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EVALUATION OF ANTI-BACTERIAL PROSPECTIVE OF MRITYUNJAYA RASA ON SALMONELLA TYPHI WITH DIFFERENT ANUPANA

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ABSTRACT

Introduction: *Vishama Jwara*, characterised by persisting fever with various intensities, diarrhoea and body ache, can be correlated with Typhoid fever where causative organism is mentioned as *Salmonella typhi*. Target towards specific micro-organisms can be achieved by administering the drug with selective *Anupana*. In *Jwara* it is mentioned to administer *Mrityunjaya Rasa* along with various *Anupana* like *Madhu, Aardraka Swarasa*, and *Jeeraka Kashaya* with *Guda*. Though *Mrityunjaya Rasa* along with *Anupana Jeeraka Kashaya* and *Guda* is indicated in *Vishama Jwara*, extent of susceptibility of *Salmonella typhi* may change with selective *Anupana*. **Aim:** The aim of the study was to evaluate the Anti-bacterial action of *Mrityunjaya Rasa* on *Salmonella typhi* (M_1), *Mrityunjaya Rasa* with *Madhu anupana* (M_2), *Mrityunjaya Rasa* with *Aardraka Swarasa anupana* (M_3), *Mrityunjaya Rasa* with *Jeeraka kashaya* & *Guda anupana* (M_4). **Materials and Methods:** The study was carried out at S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi by opting well diffusion method on *Salmonella typhi*. **Results:** The results showed that Sample M_1 (test) *Mrityunjaya Rasa* and sample M_3 (test) *Mrityunjaya Rasa* with *Aardraka Swarasa Anupana* exhibited nil activity against bacteria *Salmonella typhi.* Sample M_2 (test) *Mrityunjaya Rasa* with *Madhu anupana* exhibited activity in three concentrations while sample M_4 (test) *Mrityunjaya Rasa* with *Jeeraka Kashaya* and *Guda anupana* exhibited activity in higher concentration only. **Conclusion:** *Mrityunjaya Rasa* with *Madhu* as *Anupana* possesses *Jwaraghna* and *Krimighna* action, if *Salmonella typhi* is

taken into consideration. So, by comparing with other *Anupana*, it can be concluded that *Madhu* is proved better *Anupana* to be administered with Drug *Mrityunjaya Rasa* as it exhibited activity in lower concentration too.

Keywords: *Mrityunjaya Rasa*, *Madhu Anupana, Salmonella typhi, Vishama jwara.*

INTRODUCTION

One can get an elaborative description of the drugs and formulations which are indicated in *Jwara Roga*. There are diversities of *Rasaaushadhi* which are mentioned in *Ayurvedic* classics for treatment of *Jwara Roga*. *Mrityunjaya Rasa* is one among those medicines which is a Herbo–Mineral formulation mentioned under *Jwarachikitsa in Rasendra Sara Sangraha*[1] along with different *Anupana*. *Shudha Hingula, Shudha Gandhaka, Shudha Vatsanabha, Shudha Tankana, Pippali and Maricha* as ingredients with properties of *Tikta, Katu Rasa Teekshna Guna and Deepana-Pachana, Swedajanana, Yogavahi and Jwaraghna* action, show significant result on various types of fever. *Vishama Jwara* characterised by persisting fever with various intensities, diarrhoea and body ache, can be correlated with Typhoid fever where causative organism is mentioned as *Salmonella typhi. In Rasendra Sara Sangraha* under *Jwara chikitsa* it is mentioned to administer *Mrityunjaya rasa* along with various *Anupana like Madhu, Aardraka swarasa and Jeeraka kashaya with Guda.* In *Vishama jwara*, *Mrityunjaya rasa* is indicated along with *Anupana* of *Jeeraka kashaya with Guda*. *Anupana* acts by synergistic action in which concomitant use of two or more drugs results in a total effect greater than the sum of their independent actions[2]. Though *Mrityunjaya rasa* is indicated in *Vishama Jwara* with *Anupana Jeeraka kashaya* and *Guda*, extent of susceptibility of *Salmonella typhi* may change with other selective *Anupana*. Target towards specific microorganisms can be achieved by administering the drug with selective *Anupana*. Thus, to understand and interpret the Anti-bacterial action of *Mrityunjaya Rasa* along with different *Anupana* against *Salmonella typhi*, the study was carried out.

