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Research Paper

Development and Antifungal Assessment of a *Tridax procumbens* Linn Herbal Cream: An *In Vitro* Study

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ABSTRACT

This study aims to formulate and evaluate creams from extract of *Tridax procumbens* Linn. The present study ethanolic extract of *Tridax procumbens* Linn, was extracted using Soxhlet extraction. The extract was screened for phytochemicals like alkaloids, flavonoids, tannins. The antifungal activity of the cream against *Aspergillus niger* was tested by studying the zone of inhibition using dextrose agar media and has showed strong antifungal activity against the chosen fungus strain. *T. procumbens* leaf extract and its cream could be used as a safe and effective choice of drugs to treat skin infections because of its potent antifungal efficacy, less harmful, and less likely to lead drug resistance. Further assessment was conducted to determine the physical properties and stability of the cream. The study aims to identify the optimal formulation of the cream.

INTRODUCTION

Fungal infections represent a considerable risk to human health, especially for individuals with compromised immune systems. The increasing prevalence of antifungal resistance, coupled with a scarcity of effective treatment options, highlights the urgent need for innovative and powerful antifungal agents^[1,2,3,4]. Fungal pathogens possess the ability to manipulate the host immune

response, either by suppressing or activating it, to enhance their survival. Infections caused by fungi such as *Candida* and *Aspergillus* can lead to life-threatening conditions in immunodeficient patients. Additionally, fungal spores may provoke allergic reactions, asthma, and various respiratory problems. Skin infections caused by fungi like ringworm and athlete's foot can result in significant pain and discomfort. Agricultural diseases are primarily attributed to pathogens such as powdery mildew, rust, and leaf spot.

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Furthermore, *Aspergillus* and *Fusarium* species can produce mycotoxins that contaminate food supplies [2]. Severe pneumonia is often linked to *Cryptococcus*. Antifungal medications, including ketoconazole, miconazole, and econazole, are among the active treatments available. *Tridax procumbens* L is recognized for its chemical constituents, including flavonoids, alkaloids, tannins, carotenoids, and fumaric acid. These compounds exhibit beneficial properties such as anti-inflammatory, antimicrobial, antioxidant, anticancer, and anti-diabetic effects. This plant is particularly noted for its potent antibacterial activity, attributed to the phenolic compounds it contains [3,4,5,6].

MATERIALS AND METHODS

Materials required:

Tridax procumbens Linn was collected from medicinal garden of Gokaraju Rangaraju college of pharmacy, Bachupally, Hyderabad. Ethanolic extract of *Tridax procumbens*, absolute ethanol, bees wax, lanette wax, Cetyl alcohol, stearyl alcohol, stearic acid, white soft paraffin, methyl paraben, propylparaben, triethanolamine, propylene glycol, water [4,5,7,8].

Instrument required: Electronic weighing balance, Soxhlet extractor, Brookfield viscometer, laminar air flow, BOD incubator, autoclave, pH meter, hot air oven, magnetic stirrer, water bath [5,6,9,].

Apparatus required: Round bottom flask, condenser petri dishes, beakers, glass rod, and spatula.

Method of Extraction:

Following the collection and identification, fresh leaves of *Tridax procumbens* Linn (100g) were washed and shade dried for 24-48 hours. The dried

leaves were crushed to coarse powder and placed in 1L Soxhlet thimble attached with round bottom flask. The coarse powder with 500ml of ethanol were taken in round bottom flask. The leaf extract was obtained by Soxhlet extraction for about 6hours. The extract was collected and solvent was evaporated on water bath at 50°C. Then further dried in desiccator [9,10,12,14].

Evaluation of Herbal Extract:

Phytochemical Screening:

Standard phytochemical test was carried out for qualitative analysis of phytochemicals including, flavonoids, alkaloids, tannins, were tested by standard test and confirmed [5,6,7,8].

Test for Alkaloids: Extract (0.5 g) was shaken in a steam bath the test involves adding Dragendorff's reagent (a solution of potassium bismuth iodide) to the extract. A positive result, indicating the presence of alkaloids, is the formation of an orange or orange-red precipitate [10,12,15].

Test for Flavonoids: Add a 10% lead acetate solution to an herbal extract. If flavonoids are present, a yellow precipitate will form. This test is a simple qualitative method for detecting the presence of these phytochemicals [9,10,14].

Test for Tannins: The Ferric chloride test is a method to detect tannins in herbal extracts. A positive result is indicated by a dark blue or green-blue color when a ferric chloride solution is added to the extract. The intensity of the color can also be used to quantify the presence of tannin [9,12,16].

