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ORIGINAL RESEARCH ARTICLE

Antimicrobial study of Rasakarpura Drava and Rasakarpura Gel

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ABSTRACT

Rasakarpura is unique mercurial preparation which is abundantly used to treat the skin disorder due to its antimicrobial and antifungal properties. Present study was taken to find out the antimicrobial properties of different concentration of Rasakarpura in Drava (Solution) form (0.05%, 0.1% and 0.2% of Rasakarpura). It is also studied for its antimicrobial activity in Gel form on the therapeutic dose level i.e. concentration 0.1% of Rasakarpura. Microbial study reveals that on higher concentration, Rasakarpura Drava was found more sensitive to micro organisms. It is also found that Rasakarpura Gel is observed more sensitive than Rasakarpura Drava on same concentration of Rasakarpura.

Key words: Rasakarpura Drava, Rasakarpura Gel, micro organism, bacteria, fungi

INTRODUCTION

Rasakarpura is a Nirgandha (without using sulphur) type of Kupipakva Rasayana (sublimed preparation) of Ayurved system of medicine, which attract the attention of physician due to its small dose, quick action, effectiveness and Rasayana (adjuvant) effect. It is used to cure many skin diseases, scar of tissue and body organs. It is also used to wash surgical instruments. It indicates that Rasakarpura has antiseptic, antimicrobial and anti fungal properties. So here, study was planned to find out efficacy of anti microbial and antifungal effect of Rasakarpura in different concentration level in Drava (solution) and also different dosage form i.e. Drava (solution) and Gel.

Ancient Indian scholars were aware about the existence of micro-organism or bacteria as well as causation of diseases due to them since Vedic period. There are many references regarding Jivanu Vada (bacteriology) available in ancient literature such as Rigveda, Atharvaveda, and Mahabharata etc. which indicates familiarity of the subjects in those days. In Atharvaveda, many words like Rakshash, Pishacha etc. have been used for Krimi, which have own specific meanings. In Ayurvedic literature, Krimi have been described to be Drista (macroscopic) and Adrista (microscopic). Acharyas had recognized a number of diseases spread by Krimi. Frequent touching the body of the patient, inhaling his expired air, dining, sleeping and sitting together, wearing dress, garlands and unguents used by him (patient) by these acts diseases such as Kustha (leprosy and some skin diseases), Jwara (fever), Sosa (consumption – pulmonary tuberculosis), Netrabhisyanda (opthalmia/conjunctivitis) and Aupasargika rogas (contagious diseases) spread from one person to the other. [1]

Antimicrobial sensitivity test [2] is mainly necessary when usually effective agents fail to produce the desired effects in the treatment and control of infectious diseases which are caused by pathogens that are drug resistance. Sensitivity testing is helpful in selecting effective antimicrobial drugs. This test should not be performed on contamination or communal organisms because this would result in the patient receiving unnecessary and ineffective drug therapy. This may lead to possible side effects and resistance to other pathogenic organisms. It is

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necessary to know the effectiveness of the drugs including its rate of absorption, diffusion in tissues, metabolism, **e**xcretion, toxicity and effect on the patient's microbial flora.

These tests measure antimicrobial activity [3] against bacteria under laboratory condition (*in-vitro*) and not in the patient (*in-vivo*). Therefore, it can hardly be assured that an antimicrobial agent that kills an organism in vitro will be equally effective *in-vivo* too.

