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AN INVITRO ANTI-MICROBIAL ACTIVITY AND DRUG DIFFUSION STUDY OF DAMARU YANTRA SHODHITA, CHAKRAMARDA BHAVITA AND GODUGDHA SHODHITA GANDHAKA

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ABSTRACT

Introduction: The rising incidence of superficial fungal and bacterial infections coupled with resistance to topical agents, necessitates the development of novel dosage forms. In *Ayurveda*, *Gandhaka* and *Chakramarda* are known for their excellent antimicrobial properties, both internally and externally. This study aims to do *Bhavana* of *Damaru yantra Shodhita Gandhaka* using *Chakramarda Swarasa* and assess its antimicrobial activity and drug permeation by comparing it with *Damaru yantra shodhita Gandhaka* and *Go-dugdha shodhita gandhaka.* **Materials and methods:** *Damaru yantra shodhita gandhaka* (DYSG), fresh *Chakramarda patra*, *Godugdha Shodhita Gandhaka* (GSG), microorganisms, Franz diffusion cell, Uv-Spectrophotometry. Organoleptic, instrumental analysis and diffusion studies were conducted. **Results:** The differences in antimicrobial activity between *Damaru Yantra Shodhita/Godugdha Shodhita Gandhaka* and *Chakramarda Bhavita Gandhaka* (CBG) stem from the incorporation of phytoconstituents from *Chakramarda Swarasa* during *Bhavana*. Among all the 3 samples CBG showed better results in the drug diffusion study. **Conclusion:** CBG showed activity against different microorganisms when compared to DYSG and GSG, and highest penetration in the drug diffusion study.

KEYWORDS: *Charamarda, Damaru Yantra Shodhita Gandhaka,* Anti-microbial action, Drug diffusion study.

INTRODUCTION

Skin, the largest organ of the human body, serves as a protective barrier against the external environment. Microorganisms play a crucial role in maintaining the skin's proper functioning. The interplay between these microorganisms and their responses to environmental factors is vital, as dysbiosis can lead to serious skin disease.[1] Bacteria such as Staphylococcus, Streptococcus, Propionibacterium, Corynebacterium, and Acinetobacter, along with fungi like Malassezia, Aspergillus, and Cryptococcus, constitute the human skin microbiome.

Superficial mycosis is common worldwide. They are believed to affect 1.8 billion or 20-25% of the world's population, and the incidence continues to increase.[2] Although they can affect almost any area of the skin, they are most commonly found in warm, moist regions like the armpits and groin. The incidence of these infections has risen significantly in both developed and developing countries. This rise in prevalence and severity, coupled with growing resistance to available antifungals, underscores the urgent need for novel therapeutic options.

Gandhaka[3] *and Chakramarda*[4] both demonstrate significant antimicrobial activity on their own. The combined activity is evaluated against bacteria like *Streptococcus pyogenes, Staphylococcus aureus,* and *Propionibacterium acnes,* as well as fungi such as *Candida albicans, Malassezia furfur*, and *Aspergillus niger*.

A drug release study is an in-vitro methodology that utilizes a Franz diffusion cell to evaluate drug permeability through a membrane. Franz Diffusion Cell Study is an in-vitro method that evaluates the rate and extent of drug release from a formulation into a surrounding medium by using a two-chamber system divided by a membrane. The donor compartment holds the drug formulation, while the receiver compartment collects the drug that diffuses through the membrane.

This study aims at enhancing the antimicrobial efficacy of *Damaru Yantra shodhita Gandhaka* by triturating it with *Chakramarda patra swarasa, Damaru Yantra Shodhita Gandhaka* and *Godugdha Shodhita Gandhaka* along with drug release study of all the 3 samples.

PHARMACEUTICAL STUDY

60g of fresh *Chakramarda* leaves were washed and ground. This was filtered through a thin cotton cloth to obtain fresh *swarasa*. 30g of *Damaru yantra shodhita Gandhaka* powder was taken in a clean *khalwa yantra*. Quantity sufficient *chakramarda swarasa* was added to it, to completely immerse it. Continuous *bhavana* was done until dry powder was obtained and considered as 1 *bhavana*. The same procedure was repeated for 7 times (*Anukta Bhavana*).[5],[6] Quantity of the end product was 35g.

