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Inhibitory Effect of an Ayurvedic Eye Drop against Staphylococcus aureus and Candida albicans; an Antimicrobial Efficacy Study

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ABSTRACT:

Background

Ayurvediceyedropskeeps retains eye healthy, clean, and clear. It helps ameliorate eyesight, and has antimicrobial and anti-inflammatory properties which soothe redness, irritation and dryness in eyes.

Objectives

To check the antimicrobial efficiency of an Ayurvedic eye drop on S. aureus(ATCC 29213) and C. albicans(ATCC 10231).

Materials and Methods

The antimicrobial effect of anAyurvedic eye dropwas observed against ATCC S. aureus and ATCC Candida albicans by broth dilution method and logarithmic reduction of the growth was calculated.

Result

The anti-microbial efficacy of an Ayurvedic eye drop against S.Aureusshowed 5 log reduction at 30 minutes comparedto Candida albicans.

Conclusion: The tested Ayurvedic eye drop has shown good antimicrobial activity against S. aureus compared to that of Candida albicans. This study would help the medical practitioners and users to be aware of how the eye drop solutions are susceptible to the growth of microorganisms and also to know the efficacy of some of the Ayurvedic eye drops.

Keywords: S. aureus, Candida albicans, Ayurvedic eye drop, Broth dilution, log reduction.

I. INTRODUCTION

Infections associatedwiththe eye are oftenseenin clinical practice. Hence the ocular formulations are prepared in favour to eliminate inflammatory and allergic conditions of the eyes. These formulations must be efficacious in impeding the growth of pathogens and protecting its users from infections [1][2].

Microbial infections such as microbial keratitis, scleritis, orbital cellulitis, endophthalmitis are caused by microorganisms such as Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans, Aspergillus species and so on[1]. Such infections can be treated with a variety of medications including topical antihistamines, mast cell stabilizers, corticosteroid and surgical intervention may also be indicated in severe cases. All these medications do not have any outcome on basic pathogenesis; they provide only symptomatic comfort and might result in delayed wound healing, secondary infection and formation of cataract [3].

Hence,Ayurvedic herbal eye drops are said to be potent, safe and cost-effective.Ayurvedic herbal formulations are prepared by single or multiple plant ingredients. The products available in the markets can be different in their quality and therapeutic efficacy due to lack of standards of formulation and differences in composition of the product, methods of manufacturing and also their storage methods. The objective of this study is to evaluate the anti-bacterial and anti-fungal activity of Ayurvedic eye dropagainst American Type Culture Collection(ATCC)microbial strains.

The composition (each 10ml) of the Ayurvedic eye drop are;

- Juice of allium-cepa(onion)-1.68ml
- Juice of Zinger officinale (Ginger)-1.66ml
- Juice of citrus aurantifolia(Key Lime)-1.66ml
- Honey-5.00ml

Preservative used for this solution are Benzalkonium Chloride solution 0.1% v/v.

II. MATERIALS AND METHOD

Ayurvediceye drop, commercially available in the market were tested for their antimicrobial effect in the microbiology lab at Padmashree Institute of Medical Laboratory Technology,Bangalore. It is a prospective, experimental study. Standard strains of Staphylococcus aureus ATCC 29213 and C. albicans (ATCC 10231) were used for this test.

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ATCC 29213 strainof Staphylococcus aureuswhich was sub cultured on a Nutrient agar was inoculated into a nutrient broth and incubated at 37ºc for 12 hours.ATCC 10231strains of C.albicanswhichwassub cultured on a Sabouraud's dextrose agar was inoculated into the Brain Heart Infusion broth and incubated at 25ºc for 48 hours.

Eye drop was transferred aseptically into small beaker. To determine the microbial killing rate, the eye drop was inoculated with challenging microorganisms at a final concentration of 10^5-10^6 CFU ml^{-1} and the viable organisms were determined 30, 90 and 180 min after inoculationfor bacteria and 24 hrs for fungi.

The 0.1 ml and 0.5ml of the challenging organisms was inoculated into 9.9ml and 4.95 ml of eye drops in a two different test tubes. From each test tubes 1ml of suspension was transferred to 9 ml of thioglycollate broth taken in two different test tubes to neutralize the preservative in 1:10 dilution and incubated at 37ºc. After incubation period, 1 ml of each bacterial suspension was poured onto the Nutrient agar plate and wasspread by using **'L'** shaped glass spreader at 0 minute, 30 minute, 90 minute and 180 minute time interval and incubated at 37ºc for 24 hours to determine the number of viable organisms on each plate [1][5][6].

