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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF RED OCHRE TRITURATED WITH *PUNICA GRANATUM* (DADIM BHAVIT GAIRIKVATI)

Avinash Ashok Shete^{*}, Jayashree Patil, Ashwin Ashok Shete and Arati Prabhakar Dubewar

Department of Streerogaevam Prasutitantra, Dr. D. Y. Patil College of Ayurveda and Research Centre, Pimpri, Pune - 411018, Maharashtra, India.

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Correspondence to Author:

Dr. Avinash Ashok Shete

Ph D, Scholar,
Department of Streerogaevam
Prasutitantra, Dr. D. Y. Patil College
of Ayurveda and Research Centre,
Pimpri, Pune - 411018, Maharashtra,
India.

E-mail: dr.avinashshete3737@gmail.com

ABSTRACT: Antibiotic or antifungal drug resistance is a major challenge in this era. Because the discovery and development of new drugs takes a long time. So, ancient medicine formulations are being checked for antimicrobial properties. Red ochre triturated with *Punica granatum* (*Dadim bhavit gairikvati*) is herbo-mineral formulation prepared by red ochre (*Gairika/Ferric Oxide* Fe_2O_3) triturated with *Punica granatum* (*Dadima-pomengrate* juice). The process is done according to the classical text *Sharangadhara Samhita*. This formulation is useful in nausea, vomiting, hyperacidity, anemia, piles, anorexia, diarrhea, burning micturation, etc. Analytical testing shows normal results within the standard range. The formulation is tested with reference to standard drugs for antibacterial study as two types of gram positive bacteria, viz. *Staphylococcus aureus* and *Bacillus subtilis*, and two types of gram negative bacteria, viz. *E. coli* and *Proteus mirabilis*. Two fungi, named *Candida albicans* and *Aspergillus niger* are also tested again with the same formulation. Five batches of samples for each organism are tested. A statistical tool such as the mean (a measure of central tendency) is used for analysis. *Staphylococcus aureus* (14.6mm), *Bacillus subtilis* (16mm), *Escheria coli* (15mm), *Proteus mirabilis* (16.4mm), *Candida albicans* (17.2mm) and *Aspergillus niger* (19.2mm) were found to have a zone of inhibition in the formulation. The positive result shows about 50% antibacterial and antifungal activity as compared with standard drugs (*Streptomycin* and *fluconazole*).

INTRODUCTION: Since their discovery, antibiotics have saved countless lives¹. Many antibiotics found effective previously are not potent in the present era². Antimicrobial drugs that are available on the market are being challenged by microbes. Antibiotics show resistance or fewer effects. Antibiotics are misused worldwide³. Microbial resistance to present antimicrobial drugs causes death or health calamities⁴.

New drug discoveries are being carried out, but it may need more time in modern medicine. To overcome this challenge, alternative but traditional systems of medicine can show rays of light. The Herbo-minerals formulations are being tested in this study⁵. Herbs show antibacterial activity with the presence of phytochemicals and alkaloids⁶. Herbo-mineral preparations were also found to be effective as antimicrobial drugs⁷.

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Red ochre triturated with *Punica granatum* (*Dadimbhavitgairikvati*) is herbo-mineral formulation prepared by red ochre (*Garika / Ferric Oxide* Fe_2O_3) triturated with *Punica granatum* (*Dadima-pomengrate* juice). Then it was dried in shadow and subjected to tableting of 250mg. The process is done according to the

classical text *Sharangadhara Samhita*⁸. This formulation is useful in nausea, vomiting, hyperacidity, anemia, piles, anorexia, diarrhea, burning micturation, etc. The formulation has not been assessed as an antibiotic or antifungal. Hence, the study was done to find out the antimicrobial activity of the formulation. The formulation was tested for antibacterial study as two types of gram positive bacteria viz. *Staphylococcus aureus*⁹ and *Bacillus subtilis*¹⁰ and two types of gram negative bacteria viz. *E. coli*¹¹ and *Proteus mirabilis*¹². Two fungi named *Candida albicans*¹³ and *Aspergillus niger*¹⁴ are also tested again with the same formulation.

The standard antibiotic range is used for reference. *Staphylococcus aureus* causes pneumonia, endocarditis, *Bacillus subtilis* causes bacteremia, endocarditis, pneumonia, septicemia etc. *E. coli* is the cause of watery or bloody diarrhea, fever, abdominal cramps, nausea and vomiting. *P. mirabilis* is capable of causing urinary tract infections, including cystitis and pyelonephritis¹⁵. *Candida albicans* causes candidiasis and *Aspergillus niger* causes aspergillosis.

