

Formulation and Evaluation of Polyherbal Antifungal Transdermal Patches Containing *Tridax procumbens* Linn and *Azadirachta indica* Extracts

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ABSTRACT

This study aimed to formulate and evaluate polyherbal antifungal transdermal patches containing extracts of *Tridax procumbens* Linn and *Azadirachta indica*. These medicinal plants, known for their antimicrobial and anti-inflammatory properties, were extracted using methanol and incorporated into patches via solvent evaporation¹. Physical parameters such as pH, thickness, weight uniformity, folding endurance, and moisture content were assessed. Antifungal activity was tested against *Aspergillus niger* using the agar well diffusion method. Phytochemical screening confirmed the presence of alkaloids, tannins, flavonoids, and saponins². Results revealed that all formulations exhibited significant antifungal activity, with Patch 2 demonstrating superior performance (zone of inhibition: 19.5 mm) along with optimal physical properties. The findings suggest that polyherbal transdermal patches are a promising approach for topical antifungal therapy, offering sustained drug release, better patient compliance, and ease of application.

Keywords: Transdermal patch, Polyherbal formulation, *Tridax procumbens*, *Azadirachta indica*, Antifungal activity, *Aspergillus niger*.

I. INTRODUCTION

Transdermal patches are medicated adhesive systems designed to release active ingredients at a controlled rate over several hours to days following application to the skin. Incorporating herbal-derived drugs into transdermal drug delivery systems (TDDS) can enhance therapeutic efficacy while ensuring sustained drug release^{3,4}. TDDS offers multiple advantages, including constant drug permeation through the skin,

maintenance of steady plasma levels similar to intravenous infusion, and the ability to quickly terminate therapy by removing the patch in case of toxicity. Additionally, it provides ease of application, improves patient compliance, and bypasses first-pass metabolism, thereby enhancing bioavailability. The integration of herbal actives into TDDS thus represents a promising, patient-friendly approach to effective drug delivery^{2,4,5}.

Herbal formulations are increasingly preferred over synthetic alternatives in cosmetic applications due to their natural origin, safety profile, and added skin benefits. Derived entirely from herbs and shrubs, these products are less likely to cause adverse effects and may provide essential minerals and nutrients that enhance skin health. Unlike many synthetic cosmetics, herbal formulations possess complex bioactive components capable of repairing, protecting, and soothing the skin. Growing scientific evidence supports their efficacy, highlighting their potential as effective, skin-friendly alternatives to conventional synthetic products^{6,7}.

Fungal infections represent a serious health concern, particularly for immune compromised individuals, due to rising antifungal resistance and limited treatment options. Conventional antifungal drugs, including azoles, polyenes, echinocandins, and heterocyclic benzofurans, face diminishing efficacy, necessitating the search for novel therapeutic strategies. Herbal medicines, particularly *Tridax procumbens* Linn and *Azadirachta indica* (neem), have emerged as promising alternatives owing to their diverse bioactive compounds such as flavonoids, alkaloids, tannins, carotenoids, saponins, and phenolic compounds^{5,6,7}. These phytochemicals possess antimicrobial, anti-inflammatory, antioxidant, and antifungal properties, enabling broad-spectrum activity against pathogens including *Candida*, *Aspergillus*, and dermatophytes. Scientific evidence supports their potential in inhibiting fungal growth, modulating host immunity, and preventing toxin production. This review highlights the urgent need to explore herbal-based antifungal agents as sustainable, effective strategies to combat resistance and improve therapeutic outcomes^{4,8}.

II. MATERIALS AND METHODS

Materials

The primary materials included methanolic extracts of *T. procumbens* and *A. indica*, carboxymethyl cellulose

(CMC), polyethylene glycol 400 (PEG 400), chloroform, methanol, distilled water, and oleic acid^{7,8,9}.

Instruments

An electronic weighing balance, pH meter, vernier calipers, Soxhlet apparatus, laminar airflow cabinet, B.O.D. incubator, autoclave, and mechanical stirrer were used^{5,6,7}.

Microorganism

Aspergillus niger was obtained from the Microbiology Laboratory, Gokaraju Rangaraju College of Pharmacy, Hyderabad, Telangana.

Extraction of Plant Materials

Tridax procumbens: Shade-dried leaves were powdered and extracted with methanol in a Soxhlet apparatus at 64 °C for 24 hours^{4,8}.

Azadirachta indica: Powdered leaves underwent cold maceration in 70% methanol for 7 days with intermittent shaking. Extracts were filtered and concentrated under reduced pressure^{8,9}.



Figure1: Extraction process of herbs.

