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In vitro evaluation of indigenous formulation of *Blepharis maderaspatensis* (L.) B. Heyne ex Roth in the treatment of acute conjunctivitis

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Abstract
Ethanobotanical studies have revealed that eye drops prepared from *Blepharis maderaspatensis* (L.) B. Heyne ex Roth have been used for treating eye diseases. It was decided to test for the efficacy of both the hydro alcoholic extract as well as indigenous eye drop formulations in three different concentrations on the bacterial smears of acute conjunctivitis. The extracts of the concentration of 250,500,1000 ppm were prepared and evaluated for antibacterial activity. The indigenous formula using 200g of dry powdered plant drug powder for 400ml (0.5g/ml) of sesame oil did not show a significant anti microbial activity. So doses of 1g /ml and 1.5g/ml were prepared and evaluated. It was seen that the eye drops containing the second dose did show some activity but were not comparable to the standard .The eye drops of the next higher dose showed activity comparable to that of the standard drug .The extracts showed comparable activity at the concentration of 1000µg/ml. So, thereby authenticating the concept of use of eye drops of *Blepharis maderaspatensis* (L.) B. Heyne ex Roth for eye diseases like acute bacterial conjunctivitis.

Keywords: *Blepharis maderaspatensis* (L.) Heyne ex Roth, eye disease, acute bacterial conjunctivitis, antimicrobial activity, indigenous formulation

1. Introduction
The increasing incidence of antimicrobial resistance to current day antibiotics due to misuse has opened avenues for search of new antimicrobials. So new plant products can be searched out from literature [1] survey to confirm practical evidences of antibacterial activities. *Blepharis maderaspatensis* (L) B. Heyne ex Roth has been repeatedly reported in many ethanaobotanical [2-5] studies to be used as a potential medicinal herb which possess many curative activities. Since the reports by shivakami *et al* have shown that ethanolic extracts of *Blepharis maderaspatensis* (L.) B. Heyne ex Roth have antimicrobial activity it was decided to take up the study to confirm the utility of indigenous formulation against the bacterial smear of acute conjuctivitis.

2. Methodology
The leaves of *Blepharis maderaspatensis* (L.) B. Heyne ex Roth were collected from the medicinal herbal gardens of Anugraha Herbals, Kurupanthara, Kottayam. The plant was authenticated by Prof. Rojimon Thomas, CMS College kottayam, and the voucher specimen stored in the Pharmacognosy laboratory in Department of Pharmaceutical Sciences, Cheruvandoor Campus, M G University. The leaves were washed and dried in shade and powdered using a grinder to obtain the coarse powder of about one kilogram. The hydro alcoholic mixture was prepared by using a mixture of alcohol: water mixture (50:50) with intermittent shaking of the macerated product at every 8 hour interval. The extract was filtered, dried by using rotary vacuum filtration techniques after cold maceration for 72hrs. The final extract weighed about 280 grams and it was slightly sticky in nature. The anti-bacterial studies were carried out in both the extract as well as the indigenous formulation mentioned in the study. The stock solution was prepared by dissolving 100mg of extract in 100ml of ethanol, from which different concentrations of test solutions, 250, 500 and 1000 PPM were prepared and used for antibacterial assay by Disc diffusion method [6]. The indigenous formulation was prepared as per the journal reference [7] by macerating 200g of dry powdered leaf drug in 400ml of sesame oil in an air tight container, which was kept in sun for 15 days and then filtered. Two higher doses having 400g of dry powdered leaf drug in 400ml as well as 600g dry powdered leaf drug in 400ml were also prepared as the first dose did not give the expected

results. The microbial assay was done by disc diffusion method using discs of Ciprofolxicin 20µg as standard .The discs of test drug were prepared on discs of 6 mm of Wattmann filter paper no.1 by absorbing the 100µl of indigenous drug formulation by slowly pouring on to the disc with the help of a micropipette, the discs were air dried before use.

The microbial smears of left and right eyelid as well as conjunctiva were collected with the help of a clean sterile swab previously moistened in sodium alginate from the pathological laboratory of Bharat Hospital, Kottayam under the supervision of ophthalmologist Dr. Appu Kuttan Nair on to trypticase soy agar with 5% sheep blood agar medium. Bacterial inoculums were prepared in peptone water (Himedia, Mumbai, India) after overnight incubation .The microorganisms were inoculated and incubated at 35 ± 2ºC overnight and the disc diffusion assay was carried out on the Muller Hinton agar medium under sterile conditions in an aseptic area. Aliquots of the bacterial inoculums were spread over the sterile agar plates with the help of sterile glass rod. These plates were incubated for 24hrs at 37ºC in an incubator. These experiments were carried out in triplicate and mean values were taken. The antimicrobial activity was measured in diameter as the zone of clear inhibition (ZI) of bacteria around the discs. In the primary study the following bacterial strains were obtained from the laboratory of DDRC SRL, Kottayam, and were evaluated for antimicrobial activity as they are present among the primary flora in acute conjunctivitis, aerobic gram positive bacteria *Staphylococcus aureus*, *Streptococcus pneumonia,* gram negative bacteria *Escherichia coli, Neisseria gonorrhoeae.*

