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Evaluation of Antibacterial Activity of *Tuttha Drava*

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Abstract

Introduction: Antimicrobial agents are commonly used nowadays for treating various bacterial and fungal infections. But still most of the physicians are unable to treat these infections appropriately due to hindrances like development of resistance, adverse effects, patient affordability etc. *Tuttha Drava* (Copper sulphate solution) formulation mentioned in Ayurvedic Texts can be an ideal medicine for treating various infectious diseases. Assessment of its antibacterial activity may provide scientific evidence for the study. *Method: Tuttha Drava* prepared with classical reference was subjected for analytical study and in vitro antibacterial activity by MIC (Minimum inhibitory concentration) method. The formulation was tested against bacteria like Gram + ve Staphylococcus aureus, Streptococcus pyogens, and Gram –ve Pseudomonas auregenosa and Fluconazole as standard for comparison. *Results: Tuttha Drava* showed a significant zone of inhibition against two strains of bacteria namely Staphylococcus aureus, Streptococcus pyogens. As *Tuttha* contains Fe (4.39%), Cu (15.74%), S (4.36%) it may contribute to promote positive health and vigour by increasing the immunity, thus making the body resistant against disease causing factors. *Conclusion: Tuttha Drava* showed a significant antibacterial activity ie *Tuttha Drava* shows good Minimum inhibitory concentration (MIC) affect on Streptococcus pyogens, Pseudomonas auregenosa.

Keywords: *Tuttha Drava*; Antibacterial Activity.

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. *Tuttha* (Copper Sulphate) is one among *Maharasas.* It is a compound of copper and sulphur. *Tuttha*- Copper Sulphate [$CuSO_4 7H_2O$] is the artificially prepared and presently available form of *Sasyaka* (Copper Sulphate). *Tuttha* is also considered as the *upadhatu* of *Tamra* (Copper) and have the properties of *Tamra* and *Sasyaka* both. The *Tuttha* is also called by *Tutathaka, Sikhigreeva, Hemarasa* and *Mayoorakam* in Ayurveda. *Tuttha* is a chemical compound obtained though processing the Sulphuric acid over the copper. It reflects the color similar to the neck of a peacock and is heavy in weight. *Tuttha* is capable to penetrate into the body and to remove ringworms [1]. *Tuttha* is easy to digest and indicated in many diseases like *kushta* (skin disease), *putigadhita vrana* (wound), *phirangaja vrana* (chancre), *upadanshaja vrana (*wound), *netravartma* (diseases of eyelids) *and krimi* (worm infestation) etc. It is used in different forms like *drava (solution)*, vati (tablet), malahara (ointment), bhasma (calx) etc. Rasatarangini explained the external use of *Tuttha* in the form of *Tuttha Drava.* It is indicated externally for *dhawana* (douching) purpose in *kushta, krimi roga* and in various kinds of *vrana* due to its *krimigna* (de-worming) and *kushtagna* properties [2]. Based on these factors the present study was under taken to evaluate the antibacterial effect of *Tuttha Drava*, assessment of its antibacterial activity may provide scientific evidence for the study.

Antibacterial study: The antibacterial activity of a drug is generally expressed as its inhibiting affects toward the growth of the bacterium in nutrient broth or nutrient agar. For this study, the following conditions are required.

1. The substance or test drug must be in contact with the test organisms.

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2. Conditions must be favorable for the growth of microorganisms in the absence of antimicrobial substances.
3. There must be a means of estimating the amount of growth and thereby percentage of growth of inhibition.
4. The activity of test drug should be observed and determined by the growth response of microorganisms [3].

Aims and Objectives

1. *Tuttha Shodhana* and preparation of *Tuttha Drava* as per classical reference
2. Physical and chemical analysis of *Tuttha Drava.*
3. Anti-bacterial activity of *Tuttha Drava*.

Materials and Methods

A three part methodology was used

1. Pharmaceutical part: Preparation of *Tuttha Drava*
2. Analytical Part: Subjecting *Tuttha Drava* to Physico-chemical analysis, AAS & X-RD study.
3. Experimental part: Subjecting *Tuttha Drava* to Anti-bacterial activity study.

1. Pharmaceutical study:

Materials (Ingredients) Required:

Raw material *Tuttha* for the preparation of *Tuttha Drava* was procured from Dorle and Suns professional supplier in Kolhapur and was authenticated by HOD Rasashastra department of Shri J.G.C.H.S Ayurvedic Medical College, Ghataprabha. Karnataka. Gomutra (cow's urine) was collected from local goshala(cow ranch).

Instruments (yantra): *Tula yantra* (weighing machine), *kalva yantra* (mortar and pistil) and *dolayantra* (vessel). Equipments: Steel vessels, measuring cylinder, knife, starrer, glass vessel, filter paper.

Preparation of *Tuttha Drava:* Shodhana (purification) of *Tuttha* [4]: *Tuttha* was taken in *khalva yantra* and powdered it. After that *Tuttha powder* (550 gm) was collected in the three layered cloth and made *potali*. Collected *gomutra* was filtered in steel vessel by filter paper. Then *gomutra* was added in *dolayantra* (8 lit required) and *potali* was dipped in gomutra 3 fingers above from bottom of vessel. *Dolayantra* was kept on LPG gas stove and mandagni (mild heat) was given to boil *gomutra* for 9 hrs (Three *yama*). When the level of *gomutra* decreased, again extra 500 ml of *gomutra* was added to it. After completion of process, heating was stopped and left for self cooling and allowed to settle down for re- crystallization for 24 hours under room temperature. The crystals of *Tuttha* were formed at bottom of the *dolayantra* (vessel) and supernant liquid was removed. *Tuttha* crystal were collected and allowed to dry in shade.