Table 1: Shows observations during the preparation of *Chakramarda Bhavita Gandhaka.*

Date	*Bhavana*	Quantity of *Chakramarda swarasa* used	Time taken to complete one *Bhavana*	Observations
05/03/2024	1st	20ml	35 mins	The characteristic odour of *Chakramarda swarasa* was appreciated. The colour changed to green.
06/03/2024	2nd	15ml	30 mins	The quantity of *swarasa* required was reduced and the time taken for *mardana* was reduced.
06/03/2034	3rd	13ml	30 mins	Same observation
07/03/2024	4th	13ml	27 mins	Same observation
07/03/2024	5th	10ml	25 mins	Same observation
08/03/2024	6th	10ml	25 mins	Same observation
08/03/2024	7th	8ml	25 mins	Same observation

Fresh *Chakramarda leaves* **After grinding** ***Chakramarda swarasa***

Fig. 1: *Chakramarda Swarasa.*

Damaru yantra shodhita Gandhaka	*Bhavana with chakramarda swarasa*	*Chakramarda bhavita Gandhaka*

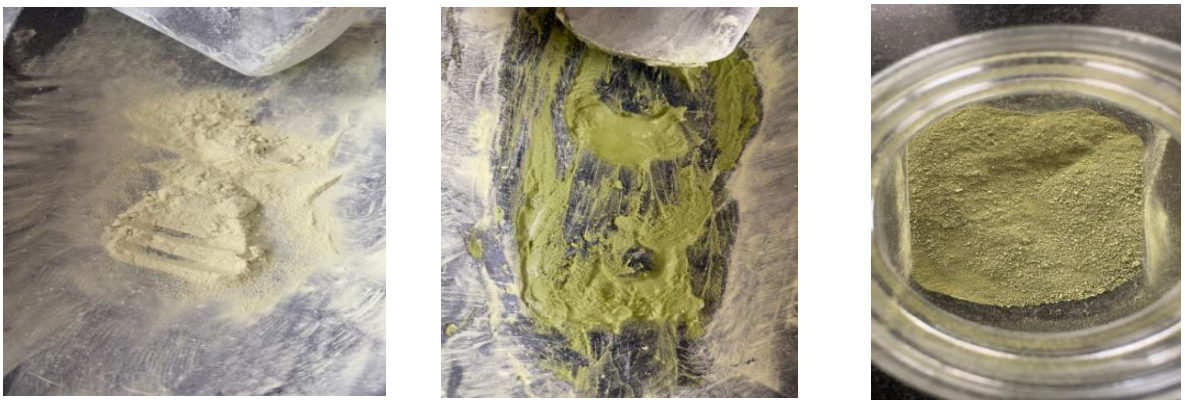


Fig. 2: ***Chakramarda bhavita Gandhaka.***

ANTI-MICROBIAL STUDY[7]

Damaru yantra shodhita Gandhaka, *Chakramarda bhavita Gandhaka*, and *Godugdha shodhita Gandhaka* were subjected to evaluate their antimicrobial activity against the following organisms: Bacteria (Streptococcus pyogenes, Staphylococcus aureus, Propionibacterium acnes) and Fungi (Candida albicans, Malassezia furfur, Aspergillus niger).

A loopful culture of Streptococcus pyogenes, Staphylococcus aureus, Propionibacterium acnes, Candida albicans, Malassezia furfur, and Aspergillus niger were grown on growth media and incubated at 35 ± 2°C for bacteria (2 days) and 23±2°C for fungi (5 days) respectively. The total numbers of cells were adjusted to 106CFU/ml at 620nm in digital photo colorimeter counting. 100% of the Sample was taken and tested as per the procedure.

Sterilized Muller Hinton agar (25 ml was poured into sterile Petri plates and let it solidify. A ditch (15 mm x 70 mm) was cut into a sterile MH agar plate. The test sample was added to the ditch and allowed to solidify. The ditch was made level with the rest of the agar by pouring the mixture. The different bacterial cultures were streaked perpendicular to the ditch using a nichrome wire loop. The plate was then incubated at 37°C for 24 hours. The results were observed as inhibition of bacterial growth on the ditch as well as adjacent to the ditch**.**

DRUG DIFFUSION STUDY

This involves the determination of a particular wavelength at which Sulphur is being absorbed (λ_{max}), developing a calibration curve of Sulphur, and conducting a drug diffusion study to determine the amount of drug permeation at a stipulated period of time.