For Candida albicans same procedure was followed with the Sabouraud's Dextrose Agar and the time intervals were 0 minute, 30 minutes, 90 minutes, 180 minutes and 24 hours. Plates were incubated at 37ºc for 48 hours to determine the number of viable organisms on each plate [5].For control,0.1 ml suspension ofS. aureus was inoculated to 9.9ml ml of Nutrient broth and directly poured into the Nutrient agar at 0, 30, 90 and 180 minutes time interval and spread by using glass spreader, incubated at 37ºc for 12 hours and 0.1ml suspension of Candida albicans was inoculated to 9.9 ml of Brain heart infusion broth and directly poured into the Sabouraud'sDextrose agar plate at 0minute, 30 minutes,90 minutes and 180 minutes and 24 hours interval and incubatedfor 48 hours at 25ºc.

The determination of the Logarithmic reduction of the growth in the selected Ayurvedic solution was calculated by following equation:
Log reduction=log10 (initial CFU/ml)-log10 (final CFU/ml) [1].

III. RESULT

The present study involves antimicrobial efficacy of anAyurvediceye-drop against ATCC Staphylococcus aureus and ATCC Candida albicans.
The efficacy of an Ayurvedic eye drop with challenged microorganisms is depicted inthe**Table 1 & 2.**
The initial microbial load for the reference organisms in Ayurvedic eye drop was 1x 10^5 cfu/mlforS. aureusand2.5 × 10^4 cfu/ml for C. albicans.
Ayurvedic Eye drop exhibited bactericidal and fungicidal activities at a rate which depended on the species of pathogens and the constituents of the eye drop.
S. aureusshowed 5 log reduction at 30minutes whereasin Candidaalbicans1 log reduction was seen after 24 hrs.
The mean logarithmic reduction in the minimum recommended disinfection timefor eye drop against the Candida albicansstandard strain is shown in **Table 3,Graph 1 &2.**
This shows that the chosen Ayurvedic eye drop was much effective towards S. aureus compared to C. albicans.

Time	**0.1 ml**	**0.05 ml**
0 minute	100,000	100,000
30 minutes	No growth	No growth
90 minutes	No growth	No growth
180 minutes	No growth	No growth

Table 1: Colony counts obtained in the solutions at different time intervals for Staphylococcus aureus.

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Time	0.1 ml	0.05 ml
0 minute	250,000	250,000
30 minutes	100,000	100,000
90 minutes	100,000	100,000
180 minutes	5,000	3,000
24 hours	350	1,340

Table 2: Colony counts obtained in the solutions at different time intervals for Candida albicans

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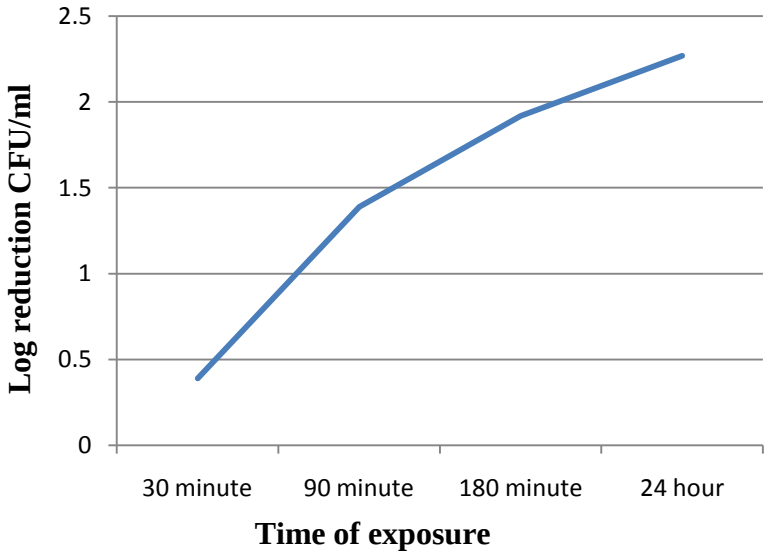
Time	0.1 ml	0.05 ml
30 minute	0.39	0.39
90 minute	1.39	1.39
180 minute	1.7	1.92
24 hour	2.85	2.27

Table 3: Log reduction of the solution per time interval for Candida albicans



GRAPH 1: Effects of 0.1 ml of Ayurvedic eye drop solution and length of exposure on the concentration of *Candida albicans.*