Objectives: To Evaluate and compare the Anti-bacterial action of *Mrityunjaya Rasa* along with different *Anupana* on *Salmonella typhi*.

Materials and Methods

Sample Preparation: Preparation of *Mrityunjaya Rasa* with all samples *Mrityunjaya Rasa* (M_1), *Mrityunjaya Rasa* with *Madhu* (M_2), *Mrityunjaya Rasa* with *Aardraka Swarasa* (M_3), *Mrityunjaya Rasa* with *Jeeraka kashaya* & *Guda* (M_4) was carried out at Practical hall, Department of *Rasashastra* and *Bhaishajya Kalpana*, Shri Dharmasthala Manjunatheshwara College of Ayurveda, Kuthpady, Udupi, Karnataka.

Source of Data: The study was carried out at S.D.M. Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka.

Pathogen selected for the study: The references of micro-organisms are available in *Ayurvedic* classics in the names of *Krimi, Bhoota, Jantu* etc, which affect the human beings and cause serious disease. While describing *Vishama Jwara Aacharya Charaka*[3] has accepted this theory. *Aacharya Sushruta*[4] has also accepted the involvement of *Agantuja Nidana* for the cause of *Vishama Jwara*.

Vishama Jwara, type of *Sannipataja Jwara* characterised by persisting fever with various intensities, diarrhoea and body ache, can be correlated with Typhoid fever where causative organism is mentioned as *Salmonella typhi*[5], so bacteria *Salmonella typhi* was selected for the study.

Materials Required: Test strains, Distilled water, Test tube, Incubator, and Laminar air flow, Hot air oven and graduated micropipettes- 20, 50, 100µl sterile tips, Growth medium: Nutrient agar, Samples of M_1, M_2, M_3, M_4, Samples of *Anupana*: Madhu, *Ardraka swarasa, Jeeraka kashaya* and *Guda.*

Method for Anti-bacterial Study: The whole procedure can be sub-divided in following steps- Preparation of different dilutions of samples M_1, M_2, M_3, M_4, Preparation of Nutrient Medium., Preparation of Inoc-

ulum. Well diffusion method for testing the antibacterial action and reading of Zone of Inhibition (ZOI).

Preparation of different concentrations for sample M_1, M_2, M_3, M_4

0.066mg of *Mrityunjaya rasa* was taken and mixed to a volume of 1ml with distilled water and shaken well. With help of micro-pipette, 40µl of this suspension was taken for antibacterial study. Here drug stands 0.00264mg/40µl. Similarly, 2mg, 62.5mg, and 125mg concentrations of *Mrityunjaya rasa* were prepared with distilled water. In 2mg concentration, drug stands 0.08 mg/40µl. In 62.5mg concentration, drug stands 2.5mg/40 µl and in 125mg concentration, drug stands 5mg/40 µl. With help of micro-pipette, 40µl of these suspensions was taken for Antibacterial study. Ampicillin as positive control was taken in 1mg amount and made to a volume of 1ml distilled water and shaken well. With help of micro-pipette, 40µl of this solution was taken. For Negative control distilled water was taken. Same methodology was opted for sample M_2, M_3, M_4. Instead of distilled water in case of sample M_2 *Madhu Anupana*, with sample M_3 *Aardraka Swarasa Anupana* with sample M_4 *Jeeraka Kashaya* & *Guda Anupana* were taken for the study.

Preparation of Nutrient Medium

a. **Preparation of Nutrient agar media:** Beef extract (1g), yeast extract (2g), peptone (5g) and Sodium Chloride (5g) were dissolved in 990ml of distilled water. The pH was adjusted to 7.2 and the volume was made up to 1000ml. Finally, 15g agar was added to the media and autoclaved at 121ºC for 20 minutes.
b. **Sterilization:** Sterilization of Nutrient Agar, spatula, pipettes and flasks was done by autoclaving at 121˚C for 20 minutes.

