



FORMULATION AND EVALUATION OF HERBAL OINTMENT CONTAINING AZADIRACHTA INDICA AND CURCUMA LONGA FOR ANTIMICROBIAL ACTIVITY AND ANTI FUNGAL ACTIVITY

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ABSTRACT

The present work is to formulate and evaluate the ointment of neem and turmeric extract for antimicrobial activity and anti fungal activity. The ethanolic extract was prepared by maceration method. The ointment base was prepared and three formulations of ointment were done by incorporating extract in the base by levigation method. All the formulation were evaluated for their physicochemical parameters like colour, odour, pH, spreadability, extrudability, consistency, diffusion study, solubility, washability. Also the formulation was evaluated for its stability at various temperature conditions which shows no change in the irritancy, spreadability and diffusion study. From three ointments, F3 was found to be the best formulation as it shows 98% drug release within 6hrs, drug content 98.8% and it shows more zone of inhibition against bacillus as compared to other three formulations.

KEYWORDS: The present work is to formulate and evaluate the herbal ointment.

INTRODUCTION:

NEEM:

The plant product or natural products show an important role in diseases prevention and treatment through the enhancement of antioxidant activity, inhibition of bacterial growth and modulation of genetic pathways. The therapeutics role of number of plants in disease management is still been enthusiastically researched due to their less side effect and affordable properties. It has been accepted the drug based on allopathy are expensive and also exhibit toxic effect on normal tissues and on various biological activities. It is a largely accepted fact that numerous pharmacologically active drugs or derived from natural resources including medicinal plants.

ACTIVE COMPOUNDS OF NEEM:

Neem shows therapeutic roles in health management due to rich source of various types of ingredients. The most important active constituent is Azadirachtin and:

- Nimbolinin
- Nimbin
- Nimbidin
- Nimbanene
- 6-desacetylnimbanene
- Nimbandiol
- Nimbolide
- Ascorbic acid
- N-hexacosanol and amino acid
- 7-desacetyl-7-benzoylazadiradione
- 7-besacetyl-7-benzoylgedunin
- 17-hydroxyazadiradione
- Nimbiol
- Polyphenolic flavonoids

These are purified from neem fresh leaves and were known to have antibacterial and antifungal activity properties and seeds hold valuable constituents including Gedunin and Azadirachtin.¹

TURMERIC:**INTRODUCTION:**

- Natural plant products have been used throughout human history for various purposes. Having co-evolved with animal life, many of the plant from which these natural products are derived are billions of years old. Tens of thousands of these products are produced as secondary metabolites by higher plants as a natural defense mechanism against disease and infection.
- Many of these natural products have pharmacological or biological activity that can be exploited in pharmaceutical drug discovery and drug design.
- Medicines derived from plants have played a pivotal role in the health care of many cultures, both ancient and modern.
- The Indian system of holistic medicine known as “Ayurveda” uses mainly plant based drugs or formulations to treat various ailments, including cancer.
- Although many synthetic drugs are produced through combinatorial chemistry, plant based drugs are more suitable, atleast in biochemical term, for human use.
- Turmeric is a plant that has a very long history of medicinal use, dating back nearly 4000 years.
- Turmeric is also known as Indian saffron, because of its bright yellow colour.

COMPOSITION OF TURMERIC:

- More than 100 components have been isolated from turmeric.
- The main component of the root is a volatile oil, containing turmerone, and there are other colouring agent called curcuminoids in turmeric.
- Curcuminoids consist of curcumin demethoxycurcumin, 5-methoxycurcumin, and dihydrocurcuin, which are found to be natural antioxidants.
- In a standard form, turmeric contains moisture (>9%), curcumin (5-6.6%), extraneous matter (<0.5% by weight), mould (<3%) and volatile oils (<3.5%).
- The components responsible for the aroma of turmeric are turmerone, arturmerone, and Zingiberene.
- The rhizomes are also reported to contain four new polysaccharides-ukonans along with stigmasterole, β -sitosterole, cholesterol, and 2-hydroxymethyl anthraquinone.
- Turmeric is also a good source of the fatty acid and α -linolenic acid.²

Collection of plant material

The Azadirachta Indica A.Juss leaves were collected from in and around Perambalur. Dried rhizomes of turmeric were collected from in and around Perambalur. These are authenticated by botanist, department of botany, national college, Trichy. Then the leaves cleaned properly and shade dried at room temperature.

