



## Formulation, Development & *In vitro* Assessment of Nimbidin & Tree Tea Oil Loaded Emulgel in the Treatment of *Tinea corporis*

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### KEYWORDS

Emulgel, Nimbidin, Tree Tea Oil, In vitro assessment, Tinea Corporis, Trichophyton rubrum

### ABSTRACT:

#### Background:

*Tinea corporis* is a common superficial fungal infection of the skin caused by dermatophytes such as *Trichophyton*, *Microsporum*, and *Epidermophyton*. It is characterized by annular, erythematous, scaly lesions associated with itching and inflammation. Nimbidin, a major bioactive tetranortriterpenoid found in seeds & leaves of Neem (*Azadirachta indica*) and, exhibits antifungal activity. Tree Tea Oil, is volatile oil isolated from the leaves of *Melaleuca alternifolia*, shows its potentials in the treatment of *Tinea corporis* with its potent antifungal, antibacterial, and anti-inflammatory properties. However, its volatility, poor aqueous solubility, and potential skin irritation limit its direct topical use.

#### Objective:

Emulgel, a novel topical drug delivery system combining the advantages of emulsions and gels, provides improved stability, enhanced skin penetration, controlled drug release, and better patient compliance for Hydrophobic drugs like essential oils. In present research work attempt was made of formulation, development and In vitro assessment of Nimbidin and Tree Tea Oil loaded Emulgel in the treatment of *Tenia Corporis*.

#### Methods:

Neem seeds (*Azadirachta indica*) were collected, dried, and coarsely powdered. The powdered material was subjected to Soxhlet extraction to obtain a Nimbidin-rich extract. The Emulgel formulation was developed by incorporating Nimbidin extract along with Tree Tea Oil (*Melaleuca alternifolia*) into an oil-in-water (O/W) emulsion system. The aqueous phase containing a suitable gelling agent such as Carbopol 934 was prepared separately and neutralized. The oil phase, consisting of Tree Tea Oil and appropriate emulsifiers, was then gradually combined with the aqueous phase under continuous stirring to form a stable emulsion, which was subsequently incorporated into the gel base to obtain the final Emulgel formulation.

The formulated Emulgel was underwent for its evaluation such as colour, consistency, homogeneity, appearance, greasiness, washability, pH, and spreadability. The In vitro antifungal activity was evaluated against *Trichophyton rubrum* using the agar Well diffusion method by determining the zone of inhibition. The drug release profile was investigated using a Franz diffusion cell equipped with a suitable membrane, with samples withdrawn at predefined time intervals for analysis.

#### Results:

Using the well diffusion method, the formulated Nimbidin and Tree Tea Oil loaded Emulgel (F2) shown strong antifungal efficacy against *Trichophyton rubrum*. The formulation effectively suppressed fungal growth, as evidenced by the significant zone of inhibition it produced. It was discovered that its activity was similar to that of the common antifungal drug Miconazole. Because



of its dual delivery method, the Emulgel demonstrated increased stability and better drug release. Overall, the combination of Tree Tea Oil and Nimbidin showed good topical adaptability and synergistic antifungal activity.

#### Conclusion:

The Emulgel containing Tree Tea Oil & Nimbidin showed effective antifungal activity against *Tinea corporis*. Overall treatment efficacy was improved by the combination's synergistic effect. The Emulgel improved skin penetration, stability, and spreadability. The outcomes *In vitro* were similar to those of conventional antifungal formulations. It was discovered to be non-irritating, harmless, and appropriate for topical use.