This is done when a particular drug/ compound is unknown and the maximum wavelength at which the drug is absorbed has to be determined. This is further utilized in drug penetration studies. Isopropyl alcohol was used to dissolve the Sulphur, which was then further diluted and tested for maximum absorbance in the range of 200-400nm using a Shimadzu 1800 UV-visible spectrophotometer. **It was discovered that the λ_{max} of Sulphur was 290 nm.**

Isopropyl alcohol was used as a solvent to dissolve sulphur. 100mg of Sulphur was dissolved in 100ml of isopropyl alcohol in a volumetric flask. A magnetic bead was placed it and was placed on a magnetic stirrer for 10 minutes at 450rpm to ensure homogenization.

5 distinct 10ml volumetric flasks were filled with 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, and 1 ml of stock solution. Phosphate buffer of pH – 6.8 was added to the volume of up to 10 ml in order to reach the desired final concentrations.

2 samples (blank) of phosphate buffer solution of pH 6.8 were filled in cuvettes. The cuvettes were placed in the cuvette holder of the UV-spectrophotometer. The auto-zero function was initiated to set the baseline to zero. The 2nd cuvette was replaced with the stock solution to obtain the maximum peak of absorbance at a particular wavelength (λ_{max}). λ_{max} of 290nm was recorded. As the maximum absorbance was seen at 290nm, 5 samples of solutions of serial dilutions were scanned at the same wavelength to measure the absorbance in comparison to the blank. A graph of the absorbance calibration curve against concentration was plotted, which was linear in nature and the R^2 value was calculated.

Damaru yantra shodhita Gandhaka, Chakramarda bhavita Gandhaka, and *Godugdha shodhita Gandhaka* were subjected to evaluate their drug diffusion study.

Procedure: The Dialysis Membrane was soaked 12 hours prior to the study. A fresh Phosphate Buffer of pH 6.8 was prepared on the day of the study. A Franz Diffusion Cell was utilized, and the soaked dialysis membrane was securely placed at the base of the donor compartment and carefully placed in the receptor compartment. The receptor compartment was carefully filled with 55 mL of buffer solution to ensure it just made contact with the membrane, facilitating proper diffusion without introducing any air bubbles. 100mg of the drug was placed on the dialysis membrane towards the donor compartment. A magnetic bead was placed inside the receptor compartment. The apparatus was placed on a magnetic stirrer which was set at 50rpm. At intervals of every 30 minutes 1ml of sample was drawn from the

receptor compartment using a pipette and collected in a test tube. An additional 1 mL of buffer solution was added to the receptor compartment to replace the previous volume. The procedure was carried out for 4 hours. The 1ml sample collected was subjected to UVS analysis at a wavelength of 290nm at pre-determined intervals. 'J - flux' was calculated to determine the drug permeated through the below formula. J-flux value is the quantity of drug absorbed through a given surface in 1 hour.

$$J = \frac{dQ}{A * dt}$$

Where, J – flux value

dQ – quantity of substance penetrated through the membrane

A – the surface area of membrane

dt – total time of drug permeation

RESULTS

Physico-chemical analysis

A total of 3 trials were done for the sample and the mean was taken into consideration.

pH of *chakramarda swarasa* – 8.20.

Ashuddha Gandhaka: pH – 7.24.

Damaru yantra shodhita Gandhaka: pH – 3.4; Loss on Drying – 0.2143%.

Chakramarda bhavita damaru yantra shodhita gandhaka: pH – 6.20; Specific gravity – 1.85; Loss on Drying – 3.93%; Total Ash – 4.84%; Acid-insoluble Ash – 2.24%; Water-soluble Ash – 1.88%.

Godugdha shodhita Gandhaka: pH – 6.88; Loss on Drying – 0.9149%.

Instrumental analysis

XRD Analysis revealed several peaks of Sulphur (S_8). 3 strong peaks from each sample with high relative intensity were chosen and compared with a standard X-ray powder diffraction file (XPDF). All samples had a Crystalline Orthorhombic Structure. The d-space value of all samples was nearly identical to the d-space value of standard Sulphur. This confirms that *Ashuddha Gandhaka* is in S_8 form and a genuine product that is suitable for further preparations. Likewise, DYSG, CBG, and GSG also exhibited similar intensities and d-space values compared to the standard Sulphur. This consistency further supports the conclusion that these samples maintain the S_8 form.

