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Formulation and evaluation of anti-bacterial and anti-fungal activity of a herbal ointment containing *Aloe-vera, Azadirachta indica* and *Curcuma- longa*

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ABSTRACT

In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. The scientific evidence has brought about the possibility of utilization of herbal plant in the treatment of fungal and bacterial infections and the development of anti-bacterial and anti-fungal products. In the present study, herbal ointment containing Aloe vera, Neem and Turmeric was formulated and evaluated to study antibacterial and antifungal activity. The evaluation is done using cup plate method for zone of inhibition and two fold dilution method for MIC (Minimum Inhibitory Concentration).The study showed that Aloe ointment is exhibiting broad-spectrum antifungal activity against A. varies and antibacterial activity against E.coli. The overall experiment showed that Aloe ointment and Turmeric ointment showed more anti-fungal activity than Neem ointment. Also it was found that ointment containing mixture of Aloe vera, Neem, Turmeric showed prominent antifungal activity than antibacterial activity. This ointment can be used in the treatment of sun burns, rashes, burns, wounds and other skin infections. It can also be used in the treatment of superficial mycosis.

Key words: *Aloe vera, Azadirachta indica, Curcuma longa,* Anti-bacterial activity and anti-fungal activity.

INTRODUCTION

Plants are the oldest source of pharmacologically active compounds and have provided human kind with many medicinally useful compounds from centuries. Today more than two thirds of the world's population relays on plant derived drugs. The origin of many effective drugs is found in the traditional medicinal practices and in view of this it is very important to undertake studies

pertaining to screening of the medicinal plants for their proclaimed biological activity. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new, antimicrobial compounds [1-3]. *Aloe vera* (family- Liliaceae) is a stem less plant. Aloe vera juice is of great medicinal importance and traditionally used as anti-inflammatory agent and in cosmetic industry. It is claimed to be useful in the treatment of Burns, Heat rashes, Allergy, Eczema, Psoriasis, Dermatitis as well as balnea, vaginal yeast infection. If *Aloe vera* juice is consumed directly or with any other type of liquid it gives relief from many kinds of stomach ailments like irritable bowel, reflux, Crohn's disease, indigestion and heartburn [4]. *Azadirachta indica* (Family-Meliaceae) known as Neem is well known for its medicinal properties. Its leaves possess broad spectrum of activity against Gram +ve and Gram – ve bacteria including M.tuberculosis, Vibrio cholera [5]. *Curcuma longa* (Family-Zingiberaceae) is a rhizomatous plant known as Turmeric. It is used for the treatment of wounds, cuts, burns, galactose induced cataract formation, ulcer etc. It is also used in protection against vascular dementia due to antioxidant activity. Both curcumin and the oil fraction suppress growth of several microbes like Streptococcus, Staphylococcus, Lactobacillus. A.flavus, P.digitatum, A.parasiticus, etc [6]. This ointment can be used in the treatment of sun burns, rashes, burns, wounds and other skin infections. It can also be used in the treatment of superficial mycosis.

EXPERIMENTAL SECTION

Plant Material:

Plant material used for this study was collected from college Botanical Garden, Pune, India.

Preparation of plant extract:

The aloe vera leaf was cut at the base of the plant, lower leaf was sliced opened and juice was collected. The turmeric rhizome was dried in shade and powdered with help of electric grinder. The neem leaves were also dried and powder of both were percolated with 250 ml of 80% ethanol.

Formulation of Ointment:

Emulsifying Wax (6gm)	
Ingredient	**Quantity Taken**
Cetosteryl alcohol	5.4gm
Sodium Lauryl Sulphate	0.6gm
Water	6ml

Emulsifying Ointment (20gm)	
Ingredient	**Quantity Taken**
Emulsifying Wax	6gm
Soft Paraffin	10gm
Liquid Paraffin	4ml

Aloe vera Ointment	
Ingredient	**Quantity Taken**
Aloe vera	0.1gm
Emulsifying Ointment	5gm

Turmeric Ointment	
Ingredient	**Quantity Taken**
Turmeric	0.1gm
Emulsifying Ointment	5gm

Neem Ointment	
Ingredient	**Quantity Taken**
Neem	0.1gm
Emulsifying Ointment	5gm

Aloe vera, Turmeric and Neem Ointment	
Ingredient	**Quantity Taken**
Aloe vera	0.03gm
Turmeric	0.03gm
Neem	0.03gm
Emulsifying Ointment	5gm

Determination of the pH:
pH of the prepared formulation was measured using digital pH meter.

