



MODIFICATION OF HARIDRADI VARTI EXTRACT INTO EYE DROPS AND EVALUATION OF ITS IN VITRO ANTIBACTERIAL ACTIVITY

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ABSTRACT

Anjana, one of the *Kriyakalpa* explained in the Ayurvedic classics is indicated in various eye conditions. *Netra varti* in the form of *yavaakriti* (wick shaped) is utilized as *Anjana* by rubbing on a clean surface to make a paste and applied over the lower eyelids. As it possesses certain inconvenience while handling, application and storage of *Anjana varti*, an attempt has been made to modify and develop a new dosage form of eye drops where patient compliance, dose fixation, easy handling and storage could be achieved. *Haridradi varti* is a *netravarti* available in *Chakradatta* text 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. *Haridradi varti* is extensively indicated in various conditions of eyes and termed as *sarvanetramayapaham* (effective in all eye diseases). *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. Antibiotics serves the prime base for the treatment of bacterial infections. However, misuse and overuse of antibiotics have indeed led to the emergence of resistant strains of bacteria. Developing new antimicrobial agents with targeted drug delivery systems is a promising approach to address this challenge. Hence an attempt has been made to develop eye drops from *Haridradi varti* extract and evaluate its in-vitro antibacterial activity. The results showed promising antibacterial activity by assessment with zone of inhibition.

KEYWORDS: *Haridradi varti* extract, *Haridradi varti* eye drops, Antibacterial, Antibiotics.

INTRODUCTION

"*Netra Kriyakalpas*" refers to therapeutic procedures or formulations specifically designed for the treatment of the eyes (*Netra*).^[1] Ayurveda places great emphasis on maintaining the health of the eyes as a vital sense organ. *Netra Kriyakalpas* include various treatments and formulations aimed at promoting eye health, preventing eye disorders, and managing specific eye conditions. It includes procedures like *Aschyotana*, *Anjana*, *Seka*, *Tarpana*, *Putapaka*, *Pindi* and *Bidalaka* according to *Acharya Sharangadhar* which is indicated different conditions of the eye.^[2] Numerous formulations have been explained in the Ayurvedic classics for the treatment of different eye diseases, *Haridradi varti*^[3] is one such preparation mentioned in *Chakradatta* termed as *sarvanetramayapaham* (effective in all eye diseases). It contains *Haridra* (*Curcuma longa*), *Daruharidra* (*Berberis aristata*), *Haritaki* (*Terminalia chebula*),

Vibhitaki (*Terminalia bellerica*), *Amalaki* (*Emblca officinalis*), *Lodhra* (*Symplocos racemose*), *Yashtimadhu* (*Glycyrrhiza glabra*) and *Raktachandana* (*Pterocarpus santalinus*) in equal proportion. Fine powder of these drugs should be triturated with *Bhringaraj swarasa* (juice of *Eclipta alba*) in iron and copper vessel for 7 days each.

Abhishyanda is classified under *Sarvagata Netraroga* which affects eye in all ways. It is considered as root cause of almost all the eye diseases.^[4] It 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.^[5] Thus, there has been a continuing search for new and more potent antibiotics. As it possesses certain

inconvenience while handling, application, and storage of *Anjana varti*, and it may also might cause ocular irritation and less contact time. Hence, an attempt is made to modify and develop a new dosage form of *Haridradi varti* extract into eye drops where easy handling, storage, increased shelf-life could be achieved considering its in-vitro antibacterial activity.

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.^[6] It was then dried and stored in an air tight container. *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 extract

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°C to evaporate the solvent. Later, the concentrated liquid was placed in Hot air oven at 60°C till all the liquid portion evaporates and semisolid thick paste is obtained.

Development of Haridradi varti eye drops

Apparatus required- Analytical balance, beaker, spatula, glass rod, measuring cylinder, pH meter, Whatman's filter paper, conical funnel.

Chemicals required- Phenyl ethyl alcohol, boric acid, borax, sodium chloride, Benzalkonium chloride, Phenylmercuric nitrate.

Active Pharmaceutical Ingredient- *Haridradi varti* extract

Vehicle – Distilled water

Trial I – using Benzalkonium chloride as preservative

- 0.5 gm of *Haridradi varti* extract was dissolved in around 200 ml of distilled water with the help of glass rod. The pH of the solution was checked. (Range 4.179). Then 500 µl of phenyl ethyl alcohol was added. 300 ml of distilled water was added, mixed well and pH was checked. The buffering was done with Boric acid, Borax and NaCl to adjust the pH to 7 to 7.4 range. Preservative Benzalkonium chloride 200µl was added and the solution was stirred well.