3. Results and discussion

The results of the Disc diffusion assay technique of the hydro alcoholic plant extract on various microorganisms at different doses are shown in table no.1

The result showed that the plant extract at a concentration of 1000 PPM showed a remarkable antibacterial activity as comparable with that of the standard. The zone of inhibition (ZI) of the extract at a concentration of 1000µg/ml for *Staphylococcus aureus* is 2.5cm, *Streptococcus pneumonia* is 2.5cm, *Escherichia coli* is 2.4 cm, *Neisseria gonorrhoeae* is 2.2 cm. Thus the least sensitivity was shown with *Neisseria gonorrhoeae.* The conjuctival smear showed the ZI of 2.5 cm. The assay was repeated wih the indigenous formulation for which the concentration of dry plant leaf drug (600 g dissolved in 400ml) was seen to have a remarkable ZI of 2.5 cm for S.aureus, 2.4 cm for *S. pneumonia*, 2.5cm for E.coli 2.5 cm for *N. gonorrhoeae*. The conjunctival smear showed a ZI of 2.5 cm.

Table 1: Antibacterial activity of hydroalcoholic plant extract of *Blepharis maderaspatensis (L.)* Heyne ex Roth

Organism.	Std (cm)	Control	Plant extract 250 PPM	Plant extract 500 PPM	Plant extract 1000 PPM
S. aureus	2.7	-	1.9	2.2	2.5
S. pneumonia	2.9	-	2.0	2.3	2.5
E.coli	2.7	-	1.9	2.2	2.4
N. gonorrhoeae	3.1	-	1.8	2.0	2.2
Conjuntival smear	2.9	-	1.8	2.2	2.5

The results of the Disc diffusion assay technique of indigenous formulation of eye drops are shown in Table no.2

Table 2: Antibacterial activity of indigenous formulation of *Blepharis maderaspatensis* (*L.)* Heyne ex Roth eye drops

Organism.	Std (cm)	Control	Plant Drug dose (200g in400ml) ZI(cm)	Plant Drug dose (400g in400ml) ZI(cm)	Plant Drug dose (600g in400ml) ZI(cm)
S.aureus	2.8	-	1.8	2.1	2.5
S.pneumonia	2.7	-	1.9	2.3	2.4
E.coli	2.8	-	2.1	2.2	2.5
N. gonorrhoeae	3.0	-	2.0	2.3	2.5
Conjuntival smear	2.8	-	1.3	1.8	2.2

The assay was repeated for the indigenous formulation of different doses. The eye drops prepared by macerating 600g of powdered plant material in 400ml of sesame oil showed ZI comparable to that of standard as 2.5 cm for *S. aureas*, 2.4 cm for *S. pneumonia*, 2.5 cm for *E.coli*, 2.5 cm for *N. gonorrhoeae*. The conjunctival smear showed slightly lesser ZI of 2.2 cm.

4. Conclusion

The potential for the use of *Blepharis maderaspatensi*s (L.) Heyne ex Roth in the treatment of acute bacterial conjunctivitis was inevitably proved. The drug may not show much potential as a single use dosage form but shall be effective in multi dose administration. This paves way for the further exploration of the therapeutic efficiency of the extracts of *Blepharis maderaspatensi*s (L.) B. Heyne ex Roth for other eye diseases as well.

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6. References

1. T Shivakami, M Jayakumar. Antimicrobial activity of ethanolic leaf extracts of selective medicinal plants against food borne pathogenic bacteria. International Journal of Current Treands in Pharmacobiology and Medical sciences. 2016; 1(1):51-55.
2. S Ignacimuthu, M Ayyanar, Sankara Sivaraman K. Ethanobotanical investigations among tribes in Madurai district of Tamil Nadu (India), Journal of Ethnobiology and Ethanomedicine. 2006; 2:25.
3. Prabu M, Kumuthakalavalli R. Folk remedies of medicinal plants for snake bites, scorpion stings and dog bites of Eastern Ghats of Kholli hills, Tamil Nadu, India. International Journal of Research in Ayurveda and Pharmacy. 2012; 3(5).
4. M Ayyanar, Sankara Sivaraman K. Signacimuthu, Traditional Healing potential of Palliyars In Southern

India. Ethanobotanical leaflets. 2008; 12:311-317.
5. Karuppsamy Arunachalam, Thangaraj Parimelazhgan. Ethanomedicinal Observations among Hooralis Tribe in Kadambur Hills, (Kal Kadambur) Erode Dist., Tamil Nadu. Global Journal of Pharmacology. 2011; 5(3):117-121.
6. Bauer AW, WMM Kirby, JC Sherris, M Turck. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. 1966; 45:493-496.
7. JN Loganathan, S Balu. Treatment of eye diseases by the healers of Marakanam bioregion. Ancient Science of Life. 1997; 16(3):215-221.