IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 5, Issue 3, January 2025

A Review on Polyherbs for Eye Disease

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Abstract: *In this work we have formulated an herbal alternative for ophthalmic disorders. The number of ocular disorders is increasing daily due to rapid development of human although there are synthetic formulations available still the demand for healternative*
This demand for the development of formulation. We have used herbs like NeemAmla, Chakshushya, Mamira as their use is well known for reducing inflammation other issue .
The formulation was prepared in sterile condition and all the necessary practices were followed.Various pre-evaluation and post evaluation of the product were conducted as per the instructions provided by various literatures, ensuring safe and efficient formulation.
All the specifications were met although it's use for humans is still subject to further animal toxicology studies.

Keywords: Neem, Sterile, Formulation development, Evaluation of formulation, Stability studies

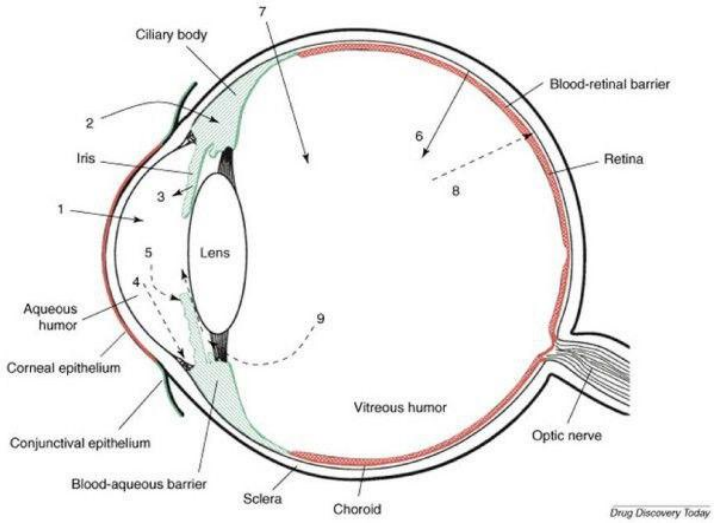
I. INTRODUCTION

Eye drops and ophthalmic products are the pharmaceutical products instilled in eye that are used to treat and prevent several ophthalmic disorders. Eyes are one of the main sensory organs of the living world. Even technologically so much advanced human too, depend on eyes for many of the chores.

The modern world is so much depended on screens and the number of screens is increasing daily, but the effects of same can be seen as people withophthalmic disorder is increasing and the effectiveness of ophthalmic product is promising in these cases.

The alternative of using herbal products in eye drops is one of the best substitutes as herbs are easy to cultivate and contain several active pharmaceutically important constituents. It is a challenging task to tackle the issue and maintain a certain specific std required.

Plants contain these ingredients in various parts and can treat several disorders. Too much of screen time can cause potential hazardous effects on eyes.



DOI: 10.48175/IJARSCT-23158

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Spending too many hours staring at a screen can cause eye to strain. One tends to blink less while staring at the blue light from a screen, and the movement on the screen makes one's eyes work harder to focus.
Many people typically do not position the screen at an ideal distance or angle, which can cause added strain.
All these issues add up and lead to lasting effect on vision especially in children. The pandemic and digitalization have shifted daily routine of people towards screens.
Even schools, official government offices and general meeting board has shifted their work to online and digital process. All these activities have increased the screen time for all type of age grouped people.

Pharmacokinetic considerations

The main routes of drug administration and elimination from the eye have been shown schematically in Figures 1

1. Transcorneal permeation from the lachrymal fluid into the anterior chamber
2. Noncorneal drug permeation across the conjunctiva and sclera into the anterior uvea
3. Drug distribution from the blood stream through blood-aqueous barrier into the anterior chamber,
4. Elimination of drug from the anterior chamber by the aqueous humor turnover to the trabecular meshwork and Sclemm's canal
5. Drug elimination from the aqueous humor into the systemic circulation across the blood-aqueous barrier
6. Drug distribution from the blood into the posterior eye acrossthe blood-retina barrier,
7. Intravitreal drug administration
8. Drug elimination from the vitreous via posterior route across the blood-retina barrier
9. Drug elimination from the vitreous via anterior route to the posterior chamber.

AIM AND OBJECTIVE

Aim

To develop and evaluate a polyherbal eye drop formulation combining NeemAmla ChakshushyaMamira extracts for effective management of ocular infections and inflammation

Objectives:

To formulate polyherbal eye drops using with optimal physical characteristics, pH, and viscosity.
To evaluate the antimicrobial efficacy of the formulated eye drops
To assess the anti-inflammatory activity of the polyherbal eye drops in vivo using the inflammation model.