MATERIALS AND METHODS

Preparation of test drugs:

Rasakarpura, Rasakarpura Drava and Rasakarpura Gel were prepared at department of Rasashastra and Bhaishajya Kalpana, I.P.G.T. & R.A., G.A.U., Jamnagar**.** As per the reference of Rasatarangini,[4] Parada (mercury) and Gandhakamla (sulphuric acid) was heated till they convert into white moisture less powder. Saindhava Lavana (rock salt) was added and mixture was sublimed into Valuka Yantra to collect the Rasakarpura. Rasakarpura Drava was prepared in different concentration by dissolving Rasakarpura in distilled water. [5] Rasakarpura Gel was prepared by using Rasakarpura, Triethanolamine, Carbopol – 940 and distilled water. [6]

Antimicrobial study:

Antimicrobial and antifungal activity study of different concentrated Rasakarpura Drava was analysed on *Staphylococcus*, *E - coli* bacteria and Candida respectively. Antimicrobial and antifungal activity of Rasakarpura Gel was carried out on *Staphylococcus Aureus, Streptococc*i, *E. coli, Klebsiella* bacteria and *Candida Albicance* & *Aspergillus Flavus* respectively.

Principles [7]:

Whatman no. 2 filter paper circular disc (6 mm) impregnated with known concentration of antibiotics are placed on an agar plate, which is inoculated with a culture of the bacteria under test. The plate is incubated at 35 – 37 ^{0}C for 18 – 24 hours. During incubation, the antimicrobial agent diffuses through the agar. Susceptibility effectiveness is proportional to the diameter of the inhibition zone around the disc. Organisms which grow up to the edge of the disc are resistant.

Requirement:

Normal saline, Muller Hinton agar plates, Sterilized cotton swab, sample of 0.05%, 0.1% and 0.2% solution of Rasakarpura and 0.1% concentrated Rasakarpura Gel

Procedure [8]:

Preparation of inoculums:

For a pure culture – subculture bacteria from the isolated colonies was placed in 1.0 ml of TSB (Tripticase Soya Broth) or nutrient growth for 2 – 4 hours at 37^{0}C. This subculture was used as the inoculum for seeding the antibiotics disc diffusion plate.

Preparation of Mac Farland Standard:

Mix 0.5 ml solution of $BaCl_2 \cdot 2H_2O$ (1.175%w/v) in 99.50 ml of H_2SO_4 (1%v/v) solution. Mixed well and the resultant suspension was equivalent to 1 x 10^6 cells/ml.

Inoculation of Test Plate:

Divide the plate into sections, according to the number of antibiotics. Inoculate properly the plate by using a sterile cotton swab so as to obtain uniform distribution of the inoculums. Put sample drugs on the inoculated plate aseptically (by using sterile forceps). Incubate the plate overnight at 37^{0}C. Measure the diameters of the zones of inhibition of growth in mm.

Test organism:

Staphylococci are Gram positive cocci, approximately 1 μm in diameter that occurs in grape like clusters. They grow readily on ordinary media within a temperature range of 10 – 42^{0}C, the optimum being 37^{0}C and pH 7.4 – 7.6. They are aerobes and facultative anaerobes. On nutrient agar, after incubation of 24 hours, the colonies are large (2 – 4 mm diameter), circular, convex, smooth, shiny, opaque and easily enulsitiable. They are the commonest cause of localized superlative lesions in human beings. [9]

The individual cocci are spherical or oval 0.5-1.1 um in diameter. They are arranged in chains. Streptococci are nonmotile and nonsporing. Culture is an aerobe and a facultative anaerobe, growing best at a temperature of 37^{0}C (range 22-42 ^{0}C). Growth occurs only in media containing fermentable carbohydrates or enriched with blood or serum. On blood agar, after incubation for 24 hours, the colonies are small (0.5-1.0 mm) circular, semitransparent, low convex discs with an area of clear haemolysis around them. Streptococci are the commonest cause of respiratory infections, skin and subcutaneous infection, genital infections, other superlative infections like abscesses in internal organs such as brain, lung, liver and kidney and also septicemia, nonsuppurative complications like acute rheumatic fever and acute glomerulonephritis. [10]

Escherichia coli are a Gram negative, straight road measuring 1 – 3 x 0.4 – 0.7 μm arranged singly or in pairs. It is motile by peritrichate flagella, though some strains may be non-motile. Spores are not formed. It is an aerobe and a facultative anaerobe. The temperature range is 10 – 40^0C (optimum 37^0C). Good growth occurs on ordinary media. Four main types of clinical symptoms are caused by E. coli. Those are urinary tract infection, diarrhoea or gastroenteritis, pyogenic infection and septicaemia. [11]

