

**Research Article****ANTIMICROBIAL STUDY OF *VYADHIVIDHWANSANA RASA* (AN HERBOMINERAL PREPARATION):
IN VITRO STUDY****Prasad Anjali Baijnath^{1*}, Agrawal Sachin², Mohammad Aslam³, Baheti Sandip¹, Dighe Dattatray⁵**¹Research Officer (Ay.), CARIRD, Patiala, Punjab, India.²Associate Professor, Rasa Shastra & Bhaishajya Kalpana, SGCAS & Hospital, SGNR, Rajasthan, India.³Medical Officer (Ay.), Dharnawat, Kota, Rajasthan, India.⁵Research Officer (Ay.), Ministry of AYUSH, New Delhi, India.**ABSTRACT**

Ayurveda-The traditional medicinal science in India has holistic approach and practiced widely in the subcontinent. Large parts of the Ayurvedic drugs are heromineral preparations. These preparations are broad spectrum as far as its indications are concerned. Definite mode of action of these drugs are inexplicable. *Vyadhividhwansana Rasa* is an herbomineral preparation used for treating acute fever, interrupted fever, fever of unknown origin, ascites, splenomegaly, colic pain, *Vata vyadhi*, *Aama jwara* (first stage of fever) and *Vishama jwara* (irregular fever). In view of its therapeutic indications an antimicrobial study was designed to ascertain its probable mode of action. The present study aims to evaluate the antimicrobial activity of *Vyadhividhwansana Rasa* against test organisms at different concentration. *Vyadhividhwansana Rasa* was prepared as per Ayurvedic texts. Positive control Streptomycin [5mg(w/v)] and negative control 20% dimethyl sulfoxide (DMSO) were prepared. Solution of *Vyadhividhwansana Rasa* were prepared in three concentrations at 50 mg/ml, 100 mg/ml and 150 mg/ml in DMSO and was tested for its antimicrobial activity against *Escherichia coli*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi* by agar-well diffusion method. The measured Inhibition Zone ranged from 4-34 mm for all the sensitive bacteria. All the bacteria except *Salmonella typhi* were found Susceptible (S) against *Vyadhividhwansana Rasa* compared to standard. *Salmonella typhi* was found Intermediate sensitive (I). The antimicrobial activity of the extracts increased when the solution is more concentrated.

KEYWORDS: Ayurveda, *Vyadhividhwansana Rasa*, Anti-microbial, Herbomineral preparation.**INTRODUCTION**

Ayurveda-a complementary and alternative medicine (CAM) in India is practiced widely in south east Asia. It includes both preventive as well as curative means of treatment. Etiopathogenesis of disease is well explained along with treatment. '*Krimi*' is one such cause attributed for production of diseases, the word is used as a generic term with meaning of visible or invisible minute animals that dwells on living & nonliving things. Agnivesha has enumerated precise description of *Adrishta* (Invisible) *Krimi*, while describing the *Raktaja Krimi*.^[1] It is quite remarkable to spot the knowledge of communicable and infectious diseases held by the centuries old ancient Ayurvedic authorities. Diseases caused by *Krimi* are described as '*Sankramaka*' and '*Aaupsargika Rogas*' i.e. communicable and its modes of transmission are indulgence in physical contact, expired air, eating together in same plate, sharing

bed, using others cloth and accessories.^[2] also while describing the qualities of a perfect physician the practical proficiency of physician towards drugs and medicinal procedure is stated.^[3] The complete understanding of science will never be attained by the knowledge of only one part of science^[4] thus it becomes obligatory to understand the pathophysiology of the disease and to test the pharmacology of the drugs for its possible mode of action to validate, produce evidence and suggest alternative to the contemporary line of treatment.

While describing characteristics of noble medicine properties like easily digestible, palatable, cause evacuation of *Dosas*, having small dosage with high potency, satisfying, improves strength, causes little side effects are attributed and should possess merits of being prepared in to various forms.^[5] Even a small dose of drug may give powerful action.