## INTRODUCTION:

*Tinea corporis* is a common superficial fungal infection of the skin caused by dermatophytes such as *Trichophyton*, *Microsporum*, & *Epidermophyton*, characterized by annular, erythematous, scaly lesions associated with itching and inflammation, and is highly prevalent in warm and humid climates.<sup>[1]</sup>

Conventional antifungal therapies often require prolonged treatment and may lead to adverse effects and drug resistance, creating a need for safer and more effective alternatives.<sup>[2]</sup>

Nimbidin, a major bioactive tetranortriterpenoid found in Neem (*Azadirachta indica*) seed oil and leaves, exhibits antifungal activity primarily by disrupting fungal cell membrane integrity and causing the leakage of essential cellular contents.<sup>[3]</sup> It acts as a fungicidal or fungistatic agent against various pathogenic fungi like *Trichophyton rubrum*.<sup>[4]</sup>

Tree Tea Oil, is volatile oil isolated from the leaves of *Melaleuca alternifolia*, showed its potentials in the treatment of *Tinea corporis* with its potent antifungal, antibacterial, and anti-inflammatory properties.<sup>[5]</sup> However, its volatility, poor aqueous solubility, and potential skin irritation limit its direct topical use.

Combination of Tree Tea Oil and Nimbidin gives synergistic antifungal activity against dermatophytes like *Trichophyton rubrum* in the treatment of *Tinea Corporis*.<sup>[6]</sup>

Emulgel, a novel topical drug delivery system combining the advantages of emulsions and gels, provides improved stability, enhanced skin penetration, controlled drug release, and better patient compliance for Hydrophobic drugs like essential oils.<sup>[7]</sup>

In present research work attempt will be made of formulation, development and *In vitro* assessment of

Tree Tea Oil loaded Emulgel in the treatment of *Tenia Corporis*.

## MATERIAL AND METHODS:

Nimbidin was isolated using the seeds of *Azadirachta indica* through Soxhlation. Marketed sample of Tree Tea Oil was procured. All chemicals and reagents used were of good analytical grade with the purity of 99%.<sup>[8]</sup>

## PLANT MATERIAL AND EXTRACTION:

Seeds of *Azadirachta indica* were collected, authenticated, shade-dried, and coarsely powdered. The powdered material was subjected to Soxhlet extraction with *n*-hexane for 48–72 hours. The resulting extract was filtered and concentrated using a water bath, yielding a semi-solid mass rich in Nimbidin.<sup>[9]</sup>

The crude extract was then dissolved in ethanol for further fractionation and purification. Water was added gradually to induce precipitation, and the mixture was transferred to a separating funnel for partitioning with chloroform.<sup>[10]</sup> The chloroform layer containing Nimbidin was collected, concentrated, and dried using electric water bath to obtain a brownish, bitter semi-solid. This substance was further purified using recrystallization.<sup>[11]</sup>

## PHYTOCHEMICAL ANALYSIS:

Preliminary phytochemical analysis of the Neem seeds extract was performed using various chemical tests.<sup>[12]</sup> The results of the phytochemical analysis are depicted in Table no. 1

## FORMULATION OF EMULGEL:

Accurately weighed Carbopol 934 was slowly dispersed in distilled water under magnetic stirring at 250 rpm and 37 °C for 30 minutes until a viscous gel was formed. The



oil phase was prepared by dissolving Span 20 and Tree Tea Oil in liquid paraffin to form an emulsion base.<sup>[13]</sup>

A preservative solution was prepared separately by dissolving methyl paraben and propyl paraben in propylene glycol. For the aqueous phase, Tween 20 and neem seed extract were added to water. The aqueous phase was then gradually incorporated into the oil phase under magnetic stirring to form an emulsion.

Finally, both phases were blended into the Carbopol 934 dispersion with continuous stirring to obtain a smooth, uniform, and homogeneous Emulgel.<sup>[14]</sup>

#### EVALUATION OF FORMULATED EMULGEL:

The formulated Emulgel was evaluated and compared with the standard criteria for its colour, consistency, homogeneity, appearance, greasiness, washability, pH, and spreadability, viscosity.<sup>[15]</sup> A digital pH meter was used to measure the Emulgel pH at room temperature in order to determine its skin biocompatibility. A Brookfield viscometer was used to measure viscosity.

One gram of the Emulgel was sandwiched between two glass slides ( $7.5 \times 2.5$  cm), and left undisturbed for a minute in order to access spreadability by keeping 100gm weight on the upper slide.