MATERIAL AND METHOD:

Materials:

Punica granatum (Dadima-Pomegranate): This is an ancient fruit described in classical texts¹⁶. Its antioxidant activity is already known¹⁷. Classical texts explained it for digestion and nutrition along with a therapeutic effect¹⁸. It paliates all *tridosha* (three vital elements of the body) and provides vigour with vitality¹⁹. Red ochre (*Gairika-Ferric Oxide* fe_2O_3) is indicated for hiccups, vomiting, nausea and hyperacidity²⁰. Red ochre (*Gairika-Ferric Oxide* fe_2O_3) is purified by frying with cow ghee as per the classical text *Rasa tarangini*²¹. In addition, cow ghee is also mentioned with medicinal properties in classical texts²².

Method: Raw material authentication is done at Agharkar Research Institute, Pune (an autonomous body under the department of science and technology, Govt. of India) with authentication voucher no. AUTH 22-49 dated 10/05/2022. Raw Red ochre (*Gairika-Ferric Oxide* fe_2O_3) is shallow fried with 1/4th cow ghee as a process of purification. This is done with reference to *Rasa*

Tarangini. Red ochre (*Gairika-Ferric Oxide* fe_2O_3) in *shudhha* or purified form is trichurated with *dadim* (pomegranate) juice. This mixture is subjected to tableting of 250mg. Its physico-chemical analysis is done **Table 1**. Macroscopic studies or organoleptic properties are assessed as color, odour and taste. Analytical studies such as pH, loss on drying, hardness, disintegration, average weight, friability, etc. are tested with standard methods and instruments **Table 1**.

Antimicrobial Study:

Antimicrobial Activity Test (Kirby-Bauer Method): An antimicrobial activity test was performed by the commonly used agar diffusion method, which is designed to determine the smallest amount of the antibiotic needed to inhibit the growth of microorganisms²³.

Requirements;

- ❖ 18 hr nutrient broth culture of the test organism
- ❖ Standard Chloramphenicol
- ❖ Mueller-Hinton agar plates
- ❖ Cork borer
- ❖ Sterile standard bioassay filter paper disc²⁴.
- ❖ Sterile cotton swabs
- ❖ Alcohol
- ❖ Ruler
- ❖ Laminar flow chamber
- ❖ Test samples of which the activity has to be

Procedure²⁵: A sterile cotton swab was taken and dipped into a culture of test organism suspension. Inoculated the entire agar surface of each plate first in a horizontal and then in vertical direction to ensure the even distribution of the organism over the agar surface using the swab. The agar surface was allowed to dry for 5 minutes. A cork borer was sterilised by autoclaving. Mueller-Hinton agar plates were obtained and holes (4 mm) in the agar were aseptically punched with a cork borer. Using a wax pencil, marks were made on the underside of the Petri to label the wells. With the help of a micropipette, the test solution was added to the well. Repeat the procedure for all wells. In an incubator, all plates were incubated at 370°F for

24-48 hours. Observations are recorded and a statistical study is done.

RESULTS: A pharmaceutical study of the formulation results in a brownish-red, odourless,

astringent sweet tablet of 250mg being formed. **Table 1.** The pH is 7.04, with 30% LOD, hardness of 1.7kg/cm, disintegration time of 2.1 minutes, and zero percent friability **Table 1.**

TABLE 1: PHYSICOCHEMICAL ANALYSIS OF DADIMBHAVITGAIKIVATI

Sr. no.	Parameter	Test observation	Method/equipment
1	Color	Reddish brown	Organoleptic method
2	Odour	Odourless	Organoleptic method
3	Taste	Kashay, Madhur (Astringent sweet)	Organoleptic method
4	pH	7.04	Electronic pH meter
5	Colour on Aqueous Extract (10%)	reddish	Petri plate
6	Loss on Drying	30%	Porcelain dish
7	Hardness	1.7 kg/cm	Monsanto Hardness Tester
8	Disintegration	2.1min	Disintegration test apparatus
9	Average weight	500mg	Electronic precision balance machine
10	Friability	0%	Friability Apparatus

Examined all the plates for the clear zone of inhibition surrounding the disc. The diameter of

zone of inhibition is measured in using a ruler on the underside of the plate **Fig. 1, 2, 3.**