FTIR analysis: The leaves of *Chakramarda* are characterized by a rich composition of phytochemicals, encompassing amines, anthraquinones, carboxylic acids, phenolic compounds, fluorinated derivatives, and aliphatic primary amines, all of which have been shown to possess significant antimicrobial activity, as evidenced by prior studies.[97] Similar phytochemicals along with chrysophanol were identified in CBG.

Mode of action of functional groups present in CBG exhibiting antimicrobial activity[99],[100]

They can interact with the phospholipid bilayer of microbial cell membranes, disrupting their structure and leading to cell death. They can denature proteins essential for microbial survival, rendering them inactive. They can alter the pH of the microbial environment, creating conditions that are unfavourable for growth and survival. Some phenolic compounds act as oxidizing agents, damaging microbial cells.

Table 2: Showing Anti-microbial activity of all samples.

ORGANISM SAMPLE NAME	Staph. aureus	Strep. pyogenes	P. acnes	C. albicans	M. furfur	A. niger
Damaru yantra shodhita Gandhaka	+	-	-	+	-	+
Chakramarda bhavita Gandhaka	-	+	-	+	+	-
Godugdha shodhita Gandhaka	+	-	-	+	-	+

Fig. 3: Antimicrobial study - *Damaru yantra shodhita Gandhaka.*

Fig. 4: Antimicrobial study - *Chakramarda bhavita Gandhaka.*

Fig. 5: Antimicrobial study - *Godugdha shodhita Gandhaka.*

Standard calibration curve of Sulphur (*Gandhaka*)

Beer-Lambert law was used to plot the absorbance against concentration at 290 nm in order to determine the standard calibration curve for Sulphur in Phosphate Buffer of pH 6.8. The calibration curve was linear and the R^2 value was 0.9958.

Table 3: Shows the Values of the Standard Calibration curve of Sulphur (*Gandhaka*).

WAVELENGTH = 290nm		
Sl. no	**Concentration (µg/ml)**	**Absorbance**
1	0	0
2	0.2	0.051
3	0.4	0.1
4	0.6	0.15
5	0.8	0.192
6	1	0.278

Percentage of drug permeation

The percentage of DYSG, CBG, and GSG that penetrated through the dialysis membrane in the first hour was 45.68%, 43.66%, 38.44% and in the four hours was 84.25%, 88.53, 83.22% respectively.

The in vitro permeation data revealed that CBG exhibited the highest drug penetration rate at 88.53%, followed closely by DYSG at 84.25% and GSG at 83.22% across the membrane.

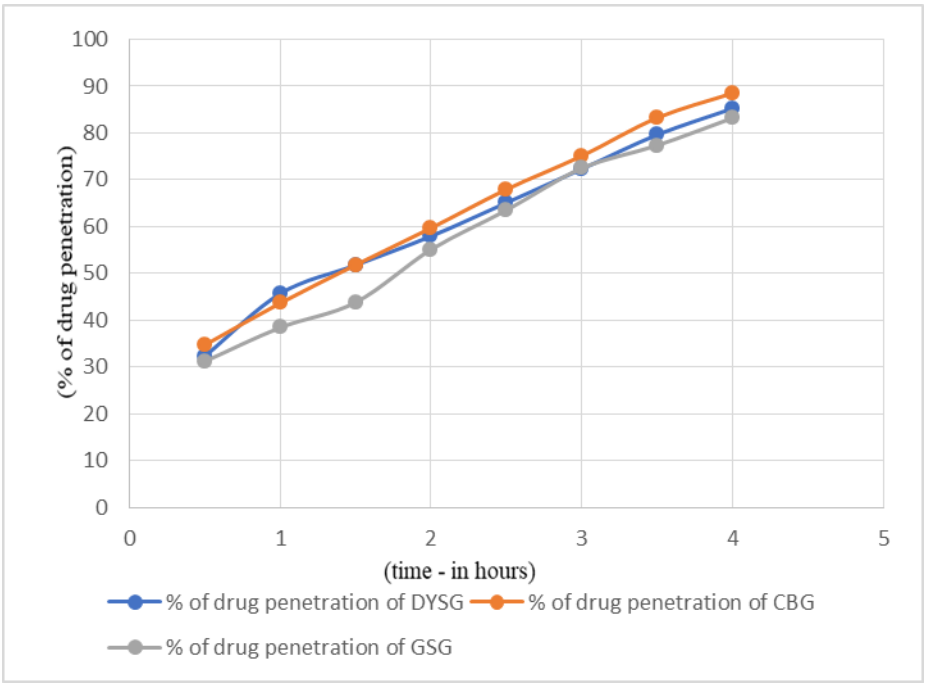
Table 4: Shows the Percentage of drug permeation in all samples.