Determination of spreadability:
For the determination of spreadibility excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000gm weight for 5minutes. Weight was added to the pan. The time required to separate the two slides, i.e. the time in which the upper glass slide moves over the lower plate was taken as measure of spreadibility.

$S = m * l / t$
Where,
m = weight tide to upper slide
l = length moved on the glass slide
t = time taken.

Test Microorganism:
Bacteria: E.coli, S.aureus, Pseudomonas, B.subtilis.
Fungi: A.varis, A.niger, P.notatum.

Standards used:
Anti-bacterial: Povidone iodine ointment.
Anti-fungal: Miconazole and Flucinolone ointment.

Anti-bacterial and Anti-fungal activity:
Determination of zone of inhibition:
Anti-microbial activity was checked by agar gel diffusion method. The cultures were grown in nutrient broth and incubated at 37ºc, for 24 hrs. After incubation periods was over, 0.1 ml of culture was seeded in 25 ml molten nutrient agar butts, mixed and poured into sterile petri plates and allowed to solidify. The well was bored with 6 mm borer in seeded agar. 0.1 g of each ointment sample was added in each well. Plates were kept at 10ºc as a period of pre diffusion for 30 minutes. After it normalized to room temperature; the plates were incubated at 37ºc for 24 hrs in case of bacteria and at 27ºc for 48 hrs in case of fungi. After incubation period was over, the zone of inhibition was measured with help of Hi-antibiotic zone scale.

Determination of minimum inhibitory concentration (MIC):
Various concentrations of the extracts were prepared by dissolving extracts in DMSO. MIC was performed by serial two fold dilution method at conc. ranging from 0.1µg/ml to 20µg/ml. Extracts were first diluted to the highest concentration (20 µg/ml) to be tested, and then serial

two fold dilution was made. The culture plates were again seeded with test bacterial and fungal organisms and allowed to solidify and thereafter punched with a sterile cork borer (6.0mm diameter) to cut uniform wells. The open wells were filled with 0.1ml of the sample extract. The

Sr. No.	Ointment	Zone of inhibition (mm)						
		E.coli	S.aureus	B.subtilis	P.aeruginosa	A.niger	A.varis	P.notatum
1	Aloe vera	20	25	28	20	23	30	28
2	Neem	20	22	25	20	20	20	20
3	Turmeric	20	40	35	25	30	30	30
4	Mixture	20	15	22	15	20	17	20
5	Standard	40	35	30	40	28	25	50

plates were then incubated at 37^0C for 24h. The lowest concentration of the extract that showed inhibition of growth of the test organisms was read. The least concentration of dilution showing clear zone of inhibition was taken as Minimum inhibitory concentration.

RESULT AND DISCUSSION

Table 1: pH and Spreadability value of Herbal Ointment

Ointment	pH	Spreadability
Aloe vera	6.2±0.057	34.7
Neem	6.22±0.202	32.2
Turmeric	6.3±0.045	26.8
Aloe vera, Neem and Turmeric	6.18±0.170	32.6

Table 2: Results of in vitro anti-bacterial and anti-fungal activity against various micro-organisms:

Table 3: Results of Minimum inhibitory concentration against various micro organisms:

Sr. No.	Ointment	MIC (in µg/ml)						
		E.coli	*S.aureus*	*B.subtilis*	*P.aeruginosa*	*A.niger*	*A.varis*	*P.notatum*
1	Aloe vera	0.1-20	1-20	1-20	1-20	0.1-20	0.1-20	0.1-20
2	Neem	1-20	0.1-20	1-20	2-20	1-20	1-20	2-20
3	Turmeric	0.1-20	1-20	1-20	1-20	0.1-20	0.1-20	0.1-20
4	Mixture	2-20	1-20	2-20	1-20	0.1-20	1-20	0.1-20

Concentrations used are 20µg/ml, 2µg/ml, 1µg/ml & 0.1µg/ml

CONCLUSION

General ointment containing Aloe vera, Neem and Turmeric was formulated. All the ointments exhibited broad spectrum anti-bacterial and anti-fungal activity against all the tested micro-organisms. It was found that bacteria are more sensitive as compared to fungi to all of the ointments. Specifically Aloe ointment is showing more Anti-bacterial and anti-fungal activity than the others. Among the all bacteria tested, E.coli is more sensitive to Aloe ointment. This is because aloe vera might be dissolving lipid content which is present in Gram –ve E.coli. In general fungi are less sensitive than bacteria. But in present study it was observed that fungus A.varis shows more sensitivity to Aloe and Turmeric ointment.

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