#### *In vitro* Assessment of Nimbidin & Tree Tea Oil Loaded Emulgel in the Treatment of *Tinea Corporis*:

The inoculum of the microorganism was prepared using fungal cultures. Sterile Petri dishes were poured with 15 mL of Sabouraud Dextrose agar (Hi Media) and allowed to cool and solidify. Subsequently, 100  $\mu$ L of fungal broth culture was pipetted onto the agar surface and evenly spread using a sterile spreading rod until completely absorbed.

Wells of 6 mm diameter were then created in the agar using a sterile corkborer. Test and standard solutions (100  $\mu$ L/mL) were prepared in methanol, and 100  $\mu$ L of each solution was carefully introduced into the respective wells. The plates were incubated at 37 °C for 24 hours.

Dimethyl sulfoxide (DMSO) served as the negative control, while miconazole (1 mg/mL) was used as the positive control. Antifungal activity was evaluated by measuring the diameter of the zones of inhibition (ZI).

## RESULT AND DISCUSSION:

### RESULT:

#### EXTRACTION:

Bioactive compounds such as flavonoids, alkaloids, and tannins were extracted from plant materials, seeds, and herbs using a Soxhlet apparatus with suitable solvents under controlled conditions.



Figure 1: Soxhlet apparatus

The choice of solvent is determined by the target compound's polarity (e.g., hexane for lipids, ethanol or methanol for polar molecules).



Figure 2: Neem seeds extract (Nimbidin)

A major bitter terpenoid found in *Azadirachta indica* (Neem) seed oil, Nimbidin exhibits strong antifungal properties.

## PHYTOCHEMICAL ANALYSIS:

Table No.: 1

### PHYTOCHEMICAL TEST FOR NIMBIDIN

Sr. No.	Test	Inference	Result
1.	Salkowski Test	Appearance of reddish-brown colour in upper layer	+



2.	Thin Layer Chromatography	RF range = 0.4 to 0.6	+
3.	Liebermann-Burchard Test	formation of a Blue /Green colour ppt	+

Important phytoconstituents were found in Nimbidin, according to preliminary study. Thin Layer Chromatography confirmed the presence of distinctive chemicals based on Rf values, whereas the Salkowski and Liebermann-Burchard tests revealed triterpenoids

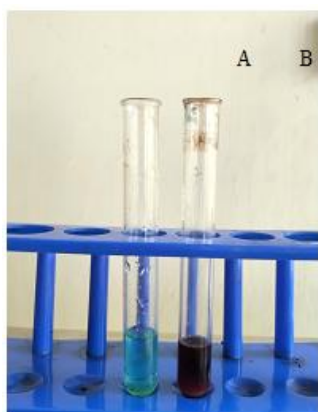


Figure 3: Preliminary test of Nimbidin

- A. Liebermann-Burchard Test indicates the bluish-green color of ppt  
 B. Salkowski Test indicates the reddish-brown ring appear junction of two layer

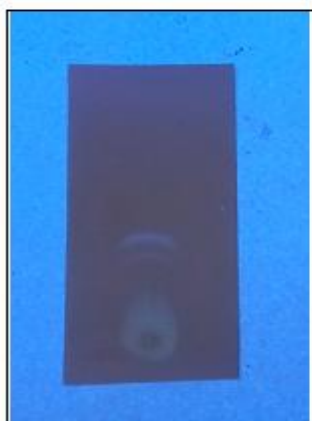


Figure 3: Thin Layer Chromatography

Distance traveled by solute= 3

Distance traveled by solvent front= 5

Rf value = 0.6 Standard RF range = 0.4 to 0.6

## FORMULATION OF EMULGEL:

Table No.: 2

### Formula for Emulgel preparation

Sr. No	Ingredients	Low(F <sub>1</sub> )	Moderate(F <sub>2</sub> )	High (F <sub>3</sub> )
1	Tree Tea Oil	0.30 ml	0.60.ml	0.90 ml
2	Nimbidin	0.15 g	0.30 g	0.45 g
3	Carbopol 934	0.30 g	0.30 g	0.30 g
4	Liquid paraffin	1.27 ml	1.27 ml	1.27 ml
5	Span 20	0.30 ml	0.30 ml	0.30 ml
6	Tween 20	0.33 ml	0.33 ml	0.33 ml
7	Methyl paraben	0.03 g	0.03 g	0.03 g
8	Propyl paraben	0.006 g	0.006 g	0.006 g
9	Triethanolamine	0.2 - 0.3ml	0.2 - 0.3ml	0.2 - 0.3ml
10	Propylene glycol	1.56 ml	1.56 ml	1.56 ml
11	Purified water	qs.to 30 g	qs.to 30 g	qs.to 30 g