MATERIALS AND METHODS

Some of the herbs used for formulation of our product are Neem, Amla, Chakshushya, Mamira the reason for using them is very simple as they all show some sort of beneficial properties to humans to reduce inflammation, kill microbes and avoid synthetic toxicities.
Neem also known as Margosa or Indian lilac is obtained from leaves, stem and other parts of the tree belonging to family meliaceae.
The active constituent of neem is azadirachtin which is complex terpenoid with insect repellant properties, the tree also shows antileprotic andantipruritic activities. Obtained by crushing the leaves and stems in mortar and using fine powder after passing it from
Amla also known as Indian Gooseberry obtained from plant emblica officinalis belonging to family Phyllanthaceae is rich in vitamin C and gives immunomodulatory, antioxidant and anti-inflammatory properties. Obtained by crushing the amla fruits and stem and passing it through the mesh
Chakshushya also known as Chinola obtained from seeds of cassia absus linn belonging to family caesalpinaceae, contain rutin and quercitin giving astringent and anti-inflammatory properties and used as collyrium to decrease ophthalmic disorders was obtained by crushing the seeds of plant and passing it through
Mamira also known as Golden thread herb obtained from leaves and aerial part of plant coptisteeta wall belonging to family ranunculaceae containing berberine, coptin and showing antimicrobial properties was also used, which was again obtained by crushing the leaves and stems of the plant and passing the powder to ensure same powder size. 0.9%

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NaCl was prepared and used as the vehicle to ensure isotonicity of the product and phosphate buffer was used to reduce the Ph changes, also rose water was used to provide essence to the product. Phenylethylalcohol was used as a preservative as the product contained herbs, so is prone to microbial contamination thus to avoid such deterioration it was used.

Neem

CommonN ame : Neem, Indian Lilac, Nimtreea Amla
Family: Meliac Common Name: Amla, Indian Gooseberry, Amalaki
Family: Euphorbiaceae
Part Used: Fruit, Leaves.

Chakshushya

Mamira:

Common Name:Indian Senna, Chakshushya
Common Name: Curry Leaf, Sweet Neem
Family: Fabaceae Leguminosae
Family: Rutaceae
Part Used: Leaves
Part Used: Leaves, Roots

EXPERIMETAL WORK

Selection of herbs More than 59 herbs show actions useful in the treatment of opthalmic disorders but there are only a few suggested by the literature that are beneficial and compatible with different excipients to be used. It was an interesting yet challenging task to select the ingredients of best therapeutic effect and compatible parameters. Thus Neem, Amla Mamira and Chakshushya were selected

Selection and Procurement of ingredients Raw ingredients viz. Neem powder, Amla powder, Chakshushya powder, and Mamira powder was procured. The identity was confirmed with compliance of microscopic,macroscopic parameters of Ayurvedic pharmacopoeia of India (API) through pharmacognostic studies. The purity and strength were also confirmed through physicochemical studies done as per various official literature

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Pharmacognostic and Phytochemical evaluation of herbs

Extraction

The method employed was Maceration method, the drugs were allowed to be soaked in 250 ml distilled water for overnight and then they were extracted using methanol or water via distillation apparatus.

Evaluation by preliminary tests

Preliminary tests for primary constituents

Tests for carbohydrates

For reducing sugars

Fehling's test: - Mix 1 ml Fehling A and Fehling B solution, boil for 1 min. add equal volume of test solution. Heat in boiling water bath for 5-10 min. First yellow, then brick red ppt is observed.

Benedict's test: - Mix equal volume of Barfoed's reagent and test solution. Heat for 1-2 min. in boiling water bath and cool. Red ppt is observed.

For non- reducing sugar

Test solution does not give response to Fehling's and Benedict's test.

Hydrolyse test solution. Fehling's and Benedict's test are negative.

Tests for proteins

Biuret test: - To 3 ml test solution add 4 % NaOH and few drops of 1% $CuSO_4$ solution. Violet or pink color appear.

Million's test: - Mix 3 ml test solution with 5 ml Million's reagent. white and warm ppt turns brick red or the ppt dissolves giving red colored solution.

Tests for amino acids

Ninhydrin test: - Heat 3 ml extract, add 3 drops 5% Ninhydrin solution in boiling water bath for 10 min. purple or bluish color appears.

Preliminary tests for secondary metabolites

Tests for alkaloids

Dragendroff test: - To 2-3 ml filtrate add 2-3 drops of Dragendroff reagent (Potassium bismuth iodide solution).

