



Research Article

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ANTI INFLAMMATORY, ANTI OXIDANT AND ANTI MICROBIAL ACTIVITY OF A NEW HERBAL EYE DROP

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ABSTRACT

Many antibiotic and steroid preparations are available for the management of Ophthalmic disorders. However prolonged use may have potential side effects. Ayurvedic Science has described many medicinal plants for treatment of ocular disorders. The aim of this study was to develop a new poly herbal formula with good anti inflammatory, anti oxidant and anti bacterial activity. A new poly herbal formula was developed from the aqueous distillates of a mixture of herbs like Padmakam (*Caesalpinia sappan*), Jati (*Jasminum grandiflorum*), Tulsi (*Ocimum sanctum*) and Durva (*Cynodon dactylon*) which have proven anti-inflammatory, antioxidant and antibacterial activities. The distillate was mixed with Saindhava lavana (Rock Salt), Honey, Rose water and Bhimseni kapur (Borneol). The anti-inflammatory activity of this formulation was evaluated in Turpentine liniment induced ocular inflammation in rabbits for chemosis, eye discharge and conjunctival congestion. Results for the biological evaluation of the formula showed that its anti inflammatory activity was comparable to that of standard betamethasone. The formula also exhibited good antioxidant potential and which was comparable to that of curcumin. It showed antimicrobial activity and the zone of inhibition against *Staphylococcus aureus* and *Escherichia coli* that is comparable to Gentamycin eye drops.

Keywords: Poly herbal formula, Anti-inflammatory, Anti oxidant and antimicrobial activity

INTRODUCTION

Inflammation of the conjunctiva is one of the common ocular disorders encountered in ophthalmology. There are many medicinal plants generally used in ocular ailments which are easily available and possess biological activity. The efficacy of many traditional medicines in curing ocular disorders is recognized by modern science also. Poly herbal eye drops have little side effects. A new poly herbal eye drop was developed using ingredients like Padmakam (*Caesalpinia sappan*), Jati (*Jasminum grandiflorum*/ Jasmine flowers), Tulsi (*Ocimum sanctum*), Durva (*Cynodon dactylon*), Honey, Saindhava Lavana (Rock salt), Rose water and Bhimseni kapur (Borneol). Several triterpenoids and flavonoids and oxygen heterocyclic compounds have been isolated from the heart wood of *Caesalpinia sappan*. It is reported that the heartwood extracts of *Caesalpinia sappan* showed antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and antifungal activity against *Aspergillus niger* and *Candida albicans*^{1,2}. Study results also reported significant antioxidant activity³. It is reported that the aqueous extract of *Ocimum sanctum* Linn, commonly referred to as Tulsi (Holy basil) has anti microbial and anti oxidant activity.^{4,6} Wound healing activity of the floral extracts of *Jasminum grandiflorum* is also reported⁷. Ancient Ayurvedic texts have reported that inflammation and itching of the eye

can be reduced by applying a paste prepared from Jati (*Jasminum grandiflorum*), Saindhava Lavana (Rock salt), *Cedrus deodara* and *Zingiber officinale*⁸ called Jathimukuladi Varti. The aqueous and alcoholic extracts of *Cynodon dactylon* exhibited wound healing activity and is a natural crude drug having widespread biological activities⁹.

MATERIALS AND METHODS

Method of Preparation

The drugs were identified by the Botanist in R and D laboratory of Sreedhareeyam and a voucher copy was preserved. *Ocimum sanctum* leaves (RMTP - 81), *Cynodon dactylon* (RMTP - 456) and *Caesalpinia sappan* (RMTP - 476), were procured from the Herbal garden of Sreedhareeyam. *Jasminum grandiflorum* flowers (RMTP - 286) were purchased from Coimbatore market in Tamil Nadu, India. Rose water manufactured by M/s Dabur India was used. Bhimseni kapur (Borneol), Saindhava Lavana (Rock salt) and Honey were purchased from market and identified. The herbs *Caesalpinia sappan*, *Ocimum sanctum*, *Cynodon dactylon* and *Jasminum grandiflorum* was cut to small pieces, suspended in water and kept overnight; refluxed the mixture for 2 hours. Decanted and the extract was distilled slowly and the distillate was collected. Borneol was added to the distillate and kept overnight. Stirred and dissolved Rock

Salt in the aqueous distillate. When completely dissolved, mixed with required Honey. Buffered and adjusted the pH to around 7. The compounded aqueous extract was filtered through a membrane filter to achieve sterility and was filled in sterile droppers under aseptic conditions.

