Preparation and \textit{Invivo} Screening of Eye Wash Suspension

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\textbf{ABSTRACT}

Human eyes are precious organs in the body that enable us to see and perceive things. The eyes secrete a natural lubricant called eye tears. This was produced to keep away the exposed part of the eye from microorganisms and other diseases causing microbes and irritants. The eyes are the most sensitive and affected parts when there is a slight change in the irritation level and allergies. Taking this into consideration, the extracts of the plants like Turmeric and Tulsi have been prepared into a suspension that can be used in the eye for its antimicrobial activity and also give the eyes that cooling and lubricating effect. The eye washes that were prepared were investigated for the antibacterial activity and were found effective against the three tested bacteria. They were also proven safe and non-irritant to the eyes.

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\textbf{INTRODUCTION}

Human eyes are precious organs in the body that enable us to see and perceive things. The eyes secrete a natural lubricant called eye tears. This was produced to keep away the exposed part of the eye from microorganisms and other diseases causing microbes and irritants. The eyes are the most sensitive and affected parts when there are a slight change in the irritation level and allergies [1]. The microorganisms sometimes attack the eyes and cause ocular infections due to which the eyes become swollen, red and irritated. There are synthetic drugs that are used to treat eye infections, but they are toxic to the body in various ways. The herbs are being in the usage for the ophthalmic uses like to treat the ocular infections and other diseases of inflammation to the eye [2].

Some herbs like Neem, Henna, Turmeric have many properties that are investigated for the antibacterial properties and are termed as powerful antimicrobial agents. The list was also added with the commonly available plants like coriander, mint and ginger [3–5]. There are few essential oils containing drugs that have the above properties like the lavender oil, lemon oil, citronella oil which have been proved for their antibacterial activities [6]. Taking this into consideration, the extracts of the plants like Turmeric and Tulsi have been prepared into a suspension that can be used in the eye for its antimicrobial activity and also give the eyes that cooling and lubricating effect.

\textbf{PREPARING THE OPHTHALMIC SUSPENSION}

\textbf{Herbs and extraction}

Fresh leaves of Tulsi were taken and dried under shade for about four days, and after drying, the leaves were powdered and then the powder was passed through a sieve number 40. The powder was then used for extraction using distilled water, and the macereration method was used for extraction. The macerate was collected and evaporated to drying using an evaporator; and this paste-like extract was suspended in water at the concentration of 200mg 100 ml of the water. The similar process was carried out for turmeric also. The rhizomes of turmeric
were subjected to the extraction using double distilled water and so made into a suspension of the final concentration of 2mg/ml.

**Formulation of the Eyewash**

The eyewash was prepared by simply suspending the extract solutions into the saline solution. 0.8% of the NaCl solution in distilled water was prepared, and then the extracts solution was measured to quantities that are given in the table and then suspended in the saline solution. This was ultrasonicated for about 2hrs and filtered for the undissolved or visible particles. This clear suspension was then subjected to sterilization using an autoclave. Then the solution was cooled and stored at 4°C for future use.

**Evaluation of the prepared eyewash**

**Physical parameters**

The eyewash that is prepared was evaluated for its pH, Stability and eye irritation test. The solution was examined for the colour, clarity and odour. The pH was analyzed using a digital pH meter, and the suspensions were subjected to the freezing at 2°C for 14 days and thawing at 45°C for 14 days and kept at normal room temperature for 14 days to study the stability and sedimentation or any instabilities under stress conditions.

The eye irritation test was carried out on human beings by selecting 3 healthy human volunteers and by washing the eyes properly with fresh water to make sure the eyes are properly cleaned. They don’t have any dirt or particles that can irritate. Now two drops of the prepared wash were poured into the left eye of the volunteers and waited for 5mins. The eyes are checked for any signs of inflammation or allergy.

**Bacterial Assay**

The prepared solutions were tested for the antibacterial activity in the disc diffusion method. The test solutions were named accordingly and then segregated. The Petri plates were sterilized, and then the freshly prepared agar culture medium was prepared and sterilized using an autoclave. The medium was let in the Petri plates for solidification and after solid Petri plate was achieved with the agar solution, the discs with filter paper were cut using a sterile scissor and are dipped in the eyewash solutions to soak up. The prepared culture plates were inoculated using the freshly prepared cultures of Streptococci, E.coli and Staphylococcus. The discs were then placed on to the plates and incubated for about one day in an oven at 35°C. Then after the next day, the Petri plates were taken, and the zone of inhibition that was achieved was measured using a scale, and it was compared with standard suspension [7].

**RESULTS**

The eye washes were prepared using the herbal extracts of Tulsi and Turmeric. The herbal eye washes were observed for the physical parameters. These prepared suspensions were clear without any visible particles; the colour was clear and didn’t have any flavour or colour. The suspensions were named as EW-Tulsi and EW-Turmeric. The eyes washes were safe and did not produce any signs of irritation to the eyes. The eyes were observed after 5mins, and there was no redness or excessive tears or any other symptoms that suggest the irritation.

The pH values were determined and are tabulated. The stability studies proved that the prepared eyeboluses were safe and stable at any temperature. They tolerated the stress caused by the temperature change, and there were no instabilities like sedimentation and any visible particles. Tables 1, 2 and 3

The eye washes were subjected to the antibacterial assay using the disc diffusion method. There was a clear indication of the differences in the zones of inhibition of the bacteria. The prepared suspensions showed a better activity compared to the standard marketed eye suspension. The eye suspension that is prepared using the tulsi showed a better activity compared to the suspension that is prepared using turmeric extract. Figure 1

| S.No | Ingredients          | EW-Tulsi | EW-Turmeric |
|------|----------------------|----------|-------------|
| 1    | Tulsi Extract 2mg/ml  | 2mg/ml   | –           |
| 2    | Turmeric Extract     | –        | 2mg/ml      |
| 3    | Saline solution      | 100ml    | 100ml       |

**Table 1: Preparation of the Eyewash suspension**

**Figure 1: Anti-bacterial activity of Eye Suspensions**

This difference in the activity might be because herbal extracts contained many chemical con-
Table 2: The Antibacterial Property of the Prepared Eyewash suspension

| Organism       | Zone of inhibition (mm) |
|---------------|-------------------------|
|               | EW-Tusli | EW-Turmeric | Control solution | Marketed eye suspension |
| Staphylococcus | 36±0.9   | 29±0.47     | 15±0.21          | 23±0.51                |
| E. coli       | 31±0.42  | 27±0.13     | 12±0.10          | 16±0.25                |
| Streptococcus | 24±0.21  | 22±0.16     | 2±0.08           | 13±0.34                |

Table 3: Testing for the Physical Parameters of the Suspension

| Formulation | Day | Temperature °C | pH  |
|-------------|-----|---------------|-----|
| GNF         | 1   | 40            | 7.45|
|             |     | 250           | 7.46|
|             |     | 450           | 7.45|
|             | 7   | 40            | 7.47|
|             |     | 250           | 7.45|
|             |     | 450           | 7.44|
|             | 14  | 40            | 7.47|
|             |     | 250           | 7.46|
|             |     | 450           | 7.48|
| GAF         | 1   | 40            | 7.46|
|             |     | 250           | 7.45|
|             |     | 450           | 7.44|
|             | 7   | 40            | 7.47|
|             |     | 250           | 7.46|
|             |     | 450           | 7.48|
|             | 14  | 40            | 7.49|
|             |     | 250           | 7.48|
|             |     | 450           | 7.48|

CONCLUSION

The eye washes that were prepared were investigated for the antibacterial activity and were found effective against the three tested bacteria. They were also proven safe and non-irritant to the eyes.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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