Hager's test: - To 2-3 ml of filtrate add few drops of Hager's reagent (sodium picrate solution)

Wagner test: - To 2-3 ml of filtrate add 2-3 drops of Wagner's reagent (potassium mercuric iodide solution).[7,9,13]

Tests for tannins

Take 2-3 ml of extract and add few drops of 5% ferric chloride solution

Take 2-3 ml of extract and add a few drops of lead acetate solution.

Take 2-3 ml of extract and add a few drops of potassium permanganate solution.

Preliminary tests for flavonoids

Take 2-3 ml of sample and add dilute sulphuric acid solution.

Take 2-3 ml of sample and add few drops of Sodium hydroxide sample.

Take 2-3 ml of sample and add a few drops of lead acetate solution.

Thin layer chromatography

Thin layer chromatography of the herbs was done to check the quality and standard of the drugs and to identify the active constituents in the herbs.

Sample preparation: - The powdered drugs were allowed to soak in water overnight and after 24 hours extraction was done using methanol. The extract was then filtered and then 1 ml of sample was taken, and methanol was added to make it up to 10 ml.

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Preparation of stationary phase: - TLC plates were taken, Silica gel G was dissolved and poured on the plate then the plate was dried in oven at 105 oC for 30 minutes

Mobile phase preparation: - Toluene: ethyl acetate: Diethylamine was took in the ratio (7:2:1) for alkaloid and Toluene: acetone: Formic acid (4.5:4.5: 1) was took for tannins.

Evaluation by physico-chemical tests

Determination of ash value

Weigh and ignite flat, thin porcelain dish or silica crucible

Take 2 gm of sample in it weigh it again.

Keep it on tripod stand and ignite it at 2 cm height and keep the dish 7cm above the flame, heat till all vapours have evolved and all the carbon is burnt off. cool it in dessicator and weigh again.

Then ash value is determined using the formula

$$\%\text{ash} = \frac{wt\ of\ crucible\ with\ ash\ - wt\ of\ empty\ crucible A}{wt\ of\ sample} \times 100$$

The % ash value found must be under standard values given in the literature if not so then it is concluded that the product or herb is of substandard category.

Determination of acid insoluble ash

The ash is dissolved in 25 ml dilute hydrochloric acid.

Then it is filtered through ashless filter paper and thoroughly washed with water.

The filter paper is then ignited in the original dish, cooled in a dessicator and then weighed.

Then determine the acid insoluble ash using the formula

$$\%\ acid\ insoluble\ ash\ \frac{m2\text{-}m1}{m1\text{-}m} \times 100$$

Where,

m2 = lowest mass in gm of dish with acid insoluble ash

m1 = Mass in gm of empty dish

m = Mass in gm of dish with dried material

Determination of water-soluble ash

Total ash + add 10 ml water and boil for 5 minutes.

Filter through ashless filter paper and ignite it at t 450 oC

Cool it and weigh again.

$$\%water\ soluble\ ash = \frac{wt\ of\ water\ soluble\ ash}{Wt\ of\ sample} \times 100$$

Formulation and Development of herbal eye drop

Three different batches were prepared for the formulation containing different concentration of the ingredients.

Table no. 1: Formulation batches.

Ingredients	Formulation 1-F1 Conc.in.gm	Formulation 2-F2 Conc.in.gm	Formulation 3 - F3 Conc.in.gm
Neem	2.8	2.4	3
Amla	2.4	2	2.8
Mamira	2.4	1.8	2.2
Chakshushya	2	1.6	2.4

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Formulation development

The step wise development of eye drops encompasses the preparation of distillate, making of the distillate isotonic to lacrimal fluid and adjustment of pH, addition of preservative and packing under sterile conditions.

All coarsely powdered drugs were soaked in rose water for overnight in a beaker.

The mixture was refluxed at 60 oC for a span of 3 hours

The mixture was then cooled, filtered and Alum was added, and solution was filtered again. The resulting filtrate was collected

And then transferred to a distillation unit. Distillate was obtained by adjusting the temperature to 40 oC for 15 minutes and raising the temperature slowly to 80 oC. The first 450 ml. of distillate was collected at the rate of 20 drops per minutes in an airtight container.

0.9 % NaCl was selected as vehicle and 2.5% of extract was added.

The distillate was made isotonic to lacrimal fluid by adding 0.9% NaCl to distillate and dissolving properly and adding isotonic phosphate buffer viz.0.16 gm of monobasic Sodium phosphate and 0.76 gm of dibasic Sodium phosphate.

Finally, the pH of the eye drops was adjusted to 6.9-7.30. Phenyl ethyl alcohol was added as preservative and pH was again checked and found within the specified range of the ophthalmic drops (pH 6.9-7.30).

Test for sterility was performed after addition of preservative the preparation was observed for 48 hours and found sterile.

The packing was made in autoclaved sterilized amber glass containers of 10 ml capacity.