The branch which deals with herbo-mineral preparation is known as *Rasa Shastra* which includes a good number of medicines advocated in Ayurveda for various diseases. The qualities like rapid action, less doses, tastelessness, prolonged shelf life, better palatability of herbomineral preparation have got the edge over other herbal preparation.^[6] *Vyadhividhwansana Rasa* is one such herbo-mineral preparation categorized as 'Sagandhaniragni moorchana' used for acute fever, interrupted fever, fever of unknown origin, ascites, splenomegaly, colic pain, *Vatavyadhi*, *Aamajwara* (first stage of fever), *Vishamajwara* (irregular fever) to be given in dose of 250 mg with vehicle as sugar, *Trikatu*, ginger juice and clarified butter, buttermilk, wholesome food and juice of *Tinospora cordifolia* (Rasa-raj Laxmi1/189-195). Considering the therapeutic proficiency of the *Vyadhividhwansana Rasa* the present study was designed to evaluate its anti-microbial property.

Table 1: Contents of Vyadhividhwansana Rasa

No.	Name of content	Part used	Latin name / common name	Quantity (gm.)
1.	<i>Abhraka Bhasma</i>	-	Mica (processed)	1
2.	<i>Gandhaka</i>	-	Sulphur (Purified)	1
3.	<i>Vatsanabha</i>	Rhizome	<i>Aconitum chasmanthum</i> (Purified)	1
4.	<i>Sunthi</i>	Rhizome	<i>Zinziber officinale</i>	1
5.	<i>Maricha</i>	Fruit	<i>Piper nigrum</i>	1
6.	<i>Pippali</i>	Seeds	<i>Piper longum</i>	1
7.	<i>Parada</i>	-	Mercury (Purified)	1
8.	<i>Tankana</i>	-	Borax (Purified)	1
9.	<i>Jayapala</i>	Seeds	<i>Croton tiglium</i> (Purified)	16

The formulation was prepared in following steps (sub process):

- Preparation of *Abhrak Bhasma* consists of *Samanyashodhana* of *Abhraka* in *Triphala Kwatha*^[7], *Dhanyabhrakikarana* (rendering *Abhraka* in to fine powder)^[8,9] in *Kanji* (Sour gruel)^[10] and *Bhaskarika* of *Abhraka*.^[11,12]
- Detoxification (*Shodhana*) of Toxic contents like *Gandhak*^[13], *Vatsanabha*^[14], *Parada*^[15], *Tankana*^[16] and *Jayapala*^[17].
- Preparation of *Kajjali* ^[18]
- Preparation of powders of crude drugs
- Mixing of *Bhasmas*
- Impregnation of powder with *Bhringraja Swarasa* (Extract of *Eclipta alba*).^[19]

Drugs like *Gandhak*, *Vatsanabha*, *Parada*, *Tankana* and *Jayapala* were subjected to process of *Shodhana* literally meaning "Purification" where the drug becomes less toxic and therapeutically more effective.

The powdered *Shuddha Vatsanabha* and *Shuddha Tankana* was taken first in a mortar and trituration was carried out. After that, *Kajjali* was

OBJECTIVES OF THE STUDY

The present study was conducted with following objectives:

- To validate the standard method of preparation of *Vyadhividhwansana Rasa*.
- To evaluate the antimicrobial activity of *Vyadhividhwansana Rasa* against test organisms at different concentration.

MATERIALS AND METHODS

- Pharmaceutical preparation of the formulation.
- Preparation of DMSO solutions of *Vyadhividhwansana Rasa*.
- Antimicrobial Susceptibility Testing was done.

Pharmaceutical preparation of the formulation:

The composition of the *Vyadhividhwansana Rasa* is given in Table No. 1.

added into the mixture and mixed well till it gets properly mixed. Then, other ingredients were added one by one. The mixture was impregnated with *Bhringraja Swarasa* (*Bhawana*) till whole *Swarasa* had dried up. This process of impregnation was done for seven times. The dough thus prepared was made into pills of 250mg. Three batches of *Vyadhividhwansana Rasa V₁ V₂ and V₃* were prepared to see the consistency of the pharmacological action.

Preparation of DMSO solutions of Vyadhividhwansana Rasa: The test drug was diluted to obtain the solution at desired concentration. 20% DMSO was used as diluents/vehicle to get desired concentration of drugs to test upon standard bacterial strains. Three solutions of 50mg/ml, 100mg/ml and 150mg/ml were prepared.

