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Development and Evaluation of Antimicrobial Activity of *Durva Nishadi* Ointment: In-vitro Study

Research Article

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Abstract

Background: Synthetic antimicrobials are often associated with adverse effects as a result, there is need for the use of alternate herbal medicines is increasing with their easy availability, safety, cost effectiveness and therapeutic potentiality. In this present study *Durva Nishadi* Ointment was developed from *Durva nishadi Yoga* described in Ayurvedic classics as a *lepa* (topical application) against microbial diseases like *Kandu* (Pruritis), *Pama* (Scabies), *Dadru* (Tinea/ Ringworm infection), *Sheetapitta* (Urticarial rashes) and *Krimi* (Microbial infections). Materials and Methods: To prepare *Durva nishadi* ointment, *Durva nishadi lepa churna* was prepared by three different methods and subjected to MIC to evaluate the optimum concentration to be incorporated in the ointment. It was found that sample 2 was more effective. *Druva nishadi* with 15% w/w and 20% w/w were prepared with compatible ointment base. In vitro antimicrobial activity was performed on different strains of microorganisms to evaluate its efficacy. Result: The developed *Durva Nishadi* ointment has highly significant antifungal activity than the antibacterial activity. Antifungal activity of 20% ointment against *Candida albicans* was 28mm of zone of inhibition at 25 µg/ ml, and for *Aspergillus niger* 15 mm of zone of inhibition at 5 µg/ ml, which are highly comparable with the synthetic standard drug used for the antifungal activity i.e. Fluconazole. Conclusion: The prepared ointment showed significant antifungal activity as compared to the antibacterial activity in comparison with Fluconazole ointment However the detail study on stability is required for commercialization.

Key Words: Antimicrobial activity, *Durva Nishadi ointment,* Microbial infection, Disc diffusion method.

Introduction

Multiple drug resistance is the concerned issue of this era, due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases.(1,2) Ayurvedic pharmaceutics includes different internal and external dosage forms to be used in particular conditions of different disease like; *Panchavidha Kashaya Kalpana (Swarasa, Kalka, Kwatha, Hima, Phanta), Churna, Vati, Asava-Arista, Avaleha Lepa, Malahara* etc.(3) *Lepa kalpana* is application drug paste on the external surface of the body for its therapeutic effect. In *lepa kalpana*, the drugs are applied externally in the form of paste for particular duration and of particular thickness depending upon the nature of disease.(4) *Durva nishadi Yoga* is one of the potent herbal formulation described in Ayurvedic classics as a *lepa* (topical application) indicated in *kandu, pama, krumi, dadru and sheetapitta.* *Durva nishadi yoga* contains equal proportions of *Haridra and Durva.* (5)

Even though pharmacological industries have produced a number of new antimicrobials in the last three decades, the resistance to these drugs by microorganisms has increased. Generally, synthetic drugs have an ability to acquire resistance and are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. (6) Contrary to these synthetic drugs, plant materials play an important role in the maintenance of human health since antiquity. These plant products are the major source of drug development in pharmaceutical industry. (7) Several plants are now being used in part or as a whole to treat many diseases. Rural dwellers in most parts of the world do not depend on modern medicine for the cure of diseases and aliments because of easy availability of plant materials in the surroundings and their time tasted efficacy. As a result of this world is turning towards the use of traditional herbal medicines, which are easily available and cost effective. Two of such plants of great importance in traditional medicine since vedic period are *Durva* (*Cynodon dactylon* Linn.) and *Haridra* (*Curcuma longa* Linn). *Durva* (*Cynodon dactylon* Linn.) is a creeping grass available throughout the year; the material is used by the domestic animals as food and for pooja in all parts of India. The juice of the plant is

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astringent and is applied externally to fresh cuts and wounds.(8) Further it is also been evident by the recent researches it has potential antibacterial, antihistaminic and antioxidant activities.(9) *Haridra* (*Curcuma longa* Linn.) or Turmeric is commonly used as a spice, food preservative and food coloring agent. It also has the long history of therapeutic uses. It is been proved to have antimicrobial, anti-diabetic and anti-inflammatory activities.(10)

Druva Nishadi yoga is *lepa* formulation to be prepared freshly and applied as mentioned classical literature. The major disadvantages of classical preparation are stability and non-acceptance by the patient. To overcome these disadvantages application of modern pharmaceutics was utilized for the preparation of *Druva Nishandi lepa* in to ointment with suitable pharmaceutical excipients.