- Solution was filtered through Whatman filter paper followed by pre-filtration in 2-20 µm filter was done. pH was checked – 7.278. Then sterile membrane filtration in 0.45 µm filter was done under sterile condition in the biosafety cabinet. After filtration sterile filling of the drops was carried out.

Trial II- using Phenylmercuric nitrate as preservative

- 0.5 gm of *Haridradi varti* extract was dissolved in around 350ml of distilled water.
- The pH of the solution was checked (Range 4.1). Then 500µl of phenyl ethyl alcohol and buffering agents like Boric acid, Borax and NaCl was added for 500 ml distilled water to adjust the pH to 7 to 7.4 range. Preservative 10 mg Phenyl Mercuric Nitrite was added and stirred well thoroughly.
- After that solution was filtered through Whatman filter paper followed by pre-filtration from 2-20 µm membrane filter. pH was checked (7.2278). Then sterile membrane filtration in 0.45 µm membrane filter was done under sterile condition in the biosafety cabinet. Then, sterile filling of the drops was carried out.

Evaluation of In-vitro antibacterial study for Haridradi varti Eye drops

- **Materials** – Mueller – Hinton Agar powder, Petri dish, earbuds, sterile bore, spirit lamp, Laminar air flow, incubator, micropipette
- **Bacteria** – Gram +ve bacteria- *Bacillus cereus*, *Staphylococcus aureus* Gram -ve bacteria- *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*.
- **Standard** – Amoxicillin - 100 µg/ml
- **Samples** – *Haridradi varti* eye drops (BKC) – 100 µl *Haridradi varti* eye drops (PMN) - 100 µl

Procedure

- **Culture preparation-** Suspensions of all the organisms were prepared as per Mac-Farland Nephelometer standard. Suspensions of organisms were 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:** 0.1 ml of inoculums was spread on the agar plate by spread plate technique. A sterile borer was used to prepare wells of 6 mm diameter in the agar media spread with the microorganisms. Accurately measured (0.1 ml) solution of each sample and standard samples was added to the wells with a micropipette. All the plates were kept in a refrigerator at 2 to 8 °C for a period of two hours for effective diffusion of test compounds and standards. Later, they were incubated at 37 °C for 24 hrs. The presence of definite zones of inhibition around the

well indicated antibacterial activity. The diameter of the zone of inhibition was measured and recorded.

RESULTS

Table 01: Showing results of zone of inhibition of Haridradi varti eye drops.

Bacteria	Zone of inhibition (mm)		
	Standard (100 μ l)	Haridradi Varti Eye Drops (BKC)	Haridradi Varti Eye Drops (PMN)
<i>Serratia marcescens</i>	25	0	17.3
<i>Escherichia coli</i>	25.3	0	16.3
<i>Pseudomonas aeruginosa</i>	25.6	11.3	16.3
<i>Klebsiella pneumoniae</i>	26.3	10.6	17
<i>Bacillus cereus</i>	27	10.3	17
<i>Staphylococcus aureus</i>	36.3	11.6	16.6

Analytical Study

Table 02: Showing analytical results of Haridradi varti eye drops.

Parameters	Haridradi Varti eye drops (BKC)	Haridradi Varti eye drops (PMN)
Specific gravity	1.007	1.008
Viscosity	1.046	1.033
Sterility test	Passed	Passed
Clarity test	Clear liquid	Clear liquid
pH	7.258	7.290

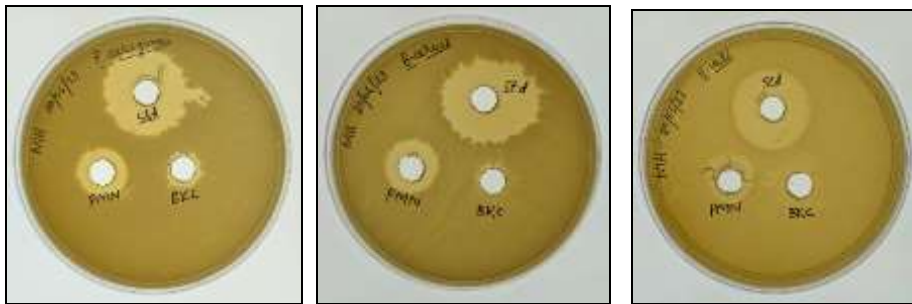
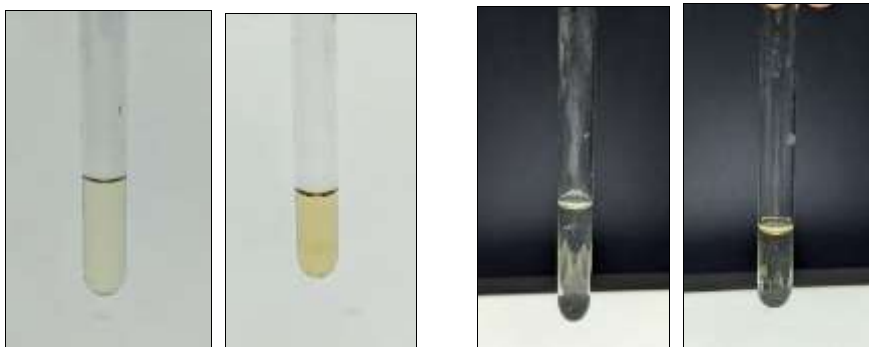