FIG. 1: ANTIBACTERIAL ACTIVITY OF DADIMGIRIJVATI WITH STAPHYLOCOCCUS AUREUS AND BACILLUS SUBTILIS



FIG. 2: ANTIBACTERIAL ACTIVITY OF DADIMGIRIJVATI WITH E. COLI, PROTEUS MIRABILIS



FIG. 3: ANTIFUNGAL ACTIVITY OF DADIMGIRIJVATI WITH CANDIDA ALBICANS AND ASPERGILLUS NIGER

The zone size recorded and prepared a table comparing the zone obtained with the known

concentration verses the zone of inhibition of standard antibiotic **Table 2.**

TABLE 2: ANTIMICROBIAL STUDY ON SAMPLE OF DADIMBHAVIT GAIRIKVATI

Sr. no.	Microorganism studied	Zone of Inhibition(mm)						Standard antimicrobial drug	
		Readings							
		1	2	3	4	5	Mean	Standard anti microbial drug	
1	<i>Staphylococcus aureus</i> (NCIM3100)	14	14	15	15	15	14.6	21.6	Streptomycin
2	<i>Bacillus subtilis</i> (NCIM2388)	15	15	18	15	17	16	26	Streptomycin
3	<i>Escheria coli</i> (NCIM 2065)	15	15	15	15	15	15	31.2	Streptomycin
4	<i>Proteus mirabilis</i> (NCIM2388)	17	15	18	16	16	16.4	25	Streptomycin
5	<i>Candida albicans</i> (NCIM3100)	17	17	18	17	17	17.2	28	Fluconazole
6	<i>Aspergillus niger</i> (ATCC504)	18	18	20	20	20	19.2	30	Fluconazole

DISCUSSION: Red ochre triturated with punica granatum (*Daim BhavitGairikVati*) formulation is prepared as per the standards of the classical text Sharangadharsamhita. Raw material authentication was done at Agharkar Research Institute, Pune with authentication voucher no AUTH 22-49 dated on 10th May 2022. Oraganoleptic characteristics are found as reddish brown in colour without odor. An astringent sweet taste is observed **Table 2.**

The colour of aqueous extract (10%) is reddish while 30% is lost on drying. So the formulation is much more stable. The tablet's hardness is 1.7kg/cm, making it easy to absorb in the body **Table 2.**

The disintegration time of a tablet is found to be 2.1 min. So, it disintegrates easily in gastric juices. The average weight is 500mg as it was made on a tableting machine. No weight variation is seen. Friability testing is a laboratory technique used to test the durability of tablets during transit. The formulation was found to be extremely durable, with no friability **Table 2.**

In an antimicrobial study, five batches of samples for each organism are made. Streptomycin is used as an antibiotic for two gram-positive and two gram-negative bacteria. The zone of inhabitation of the formulation for *Staphylococcus aureus* is 14.6mm, whereas streptomycin shows 21.6mm. *Bacillus subtilis* responded by 16 mm to the formulation as streptomycin has a 26 mm zone of inhibition. *Escheria coli* has a 15mm and 31.2mm zone of inhibition for the formulation and standard. *Proteus mirabilis* has a 16.4 mm and a 25 mm zone of inhibition for the formulation and standard.

An antifungal study is done with reference to the standard drug fluconazole. *Candida albicans* responded with 17.2 mm and 28 mm of zone of inhibition for the experimental formulation and the standard, respectively. *Aspergillus niger* has a 19.2 mm zone of inhibition for the formula and a standard of 30 mm for the standard.

Red ochre triturated with *Punica granatum* (*Dadimbhavitgairikvati*) shows antibiotic as well as antifungal activity, approximately half of the standard. Although this formulation is not meant for antimicrobial activity, the activity is still found. This activity can be enhanced by additives or synergism with other herbal drugs.

CONCLUSION: According to the classical text Sharangadharsamhita, red ochre triturated with *Punica granatum* (*Dadimbhavitgairikvati*) is herbo-mineral formulation.

Red ochre triturated with *Punica granatum* (*Dadimbhavitgairikvati*) is standardised with analytical study parameters at both the raw phase and finished product phase. That shows the drug is standardised and manufactured with Standard Operating Procedures. The results found are satisfactory as there are five batches of the drug in each plate for a single organism. The zone of inhibition in five samples was observed and the average was calculated and compared with the present antibiotic and/or antifungal. Even though the formulation is not claimed to have antimicrobial property, the formulation shows positive results as antimicrobial and antifungal. So, with therapeutic properties in the said diseases, antimicrobial action can be expected in a limited range. As a further scope of the study, potentiating

the drug with additives or synergism can enhance the zone of inhibition to function as an antimicrobial drug.

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