III.FABRICATION OF TRANSDERMAL PATCHES

Transdermal films containing herbal formulation were cast on glass slide by solvent evaporation method using HPMC (100 M) in presence of a plasticizer. PEG 400 (5%) was used as a plasticizer in all cases. Table no. 1 shows the formulae and composition for the different types of formulated patches^{8,9}. 25mg of Herbal formulation was dissolved in water-methanol solvent mixture (6:4). The drug matrix was prepared by dissolving varying concentrations (1% and 1.5% w/v) of HPMC in the same solvent system^{9,10}. The solution was kept undisturbed for 24 h. Then, with

the help of syringe, the solution was poured into a glass plate of 5.0-cm diameter placed on the glass surface. The solvent was allowed to evaporate for overnight in a thermostatically controlled oven at 60 °C. (Jyothsna et.al 2017) The patches were stored in an airtight container under ambient conditions for 7 days prior to use¹⁰.

Formulation	% of HPMC	Concentration of crude drug (mg)	% of PEG400
F1	1.5	25	4
F2	1.5	25	5
F3	1	25	5

Table 1: Formulation table for transdermal patches

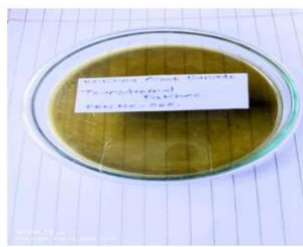


Figure1: Formulation of transdermal patches

Both extracts contained alkaloids, tannins, and flavonoids (*T. procumbens*) or saponins (*A. indica*), supporting their antimicrobial potential^{10,11}.

Phytochemical analysis of tridax extract:

Standard tests were performed to identify the presence to alkaloid, tannins, flavonoid and the test are mentioned^{11,12} in the table 2.

Evaluation Parameters

Phytochemical Screening

Sl.no	Phytoconstituent	Standard test	Method	Observation
1	Alkaloids	Dragendroff's test	1ml of ethanolic extract + ferric chloride reagent.	Blue to green colour
2	Tannins	Ferric chloride test	1ml of ethanolic extract + lead acetate reagent	Yellow colour
3	Flavonoids	Lead acetate test	1ml of ethanolic extract + Dragendroff's reagent	Orange to red precipitate

Table 2: Procedure and observation of Standard phytochemical tests of *Tridax procumbens* Linn

Phytochemical analysis of neem extract¹²:

S.no	Phytoconstituent	Standard test	Method	Observation
1	Alkaloids	Dragendroff's test	1ml of ethanolic extract + dragendroff's reagent.	Orange to red colour
2	Tannins	Ferric chloride test	1ml of ethanolic extract + ferric chloride reagent	green colour
3	Saponins	Bicarbonate test	1ml of ethanolic extract + 1ml sodium bicarbonate	Pale brown colour

Table 3: Procedure and observation of Standard phytochemical tests of *Azadirachta indica*.

Physical Appearance: All the prepared patches were visually inspected for color, clarity, flexibility, and smoothness¹².

Surface pH: Patches were kept in contact with 0.5 ml of double distilled water for 1 h in glass tubes and were allowed to swell. A combined glass electrode was brought near the surface of patch and pH readings were taken after allowing an equilibration^{10,12}.

Thickness of the Patch: The thickness of the drug loaded patches was measured by using a vernier callipers at three different points on the patches. Average values and standard deviation values of the three readings were calculated for each drug loaded patch.). Ensure the thickness falls within a specified range ($\pm 0.2 - 0.3$ mm)^{10,13}.

Uniformity of Weight: The patches were subjected to weight variation test by weighing all the patches on a digital weighing machine. The determinations were carried out in triplicate for each formulation. Average weight and standard deviation values were then calculated. Ensure weight variation does not exceed a certain percentage (e.g., $\pm 5\%$)^{12,13}.

Folding Endurance: This test was carried out to check the efficiency of the plasticizer and the strength of the patch prepared using different polymers. Folding endurance is defined as the number of folds required to break any polymeric patch. The folding endurance was measured manually by repeatedly folding a small strip of the film (2×2 cm) at the same place until it broke. The number of times the patch could be folded at the same place without breaking/cracking gave the value of folding endurance. Three patches of each type were taken for the test¹⁴.

Percentage Moisture Content: This test was also carried out to check the integrity of films under dry conditions¹⁰. The individual transdermal films (of specified area) were kept in a desiccator containing fused anhydrous calcium chloride at room temperature. During this period, the films were weighed at regular time intervals of 24, 48, and 72h. (Smith & Jones, 2012) The percentage moisture

content was determined by using the following formula¹²:

$$\% \text{ Moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Antifungal Assay

The antifungal activity of *Tridax procumbens* Linn extract, *Azadirachta indica* extract, and the prepared herbal patches was tested against the fungal strain *Aspergillus niger* using the modified agar well diffusion method^{9,12}.