Preparation of Neem Extract

Leaves of the plant were collected and washed thoroughly with distilled water and shade dried for 10 days. Dried leaves were ground into powder form. 100gm powder was imbibed with 350ml of 90% ethanol for 3hrs. and transferred to percolator with addition of 150ml of 90% ethanol for maceration for 7 days with occasional stirring. Finally ethanolic extract was collected and concentrated to get blackish green residue. The extract was stored in the airtight container at cool and dark place.³

Preparation of Turmeric extract

Dried rhizomes of turmeric were ground and the powder obtained was followed for extraction same as that for neem leaves extract. The extract with crimson red colour was obtained and stored at cool and dark place in air tight container.



Figure 1: Maceration Process



Figure 2: Crude extract of Turmeric and Neem

PREPARATION OF OINTMENT BASE

Table 1: Formation of ointment bases

S.No	Name of Ingredients	Quantity to be taken
1.	Wool fat	0.5gm
2.	Cetostearyl alcohol	0.5gm
3.	Hard paraffin	0.5gm
4.	Yellow soft paraffin	8.5gm

Table 2: Formulation of Herbal ointment.

Formulation code	Prepared neem extract (gm)	Ointment base q.s (gm)
F1	0.08	10
F2	0.10	10
F3	0.12	10

Table 3: Formulation of Herbal ointment.

Formulation code	Prepared turmeric extract (gm)	Ointment base q.s (gm)
F1	0.08	10
F2	0.10	10
F3	0.12	10

Procedure for preparation of herbal ointment

- a) Initially ointment base was prepared by weighing accurately grated hard paraffin which was placed in evaporating dish on water bath. After melting of hard paraffin remaining ingredients were added and stirred gently to aid melting and mixing homogeneously followed by cooling of ointment base.
- b) Herbal ointment was prepared by mixing accurately weighed Neem and Turmeric extract to the ointment base by levigation method to prepare a smooth paste with two or three times its weight of base, gradually incorporating more base until to form homogeneous ointment, finally transferred in a suitable container.⁴

Evaluation of antimicrobial activity of herbal ointment

Materials and methods:

Microorganism and culture media

Bacterial cultures such as *Bacillus subtilis*, *staphylococcus aureus*, *E.coli*, and *Klebseilla pneumonia* were obtained from Eumic analytical lab and Research Institute, Tiruchirapalli. Bacterial strains were maintained on nutrient agar slants at 4°C

Inoculum preparation

Bacterial cultures were subcultured in liquid medium (nutrient broth) at 37°C for 8hours and further used for the test (10^5 - 10^6 CFU/ml). These suspensions were prepared immediately before the test was carried out.

Preparation of culture media

Nutrient agar medium

Nutrient agar medium is one of the most commonly used medium for several routine bacteriological purposes:

Ingredients	Grams/Litre
Peptone	5gm
Beef extract	3gm
Agar	15gm
Sodium chloride	5gm
Yeast extract	1.5gm
pH	7.0

After adding all the ingredients into the distilled water it is boiled to dissolved the medium completely and sterilized by autoclaving at 15 lb psi pressure (121°C) for 15 minutes.

Nutrient broth

The nutrient broth was prepared by the same composition without agar. At the adding all the ingredients into the distilled water it is boiled to dissolved the medium completely and sterilized by autoclaving at 15 ib psi pressure (121°C) for 15 minutes.

Assay of antimicrobial activity

Microbial inoculum preparation:

The nutrient broth were prepared, then identified bacterial colonies were inoculated into the broth culture were used for antimicrobial activity.

Kirby bauer agar well diffusion assay

The nutrient agar medium was prepared and sterilized by autoclaving at 121°C 15 ibs pressure for 15 minutes then aseptically poured the medium into the sterile petriplates and allow to solidify the bacterial broth culture was swabbed on each petriplates using a sterile buds. Then wells were made by well cutter. The organic solvent extracts of leaves were added to each well aseptically.