Physico chemical parameters

The Physico chemical parameters of the Eye drop like color, clarity, pH etc was determined. Gas chromatography of the eye drop was also taken.

Anti inflammatory activity

The experiment was conducted in New Zealand rabbits (selected at random) having an average weight of 2.0 to 2.5 Kg. Twenty four rabbits were randomly assigned to control, test and standard groups. Ocular inflammation was induced by instilling 100 µl of Turpentine liniment IP in the left eye. Instillation of turpentine liniment resulted in eye discharge, chemosis and conjunctival congestion. The Animal Ethics Committee Reference Number of this work was EX/AI/002/10.

Antioxidant activity

Antioxidant activity was evaluated by inhibition of lipid per oxidation induced by ferric chloride. 1 ml of liver homogenate (10 % w/v) was added to the test sample of

different concentrations. The lipid per-oxidation was initiated by adding ferrous sulphate solution. After 30 minutes incubation at 37°C the reaction was terminated by the addition of butyl hydroxyl toluene. The samples were boiled for 30 minutes at 90°C after adding phosphoric acid and thiobarbituric acid the cooled and added butanol. The optical density of the butanol layer was read spectrometrically at 530 nm.

Antimicrobial activity

The Antibacterial activity of the new formulation was tested against *Staphylococcus aureus* and *Escherichia coli* using cylinder plate assay receptacle method^{10,11}. From the frozen bacteria, inoculation was done to nutrient agar media and incubated at 37°C for 24 hours. The grown bacteria were suspended in a normal saline solution (0.85 % sodium chloride w/v). The prepared bacterial suspension was used to inoculate into Muller – Hinton agar plate with a sterile non toxic cotton swab on glass petridishes having covers. Four wells were done by a sterile cork borer of 5 mm diameter in each plate. 100 µl of the new formulation was added in one well. To the other three wells 100 µl of three herbal formulas available from the market were added. Plates were incubated at 37°C for 24 hours. Plates were prepared similarly for estimating the zone of inhibition of Gentamycin eye drops against *Staphylococcus aureus* also.

Table 1: Evaluation of the Anti inflammatory activity of Poly herbal eye drop

Group	Day - 0			Day - 1			Day - 2		
	Eye Discharge	Chemosis	Conjunctival Congestion	Eye Discharge	Chemosis	Conjunctival Congestion	Eye Discharge	Chemo sis	Conjunctival Congestion
Control	3.30± 0.000	2.1487 ± 0.038	2.0	2.83± 0.167	2.0	2.0± 0.00	2.667± 0.211	2.063± 0.0104	1.66
Sreedhareeyam Herbal Eye drops	3.0± 0.000	1.918 ± 0.1002	2.0	1.833± 0.167*	1.532 0.17*	2.0	1.333± 0.211*	1.388± 0.159*	1.581
Betamethasone	2.833± 0.167	1.462± 0.041	2.0	1.00± 0.000*	1.018 0.018*	1.257	1.00± 0.000*	1.028± 0.069*	1.033