Materials and Methods

- **Materials:** Raw material viz. *Haridra* was procured from KLE Ayurved Pharmacy, Khasbag, Belgaum. Fresh *Durva* was collected from local natural habitat. Emulsifying wax BP, Liquid paraffin, white soft paraffin were purchased from authentic traders of local market. Both the herbal drugs were authenticated by the experts, at AYUSH approved drug testing lab - Central Research Facility of KLE University's Shri B.M.K. Ayurveda Mahavidhyalaya Belgaum, Karnataka, India.
- **Methodology:** Carried out in three phases Pharmaceutical part, Analytical Part and Experimental part.

Pharmaceutical part

Preformulation study: Prior entering to pharmaceutical part of *Durva nishadi* ointment, to decide in which form and at which concentration, *Durva nishadi lepa* is more efficient to exhibit antimicrobial activity, Preformulation study was carried out by three different methods of *Durva nishadi lepa churna* preparation and all the three samples were evaluated for M I C.(11)

- Sample 1 – *Bhavana* (Levigation) of fresh *durva swarasa* to *haridra churna*.
- Sample 2 – Uniform mixture of *Haridra churna* and *Durva swarasa churna* in equal quantity.
- Sample 3 – Mixture of aqueous extracts of *Haridra* and *Durva* in equal quantity. (11)

The Minimum Inhibition Concentration results of all the three intermediate preparations of *Durva nishadi lepa churna* by different methods suggested the sample 2 is having good antimicrobial activity. Based on the results, sample 2 *Durva nishadi lepa churna* was taken for the ointment preparation.

Preparation of *Durva nishadi lepa churna*:

Table No 1: Ingredients of *Durva nishadi lepa churna*

Sl. No	Name of Drug	Latin Name	Part used	Quantity
1	*Nisha*	*Curcuma longa* Linn	Rhizome	1 part
2	*Durva*	*Cynodon dactylon* Linn	Whole plant	1 part

Haridra churna preparation

The *Churna* (powder) was prepared as per the procedure explained in Ayurvedic Formulary of India. (12) Dried rhizomes of *Haridra* were made into powder in mixer grinder to reduce the particle size and are passed through 85# mesh, followed by 180# mesh to obtain finest powder.

***Durva swarasa churna* preparation**

Fresh *Durva* was collected and washed under running tap water properly to make it free from mud and dust etc. It was cut into small pieces and juice was extracted by the mixer grinder. The filtered juice was taken in a wide mouthed porcelain crucible kept in sunlight till it becomes completely dry. This dried powder was again grinded to reduce its particle size and sieved through 180 # mesh to get very fine powder.

***Durva nishadi lepa churna* preparation**

The fine powders of *Durva* and *Haridra* were taken in equal proportion and mixed well to form homogenous mixture of *Duradi lepa churna.*

Preparation of Ointment Base

Table No 2: Ointment base ratio

Sl. No	Ingredients	Percentage
1	Emulsifying wax BP	28%
2	Liquid paraffin	21%
3	White soft paraffin	50%
4	Methyl paraben	0.1%
5	Propyl paraben	0.05%

Method of preparation: Ointment base was prepared as per the above specified quantity**.** Emulsifying wax and liquid paraffin were taken in a china dish and heated at 50^0 c on hot water bath and mixed properly. To the above mixture, white paraffin was added and mixed well. This base was taken on the ointment slab levigated thoroughly to obtain ointment base.

Preparation of *Durva nishadi* ointment:

Durva nishadi lepa churna percentage in the final product of *Durva nishadi* ointment was decided on the basis of Minimum Inhibitory Concentration results and previous research works.(13) MIC results showed 1% of drug extract is sufficient to exhibit antimicrobial activity but D*urva nishadi lepa churna* in the present study is a whole drug product rather than the extract in ointment base, so the quantity of drug (*Durva nishadi lepa churna*) decided was higher in this present preparation of *Durva nishadi* Ointment. Here the *Durva nishadi* ointment has been prepared in two different concentrations i.e D. N. Ointment 1 with 15% of active drug and D. N. Ointment 2 with 20% of active drug powder.

Table No 3: *Durva Nishadi* Ointment ingredient and base ratio

Sl. No	Ingredients	Percentage
D. N. Ointment 1 (15%)	Base	85%
	Durva nishadi lepa churna	15%
D. N. Ointment 2 (20%)	Base	80%
	Durva nishadi lepa churna	20%

* D. N. Ointment – *Durva Nisha* Ointment

Method of preparation

Calculated quantity of ointment base was levigated on glass ointment slab with stain steel spatula with *Druva nishidi churna* in different ratio as mentioned in the Table 3. After complete uniform distribution of drug in to the ointment base. Prepared formulation was stored in air tight wide mouth glass container at room temperature.