Fig. No. 01. Showing zone of inhibition of Haridradi varti eye drops.



Fig. 02: Showing Clarity test of Haridradi varti eye drops.



DISCUSSION

- *Haridradi varti* was prepared as per the reference of *Chakradatta* which is explained under *Netrarogadhikara* (diseases of the eyes). 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.^[7] Extraction of *Haridradi varti* was carried out taking hydro-ethanolic solution (50:50) as the solvent media. Phytoconstituents soluble in ethanol cannot be extracted by taking purely aqueous solvent, similarly phytoconstituents soluble in aqueous media cannot be extracted by taking purely ethanol solvent. Therefore, solvent was selected as 50:50 hydro-ethanolic media. And as this extract is proposed to be used for eye drops formulation it was decided to use this solvent.
- *Haridradi varti* extract used as active pharmaceutical ingredient for the development of eye drops with addition of other excipients and pH was checked in every step. Boric acid, Borax and Sodium Chloride was added to maintain the pH in the range of 7.0-7.4. Preservatives like Phenylethyl alcohol, Benzalkonium chloride and Phenyl Mercuric Nitrite was added for maintaining the sterility of the contents throughout its intended length of use. Addition of BKC preservative in the trial I resulted in some formation of precipitate during dissolution the contents and filtration. This may be due to some reaction between chloride and phytoconstituents of the API. So trial II was conducted with PMN preservative, where such precipitation was not observed.
- pH of *Haridradi varti* eye drops was 7.2 which is towards the normal physiological pH of the ocular surface which would not cause irritation to the eyes. Specific gravity of *Haridradi varti* eye drops (BKC) and (PMN) was 1.007 and 1.008 respectively. This shows that there is not much difference in specific gravity between the two eye drops. Viscosity of *Haridradi varti* eye drops (BKC) and (PMN) was 1.046 Pa s and 1.033 Pa s respectively. This indicates the low viscosity of eye drops as it flows easily in very little friction when in motion. *Haridradi varti* eye drops both BKC and PMN when examined under black and white background shows clear liquid. This shows that there is no particulate matter present in the solution.
- According to Indian Pharmacopoeia,^[8] the sterility testing for the *Haridradi varti* eye drops (both BKC and PMN) was carried out where membrane filtration method was adopted. The prepared ophthalmic *Haridradi varti* eye drops (both BKC and PMN) passed the sterility test as there was no turbidity appearance after the completion of 14 days. It can be ensured that the eye drop product is safe, effective, and free from harmful microorganism, toxins and particulates.
- *Haridradi varti* eye drops with BKC preservative in 100 µl concentration showed zone of inhibition 11.3, 10.6, 10.3 and 11.6 mm to 11.6 mm against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus cereus* *Staphylococcus aureus* respectively. But there was no inhibitory zone observed against *Serratia marcescens* and *Escherichia coli*. *Haridradi varti* eye drops with PMN preservative in 100 µl concentration showed significant zone of inhibition against all the bacteria. The maximum inhibitory zone of 17.3 mm against *Serratia marcescens* and least 16.3 mm against *Escherichia coli* and *Pseudomonas aeruginosa*. While formulation of *Haridradi varti* eye drops with BKC preservative there was some precipitate formed, which could have reacted with compounds of the extract and resulted in low zone of inhibition.
- Hence it can be inferred that *Haridradi varti* eye drops could be very useful antimicrobial agent against different strains of bacteria.

CONCLUSION

Haridradi varti is a *netra varti* indicated in many *netra rogas*. It is used as *Anjana* which is applied to the inner part of lower eyelid. As it possesses certain inconvenience while handling, application and storage of *Anjana varti*, an attempt has been made to modify and develop a new dosage form *Haridradi varti* eye drops, considering its anti-bacterial effect, where patient compliance, dose fixation, easy handling and storage could be achieved. With the factual evidence obtained by the experimental data, it has been concluded that *Haridradi varti* eye drops is an effective antibacterial drug.

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