This procedure was repeated for each petriplates then the petriplates were incubated at 37°C for 24 hours. After incubation the plates were observed for the zone of inhibition.

SAMPLE : A, B, C, D, E Ethanol extract

CONTROL : Gentamicin antibiotic disc

Antibacterial activity

Figure 1:



Figure 2:

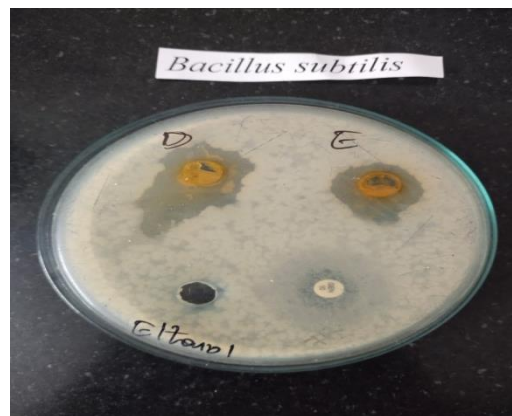


Figure 3:



Figure 4:



Table4 : screening of antibacterial activity of F1, F2 and F3

NAME OF THE MICRO ORGANISM	ETHANOL Extract 100 µl added and Zone of inhibition (mm/ml)										
	F1			F2			F3			SOLVENT	CONTROL
	A	B	C	A	B	D	A	B	E		
<i>Bacillus subtilis</i>	30	25	30	30	25	28	30	25	23	21	25
<i>E.Coli</i>	28	26	22	28	26	21	28	26	22	10	22

A: Neem extract B: Turmeric extract C: 0.08gm D: 0.10gm E: 0.12gm

The antibacterial activity of F1, F2 and F3 herbal extract are evaluated. The herbal extract are produce a better inhibition activity than prepared formulation, but prepared formulation produce better activity in minimum concentration extract. The comparison F1, F2 and F3, F1 shows greater inhibition against *Bacillus subtilis*. The prepared formulation are compared with standard.

Antifungal activity

Figure 5:



Figure 6:

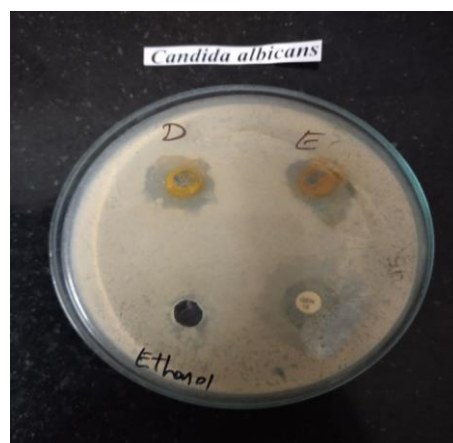


Table 5 : screening of antifungal activity of F1, F2 and F3

NAME OF THE MICRO ORGANISM	ETHANOL Extract 100 µl added and Zone of inhibition (mm/ml)										
	F1			F2			F3			SOLVENT	CONTROL
	A	B	C	A	B	D	A	B	E		
<i>Candida albicans</i>	23	22	26	23	22	22	23	22	32	12	25

A: Neem extract B: Turmeric extract C: 0.08gm D: 0.10gm E: 0.12gm

The antifungal activity of F1, F2 and F3 herbal extract are evaluated. The herbal extract are produce a better inhibition activity than prepared formulation, but prepared formulation produce better activity in minimum concentration extract. The comparison F1, F2 and F3, F1 shows greater inhibition against *candida albicans*. The prepared formulation are compared with standard.⁵

CONCLUSION

The study determine the good anti microbial activities of the ointment formulations containing the herbal extract. This could make them potential topical antimicrobial agents effective in the treatment of skin infections. The use of alcoholic herbal extract produce a effective antimicrobial property. The prepared formulations effective in both bacteria and fungi. The produce a both antifungal and antimicrobial activity, but the antibacterial activity is much better than antifungal activity. The zone of inhibition is more against microbial agent. So the prepared ointment have better antibacterial property.

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