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FORMULATION AND EVALUATION OF HARIDRADI VARTI EXTRACTS FOR ITS IN-VITRO ANTIBACTERIAL ACTIVITY

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ABSTRACT

Local treatment explained as *Netra Kriyakalpa* plays a crucial role for the treatment of eye diseases. *Netravarti* which is applied as *Anjana* belongs to one of the *Netra Kriyakalpa. Haridradi varti* is a preparation indicated in all eye diseases explained in *Chakradatta* which contains *Haridra, Daruharidra, Haritaki, Vibhitaki, Amalaki, Lodhra, Yashtimadhu* and *Raktachandana.* Extraction procedures play a crucial role in obtaining the therapeutic benefits of crude drugs, ensuring the purity and concentration of active compounds in lesser dose, and facilitating the development of various medicinal products for effective and controlled administration. *Abhishyanda* is classified under *Sarvagata Netraroga* which affects eye in all ways and is said to be the cause of all the eye diseases, which can be compared to Conjunctivitis based on its signs and symptoms. The primary base for treating bacterial infections is reliant on the use of Antibiotics. Due to inappropriate use of antibiotics, which can lead to bacterial adaptation or mutations, in turn growth of new strains of bacteria that can give rise to antibiotic resistance. Hence, there is a need to develop a new antimicrobial agent based on targeted drug delivery. Haridradi varti extracts is chosen in the present study focusing on its antibacterial effect in-vitro, against gram -ve and gram +ve bacteria by assessment of zone of inhibition and minimum inhibitory concentration using OD method. The results showed promising antibacterial activity in the lowest concentration of *Haridtadi varti* extracts.

KEYWORDS: *Haridradi varti, Haridradi varti* extract, Antibacterial, Phytochemicals, Bacteria.

INTRODUCTION

Among the five sense organs, Ayurveda gives prime importance to the eye, "*Sarvendriyaanam Nayanam Pradhanam*".[1] The local treatment procedures of *netra* are explained in the name of *netra kriyakalpas*.[2] The word *kriya kalpa* is made of two words kriya and kalpa. *Kriya* means special therapeutic procedures and kalpa means adopting a specific formulation like swarasa, ghrita, Kashaya, Anjana, etc. prepared by particular herbo-mineral drugs. So, Netra kriyakalpa refers to specific formulation used for therapy in various eye diseases.[3]

Kriyakalpa helps in alleviating the localised doshas of the eyes. It includes procedures like *Aschyotana, Anjana, Seka, Tarpana, Putapaka, Pindi* and *Bidalaka* according to *Acharya Sharangadhar* which is indicated different conditions of the eye.[4] Among these, *Anjana varti* is one important *kriyakalpa* having a vivid utility in many *netra rogas*. *Haridradi varti* is one such preparation mentioned in *Chakradatta* indicated as *sarvanetramayapaham* (in all types of eye diseases).[5]

The primary goal of the extraction process is to isolate the active constituents or therapeutic components present in the crude drug. Different plant or natural sources contain a variety of chemical compounds, and the extraction process aims to selectively obtain the substances responsible for the desired therapeutic effects. The extracted material may undergo additional processing to prepare it for incorporation into various dosage forms. This step allows for convenient administration and controlled dosing. *Haridradi varti* is a polyherbal formulation containing *Haridra* (Curcuma longa), *Daruharidra* (Berberis aristate), *Haritaki* (Terminalia chebula), *Vibhitaki* (Terminalia bellerica), *Amalaki* (Emblica officinalis), *Lodhra* (Symplocos racemose), *Yashtimadhu* (Glycyrrhiza glabra) *and Raktachandana* (Pterocarpus santalinus) in equal quantity. As per the research in modern science, all these drugs have been reported to contain multiple phytochemicals having various therapeutic activity.

Haridradi varti is indicated in *pichitta, dhumadarshi, timira,* and all types of eye diseases. *Abhishyanda* is classified under *Sarvagata Netraroga* which affects eye in all ways. It is considered as root cause of almost all the eye diseases.[6] If it is not treated in time, it may lead to complication like *Adhimantha. Acharya Sushruta* has explained the disease *Netra*

Abhishyanda as one of the *'Aupasargika Rogas'* and explained the contagious nature of disease. *Abhishyanda* can be correlated to conjunctivitis based on its signs and symptoms. Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance.[7] Thus, there has been a continuing search for new and more potent antibiotics. Therefore, *Haridradi varti extract* has been considered for its in vitro antimicrobial evaluation.