Analytical Part

Raw drug analysis: Physico chemical and Phytochemical analysis of raw drugs *viz*. *Durva* and *Haridra* were carried out as per the standard procedures. (14,15)

MIC of Intermediate product: In preformulation study, *Durva nisadi lepa churna* has been prepared in three different methods were evaluated for MIC and Sample 2 was taken based on MIC results (Table No.10). The minimum inhibitory concentrations (MICs) were determined using agar dilution method for bacteria's (*Staphylococcus aureus, E. Coli, Pseudomonas aeruginosa*) and fungi (*Candida albicans, Aspergillus niger*). Mueller-Hinton agar with the standard antibacterial drug like ciprofloxacin and Sabouraud dextrose agar with standard anti-fungal drug fluconazole was used. The MICs were recorded by observation of lowest concentration that showed no visible growth of bacteria and fungi. (16)

Experimental Part

Anti-microbial screening: Experiment was carried out in comparison with Standard drugs by Agar well diffusion method. (17)

Anti-bacterial study

Method: The antimicrobial activity was determined using the agar well diffusion method. Overnight cultures were grown at 37°C in Mueller-Hinton Broth (MHB) and diluted to contain 105 cfu/ml. Petri dishes containing 20 ml of Mueller-Hinton Agar (MHA) (Hi Media), were used. The bacterial culture was spread over the surface of the MHA plate. 4 mm diameter wells were punched into the agar and filled with 20µl of of both the ointment of various concentrations (75, 50, 25, 10 and 5 µg/ml) The plates were then incubated at 37°C for 18 hrs.

Anti-fungal study

Method: The agar well diffusion method was modified. Sabouraud dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabouraud dextrose broth. 8 mm diameter wells were punched into the agar and filled with ointment and solvent blanks (hydro alcohol, and hexane). Standard antifungal (Fluconazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37°C for 72 hours. The diameters of zone of inhibition recorded. Results were interpreted on the basis of Zone of inhibition.

Results

Physico chemical and Phytochemical analytical results of raw drugs *viz*. *Durva* and *Haridra* are as follows

Table No 4: Macroscopic description of Raw Drugs

Raw drugs	Color	Odor	Taste	Part
Durva	Yellowish green	Characteristic	Bitter, astringent	Whole plant
Haridra	Yellowish brown	Characteristic	Bitter, pungent	Rhizome

Table No 5: Physico-Chemical analysis of Durva and Haridra

Drug	Foreign matter	LOD	Total ash	Acid insoluble ash	Aqueous extractive	Alcoholic extractive
Durva	Nil	7.98%	8.52%	4.21%	12.86%	8.23%
Haridra	Nil	9.2%	7.56%	0.58%	13.25%	9.38%

Table No 6: Phytochemical analysis of raw drugs (Qualitative organic compounds)

Test /Drugs	*Durva*		*Haridra*	
	Aq	Al	Aq	Al
Carbohydrates	+	+	+	+
Reducing sugar	+	+	+	+
Proteins a.Millons Test	-	-	-	-
Amino acids	+	+	-	-
Fats and oils	-	+	-	+
Tannins & Phenolic compounds - in $FeCL_3$	+	+	+	+
Alkaloids a.Dragendroffs test	-	+	-	+
Saponin Glycosides a.Foam test	+	-	+	-
Coumarin Glycosides	-	+	-	+
Steroids	+	+	-	+
Flavonoids	+	-	-	+

*Aq - Aqueous, Al – Alcohol

Table No 7: Qualitative analysis of Inorganic elements

Drug /Element	*Durva*	*Haridra*
Iron	Present	Present
Sodium	Absent	Absent
Calcium	Absent	Absent
Potassium	Absent	Absent
Magnesium	Absent	Absent
Chlorides	Present	Present
Sulphates	Present	Present
Phosphates	Present	Present
Carbonates	Present	Absent
Nitrates	Present	Present

Table No 8: Showing TLC of Raw drugs

Drugs	Rf- Short wavelength (254nm)	Rf- Long wave length (356nm)
Durva	0.07, 0.17, 0.46, 0.52, 0.57, 0.71,0.81, 0.87	0.07, 0.11, 0.17, 0.26, 0.40, 0.46, 0.60, 0.63, 0.71, 0.81, 0.86
Haridra	0.17, 0.23, 0.33, 0.55, 0.95	0.02, 0.16, 0.23, 0.32, 0.88

Table No 9: Showing Microbial load of Raw drugs

Sr. No	Drugs	Count	Limits (As per IP)	Results
1	***Durva***	Total bacterial count	30 – 300 cfu/ml	39 cfu/ml
		Total fungal count	10 – 100 cfu/ml	47 cfu/ml
2	***Haridra***	Total bacterial count	30 – 300 cfu/ml	31 cfu/ml
		Total fungal count	10 – 100 cfu/ml	31 cfu/ml