An extractive soluble in alcohol:

100 milli-liters of 90% alcohol were added to a Stoppard conical flask containing five grams of precisely measured powdered medication. After



being continuously in an electrical shaker, the mixture was allowed to macerate overnight. The weight and percentage of the extractive were then calculated after the filter was cautiously evaporated until it was dry^[10,14,16,17].

Alcohol Soluble Extractive: Extractive Weight/ Drug Weight X 100

Total Ash

A China dish was used to weigh three grams of the drug, burn it at a temperature of no more than 450 degrees Celsius until the carbon was gone, let it cool, and then weigh it again until it stayed the same for three readings. To calculate the percentage of ash the air-dried drug was used^[10,12,18].

$$\text{Total Ash} = \text{Wt. of ash} / \text{Wt. of drug} \times 100$$

Acid Insoluble Ash

The ash was obtained after boiling it for five minutes with 25 milli-liters of diluted

hydrochloric acid in it. After that, the insoluble substance was collected in a Gooch Crucible, cleaned with hot water, and burned until its weight remained constant. The amount of acid-insoluble ash was calculated with respect to the drug that had been allowed to air dry^[10,12,18].

Method of Preparation of Cream:

Cream was prepared by oil in water emulsion method. Required ingredients for oil phase, such as Stearic acid, stearyl alcohol, cetyl alcohol, bees wax, lanette wax, were weighed accurately in a beaker and heated at 70°C on a water bath by continuous stirring^[6,8,12]. Now in another beaker propylene glycol, triethanolamine, water is heated at 70°C on a water bath to prepare water phase and. Now oil phase is added to the water phase with continuous stirring. Optimum quantity of herbal extract added to this by continuous mixing by mechanical stirrer Methylparaben, propyl paraben, perfume was added after the formulation is cooled. And the formulation table is as follows:

Table1: Formulation table of Tridax procumbens Linn cream.

Oil Phase	F1 quantity %w/v	F2 quantity %w/v	F3 quantity %w/v
White bees wax	0	0.2	0.4
Lanette wax	0.4	0	0
Stearyl alcohol	1	1	1
Liquid paraffin	0.6	0.8	0.6
Stearic acid	0.2	0.2	0.2
Cetyl alcohol	1	1	1
Water Phase	F1 quantity %w/v	F2 quantity %w/v	F3 quantity %w/v
Ethanollic extract	1	1	1
Methylparaben	0.004	0.004	0.004
Triethanolamine	0.4	0.4	0.4
Propylene glycol	1.1	1.1	1.1
Propylparaben	0.01	0.01	0.01
Water	20	20	20

Evaluation of Topical Formulations:

➤ Organoleptic evaluation:

Visual inspection is performed for their physical appearance, colour, texture, phase separation, homogeneity, texture and homogeneity were



tested by placing cream in between thumb and index finger and pressing it ^[18,19,20].

➤ **Procedure of Phytochemical Test:**

➤ **Determination of viscosity:**

Viscosity of different formulations was determined using a Brookfield viscometer using spindle S-04 at 20 rpm ^[18,19].

➤ **Stability test:**

Stability of the formulations were tested by placing the formulations in environmental stability chamber at 25-27°C for 14 days and creaming or coalescence is inspected. ^[5,16].

➤ **Determination of pH:**

Dissolve 1gram from different formulations in 10ml of distilled water and homogenised using magnetic stirrer pH was determined using digital pH metre. pH of formulation was noted and the average results were shown ^[17,18,19].

➤ **Spreadability:**

The spread ability was determined by applying 1g of each formulation on a glass plate over which another plate was placed. The sample was pressed between two plates. A weight of 100g was allowed to rest on plate for 5mins. The increased in diameter due to spreading of cream was noted ^[17,18,19].

Anti-Fungal Assay:

Test organism: Standard fungal strain of *Aspergillus niger* (grown culture).

Antifungal Assay of *Tridax Procumbens* Linn Extract:

The antifungal assay of ethanolic extract of *Tridax procumbens* Linn was evaluated using modified agar well diffusion assay. Sabouraud's dextrose agar (30ml) was poured in sterile petri dishes (n=3) and allowed to solidify. Two wells (10mm diameter) were created in 2 petri dishes containing media with a cork borer, and plates were inoculated with standardized inoculum of *Aspergillus niger* using a cotton swab. The ethanolic extract of *Tridax procumbens* Linn (1mg) were added to the wells made in two petri dishes and another petri dish was kept as control. These petri dishes were labelled and were incubated at 25°C for 7days after 1hour room stabilization period. The diameter of the zone of inhibition was subsequently measured to assess antifungal activity ^[15,16].