Antimicrobial Susceptibility Testing: Antimicrobial activity was detected by agar-well diffusion test using modified method.^[20] For this test, a culture medium, specifically the Mueller-Hinton agar, is uniformly and aseptically inoculated with the test organism and then a specific concentration of a streptomycin, is

placed on the medium. The organism grows on the agar plate while the antibiotic “works” to inhibit the growth. A “Zone of Inhibition” can be observed and measured to determine the susceptibility to an antibiotic for that particular organism. The measurement is comparable to standards by the National Committee for Clinical Laboratory Studies (NCCLS).^[21]

The sub steps followed in the study are:

- Preparation of Muller-Hinton Agar (MHA) plate: MHA was prepared according to the

manufacturer’s instructions and placed in a water bath at 50°C to prevent solidification.^[22] 25 ml of the agar was poured in standard petri dishes and left to set.^[23]

- Preparation of inoculation of Microbial Strains: ATCC- American Type Culture Collection (both Gram-positive and Gram-negative bacteria), were used (Table No.2.) The microbial strains were procured from IMTECH, Chandigarh, India and this study was done at Dr.B.Lal Institute of Biotechnology, Jaipur.

Table 2: Showing bacterial strain with their ATCC No

S.No.	Species	ATCC No.
1.	<i>Escherichia coli</i>	25922
2.	<i>Streptococcus pyogenes</i>	19615
3.	<i>Staphylococcus aureus</i>	29213
4.	<i>Pseudomonas aeruginosa</i>	27853
5.	<i>Salmonella typhi</i>	6539

- Each culture to be tested were streaked onto a non-inhibitory agar medium to obtain isolated colonies. After incubation at 35°C overnight, 4 or 5 well-isolated colonies were selected with an inoculating needle or loop, and transferred the growth to a tube of sterile saline. The Bacterial suspension was then compared to the 0.5 McFarland standards to adjust the turbidity of the inoculums for the susceptibility test.
- Positive control Streptomycin [5mg(w/v)] and negative control 20% DMSO- Di Methyl Sulfoxide were prepared.
- **Antimicrobial Susceptibility testing:** The cultures were swabbed on the surface of sterile Mueller-Hinton agar plates using a sterile cotton swab dipped into the suspension. ^[24] Pressing firmly against the inside wall of the tube just above the fluid level, rotated the swab to remove excess liquid. Streaked the swab over the entire surface of the medium rotating the plate approximately 90 degrees thrice ensure an even distribution of the inoculums and allowed to dry for 5 minutes. Agar wells were prepared with the help sterile pasteur pippete borer with 8 mm diameter. The extract of *Vyadhidhwansana Rasa* diluted to different concentration of 50 mg/ml, 100 mg/ml in 20% DMSO Solution was added to the wells of the inoculated plates. The plates were incubated in an upright position at 37±10C for 24hrs. ^[25] The extract diffuses into the agar media and inhibits the growth of microbial strain producing zone of Inhibition. The zone of inhibition (including the diameter of the antibiotic) was measured and expressed in millimeters (mm).^[26] Based on the criteria, the organism can be classified as being Resistant (R), Intermediate (I) or Susceptible (S) as given in Table No. 3.

Table 3: Showing the relation between Zone of Inhibition drug sensitivity^[27]

S.No.	Inhibition Zone (I.Z.)	Drug Sensitivity
1.	≤11mm	Sample is Resistant (R)
2.	12-14mm	Sample is Intermediate (I)
3.	≥15mm	Sample is Susceptible (S)

Results and Observations

The sample was found active at all the three concentration against all micro-organisms. The antimicrobial activity of the extracts increased with increase in concentration of extracts (mg/ml). The picture of antimicrobial test at highest concentration 125mg/ml of all the samples is shown in Fig. 1. The measurement of the Inhibition Zone of *Vyadhidhwansana Rasa* at 50 mg/ml, 100 mg/ml and 150 mg/ml in DMSO solution against different pathogens are given in Table No. 4.

Table 4: Showing Zone of Inhibition.