The finished product was tested for quality assurance and safety and the analytical specifications complied specified parameters of Indian pharmacopeia for ophthalmic preparations.

Evaluation of finished product

Various evaluation tests were performed on the product as suggested by the literature using proper standards given in specified condition so as the product obtained was sterile and estimated to be beneficiary to the consumer

Organoleptic properties

Color

Odor

Appearance

Texture.

Physico-chemical parameters of finished product

Determination of PH[14]

The pH meter was calibrated using acetate and phosphate buffer.

First the acetate buffer was dissolved having pH around 3.6 and checked under the equipment if not found then knob was adjusted

Then it was cleaned using distilled water.

Then phosphate buffer was dissolved in water and pH of 6.8 was obtained

Then the indicator electrode was cleaned using distilled water.

Then the machine was calibrated and then product was checked.

Determination of density[13,14]

10 ml density bottle was taken and the weigh balance was calibrated.

Empty bottle was weighed(w1)

Then product was taken, and its weight was determined (w2)

Then weight of product was determined

Then density was determined by formula

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Determination of viscosity[13,14]

Ostwald viscometer was used, clean viscometer was mounted in vertical position
Water was filled up to G mark, time required to flow from A mark to B mark wascounted in seconds.
Same process was repeated 3 times
Viscometer was rinsed and product was filled.
Then viscosity was determined by formula.

$$n2 = \frac{p2t2}{p1t1} \times 100$$

Where,
n1 = viscosity of std liquid
p1 = Density of std liquid
t1 = Time required by std liquid
n2 = Viscosity of test liquid
p2 = Density of test liquid
t2 = time required by test liquid.

Determination of Surface tension[13,14]

Stalagmometer and drop count method was used for determination of surface tension.
Clean the stalagmometer and mount it in vertical position.
Fill with water up to mark A, drops were counted till it reached mark B.
Then it was filled with sample and surface tension was determined using formula

$$r2 = \frac{p2n1}{p1n2} \times r1$$

Where,
n1 = Number of drops of std liquid
n2 = number of drops of test liquid
p1 = density of std liquid
p2 = density of test liquid
r1 = surface tension of std liquid
r2 = surface tension of test liquid.

Isotonicity

The product was checked for isotonicity on Red Blood Cells.
The finger was pricked, and sample was collected. Blood sample was mixed with rbc fluid and observed under microscope for shape and size.
Then formulation was added in blood sample and observed for shrinkage or swelling of the cells.

Sterility testing[13,14

The product was made sterile in autoclave at 121 oC and 115 psig for 15 mins.
The product was filtered using membrane filter and the membrane was cut in 2 halves and direct inoculation was done for presence of any microbial growth.
The product was observed under microscope for growth of any viable bacteria after 48 hrs in incubation

Stability testing[13,14]

Stability tests were performed after 1 month under ICH guidelines for ophthalmic preparation
Various tests including physico-chemical tests, organoleptic property screening, and UV spectrometry studies were conducted on the formulation to study the stability characteristics

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Physico-chemical tests

Ash values

Various ash values were determined such as ash values, Acid Insoluble ash, water soluble ash according to the literature and results are depicted in the table.

For neem

Table: Different ash values for neem

Test	Obtained	Standard
Total ash	9.7 % ± 0.2	Not more than 12 %
Acid insoluble ash	2.22 % ± 0.01	Not more than 2 %
Water soluble ash	17 % ± 0.5	Not more than 18.12 %

For Amla

Table: Different ash values for amla

Test	Obtained	Standard
Total ash	4.3 % ± 0.3 %	Not more than 5%
Acid insoluble ash	1.25 % ± 0.03 %	Not more than 1 %
Water soluble ash	2.8 % ± 0.2 %	Not more than 3.01 %

II. CONCLUSION

In present research work eye drops were formulated from herbs like neem and amla shows promising action in reduction of strain and inflammation of eyes. The eye drops were prepared and evaluated as per various literature sources available.

The herbal extracts were obtained and tested for their purity and strength ensuring good and safe product formulation. Safe and effective concentration was determined for the herbs by batch production. Different tests for constituents were performed showing promising results. As per the recommendation from various literature the drops were made sterile and isotonic to support the formulation as well as treat the disorder.

All the specifications provided were tried to be met to gain as much benefit as possible from the product. It is estimated to show beneficial health effects on eyes.

The product formulation F1 is concluded to be safe and effective as various standards were met during the preparation of the product. It can be concluded from the results that the formulation prepared is of standard quality with least possible deterioration and hazard.

The product was checked for stability and sterility to avoid any risk or chance factor. Although it is subjected to further studies for its use in humans.

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