The genus Klebsiella consists of nonmotile, capsulated rods that grow well on ordinary media forming large, dome shaped, mucoid colonies of varying degrees of stickiness. Klebsiellae are widely distributed in nature, occurring both as commendsals in intestine and as saprophytes in soil and water. It causes pneumonia, urinary tract infection, other pyogenic infection, septicemia and rarely diarrhoea. [12]

Candida albicans is an oval or spherical budding cell, which produces pseudomycelia both in culture and in tissue. *Candida albicans* alone forms chlamydospores on corn meal agar culture at 20^0C. Though candida infection is mostly confined to the skin and mucosa, it can also cause systemic disease rarely, involving any organ. Candida infections, therefore represents a bridge connecting superficial and deep mycosis. [13]

Aspergilli and Penicillia constitute the commonest mould seen on damp bread or almost any other organic matter. The fungus grows rapidly on culture media. Aspergillli have septate hyphae. Asexual conidia are arranged in chains, carried on elongated cells called 'sterigmata' borne on the expanded ends (vesicles) of conidiophores. The commonest human disease caused by aspergilla is otomycosis. Systemic aspergillosis occur in two clinical types i.e. pulmonary aspergillosis and disseminated aspergillosis involving brain, kidney and other organ**.** [14]

RESULTS

The data of (**Table 1**) shows 0.05% and 0.01% both dilutions of Rasakarpura were moderately sensitive to staphylococcus while poorly sensitive to Candida and *E. coli*. 0.02% of Rasakarpura Drava was highly sensitive to staphylococcus and moderately sensitive to Candida, while poorly sensitive to E. coli bacteria (figure 1).[15] The results of antimicrobial study of Rasakarpura Gel indicate that it is moderately sensitive for organisms like *Staphylococcus aureus*, *Streptococci*, *E. coli*, *Klebsiella* and poorly sensitive to *Candida albicance* and *Aspergillus flavus* (**Table 2**). [16]