Values are mean ± SE (n = 8), p ≤ 0.05 as compared to the control group

Table 2: Evaluation of the Antioxidant activity of Poly herbal eye drop

Effect of herbal eye drops on lipid per oxidation induced in Rat liver Tissue homogenate				
S. No.	Particulars	Concentration	Absorbance value	% Protection
1	T-1	40 µl	0.562 ± 0.043	40.8
2	T-2	60 µl	0.363 ± 0.036	61.46
3	T-3	80 µl	0.287 ± 0.034	69.53
4	T-4	100 µl	0.229 ± 0.029	75.69
5	T-5	120 µl	0.111 ± 0.020	88.21
6	S-1 (curcumin)	10 µl	0.492 ± 0.034	48.5
7	S-2 (curcumin)	20 µl	0.412 ± 0.035	56.8
8	S-3 (curcumin)	30 µl	0.029 ± 0.053	69.3
9	S-4 (curcumin)	40 µl	0.187 ± 0.052	80.9
10	S-5 (curcumin)	50 µl	0.057 ± 0.032	94.8

Table 3: Evaluation of the Antimicrobial activity of Poly herbal eye drop

Comparative Antimicrobial activity of herbal eye drops				
Microorganism	Diameter of Zone of inhibition			
	C Sample - 3	B Sample - 2	A Sample - 1	S New formula
<i>Staphylococcus aureus</i>	16.6 mm	19.0 mm	13.2 mm	31.0 mm
<i>Escherichia coli</i>	NIL	8.5 mm	NIL	16.2 mm

A: Sample - 1, B: Sample - 2 C: Sample - 3, S: New Poly herbal formula

Table 4: Comparison of Antimicrobial activity of Gentamycin and Poly herbal eye drops

Comparative Antimicrobial activity of Gentamycin and Poly herbal eye drops				
Microorganism	Diameter of Zone of inhibition			
	Gentamycin Eye Drops		New poly herbal formula	
	As is	50 %	As is	50 %
<i>Staphylococcus aureus</i>	31.0 mm	16.5 mm	31.0 mm	16.0 mm

File :E:\GCMSD\2014\December\031214\MS4.D
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 Acquired : 3 Dec 2014 19:55 using AcqMethod GENERAL PROFILING.M
 Instrument : GCMSD
 Sample Name: Aqueous Distillate (A0965)
 Misc Info :
 Vial Number: 24

