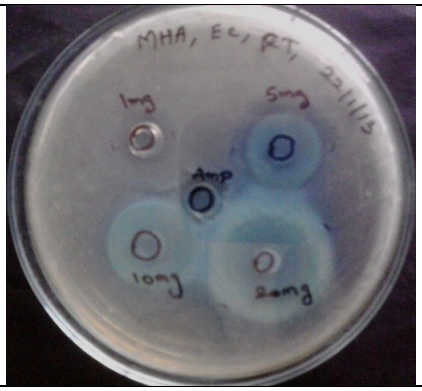
Graph No.1. Shows zone of inhibition (in mm) of Tuttha samples in Low concentration



Graph No.2. Shows zone of inhibition (in mm) of Tuttha samples in High concentration



Images from No. 1 to 12

ANTIBACTERIAL PLATES	
	
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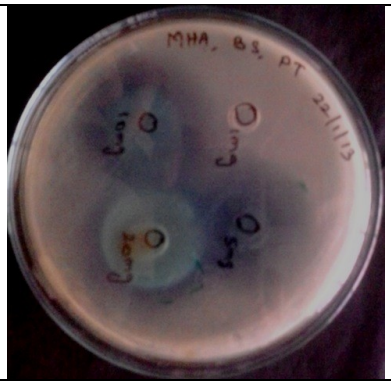	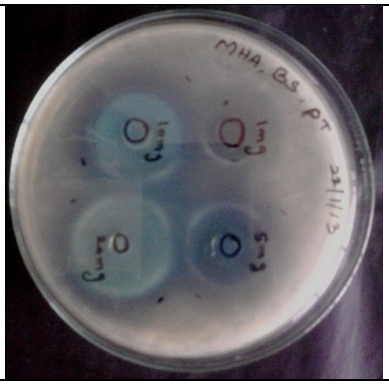
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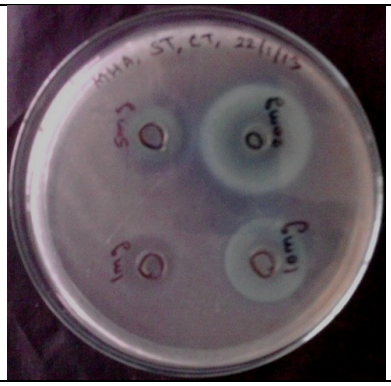
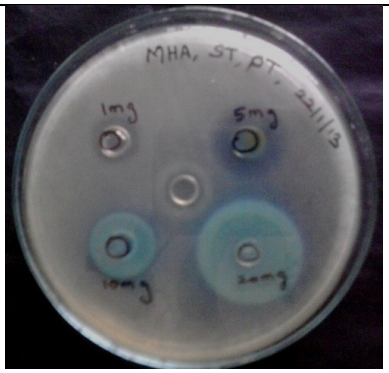
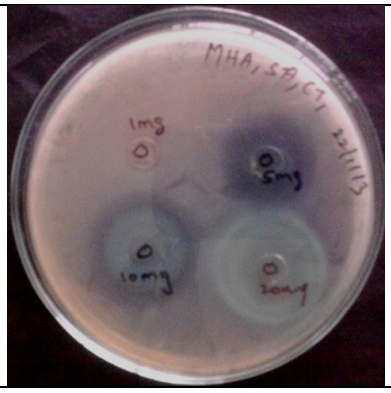
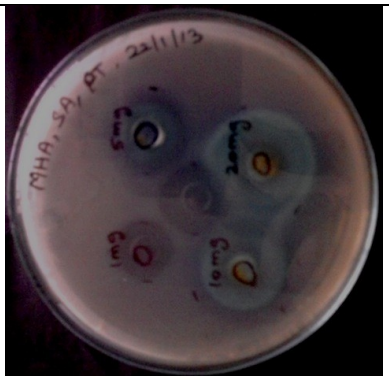
	
Salmonella typhi	
	
Staphylococcus aureus	

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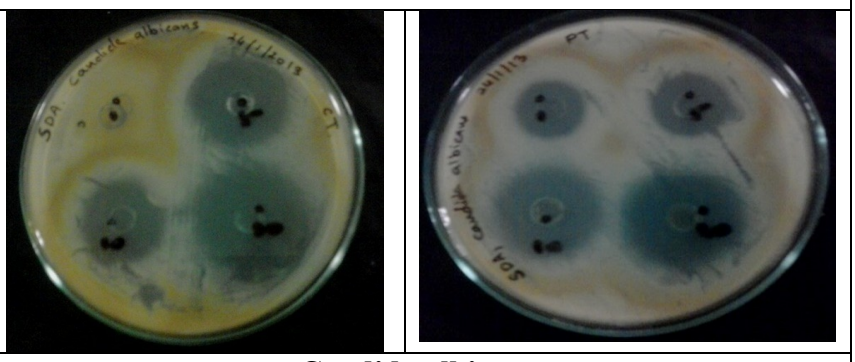
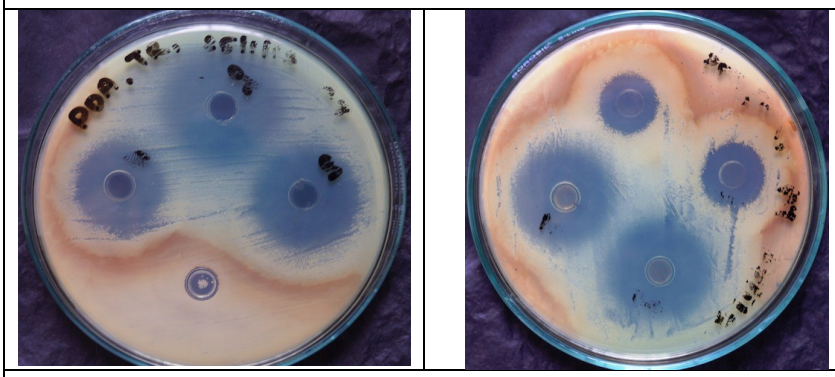
ANTIFUNGAL PLATES



Candida albicans



Trycophyton rubrum

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