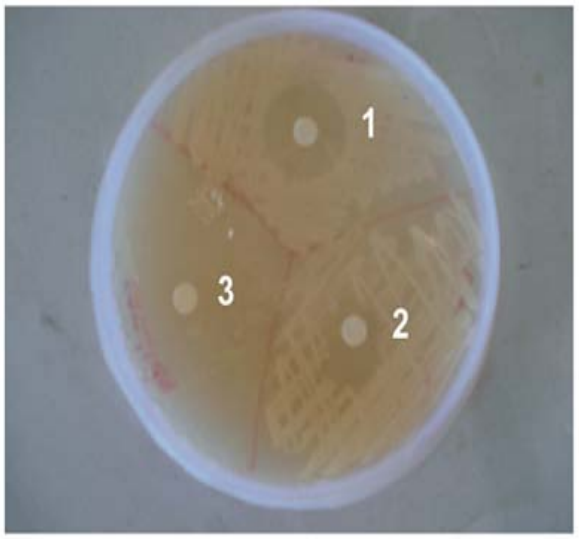

Figure 1: Showing the zone of inhibition against the Staphylococcus

Figure 2: Showing the zone of inhibition against *E. coli*

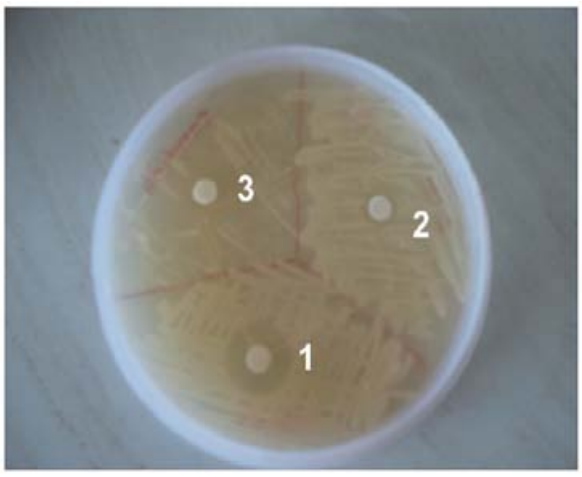

Figure 3: Showing the zone of inhibition against the Candida

Table 1: Antimicrobial Activity of Rasakarpura Drava in Different Concentrations

Antibacterial agent	Staphylococcus	*E. coli*	Candida
Rasakarpura (0.05%)	Moderately sensitive	Poorly sensitive	Poorly sensitive
Rasakarpura (0.1%)	Moderately sensitive	Poorly sensitive	Poorly sensitive
Rasakarpura (0.2%)	Highly sensitive	Poorly sensitive	Moderately sensitive

Table 2: Action of Rasakarpura Gel on Different Tests Organism

Activity conducted	Test Organism used	Action of Rasakarpura
Antimicrobial activity of Rasakarpura Gel	*Staphylococcus Aureus*	Moderately sensitive (3 mm)
	Streptococci	Moderately sensitive (3 mm)
	E. coli	Moderately sensitive (3 mm)
	Klebsiella	Moderately sensitive (3 mm)
Antifungal activity of Rasakarpura Gel	*Candida albicance*	Poorly sensitive (1mm)
	Aspergillus flavus	Poorly sensitive (1mm)

mm - millimetre

Table 3: Comparative Effect of Rasakarpura Drava and Rasakarpura Gel on Different Tests Organisms

Antibacterial agent	Staphylococcus	E. coli	Candida
Rasakarpura Drava (0.1%)	Moderately sensitive	Poorly sensitive	Poorly sensitive
Rasakarpura Gel (0.1%)	Moderately sensitive (3 mm)	Moderately sensitive (3 mm)	Poorly sensitive (1mm)

mm – millimetre

DISCUSSION

Here Rasakarpura was prepared as per the reference of Rasatarangini. Analytical parameter reveals that mercuric chloride was there as major constitute along with some trace elements in Rasakarpura.[17] As mercuric chloride is a water soluble compound of mercury, [18] it was decided to prepare Rasakarpura Drava, which is found 98% soluble in water. [19] So here to show the antimicrobial activity of Rasakarpura drug, three different concentration solutions (0.05%, 0.1% and 0.2%) was prepared in distilled water. To develop the new dosage form of Rasakarpura, which is suitable to prepare and use, it was decided to prepare Gel form of Rasakarpura. As per the need of era, both form of Rasakarpura was passed through different parameters before their clinical trial, and one of them is their antimicrobial study.

Rasakarpura has Twakroganashaka, Vranashodhaka, Bhuta-ghna properties. [20] It is externally used on different diseases which are occurring by the infections of micro organisms and also used as antiseptic purpose. So, antimicrobial study of Rasakarpura Drava and Rasakarpura Gel was carried out on *Streptococci, Staphylococcus aureus, E. coli, Klebiella, Candida albicance* and *Aspergillus flavus* because they produce various skin diseases.

The results of antimicrobial study of different concentrated Drava indicate that 0.2% solution of Rasakarpura was more sensitive to staphylococcus, poorly sensitive to *E. coli* and moderately sensitive to Candida. 0.1% and 0.05% solution of Rasakarpura were moderately sensitive to Staphylococcus and poorly to *E. coli* and Candida. These results indicate that 0.1% and 0.05% are equally sensitive for organisms, while 0.2% solution of Rasakarpura is more sensitive for the organisms. (**Figure 1, 2 & 3**)

0.1% of Rasakarpura in Gel form was observed moderately sensitive for organisms like Staphylococcus bacteria and poorly sensitive to organism like Candida fungus.