MATERIALS AND METHODS

Preparation of Haridradi varti

It was done as per the reference of *Chakradatta*. Fine powder of *Haridra, Daruharidra, Haritaki, Vibhitaki, Amalaki, Lodhra, Yashtimadu* and *Raktachandana* was taken in equal quantity. It was triturated with *Bhringaraj swarasa* (juice of Eclipta alba) in iron and copper vessel separately for 7 days each. When the mixture attained proper consistency, *vartis* were prepared of approximate 2 cm length as per AFI.[8] It was then dried in Hot air oven and stored in an air tight container. In the present study, *bhavana* (trituration with liquid media) was done with *Bhringaraj swarasa* media for 7 times each in iron and copper vessel. Hence a total of 14 *bhavana* has been done.

Preparation of Haridradi varti extracts

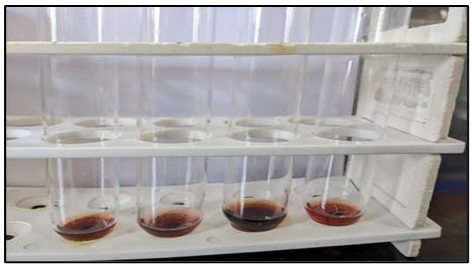
Hydroethanolic solution (50:50) was used as solvent for the extraction. Fine powder of *Haridradi varti* taken in centrifuge tubes were added with solvent and mixed well. It was placed in shaker for 10-15 mins and then in centrifuge for 10 mins. Then supernatant portion was transferred in a clean bottle. The procedure was repeated till the colour of supernatant liquid turns light. The liquid solution was transferred to round bottom flask and it was fixed to rotary vacuum evaporator. The apparatus was switched on at 55^0C to evaporate the solvent. Later, the concentrated liquid was placed in Hot air oven at 60^0C till all the liquid portion evaporates and semisolid thick paste is obtained.

Phytochemical screening of Haridradi varti extracts

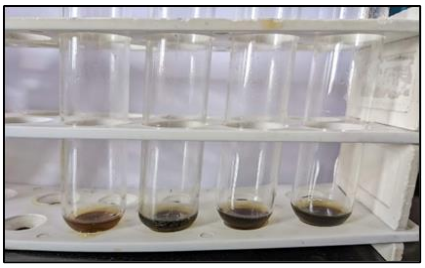
The obtained Haridradi varti extracts was screened for Phytochemical analysis and the observation are shown as:

Table No. 1: Showing qualitative phytochemical analysis of Haridradi varti extracts.

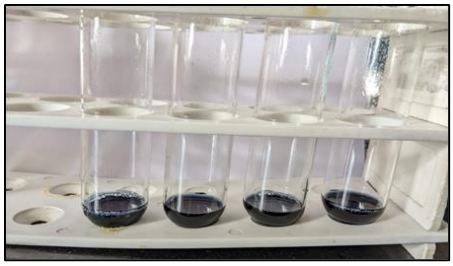
Sl. No.	Name of the test	Observations	Results
01.	Alkaloids (Mayer's test, Wager's test, Hager's test and Drangendroff's test)	No change in colour	Absent (-)
02.	Phytosterols	Colour changed to brownish red	Present (++)
03.	Tannins	Colour changed to dark green	Present (+++)
04.	Phenols	Colour changed to dark blue	Present (+)
05.	Flavonoids	Colour changed to light pink	Present (+)



Detection of Phytosterols



Detection of Tannins



Detection of Phenols

Detection of Flavonoids

Fig No. 01. Showing observations of Phytochemical screening.

Evaluation of in-vitro anti-microbial activity of Haridradi varti extracts

i) To assess Zone of inhibition

The Haridradi varti extracts was tested against following organisms-

Gram -ve bacteria- Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Serratia marcescens.

Gram +ve bacteria- Bacillus cereus, Staphylococcus aureus.

Sample- *Haridradi varti* extracts

Method – Agar well diffusion method

Procedure

Sample preparation- the extract solution was prepared with 1mg/ml concentration using Dimethyl sulfoxide (DMSO).

Culture preparation- the suspensions of all the organisms were prepared as per Mac-Farland Nephelometer standard. Suspensions of organisms were made in sterile isotonic solution of sodium chloride (0.9% w/v) and the turbidity was adjusted.