***Tuttha Drava Nirmana* (Preparation of *Tuttha Drava*) [5]:** 500 mg *gomutra shodhita Tuttha* was taken in a glass vessel and in that added 50 ml distilled water and starred till complete dissolution of *Tuttha* takes place. Then 50 ml of Brown coloured *Tuttha Drava* was obtained.

Pharmaceutical Observations and Results are given in Table 1 and 2.

Table 1: Observations during *Tuttha Shodhana*

***Shodhana* method**	**Quantity of *Tuttha***	***Dravya* (drugs) used for *shodhana***		***Shodhita Tuttha***		**Loss during shodhana**	**Time required**
		Dravya	**Quantity**	**Weight**	**Colour**		
Gomutra swedan	550 gms	Gomutra	8 liters	510 gms	Blackish brown	40 gms	3 *yama* (9 hours)

Table 2: Observations during preparation of *Tuttha Drava*

Tuttha Drava	***Dravya* (drugs) *used***		**Colour of *Tuttha Drava***	**Time required**
	Dravya	**Quantity of water**		
Prepared by *Gomutra shodhita Tuttha*	*Gomutra shodhita Tuttha* - 4 *ratti* (500 mg)	5 *phala* (50 ml)	Brown colour	5 min

2. Analytical study [6]:

Tuttha Drava was evaluated for physico-chemical properties by using standard methods like organoleptic characteristics, Ph value, Ash value, loss on drying. Qualitative and quantitative analysis of inorganic elements by AAS study, X-RD (X-ray diffraction) method.

Observations and Results

Physico-chemical analysis, qualitative and quantitative analysis were carried out, observations and Results are given in Table 3 and 4.

Table 3: Showing the Results of Analytical Study of the ***Tuttha Drava***

Physical test	
Colour	Brown
Odour	*Gomutra* smell (cows urine smell)
Touch	Smooth
Chemical test	
pH	5.47
Total ash value	44.11 %
Acid insoluble ash	2.55 %
Water insoluble ash	21. 96 %
Loss on drying	5.89 %
Assay of element	
Cu	15.74 %
Fe	4.39 %
S	4.36 %

Percentage of structure of Tuttha samples by X-RD:

X-RD Study of *shodhita Tuttha Drava* sample has been carried out at Shivaji university kolhapur. In this study sample was used in powder form. In X-RD reports D- values obtained with Theta angle, Intensity and its Graphs. Then this graph compared with Standard JCPDS data. They are represented as below.

Table 4: Observation of crystalline and amorphous structure of ***Tuttha Drava***

Sample name	Crystalline	Amorphous
Tuttha Drava	75.7 %	24.3 %

Anti bacterial Study of Tuttha Drava [7]:

Antimicrobial activities of any therapeutic agent are understood by its degree of growth inhibition of microorganisms as well as bacterial property. Usually different microbial species and strains have different degrees of susceptibility to therapeutic agents. The susceptibility of microorganisms can change with time even during therapy with a specific drug. Thus, it is essential for the physician to know the sensitivity of the pathogen before treatment.

Our ancient Acharyas were aware of the existence of microorganisms or bacteria as well as causation of disease since Vedic period. There are many references pertaining to Jivanuvada (bacteriology) in ancient literature such as Rigveda, Atharvaveda and Mahabharata etc which indicates familiarity of the subject in those days.

Antibacterial activity of *Tuttha Drava* was studied to determine Minimum inhibitory concentrations (MIC) against three selected bacteria's, namely Staphylococcus aureus (Gram + ve), Streptococcus pyogens (Gram +ve) and Pseudomonas aerugenosa (Gram –ve). MIC is the lowest concentration of the drug that inhabits growth of bacteria.

Antibacterial study observations and Results are given in Table 5

Table 5: MIC of ***Tuttha Drava***

Bacterial culture	Gram stain	M.I.C
Pseudomonas aerugenosa	Gram – ve	10 mg/ml
Staphylococcus aureus	Gram + ve	10 mg/ml
Streptococcus pyogens	Gram + ve	Not seen

Discussion

The selected *Tutha* (copper Sulphate) was first subjected to *shodhana* by gomutra using *dolayantra* to remove impurities and to increase the potency. To prepare *Tuttha Drava, shodhita Tuttha* was mixed with water to get brown coloured Tuttha Drava.

Tuttha Drava was subjected to physico-chemical study; it has gomutra like odour, smooth to touch and appears brown coloured liquid form. Quantitative estimation of inorganic elements was carried out by AAS method. *Tuttha Drava* contains Fe 4.39%, Cu 15.74 % and S 4.36%. The pH value is 5.47, Total Ash value is 44.11% and loss on drying is 5.89%.

Antibacterial activity of *Tuttha Drava* was carried out by MIC method using bacteria's namely Staphylococcus aureus (Gram +ve), Streptococcus pyogens (Gram +ve) and Pseudomonas aerugenosa (Gram –ve) and Fluconazole as standard for comparison It shows good Minimum inhibitory concentration (MIC) effect on Streptococcus pyogens, Pseudomonas auregenosa.

Conclusion

Pharmaceutical study revealed that preparation of *Tuttha Drava* is a very easy procedure. Physico-chemical analysis, AAS and X-ray diffraction study of *Tuttha Drava* helps in its standardisation. Antibacterial study revealed that *Tuttha Drava* shows good minimum inhibitory concentration (MIC) effect on Pseudomonas aeruginosa, Staphylococcus aures, while less Minimum inhibitory concentration (MIC) effect on streptococcus pyogens.

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