Sl. no	Time (hours)	% of drug penetration of DYSG	% of drug penetration of CBG	% of drug penetration of GSG
1	0.5	32.33	34.71	31.2
2	1	45.68	43.66	38.44
3	1.5	51.78	51.82	43.82
4	2	57.96	59.71	55.1
5	2.5	65.1	67.95	63.51
6	3	72.32	75.12	72.49
7	3.5	79.64	83.26	77.37
8	4	85.25	88.53	83.22

Table 5: Shows the J-Flux value of all samples.

Sample name	J-Flux value
Damaru yantra shodhita Gandhaka	10.65 mg/cm^2/hr
Chakramarda bhavita Gandhaka	11.06 mg/cm^2/hr
Godugdha shodhita Gandhaka	10.40 mg/cm^2/hr

Fig. 6: Franz Diffusion Apparatus – permeation study of DYSG, CBG, GSG.



Graph 7: Drug diffusion study graph – DYSG, CBG, GSG.

DISCUSSION

Antimicrobial study by ditch plate method was chosen to ensure the use of 100% of the sample for the antimicrobial study, thereby avoiding any drug dilution. Given the prevalence of skin diseases and infections, 6 organisms were selected for testing: 3 bacteria (*Staphylococcus aureus, Streptococcus pyogenes, and Propionibacterium acnes*) and 3 fungi (*Candida albicans, Malassezia furfur, and Aspergillus niger*). The results indicated that DYSG, CBG, and GSG demonstrated significantly greater fungicidal activity than bactericidal activity. Additionally, numerous studies have confirmed the microbicidal properties of both *Gandhaka* and *Chakramarda* (seeds and leaf extracts).[8],[9] All samples demonstrated resistance against *C. albicans*, indicating the drug's efficacy in treating it and none of the samples showed resistance against *P. acnes*. DYSG and GSG both showed

resistance against *Staphylococcus aureus* and *Aspergillus niger*, while CBG exhibited resistance against *Streptococcus pyogenes* and *Malassezia furfur*. This suggests that the *Bhavana* with *Chakramarda swarasa* has imparted phytoconstituents present in *Chakramarda* leaves to *Gandhaka*, enhancing its resistance against these other organisms. The aldehydes detected in the FTIR analysis may be the Chrysophanols (Chrysophanic acid), another anthraquinone aglycone that can be converted to an aldehyde upon oxidation. Anthraquinone glycosides are known to break down into anthraquinone aglycones upon hydrolysis, and some of these aglycones are aldehydes. These may be the aldehydes detected in FTIR.[10]

In the drug diffusion study, CBG demonstrated the highest permeation within the given time frame, followed by DYSG and GSG. This suggests that CBG is more readily absorbed through the skin at a higher penetration rate. This enhanced absorption may be attributed to the reduction in particle size of CBG, which occurred during the 7 *Bhavana* with *Chakramarda swarasa.*

Diffusion study signifies[11],[12]: Drug molecules must diffuse through the skin's multiple layers to reach deeper tissues, making diffusion properties crucial for effective formulations. Controlled-release mechanisms in topical products rely on diffusion to regulate the drug's release rate into the skin. Once the drug penetrates the skin, its diffusion through various layers affects its distribution in underlying tissues. The concentration of the drug at the target site, influenced by diffusion, determines the efficacy of topical treatments.

CONCLUSION

This study states that GSG, DYSG, and CBG possess more antifungal activity than antibacterial activity. The process of bhavana has also incorporated new potential in the drug that, it has shown resistance against other organisms. In vitro permeation studies showed that *Chakramarda Bhavita Gandhaka* had the highest drug penetration rate at 88.53%, followed by *Damaru Yantra Shodhita Gandhaka* at 84.25%, and *Godugdha Shodhita Gandhaka* at 83.22%.

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