Table No 10: Showing MIC results of Intermediate product – *Durva nishadi lepa churna* samples

Sl. No.	Samples	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ ml	31.25 µg /ml	16 µg/ml	8 µg/ml	4 µg/ml	2 µg/ml	1 µg/ml
S.aureus											
1	1	R	R	R	R	R	R	R	R	R	R
2	2	S	S	R	R	R	R	R	R	R	R
3	3	S	S	S	R	R	R	R	R	R	R
Pseudomonas											
1	1	S	S	S	R	R	R	R	R	R	R
2	2	S	S	S	S	S	R	R	R	R	R
3	3	S	S	R	R	R	R	R	R	R	R
E.coli											
1	1	R	R	R	R	R	R	R	R	R	R
2	2	R	R	R	R	R	R	R	R	R	R
3	3	R	R	R	R	R	R	R	R	R	R
Candida											
1	1	S	S	S	R	R	R	R	R	R	R
2	2	S	S	S	S	S	S	S	S	S	S
3	3	S	S	S	R	R	R	R	R	R	R
A.niger											
1	1	R	R	R	R	R	R	R	R	R	R
2	2	S	S	R	R	R	R	R	R	R	R
3	3	R	R	R	R	R	R	R	R	R	R

*NOTE: S - Sensitive R – Resistant

Table No 11: Showing MIC result of *Durva Nishadi Lepa Churna* in comparison to Standard drug

Sl.no	Organism	Standard	*Durva nishadi lepa churna*
1	*Staphylococcus aureus* (Gram +ve)	2µg/ml	250µg/ml
2	*Escherichia coli* (Gram –ve)	2µg/ml	Resistant
3	*Pseudomonas* (Gram –ve)	<4µg/ml	31.25µg/ml
4	*Candida albicans*	16µg/ml	1µg/ml
5	*Aspergillus niger*	8µg/ml	250µg/ml

Table No 12: Showing Antimicrobial activity of *Durva nishadi* ointment in two different Concentrations by disc diffusion method

Sl. No.	Samples	75 µg/ml	50 µg/ml	25 µg/ml	10 µg/ml	5 µg/ml
20% *Durva nishadi* Oniment						
1	*E.coli*	10mm	R	R	R	R
2	*Pseudomonas*	18mm	15mm	R	R	R
3	*Candida*	>30mm	>30mm	28mm	R	R
4	*A.niger*	>30mm	>30mm	25mm	18mm	15mm
5	*S.aureus*	10mm	8mm	R	R	R
15% Duravadi Oniment						
1	*E.coli*	10mm	8mm	R	R	R
2	*Pseudomonas*	15mm	10mm	R	R	R
3	*Candida*	30mm	25mm	18mm	R	R
4	*A.niger*	25mm	20mm	18mm	R	R
5	*S.aureus*	10mm	8mm	R	R	R

Table No 13 : Showing disc diffusion result of *Durva nishadi* ointment

Sl.no	Organism	Standard	*Durva nishadi lepa churna* Sample 2 (MIC)	*Durva nishadi* ointment 20%
1	*Staphylococcus aureus* (Gram +ve)	26mm at 2µg/ml	Sensitive at 250 µg/ml	10mm at 75µg/ml
2	*Escherichia coli* (Gram –ve)	32mm at 2µg/ml	Resistant	10mm at75µg/ml
3	*Pseudomonas* (Gram –ve)	>21mm at 4µg/ml	Sensitive at 31.25 µg/ml	15mm at 50µg/ml 18mm at 75µg/ml
4	*Candida albicans*	24mm at 16 µg/ml	Sensitive at 1 µg/ml	28mm at 25 µg/ml >30mm at 50 µg/ml >30mm at 75 µg/ml
5	*Aspergillus niger*	26mm at 8 µg/ml	Sensitive at 250 µg/ml	15mm at 5 µg/ml 18mm at 10 µg/ml 25mm at 25 µg/ml >30mm at 50 µg/ml >30mm at 75 µg/ml

Discussion

The present study was about modification of the *lepa* into a convenient ointment dosage form and screening of its antimicrobial activity. *Durva nishadi yoga* is a potent herbal formulation comprised of *Durva* and *Haridra*. The individual as well as a compound mixture extract of these drugs is scientifically proved for their antimicrobial activity. Raw drug phyto-chemical analysis of *Durva* and *Haridra* shows the presence of tannins, phenolic compounds, alkaloids, steroids and flavonoids the secondary bioactive plant metabolites known for their antimicrobial activity. With this background classical *Durva nishadi lepa* was developed into the *Durva nishadi* ointment for the convenient use and for the better stability.