Preparation of the inoculum

Bacterial cultures: Workplace was cleaned in laminar air flow using 70% ethyl alcohol and exposed to UV for 20 minutes. The bacterial culture was revived in Nutrient Agar media at 37ºC for at 24 hours and then stored at 4ºC

Well diffusion method[6]: The work was done in aseptic conditions in laminar air flow unit. 10ml of broth was taken and inoculated with one loop of desired organism and mixed well. 15ml of the medium was poured uniformly over the sterile petri dish, inoculated with the broth, mixed, and allowed to solidify. Seven wells were bored in the agar medium. The samples were loaded into the wells. Sterile distilled water and standard drug were loaded as controls. In six wells 40µl of mg/ml volume of 0.066mg, 2mg, 62.5mg, and 125mg/ml concentrations of sample M_1 was filled and one well was filled with 40µl of mg/ml volume of Ampicillin (positive control). In similar way this methodology was also opted for sample M_2, M_3 and M_4. All the petri dishes were incubated at 37˚C for 24 hours. The test was performed in duplicate and the presence of zone of inhibition in petri dish was observed on next day. The average value was considered as the result.

RESULTS AND DISCUSSION:

In the present study it was observed that zones of inhibition were obtained for two test samples (M_2 test) *Mritunjaya rasa* with *Madhu Anupana* and (M_4 test) *Mritunjaya rasa* with *Jeeraka Kashaya* and *Guda anupana*. But no zone of inhibition was obtained for sample (M_1 test) *Mritunjaya rasa* and (M_3 test) *Mritunjaya rasa* with *Aardraka Swarasa Anupana*. Maximum zone of inhibition was observed by positive control drug Ampicilline. Sample (M_2 test) *Mritunjaya rasa* with *Madhu Anupana* exhibited activity in three concentrations while sample (M_4 test) *Mritunjaya rasa* with *Jeeraka Kashaya* and *Guda* exhibited activity in higher concentration only but not more than compared to standard drug. The results are shown in table no 01.

Mode of action of sample (M_2 test) *Mrityunjaya rasa* with *Madhu Anupana*

It was observed that antibacterial activity of the test drug (M_2) *Mrityunjaya rasa with Madhu Anupana* showed results in concentration of 2, 62.5, and 125 mg/ml. Antibacterial activity of *Mrityunjaya rasa* with *Madhu Anupana* may be attributed by *Yogavahi* properties of *Madhu*. Definition of *Yogavahi* is well explained in *Chakrapani Teeka* in *Jwara Chikitsa* in *Charaka Samhita*[7]. It means that when one drug is administered in combination with another drug it acts similarly to that drug and increases the potency of the

drug. *Ayurveda Prakasha* also explained definition of *Yogavahi* properties[8] in context of *Parada Sevana Apthaya.* In *Vishama Jwara* involvement of *Kapha pradhana Tridoshja* vitiation, is corrected by administering *Madhu* as *Anupana* along with *Mrityunjaya rasa* by its properties of *Kashaya, Madhura rasa, Chedana karma,* and *Ruksha Guna*. In *Yoga ratnakara madhu* is indicated in *Jwara,* and *Krimi Roga*[9]. By these properties it shows activity against bacteria *Salmonella typhi* along with *Mrityunjaya rasa*. According to *Yogaratnakara* if a drug is administered in a disease along with *Tattada Rogaghna Anupana* it cures the disease by the strength of that particular *Anupana*. According to modern science the role of Honey is attributed to its antibacterial property with regards to its high osmolarity and content of hydrogen peroxide (H_2O_2) and phytochemical components like Methylglyoxal (MGO). The antibacterial agent in honey is predominantly hydrogen peroxide, of which the concentration is determined by relative levels of glucose oxidase. Most types of honey generate H_2O_2 because of the activation of the enzyme glucose oxidase that oxidizes glucose to Gluconic acid and H_2O_2, which thus attributes the antibacterial activity. Honey is characteristically acidic with pH between 3.2 and 4.5, which is low enough to be inhibitory to several bacterial pathogens. The antibacterial property of honey is also derived from the osmotic effect of its high sugar content and low moisture content, along with its acidic properties of Gluconic acid and the antiseptic properties of its H_2O_2[10].