0.1% Rasakarpura Drava is poorly sensitive, while 0.1% Rasakarpura Gel is moderately sensitive on E. coli bacteria. So Rasakarpura Gel is observed more sensitive in comparison to Rasakarpura Drava on micro organism (**Table 3**). The results also indicate that 0.1% of Rasakarpura in Rasakarpura Drava and Gel are moderately sensitive for micro organism like bacteria and poorly sensitive to organism like fungus.

CONCLUSION

Higher concentrated (0.2%) Rasakarpura Drava is found more sensitive than 0.1% and 0.05% concentrated Rasakarpura Drava. 0.1% concentrate of Rasakarpura Gel is found more sensitive to bacteria and fungus in comparison with Rasakarpura Drava of the same concentration.

REFERENCE

1. KR Srikantha Murthi. Acharya Sushruta. Sushruta Samhita. Vol. 1, Nidanasthana. 2nd ed. Varanasi: Chaukhambha Orientalia; 2004, 502.
2. Godkar P. B., Textbook of medical laboratory technology. Mumbai: Bhalani Publishing House; 1998, 395.
3. Godkar P. B., Textbook of medical laboratory technology. Mumbai: Bhalani Publishing House; 1998, 395.

4. Shastri K N., Sharma S. N., Rasa Tarangini. Delhi: Motilal Banarasi Das; 2004, 115.
5. Mehta N. J. *et al*., Pharmaceutical standardization of Rasakarpura Drava & Rasakarpura Malahara (Gel) and their effect on Kshudra Kustha. Ph.D. thesis. IPGT & RA, G.A.U., Jamnagar, 2012.
6. Mehta N. J. *et al*., Pharmaceutical standardization of Rasakarpura Drava & Rasakarpura Malahara (Gel) and their effect on Kshudra Kustha. Ph.D. thesis. IPGT & RA, G.A.U., Jamnagar, 2012.
7. Godkar P. B., Textbook of medical laboratory technology. Mumbai: Bhalani Publishing House; 1998, 395.
8. Godkar P. B., Textbook of medical laboratory technology. Mumbai: Bhalani Publishing House; 1998, 396.
9. R Anantha Narayana. Textbook of Microbiology. Hyderabad: Orient Longman; 1997, 177.
10. R Anantha Narayana. Textbook of Microbiology. Hyderabad: Orient Longman; 1997, 187.
11. R Anantha Narayana. Textbook of Microbiology. Hyderabad: Orient Longman; 1997, 254.
12. R Anantha Narayana. Textbook of Microbiology. Hyderabad: Orient Longman; 1997, 258.
13. R Anantha Narayana. Textbook of Microbiology. Hyderabad: Orient Longman; 1997, 568.
14. R Anantha Narayana. Textbook of Microbiology. Hyderabad: Orient Longman; 1997, 575.
15. Mehta N. J. *et al*., Pharmaceutical standardisation of Rasakarpura & Rasakarpura Drava, its safety profile & therapeutic effect on Kshudra Kustha. M.D. thesis. IPGT & RA, G.A.U., Jamnagar, 2007.
16. Mehta N. J. *et al*., Pharmaceutical standardization of Rasakarpura Drava & Rasakarpura Malahara (Gel) and their effect on Kshudra Kustha. Ph.D. thesis. IPGT & RA, G.A.U., Jamnagar, 2012.
17. Mehta N. J. *et al*., Pharmaceutical standardisation of Rasakarpura & Rasakarpura Drava, its safety profile & therapeutic effect on Kshudra Kustha. M.D. thesis. IPGT & RA, G.A.U., Jamnagar, 2007.
18. Vasudev M. D., Parada Vigyaniyam. Datia: Sharma Ayurveda Mandir; 1997, 277.
19. Mehta N. J. *et al*., Pharmaceutical standardisation of Rasakarpura & Rasakarpura Drava, its safety profile & therapeutic effect on Kshudra Kustha. M.D. thesis. IPGT & RA, G.A.U., Jamnagar, 2007.
20. Shastri K N., Sharma S. N., Rasa Tarangini. Delhi: Motilal Banarasi Das; 2004, 123.