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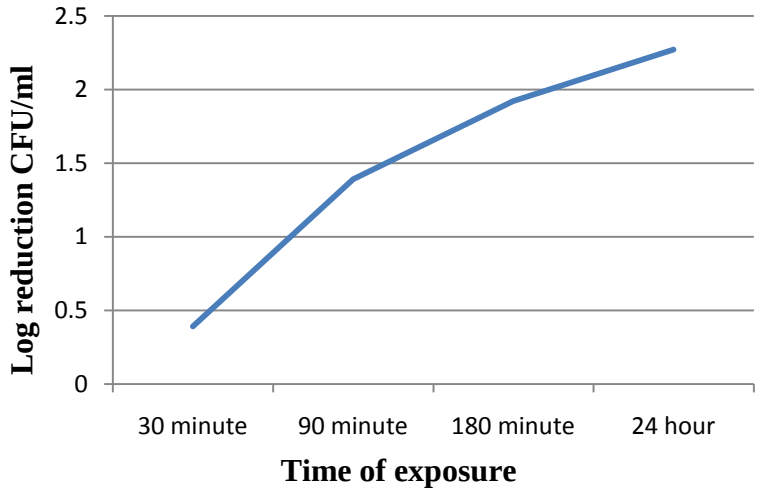

GRAPH 2: Effects of 0.05 ml of Ayurvedic eye drop solution and length of exposure on the concentration of *Candida albicans.*

IV. DISCUSSION

The single ruling factorto check the quality of all ophthalmic products is specification on its efficacy on microbes and sterility. Despite this prerequisite, researches have not been done much on theantimicrobial efficacy of commercially available eye drops. Nearly all reported studies have concentrated on patient-used preparations and microbial contaminations in these preparations [7][8]. Not many studies have been done on the efficacy of the ayurvedic eye drops against microbes.

Staphylococcus aureus and Candida albicans are the microorganism which frequently causes eye infections.

From the antimicrobial challenge test carried out using Ayurvedicherbal eyedrop, it was observed that the anti-microbial activity againstS. aureusandC. Albicanswas seen at 30minute. In which the microbial load of S. aureus was reduced to 0 at 30 minutes where as in C. Albicansthere was no complete reduction of growth until 24 hours. Accordingly in a study conducted by Ezekiel O Aet al, the microbial load of S. aureus was reduced to 0 within 1 h of inoculation of gentamicin eye drop, whereas the microbial load of C. albicans was similarly reduced to 0 within 2 h, this suggests the use of Ayurvedic eye drop over Chemical eye drop is much efficient in the case of S. aureus than C. albicans. Though gentamicin being a broad spectrum antibiotic it showed less bactericidal activity compared to Ayurvedic eye drop. [6]

According to a study conducted by Thakur et al, the herbal eye drop (Itone) was effective against bacterial strains such as S. aureus, E. coli and Listeria, whereas it was less effective againstC. albicans which is same as the present study [2]. The same findings were noted in the study done by Premanth S, et al[4].The homology inthese studies is the presence of similar herbal components. According to the researchers, these components possess outstanding antibacterial action [9].

In the present study the preservative used was benzalkonium chloride which is in line withthe study conducted by Gerard R J et al,states that the use benzalkonium chloride showed good efficacy against the bacteria and fungi when compared to the other preservatives used [10].

Kusumaet al, mentioned that the use of chloramphenicol eye drop withbenzalkonium chlorideimproved the antibacterial effect of chloramphenicol instead of when used alone,the efficacy decreased sharply over the storage time[11]. This shows the synergistic effect between essential components and the preservatives in inhibiting the growth of microbes. It also suggests the use of preservatives to avoid contamination with the microorganisms.

Hence, the present study underscores the use of an Ayurvedic eye drops over chemical based for the treatment of bacterial infections. The various studies have proved thatAyurvedic herbal eye drops have a useful role in a variety of infective, inflammatory and degenerative

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ophthalmic disorders and also observed no side effects on humans and experimental animals[12][13]. Since the antifungal activity of the Ayurvedic eye drop did not show remarkable effect compared to that of antibacterial activity. Hence, there is an urgent need to look for either strong herbal components or the combination of the different herbal and chemical components to form a single effective antifungal formulation.

V. CONCLUSION

The investigated Ayurvedic eye drop demonstrated ability to effectively inhibit the growth of S. aureus compared to C.albicans. These findings would help the clinicians as well as patients to use Ayurvedic Eye drop solution to reduce the chances of eye infections.The present study does not assess the efficacy of Ayurvedic eye drops in clinical isolates; hence, discrepancy may be noted and a sole study cannot determine the quality of an Ayurvediceye drop. Hence further studies should be conducted. It can therefore be further concluded that the effectiveness of the Ayurvediceye drops depends on the efficacy of the preservatives and its intrinsic antimicrobial activity.

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CONFLICT OF INTERESTS

All the authors have contributed equally. The authors of this study have no financial interest nor received any financial support from the company that manufactures Ayurvedic eye drop solution.

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