Tridax procumbens Linn Extract¹⁴

- Sabouraud's dextrose agar (30 mL) was poured into sterile Petri dishes (n=3) and allowed to solidify¹⁴.
- Two wells (10 mm) were made in the agar using a cork borer, and the plates were inoculated with *A. niger*.
- Ethanolic extracts at 100 ppm and 200 ppm were added to the wells; a control plate was also prepared.
- Plates were incubated at 25 °C for 7 days after 1 hour of stabilization.
- Zones of inhibition were measured to determine antifungal activity.

Azadirachta indica Extract^{12,14}

- The same method was used as above.
- Ethanolic extracts at 100 ppm and 200 ppm were tested, along with a control.
- After incubation at 25 °C for 7 days, inhibition zones were recorded.

Herbal Patch^{14,15}

- Sabouraud's dextrose agar (30 mL) was poured into sterile Petri dishes (n=4) and solidified.
- Three wells (10 mm) were created, and the plates were inoculated with *A. niger*.
- Herbal patches were soaked in sterile saline and placed into the wells; one plate served as control.
- Plates were incubated at 25 °C for 7 days after 1 hour of stabilization.
- The antifungal activity was evaluated by measuring the zone of inhibition.

IV. RESULTS AND DISCUSSIONS

PHYTOCHEMICAL ANALYSIS FOR *TRIDAX PROCUMBENS LINN* EXTRACT

Extract of tridax was used to perform standard phytochemical test¹² and the results are given below in table:4

Sl.no	Phytoconstituent	Standard test	Results
1	Tannins	Ferric chloride test	+
2	Flavonoids	Lead acetate test	+
3	Alkaloids	Dragendroff's test	+

Table 4: Results of phytochemical analysis.

PHYTOCHEMICAL ANALYSIS FOR *AZADIRACHTA INDICA* EXTRACT

Sl.no	Phytoconstituent	Standard test	Results
1	Alkaloids	Dragendroff's test	+
2	Tannins	Ferric chloride test	+
3	saponins	Bicarbonate test	+

Table 5: Results of phytochemical analysis of neem.

Physical Characterization

Formula tion	p H	Thick ness (mm)	Weight Uniformity (mg)	Foldin g Endura nce	Moist ure Conte nt
F1	8.39	0.19	198	77	3.54
F2	8.37	0.23	215	155	2.79
F3	8.32	0.21	210	123	4.81

All formulations exhibited a pH range of 8.32–8.39, falling within acceptable limits for topical application. The thickness ranged from 0.21–0.23 mm, indicating uniform film formation with less than 5% variation among formulations. Patch 2 showed the highest

folding endurance (155), reflecting superior flexibility, and recorded a moisture content of 2.79%, which helps prevent brittleness.

ANTIFUNGAL ACTIVITY

ANTI-FUNGAL ACTIVITY STUDY OF *Tridax Procumbens Linn* EXTRACT

The antifungal assay of 100ppm and 200ppm extract was performed and the results of zone of inhibition are as follows,

Strain used	Zone of inhibition (in diameter mm)		
<i>Aspergillus niger</i>	Control	100ppm Extract	200ppm Extract
	0	20.5	19.5

Table 6: Antifungal activity of herbal extracts of *tridax* (Zone of inhibition)

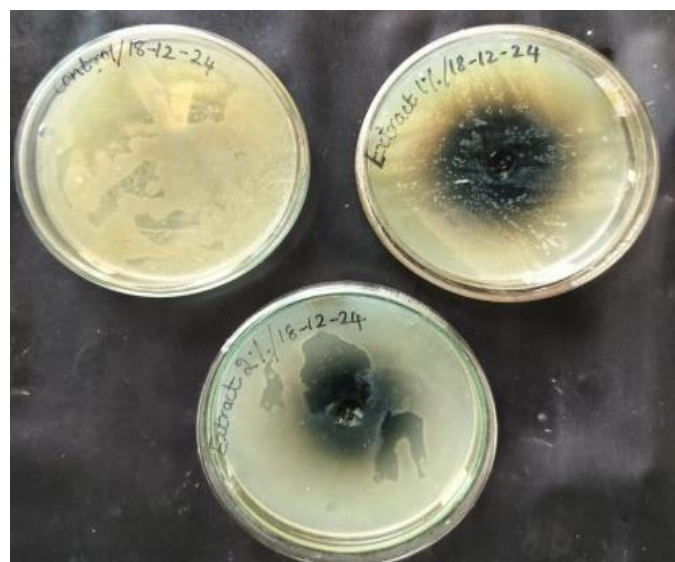


Figure 2: Zone of inhibition of *tridax* extract against *Aspergillus niger*.

Zone of inhibition is almost identical for 100ppm and 200ppm so, 100ppm extract was used for formulating a patch.