Media preparation- nutrient broth powder was dissolved in distilled water, pH was adjusted and sterilized by autoclaving. The sterilized medium was cooled and poured into petri dishes to obtain 4-6 mm thickness. Media was allowed to solidify at room temperature.

Plate preparation- when agar in petri dishes got solidified, bacterial culture which was previously prepared was pipetted and dropped over these plates at 2-3 spots. These dropped culture was swabbed in all directions with slight pressure. Two wells were made in each of these plates using sterile cork borer having 6 mm diameter. These wells were loaded with 250 µg/ml and 500 µg/ml of extract solution. The plates were incubated at 37^0 C for 24 hrs in incubator. The diameter of the inhibition zone was measured for its antibacterial activity.

ii) To calculate Minimum inhibitory concentration (MIC)

Bacteria –

Gram -ve bacteria- Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Serratia marcescens.

Gram +ve bacteria- Bacillus cereus, Staphylococcus aureus.

Sample- *Haridradi varti* extracts

Procedure- The samples with different concentrations from 100 µg/ml till 500 µg/ml were added to the test tubes containing Mueller-Hinton broth after autoclaving. Inoculums of organisms was prepared in Mueller- Hinton broth, and the turbidity was adjusted to 0.5 McFarland turbidity standard to prepare 1×10^8 bacterial /ml. 50 µl of bacterial cultures were added to each test tube except the negative controls. The test tube without the sample with only bacterial culture was used as positive control. The test tubes were incubated at 37℃ on an orbital shaker at 100 rpm for 4 hr. antimicrobial activity was assessed by measuring absorbance at 600 nm of wave length and percentage of inhibition was calculated. Based on the regression equation Minimum Inhibitory Concentration (MIC) was derived and evaluated.

Percentage of inhibition = (OD of control – OD of test) × 100

OD of control

RESULTS

Table No. 02: Showing the zone of inhibition of Haridradi varti extracts.

	Zone of inhibition (mm)	
Bacteria	**Extract 250 µg/ml**	**Extract 500 µg/ml**
Serratia marcescens	10.3	12.6
Escherichia coli	11.3	14.6
Pseudomonas aeruginosa	10.6	14.6
Klebsiella pneumoniae	10.3	11.6
Bacillus cereus	10.6	13.3
Staphylococcus aureus	10.3	12.6

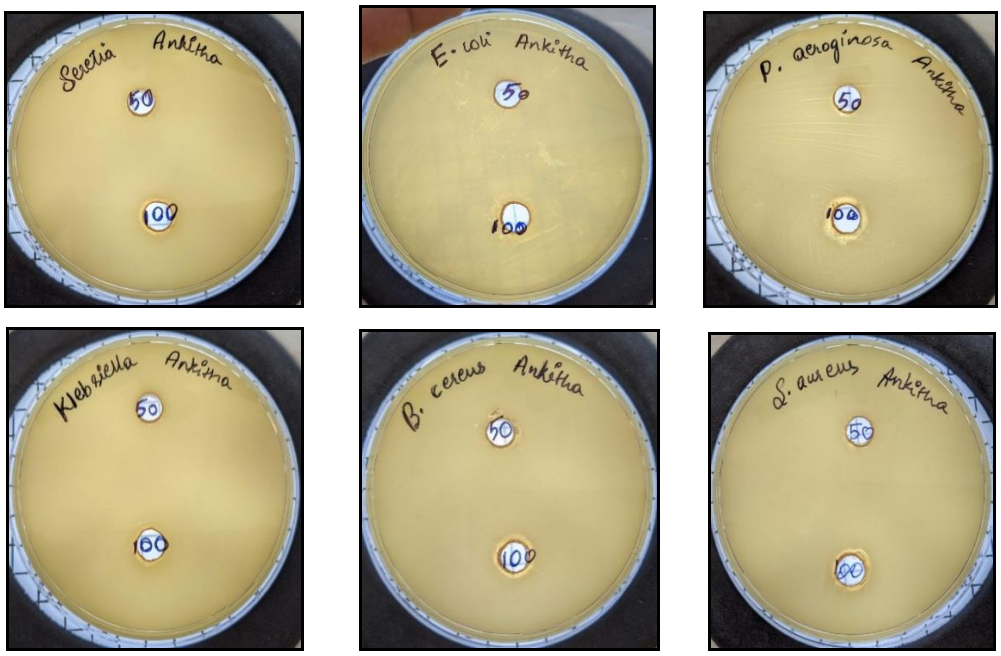


Fig No. 02. Showing observations of zone of inhibition at 250 µg/ml and 500 µg/ml concentration.