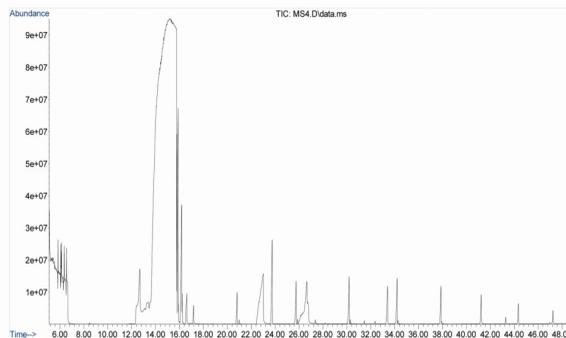


Plate 1: GC-MS Chromatogram of Sample



Normal Rabbit Eye



Eye after Inflammation

Plate 2: Anti inflammatory activity studies



Herbal Drops



Betamethasone

Plate 3: The reduction in inflammation after application for one day

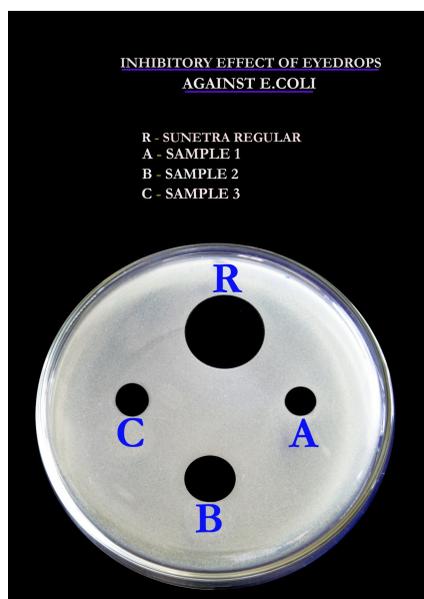


Herbal Drops

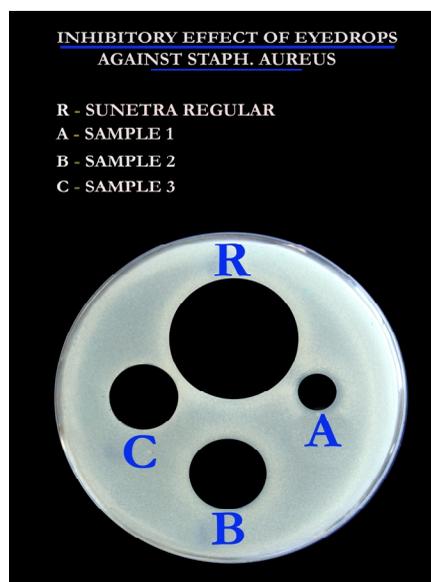


Betamethasone

Plate 4: The reduction in inflammation after application for two days

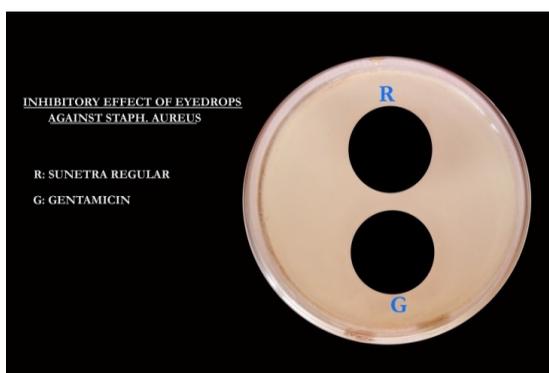


Against *Staphylococcus aureus*

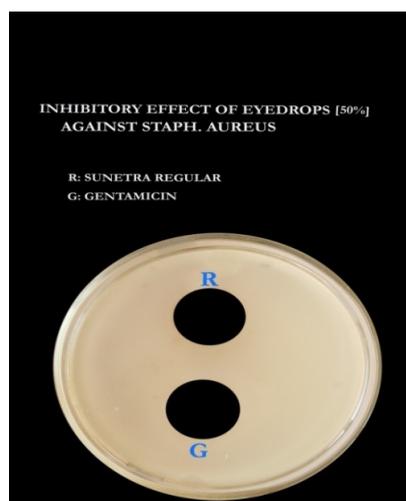


Against *Escherichia coli*

Plate 5: Inhibitory effect of poly herbal eye drop



Against *Staphylococcus aureus*



Against *Escherichia coli*

Plate 6: Comparative inhibitory effect study of poly herbal eye drops and gentamycin eye drops
G - Gentamycin Eye Drop R - Poly Herbal Eye Drop

RESULTS AND DISCUSSION

Physico Chemical parameters

It was a clear colorless solution with a pH around 7.4. The GCMS of the extract was also taken to identify the ingredients. The GC Chromatogram is given in Plate 1.

Anti inflammatory Activity

Distilled water was instilled to Group 1 rabbits which serve as control. Rabbits in Group 2 were instilled with 0.1 ml herbal eye drop and Group 3 with 0.1 ml of Betamethasone ocular eye drops. The extent of alleviation of eye discharge, chemosis and conjunctival congestion in Control, Test and Standard groups were compared and the results are tabulated in Table 1. The newly formulated eye drops showed marked protection in this model and anti inflammatory effect of test formulation was comparable to that of standard betamethasone. The parameters observed for ocular anti inflammatory activity evaluation were chemosis, eye discharge and conjunctival congestion. The formulated eye drops showed marked protection as evident from Table 1 and Plates 2, 3 and 4. The anti inflammatory activity of test formulation was comparable to that of standard betamethasone.

Antioxidant activity

The herbal formula showed significant antioxidant potential against ferric chloride induced lipid per oxidation. The antioxidant activity of the formula at different concentrations were tabulated in Table 2. The antioxidant activity of 60 µl / ml of the given sample is comparable to 20 µg / ml of curcumin. The formula showed significant antioxidant potential against ferric chloride induced lipid per oxidation. The antioxidant activity of 60 µl/ml of the given formulation was comparable to that of 20 µg/ml of the curcumin as detailed in Table 2.