Figure 4: Batches of Formulated Emulgel



## EVALUATION PARAMETERS

Sr. No	Parameter	F1	F2	F3
1.	Colour	Off white	Off white	Off white
2.	Consistency	Good	Very Good	Good
3.	Homogeneity	Good	Very Good	Good
4.	Appearance	Semi-solid	Semi-solid	Semi-solid
5.	Greasiness	Non-greasy	Non-greasy	Non-greasy
6.	Washability	Washable	Washable	Washable
7.	pH	5.8	6	6.2
8.	Spreadability	9.8 gm.cm/sec	10 gm.cm/sec	10.2 gm.cm/sec
9.	Viscosity	1400cps	1450cps	1500cps

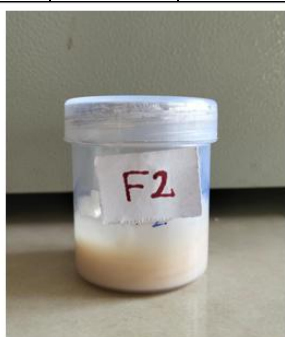


Figure 5: Selected batch of formulated Emulgel for antifungal activity

The moderate (F<sub>2</sub>) batch is selected because it provides the best balance between evaluation parameters. Ensure the minimum irritation & good formulation stability. It shows overall best balanced properties compared to low & high batch.

## IN VITRO ANTIFUNGAL ACTIVITY

Table No.4:

Evaluation of antifungal activity by using agar well diffusion method

SR.NO	SAMPLES	ZONE IN DIAMETER(mm)
1	Control	00
2	Standard (Miconazole)	16
3	F2	11



Figure 5: Anti-fungal Activity of test samples against *Trichophyton rubrum*

By using the Well Diffusion Method to measure the zone of inhibition against fungal strains *Trichophyton rubrum* (ATCC 28188), the antifungal profile of F<sub>2</sub> was assessed. Compared to the standard 'Miconazole', the compound F<sub>2</sub> showed good activity.

## DISCUSSION:

The present study focused on formulating an Emulgel incorporating Nimbidin isolated from (*Azadirachta indica*) and Tree Tea Oil (*Melaleuca alternifolia*) for the topical management of *Tinea corporis*. The Emulgel system was chosen for its capacity to improve the solubility of hydrophobic constituents, enhance formulation stability, and increase patient acceptability. Carbopol 934 was utilized to impart appropriate viscosity and bio adhesive properties, while Span 20 and Tween 20 facilitated the formation of a stable emulsion. Liquid paraffin contributed to improved spreadability, and propylene glycol functioned as a penetration enhancer.

The combined use of Nimbidin and Tree Tea Oil demonstrated a probable synergistic antifungal effect, against *Trichophyton rubrum*, primarily through disruption of fungal cell membrane integrity. *In vitro* antifungal assessment using the Agar well diffusion technique revealed significant zones of inhibition,



comparable to those of Miconazole, indicating efficient drug release and notable antifungal activity.

The optimized formulation demonstrated acceptable physicochemical properties/tests and indicated a controlled drug release pattern. Collectively, the findings suggest that the Emulgel may serve as a promising topical delivery system for the management of *tinea corporis*.

## CONCLUSION:

Effective antifungal action against *Tinea corporis* was demonstrated by the Emulgel containing Nimbudin and Tree Tea Oil. Overall treatment efficacy was improved by the combination's synergistic effect. The Emulgel improved skin penetration, stability, and spreadability. The outcomes *In vitro* were similar to those of conventional antifungal formulations. It was discovered to be non-irritating, harmless, and appropriate for topical use.

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