Anti-Fungal Assay of Cream:

The antifungal efficacy of topical agents was evaluated using modified agar well diffusion assay. Sabouraud's dextrose agar (30ml) was poured in sterile petri dishes (n=4) and allowed to solidify. Three wells (10mm diameter) were created in 3 petri dishes containing media with a cork borer, and plates were inoculated with standardized inoculum of *Aspergillus niger* using a cotton swab ^[12,13]. The topical formulations (1mg) were added to the wells made in three petri dishes and another petri dish was kept as control. These petri dishes were labelled and were incubated at 25°C for 7days after 1hour room stabilization period. The diameter of the zone of inhibition was subsequently measured to assess antifungal activity ^[12,13,18].

RESULTS AND DISCUSSIONS:

Phytochemical analysis: Extract of tridax was used to perform standard phytochemical test and the results are given below in table 2.



Table 2: Results of phytochemical analysis.

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

**Figure 1: Standard chemical tests for tridax procumbens.****Extractive solubility analysis:****Table 3: Results for Extractive solubility analysis.**

Test	Result
Total Ash Content	10.5 %
Acid Insoluble Ash Content	3.00
Alcohol Soluble Content	10.11
Water Soluble Content	1.19

Evaluation of Physical Parameters of Prepared Formulations

The physical characteristics of three formulations are presented in the table below. The results of

creaming, coalescence test indicated that the formulations exhibit excellent stability and aesthetic appearance and uniformity.

Table 4: Evaluation of physical parameters of prepared formulations.

Sr.no	Evaluation parameters	Formulation 1	Formulation 2	Formulation 3
1	Colour	Light green	Olivaceous green	Pisum green
2	Physical state	Semi-solid	Semi-solid	Semi-solid
3	Homogeneity	Homogenous	Homogenous	Homogenous
4	Texture	Uniform	Non-uniform	Uniform
5	Fluidity	Viscous	Viscous	Viscous
6	Appearance	Soft	Soft	Soft
7	Stability	Stable	Stable	Stable
8	pH	8.39	8.37	8.32
9	Spreadability (cm)	5.6	5.1	7.5
10	Viscosity (cp)	348.3	295.3	318.2
11	Loss on drying	41%	31%	20%

In Vitro 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:

Table 5: Antifungal activity of herbal extracts (Zone of inhibition)

Strain used	Zone of inhibition (in diameter mm)		
<i>Aspergillus niger</i>	Control	100ppm Extract	200ppmExtract
	0	20.5	19.2



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

Anti-Fungal Activity Study:

The antifungal assay of 3 formulations was performed and the results of zone of inhibition are as follows:

Table 6: Antifungal activity of herbal creams (Zone of inhibition)

Strain used	Zone of inhibition (in diameter mm)			
<i>Aspergillus niger</i>	Control	F1	F2	F3
	0	9	9.5	10.5



Figure 3: Zone of inhibition of different formulations.

SUMMARY:

The work aims to formulate and evaluate an antifungal herbal cream of *Tridax procumbens*

Linn extract. The various herbal creams are prepared with different oil phase ingredients and are characterized with various parameters such as pH, appearance, phase separation, viscosity,

spread ability, and antimicrobial activity. This study demonstrates the antifungal activity efficacy of *Tridax procumbens* Linn leaf extract base cream against *Aspergillus niger*. The results show that cream of *Tridax procumbens* Linn exhibits superior antifungal activity. The extract's complex chemical composition likely contributes to its broad-spectrum antifungal properties, making it a promising natural alternative for treating skin infections.

- All the creams were observed to be green and had strong odour with smooth texture.
- pH of the creams was within the range of 8.32-8.39
- The viscosity of the herbal creams was found to be in the range of 295-398 cps.
- No phase separation was observed in the 3 herbal creams.
- The spread ability of all the herbal creams was found to be in the range of 5.1 -7.9 g.cm/s.
- All the developed herbal creams showed good antifungal activity. Amongst all the herbal creams, the F3 formulation showed highest zone of inhibition against fungi as compared to F1 and F2 formulations. The zone of inhibition was higher i.e. 10.5 ± 0.1 mm in case of *Aspergillus niger*.
- The texture of F3 was uniform and the pH was 8.2 with a viscosity of 318.2 cps.
- The Spreadability of F3 was 7.5
- All the herbal creams were found to be stable at the end of storage period and can be safely used on skin. Among them F3 formulation showed better results.

Hence, it can be concluded that herbal cream of *Tridax procumbens* Linn showed promising formulation for future application.

CONCLUSION:

This study demonstrates the antifungal activity efficacy of *Tridax procumbens* Linn leaf extract base cream against *Aspergillus niger*. The results show that cream formulation of *Tridax procumbens* Linn exhibits superior antifungal activity. The extract's complex chemical composition likely contributes to its broad-spectrum antifungal properties, making it a promising natural alternative for treating skin infections.

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