Durva nishadi lepa churna was decided for final product on the basis of MIC of three different samples, prepared with three different methods. Among the three samples, sample 2 was selected, which has shown the sensitivity to the microorganisms specially *Candida albicans* at the lowest concentration of 1µg/ml.

The developed *Durva nishadi* ointment in two different concentrations of drug (*Durva nishdi lepa churna*) was evaluated for its antimicrobial activity. D. N. Ointment 1 with the drug concentration of 15% shown the antifungal activity at the concentration of 75 µg/ml for *Candida albicans* and *Aspergillus niger* with 30 mm and 25 mm of zone of inhibition respectively. Which is much higher than the standard values of fluconazole Antibacterial activity of ointment with 15% and 20% of drug have shown almost similar antibacterial activity at the concentration of 75 µg/ml for *S. aureus, Pseudomonas and E coli* with 10 mm, 15-18mm and 10mm of zone of inhibition, which were comparatively lesser than the standard values of ciprofloxacin. D. N. Ointment 2 with 20% drug concentration has shown better antifungal activity than the D. N. Ointment 1; with much lower concentration of 25 µg/ml.

Conclusion

Durva nishadi lepa sample which has been selected for this study is a combination *Durva panchanga swarasa churna* and *Haridra kanda churna* in equal quantity on the basis of MIC results. This *Durva nishadi lepa churna* has been developed into the ointment form to improve the acceptability and to increase the shelf life of the formulation. The developed *Durva nishadi* ointment was screened for its antimicrobial activity, which shows that ointment prepared with two different concentration i. e. 15% and 20% both have significant anti-fungal activity than the antibacterial activity. Antifungal activity of both ointments (15% and20%) was comparatively much higher than the standard drug used for the antifungal activity i.e. Fluconazole, with zone of inhibition of 30mm. The results of this study support the use of *Durva nishadi yoga* as a potent antifungal preparation in various fungal infections of the skin.

Abbreviations

MIC - Minimum inhibitory concentration, MHA - Mueller-Hinton Agar.

Author contributions

Dr Geeta Gadad and Dr R. S. Hiremath designed and carried out the experiments. Dr Geeta Gadad prepared manuscript. Mr U. B. Bolmal and Dr. R. S. Hiremath revised the manuscript. Dr. U. B. Bolmal and Dr. R. S. Hiremath supervised all research. All authors reviewed the manuscript.

Conflict of interest

No competing financial interests exist.

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Illustrations

Figure 1:Raw drug *Haridra*

Figure. 2 : *Durva swarasa*

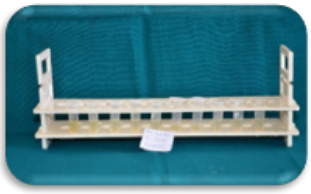

Figure. 3 : MIC of *Durva nishadi lepa churna*

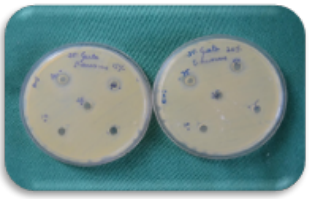

Figure 4 : Antibacterial activity aginst *S. aureus*

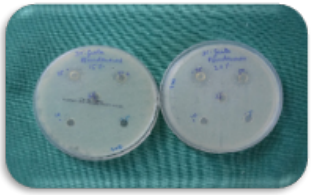

Figure 5 : Antibacterial activity aginst *Pseudomonas*

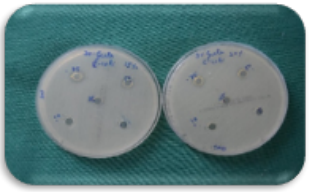

Figure 6 : Antibacterial activity aginst *E- Coli*

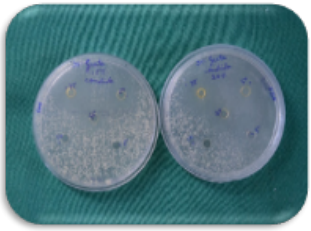

Figure 7 : Antifungal activity aginst *Candida Albicans*

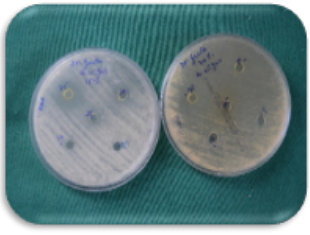

Figure 8 : Antifungal activity aginst *A. Niger*

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