



Research Article

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EFFICACY OF HERBO-MINERAL PREPARATIONS ON MULTIDRUG RESISTANCE BACTERIA (MDRB) ISOLATES FROM HUMANS

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ABSTRACT

Study includes the bacteria isolates *Pseudomonas* spp. and *Staphylococci* spp which showed resistance against the maximum number of antibiotics were selected for antibacterial assay against herbo-mineral drugs. Different concentrations of gum acacia suspension of *Rasa sindhoora*, *Vyadhihara rasayana*, *Rasa manikya* and *Gandhaka rasayana* were treated with above mentioned multidrug resistant bacteria isolates by cup diffusion method. Different concentrations of gum acacia suspension of *Rasa sindhoora*, *Vyadhihara rasayana*, *Rasa manikya* and *Gandhaka rasayana* were treated with Multidrug resistant *Pseudomonas aeruginosa*. and *Staphylococcus aureus*. To see the antimicrobial efficacy against MDRB. It was observed that the above mentioned herbo-mineral drugs were showing highly significant susceptibility. The present study concludes that these rasa preparations can be used against multidrug resistant bacteria causing infectious diseases. Antimicrobial activity of herbomineral drugs has been tested against both multidrug resistance Gram positive and Gram negative bacteria. The Minimum Inhibitory Concentration of drug against each organism was determined by agar well diffusion method. The drug showed antibacterial activity. Elevated multidrug resistance has led to renewed interest in herbal medicine. Most important aspect of herbo-mineral drugs that are combination of medicinal plants and essential minerals used in treating infectious bacterial diseases.

Key words: *Rasa sindhoora*, *Vyadhihara rasayana*, *Rasa manikya* *Gandhakarasyana*

INTRODUCTION

Ayurveda is a science that deals not only with treatment of some diseases but is a complete way of life¹. Ayurveda system of medicine are slow acting, but the Herbomineral preparations known as Bhasma are the wonder drugs. This is the real fact that these drugs have a fast pharmacological action in target site. Herbo-mineral preparations are safe in therapeutic doses and absorbs easily in the body. Bhasmas and Sindoor⁸, the unique Ayurvedic preparation for curing diseases, can easily enter into the blood stream and became more biocompatible as compare to conventional medicines. They have developed a new era in nano-medicine system due to its nano particles size and holistic approach towards disease in curing the infectious diseases caused by resistant bacteria. In India and other countries in South Asia, Manikya Rasa (MR) are such *Rasashastra* herbo-minerallic drugs were commonly used. The efficacy and the mode of action of mineral based preparations are uncertain due to the insufficient antimicrobial studies. Even after having well known traditional use of skin diseases, reported antimicrobial and mineralogical studies are few in numbers². Therefore, in this study antimicrobial activities of the drugs and their antibacterial fractions were evaluated against *Pseudomonas aeruginosa*. and *Staphylococcus aureus*,

MATERIALS AND METHODS

Collection of sample

The samples were collected using pre-sterilized sample bottles. Precautionary measures were taken to minimize the contamination. The hospital samples like urine and pus collected from person suffering from infectious diseases³.

IEC number: SDM/IEC/86/2014-2015

Source of Drugs: Rasamanikya, Rasasindhoora, Vyadhihara rasayana and Gandhaka rasayana was procured from SDM Ayurveda pharmacy (GMP-ISO 9001:2008 Certified) Lakshminarayana Nagar, Kuthpady Post. 574118. Udupi. Karnataka.

Isolation of bacteria

5ml of urine sample is taken in a clean sterilized centrifuge tubes and centrifuge the sample at 2500 RPM at 2-3 minutes. Collect the deposit for the microscopic observation. One loop full of deposit in a inoculation loop to make a primary well on MccConkey culture plate, from the primary well secondary and tertiary streaking were done on the plate incubate in an incubator at 37°C for 24 hours. After incubation plates were observed for positive growth. Colonies were picked and sub-cultured to obtain pure culture. Stock cultures were maintained on Nutrient agar at 4°C. Positive culture processed in a usual manner for identification³.

Screening of multidrug resistant bacteria

0.5 McFarland standards turbidity inoculums were lawn cultured on a sterilized plates containing 10 to 15 ml of solidified culture media by Kirby-Bauer disc diffusion method. With help of sterilized forceps Place the appropriate antimicrobial-impregnated disks on the surface of the culture media. Each organism is tested with 10 to 11 antibiotics to screen the multidrug resistance pattern of each organism. Plates were incubated in an incubator at 37°C 24 hours. After incubation, the diameter of the zone of inhibition around the disc is measured with ruler compare with HIMEDIA antibiotic chart and the bacteria showing resistance to more than 5-6 antibiotics were taken for the study. Multidrug resistant strains like *Staphylococci* spp. And *Pseudomonas* spp were selected for further experiment⁹

Characterization of bacteria

Multidrug resistant *Staphylococci* spp and *Pseudomonas* spp are isolated from Pus and urine. Cultured on MecConkey and Blood agar cultural characteristics studied, microscopic observation did by gram's staining technique confirms the organisms is gram's +ve are gram's -ve and IMVIC reactions, catalase, and string test confirms the bacterial isolates biochemically and serologically⁹.