Antimicrobial activity

At 100 µl concentration, the herbal formula showed an inhibition zone diameter of 31.0 mm. Against *Escherichia coli* the herbal formula had a zone of inhibition of 16.2 mm. The other three samples procured from the market showed varying zones of inhibition. The diameter of inhibition zone exhibited by Gentamycin eye drops against *Staphylococcus aureus* was also around 31 mm. The herbal formula and Gentamycin eye drops were diluted to 50 % and then again evaluated against *Staphylococcus aureus*. Both of them exhibited a zone of inhibition having diameter of 16.0 mm and 16.5 mm respectively. The poly herbal formula exhibited significant Anti microbial activity against *Staphylococcus aureus* and *Escherichia coli* as detailed in Table 3. The

diameter of the zone of inhibition exhibited by the poly herbal formula and other market samples against *Staphylococcus aureus* and *E. coli* are shown in Plate 5. The diameter of the zone of inhibition exhibited by Gentamycin eye drops and the poly herbal formula against *Staphylococcus aureus* is shown in Plate 6. Zone of inhibition of the poly herbal formula was comparable to Gentamycin as detailed in Table 4 and Plate 6.

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REFERENCES

1. R Sreenivasan, GG Selvam, S Karthik, K Mathivanan, R Baskaran, M Karthikeyan et al, *In vitro* antimicrobial activity of *Caesalpinia sappan*. Asian Pacific Journal of Tropical Biomedicine 2012; S 136-139.
2. Hong Xi Xu and Song F Lee. The Antibacterial principle of *Caesalpinia sappan*, Phytotherapy Research 2004; 18: 647-651. <http://dx.doi.org/10.1002/ptr.1524>
3. Badami S, Moorkoth S et al. Antioxidant activity of *Caesalpinia sappan* heart wood, Biological and Pharmacological Bulletin 2003; 26(11): 1534. <http://dx.doi.org/10.1248/bpb.26.1534>
4. Surender Singh, Manish Taneja, Dipak K Majumdar. Biological activity of *Ocimum sanctum* L fixed oil-An overview, Indian Journal of Experimental Biology 2007; 45(05): 403-412.
5. J Sethi, S Sood, S Seth and A Talwar. Evaluation of hypoglycemic and anti oxidant effect of *Ocimum sanctum*, Indian Journal of Clinical Biochemistry 2004; 19(2): 152-155. <http://dx.doi.org/10.1007/BF02894276>
6. AR Singh, VK Bajaj, PS Sekhvat and Kapil Singh. Phytochemical Estimation and Antimicrobial activity of Aqueous and Methanolic Extract of *Ocimum sanctum* L, J. Natural Prod. Plant Resour 2013; 3(1): 51-58.
7. RK Kath and RK Gupta. Antioxidant activity of Hydroalcoholic leaf extract of *Ocimum sanctum* L in animal models of peptic ulcer, Indian J of Physiology and Pharmacology 2006; 50(4): 391-396.
8. Anonymous, Sahasrayoga, Kendriya Ayurveeya Vijnan Anusandhan Parishad, Govt. of India, AYUSH Department, New Delhi; 2011. p. 394.
9. K Ashok Kumar, K Selvaraj and M Saradha Devi. *Cynodon dactylon*- An updated Review of its Phytochemistry and Pharmacology. J. Med. Plants Res 2013; 7(48): 3477-3483.
10. Anonymous, Indian Pharmacopoeia 1996, Vol - 2, The Controller of Publications, Ministry of Health and Family Welfare, Govt. of India, New Delhi 1996; Ap. 9: A - 105.
11. A Karthikeyan, KS Sivasaravanan, IS Gnanavel, D Prakash and V Velpandiyam. Screening of antimicrobial activity of Siddha herbal drug Milagaranai ver choornam (Root of *Toddalia asiatica*), Int. J Res Ayurveda Pharm 2014; 5(3): 347-351. <http://dx.doi.org/10.7897/2277-4343.05372>

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