Bacteria culture	Samples	Zone of Inhibition (mm) @		
		50 mg/ml	100 mg/ml	150 mg/ml
<i>Escherichia coli</i>	V1	15	28	30
	V2	17	22	32
	V3	8	21	34
	Streptomycin	24	34	39
<i>Streptococcus pyogenes</i>	V1	8	12	19
	V2	4	16	20
	V3	13	17	21
	Streptomycin	15	22	31
<i>Staphylococcus aureus</i>	V1	6	21	30
	V2	13	18	30
	V3	7	19	30
	Streptomycin	16	28	36
<i>Pseudomonas aeruginosa</i>	V1	11	18	25
	V2	12	15	24
	V3	6	13	25
	Streptomycin	2	31	41
<i>Salmonella Typhi</i>	M1	4	11	14
	M2	6	11	14
	M3	8	12	14
	Streptomycin	19	31	38

DISCUSSION

Abhraka Bhasma does not have any antibacterial effect, by itself.^[28] Antimicrobial activity in this formulation may be improved with organic matter or other mineral present in the formulation in comparison with the processed mica. The water soluble cations present on the surface of the *Abhraka Bhasma* may contribute to the antimicrobial activity.

Ayurvedic texts also describes *Shuddhagandhakpamari*, *Dadrughna*.^[29] It is an effective antimicrobial agent.^[30] Sulphur has been associated as an important constituent in some antibiotics and is used both topically or systemically as an antimicrobial agent. Soluble Sulphur present in formulation possibly be transformed into pentathionic acid and may be responsible for its antimicrobial activity.^[31]

Vatsanabha (*Aconitum chasmanthum*) is a poisonous drug known for its cardio toxic activity. In Ayurveda it has been used in many formulations for *Jwara* and *Kaphajvyadhi*. It also exhibits antimicrobial, antioxidant, antipyretic, anti-inflammatory and analgesic activities.^[32] It is also known for its stimulant activity, antitussive, antidiarrhoea, dyspepsia and anti-poisonous activity.^[33]

The Ginger possesses potent antimicrobial activity against some food borne pathogens which may be due to presence of phenolic compounds.^[34] Thus ginger may provide protection against bacterial pathogens.^[35]

Piperine extracted from *Piper nigrum* showed antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli*. Gram negative bacteria are less susceptible towards the pepper extracts than gram positive bacteria.^[36] The isolated constituents particularly piperine from *Piper longum* tested for antimicrobial activity against *Klebsiella pneumoniae*, *Salmonella typhi* and *Staphylococcus aureus* has antimicrobial activity against shown the better activity profile against both Gram positive and Gram negative bacteria.^[37] The extract of *Trikatuchurna* was more effective against test organisms than its component.^[38]

Kajjali is a *Kharaliya Rasayana*, it should pass the tests like *Rekhapurnata* (fills the crevices of the finger), *Slakshnata* (smoothness), *Nischandrata* (lustreless) and *Tamrapareeksha* (*Kajjali* rubbed over copper foil should not leave any white streak). These tests strike out the chances of free mercury. *Parada* acts as *Rogaghna*, *Rasayana*, *Yogavahi*.^[39]

Tankan (Borax) is an important compound of Boron. It is effective against bacterial strains *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes*.^[40] *Tankan* is used in the process of repair that follows injury to the skin & other soft tissues.^[41]

The seed extracts of *Croton tiglium* possesses antimicrobial activities against skin disease causing microbes.^[42] A protein was purified from the plant that exhibited strong and broad spectrum antimicrobial activity.^[43]

Physical and chemical characteristics, such as small particle sizes and higher water solubility of the constituents of the formulation contribute to enhance the antimicrobial activity. This antimicrobial property of the drug may be due to the antimicrobial property of contents or due to the formation of organometallic complexes. The metal ingredients act as the carrier of the herb derived organic matter used during the pharmaceutical processing.^[44]

CONCLUSION

At the given concentration sample was found active against all micro-organisms. The antimicrobial activity of the extracts increased linearly with increase in concentration of extracts (mg/ml). The inhibition zone measured ranged from 4-34 mm for all the sensitive bacteria. All the bacteria except *Salmonella typhi* were found Susceptible (S) against *Vyadhividhwansana Rasa* compared to standard. *Salmonella typhi* was found Intermediate sensitive (I). The encouraging results obtained from antimicrobial study of *Vyadhividhwansana Rasa* are purely based on in-vitro antimicrobial method (well agar diffusion method), all the bacteria except *Salmonella typhi* were found susceptible against *Vyadhividhwansana Rasa* compared to standard. Further in-vivo studies are suggested for the authentication of the result for practical applicability.