ANTIFUNGAL ASSAY OF *Azadirachta indica* EXTRACT

The antifungal assay of 100ppm and 200ppm extract was performed and the results of zone of inhibition are shown below in table no. 7

Strain used	Zone of inhibition (in diameter mm)			
	Control	F 1	F 2	F 3
Aspergillus niger	0	19	19.5	18.5

Table 7: Antifungal activity of herbal extracts of neem (Zone of inhibition)

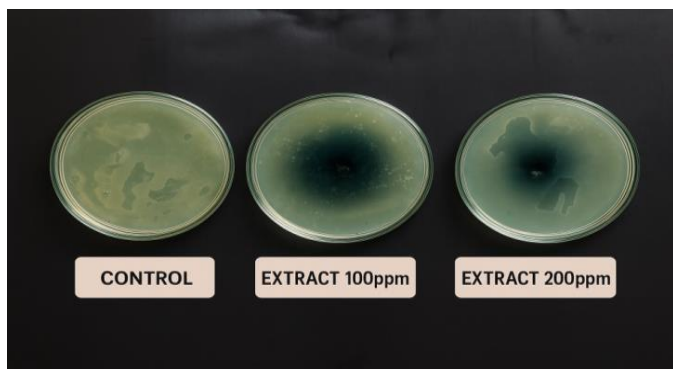


Figure 3: Zone of inhibition of tridax extract against *Aspergillus niger*.

ANTIFUNGAL ASSAY OF HERBAL PATCH

Strain used	Zone of inhibition (in diameter mm)			
	Control	F 1	F 2	F 3
Aspergillus niger	0	19	19.5	18.5

Table 8: Antifungal activity of herbal patch (Zone of inhibition)

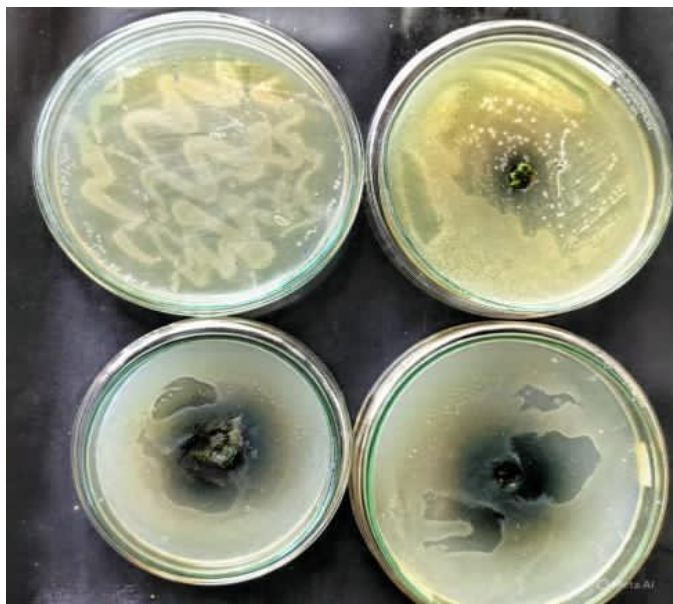


Figure 4: Zone of inhibition of polyherbal transdermal patch against *Aspergillus niger*.

T. procumbens (100 ppm) produced a 20.5 mm inhibition zone, while *A. indica* (100 ppm) showed an 18 mm inhibition zone. Among the patches, inhibition zones were recorded as follows: Patch 1 – 19 mm, Patch 2 – 19.5 mm, and Patch 3 – 18.5 mm.

V. FUTURE SCOPE:

The study highlights the antifungal potential of *T. procumbens* and *A. indica* extracts, individually and in patch form, showing promise for topical use. Future research should optimize formulation, assess in-vivo efficacy, evaluate stability, and ensure safety, while exploring synergistic combinations and commercial scalability for developing effective, eco-friendly herbal antifungal products.

VI. CONCLUSION:

In conclusion, the study successfully formulated and evaluated herbal antifungal patches incorporating *Tridax procumbens* Linn and *Azadirachta indica* extracts, both of which demonstrated notable antifungal activity against *Aspergillus niger*. Phytochemical analysis confirmed the presence of bioactive compounds with antimicrobial potential, supporting their therapeutic relevance. Among the three developed patches, all met acceptable physicochemical criteria for topical application; however, Patch 2 exhibited superior performance, reflected by the highest inhibition zone, optimal pH, consistent thickness, excellent folding endurance, and favorable moisture content. These findings indicate that herbal antifungal patches, particularly the formulation represented by Patch 2, hold significant promise as effective, eco-friendly alternatives to conventional antifungal treatments.

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Competing Interests: The authors declare that they have no conflict of interests.

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