Source of Antibiotics: Antibiotics used were Amikacin (30µg), Azithromycin(15µg), Amoxicillin (10µg), Cefoxitin (30mcg), Ceftazidime (30µg), Cefepime / Tazobactam (30mcg) Ceftriaxone (30mcg) Cefuroxime (30mcg), Colistin (10µg), Ciprofloxacin (5µg), Co-Trimoxazole (25µg), Gentamicin (10µg), Imipenem (10µg), Methicillin (5µg), Meropenem (10µg), Ofloxacin (5mcg), Polymyxin-B (300units)⁴

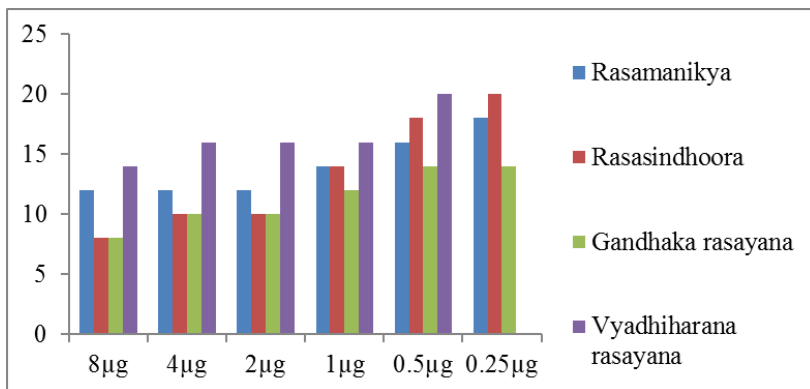
Antibacterial Assay of Herbo-mineral Preparation Cup diffusion Method

40mg of each Rasasindoora, Rasamanikya⁵, Gandhakarasyana⁶ and Vyadhihara rasayana⁷ and 25mg of gum acacia were weighed and dissolved in 5ml of distilled water allowed to dissolve completely to make stock solution carrying 8µg/ml of drug concentration. With the help of distilled water prepared different drug concentrations- 4mg/ml, 2µg/ml, 1µg/ml, 0.5µg/ml, 0.25µg/ml,

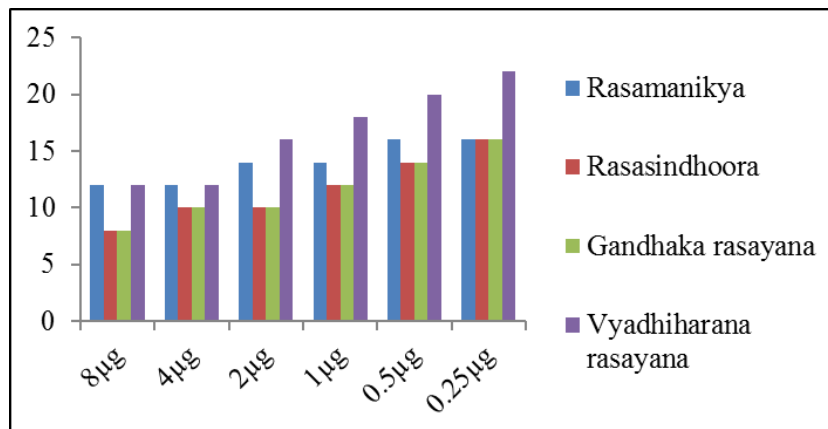
The different concentrations of Herbomineral drugs were subjected to Antimicrobial sensitivity test. By well diffusion method, Muller Hinton Agar plate was swabbed with standard McFarland inoculums Replace the lid of the dish leave it for 5 minutes. Make 8 equidistant wells on the plates with the help of sterile cork borer. Add 100 µl of control (Gum acacia) standard (Ampicillin 10µg) and drug extracts of different concentration (8µg, 4µg, 2µg, 1µg, 0.5µg, 0.25µg) onto the labeled wells. Incubate all the plates at 37 °C for 24 hours. After incubation period, the zone of inhibition was measured with a ruler millimeters⁹.

RESULTS

Different concentrations of gum acacia suspension of *Rasa sindhoora*, *Vyadhihara rasayana*, *Rasa manikya* and *Gandhaka rasayana* were treated with Multidrug resistant *Pseudomonas aeruginosa*. and *Staphylococcus aureus*. To see the antimicrobial efficacy against MDRB. The results obtained were tabulated below.



Graph: 1 antimicrobial assay of Pseudomonas spp.