Table No. 03: Showing MIC of Haridradi varti extracts.

Bacteria	**MIC**
Serratia marcescens	510.46 µg/ml
Escherichia coli	363.74 µg/ml
Pseudomonas aeruginosa	759.37 µg/ml
Klebsiella pneumoniae	489.22 µg/ml
Bacillus cereus	509.34 µg/ml
Staphylococcus aureus	308.40 µg/ml

DISCUSSION

Equal quantity of fine powder of each drug was taken by passing it through sieve No. 120 and filtering again through clean white cloth. Whole plant of Bhringaraj was used for swarasa extraction. When swarasa was added to the mixture of drugs, it gets properly mixed and helps to bring the fine powder of mixture in contact with each other. Quantity of *swarasa* required for *bhavana* decreased with each successive *bhavana*. Wedelolactone present in *Bhringaraj* is proved for Anti-oxidant activity and Immunomodulator activity. *Bhringaraja* mainly acts on *Rasavaha srothas* and can be used as *Rasayana dravya* which means it helps to delay aging, prevent diseases and restoring energy.[9]

Churnakriya involves the levigation of juice/decoction of one drug to the other having similar attributes, which not only will yield a combined effect of all ingredients but can change the effect of the finished drug due to synergistic, antagonistic, or change in action or addition of new action.[10] This may be explained by collision theory. It states that when suitable particles of the reactant hit each other, the successful collisions contain activation energy at the moment of impact, to break the pre-existing bonds and form all new bonds which helps in formation of new compound. The medication enriched new properties when it contacts with a particular metal. Here iron and copper vessels are used for the trituration. Indication of specific metal container for mixing of specific drug, increases the potency by chemical reaction between the metal and the drug.

Extraction of medicinal drugs is a process of separating active plant material or secondary metabolites such as alkaloids, flavonoids, phenols, phytosterols, tannins, etc from inert or inactive material using an appropriate solvent.[11] Haridradi varti was prepared as per the reference of Chakradatta which is explained under Netrarogadhikara (diseases of the eyes). Extraction of Haridradi varti was carried out taking hydro-ethanolic solution (50:50) as the solvent media. Phytochemical screening of the extracts revealed the presence of phenols, tannins, phytosterols and flavonoids. Different studies reported the therapeutic potential of phytochemicals as:

Phytosterols regulate the membrane fluidity and the activity of membrane bound enzymes. They are active in reducing the proton and sodium leaks from cell membrane and reported to be anti-inflammatory and to enhance immune response.[12]

Polyphenolic compounds can help prevent photoreceptor cell damage caused by Reactive oxygen species and thus they have beneficial effects on visual function in retinal degenerative diseases.[13]

Flavonoids acts on various etiological factors responsible for the development of ocular diseases. In-vitro studies demonstrate that flavonoids interact directly with rhodopsin and modulate visual pigment function and that flavonoids protect retinal cells from oxidative stress induced cell-death.[14]

Haridradi varti extracts showed significant zone of inhibition in 500 µg/ml concentration than 250 µg/ml concentration against four gram-ve and two gram +ve bacteria. Maximum zone of inhibition (14.6 mm) was seen against P. aeruginosa and E.coli and least was seen against K.pneumoniae.

OD method was adopted to determine the minimum inhibitory concentration of *Haridradi varti* extract taken in concentration from 100 to 500 µg/ml against six different bacterial culture. It has shown excellent antibacterial activity against six strains of bacteria with lowest concentrations. *Haridradi varti* extracts showed the presence of Tannins which have been reported to have antimicrobial activity. The antibacterial effectiveness of tannins is explained by their ability to pass through the bacterial cell wall up to the internal membrane, interference with the metabolism of the cell, and as a result their destruction.

The primary study has yielded the positive outcomes, indicating that *Haridradi varti* extracts have significant antibacterial properties. This is an encouraging finding, suggesting the potential for the development of a new therapeutic agent using modern technology which can have a similar effect.

CONCLUSION

Antibiotic resistance is a complex and pressing global health issue. Addressing it requires a multifaceted approach through exploring new antibacterial agent and development of new practical and user-friendly forms using integrated system. In the present study affirmative results of *Haridradi varti* extract could help up for new possibilities for the treatment of infections to optimize the formulation and delivery of *Haridradi varti* extracts for better therapeutic outcomes.

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