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Cite this article as:

Prasad Anjali Baijnath, Agrawal Sachin, Mohammad Aslam, Baheti Sandip, Dighe Dattatraya. Antimicrobial Study of Vyadhividhwansan Rasa (An Herbomineral Preparation): In Vitro Study. International Journal of Ayurveda and Pharma Research. 2020;8(1):30-36.

Source of support: Nil, Conflict of interest: None Declared

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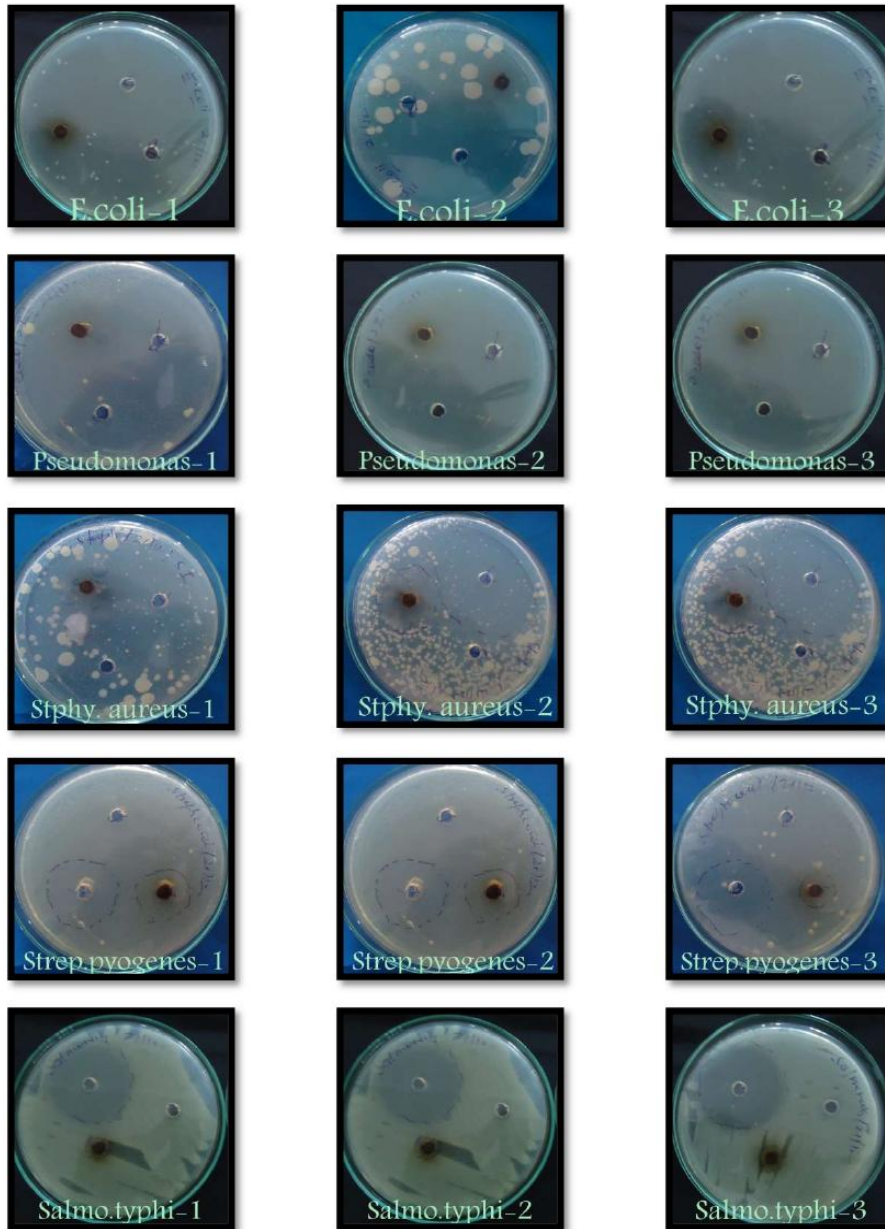


Fig. 1 Antimicrobial Activity of Vyadhividhwansa Rasa at 150 mg/ml in DMSO solution.