Graph: 2 Antimicrobial activity of Staphylococci spp

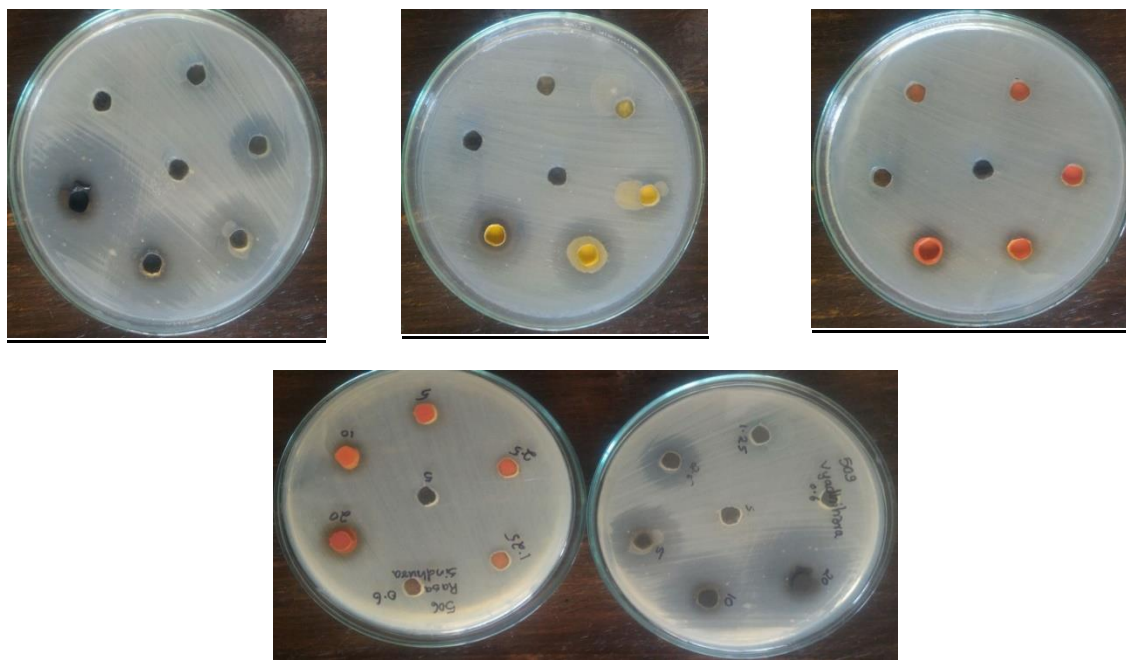


Figure: 1 Antimicrobial activity of Herbomineral drugs of MDRB

DISCUSSION

Antimicrobial activity of herbomineral drugs has been tested against both multidrug resistance Gram positive and Gram negative bacteria. The Minimum Inhibitory Concentration of drug against each organism was determined by agar well diffusion method. The drug showed antibacterial activity. The MIC of herbomineral drugs was determined against multi drug resistance *Pseudomonas* spp. and *Staphylococcus* spp. (Graph 1,2 and 3) The study of antimicrobial susceptibility test using herbomineral drugs exhibited strong effectiveness against the selected microbial strains. Present study showed the effectiveness against multidrug resistance *Pseudomonas* spp. and *Staphylococcus* spp. (Figure 1)

***Pseudomonas* spp:** Multidrug resistance *Pseudomonas* spp is a Gram negative bacterium. Rasasindhoora showed maximum zone of inhibition against *Pseudomonas*.spp i.e., 20mm at the concentration of 0.25µg. Rasamanikya showed maximum zone of inhibition against *Pseudomonas*.spp. i.e., 18 mm at the concentration of 8µg. Gandhakarasyaan showed maximum zone of inhibition against *Pseudomonas*.spp. i.e., 14 mm at the concentration of 0.25µg. Vyadhihara rasayana showed maximum zone of inhibition against *Pseudomonas*.spp. i.e., 22mm at the concentration of 0.25µg.

***Staphylococci* spp.:** *Staphylococci* spp. is a genus of Gram positive bacteria. Rasasindhoora showed maximum zone of inhibition against *Staphylococci* spp i.e., 16 mm at the concentration of 0.25µg. Rasamanikya showed maximum zone of inhibition against *Staphylococci* spp i.e., 16 mm at the concentration of 0.25µg. Gandhakarasyaan showed maximum zone of inhibition against *Staphylococci* spp i.e., 14 mm at the concentration of 0.25µg. Vyadhihara rasayana showed maximum zone of inhibition against *Staphylococci* spp i.e., 22mm at the concentration of 0.25µg.

CONCLUSION

Treating of infectious diseases has become more challenging with each passing year with respect to the multidrug resistance

bacteria. It limits therapeutic options and leads to increased mortality and morbidity. Alternative medicine can be used for the treatment of these multidrug resistance bacterial strains. In Ayurvedic system of medicine here are three major classes of drugs belonging to plant, animal and mineral origin. Herbomineral formulations of Ayurveda, constituting bhasma as an ingredient, are the superior forms of administration of Nano medicine. In the medieval period, it was widely accepted because of its minimum dose schedule and higher efficacy. As there is flaring up of multidrug resistant organisms in present day, there is a need for finding safe, cost effective drug, in which Rasa sindhoora, Vyadhihara rasayana, Rasa manikya Gandhaka rasayana may be an ideal replacement in certain multi drug resistance bacterial infections.

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