Mrityunjaya rasa is a herbo-mineral formulation mentioned under *Jwara chikitsa* along with different *Anupanas*. It is having ingredients like *Shudha Hingula, Shudha Gandhaka, Shudha Vatsanabha, Shudha Tankana, Pippali, and Maricha* which show significant result on various types of fever. *Hingula and Vatsanabha* are having *Jwara shamana* properties. *Gandhaka, Pippali, Maricha,* and *Tankana are having Katu rasa, Ushna Veerya and Deepana–Pachana action.* They all have proved antibacterial action. Piperine is a proven bio enhancer. It can enhance the bioavailability of companion drugs.

Here *Anupana* acts by synergistic action in which drugs having similar properties, show better results. *Mrityunjaya Rasa* is a compound formulation and its antibacterial action along with *Madhu Anupana* against bacteria *Salmonella typhi* is due to synergistic and additive action.

CONCLUSION

Target oriented therapeutic action can be achieved by administering the drug with selective *Anupana*. It acts by synergistic action that means, when concomitant use of two or more drugs results in a total effect greater than the sum of their independent actions. *Mrityunjaya rasa* contributes better results when administered with suitable *Anupana* which are also withholding *Jwaraghna action*. It is drug to drug interaction which is valued here in the present study. *Mrityunjaya rasa* with *Jeeraka kashaya and Guda anupana* shows maximum zone of inhibition only in higher concentration against bacteria *Salmonella typhi*. *Mrityunjaya rasa* with *Madhu Anupana* shows maximum number of susceptibilities in three concentrations lower to higher because *Madhu* increases the action of *Mrityunjaya rasa* by its *Yogawahitwa* and *Krimighna* property. Hence it can be concluded that *Madhu* is proved better *Anupana* to be administered with *Mrityunjaya Rasa* in *Jwara Roga*. The study comforts in the verification of the principles of *Ayurveda* on the recent scientific basis.

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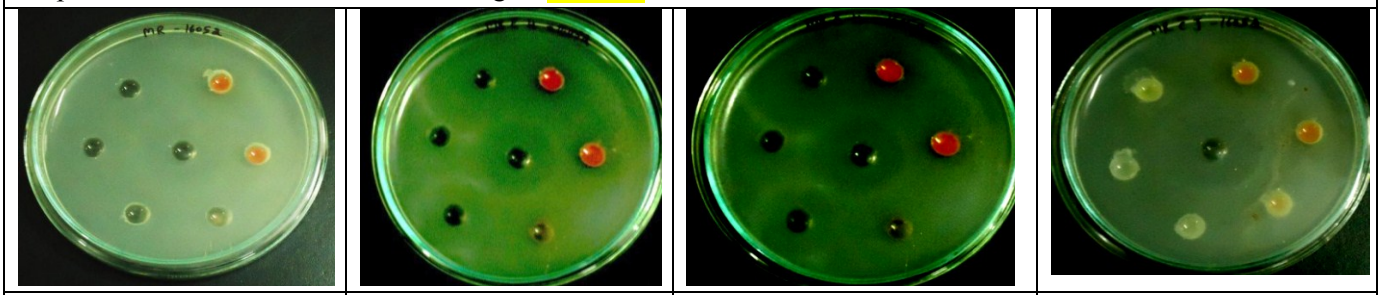
Table 1: Comparison of ZOI of test samples

Conc. (40µl of mg/ml)	Zone of inhibition (radius mm) of test samples			
	M_1	M_2	M_3	M_4
125	0	09	0	10
62.5	0	07	0	0
2	0	05	0	0
0.066	0	0	0	0

Figure 1: Pictures of Anti-Bacterial Study

Preparation of Nutrient Media & Loading of Incolum



Action of M_1 Test	**Action of M_2 Test**	**Action of M_3 Test**	**Action of M_4 Test**

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