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Formulation, Evaluation, And In Vitro Antifungal Assessment of A Topical Preparation Using Leaf Extract From Tridax Procumbens L.

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ABSTRACT:
Background and Objectives: Most of the time, these fungi affect the skin and cause skin conditions like eczema, rashes, candidiasis, tinea pedis, and tinea corporis. Fungal diseases, which have become a major problem, can be caused by many different types of fungi. The study's goal was to create a safe and effective antifungal cream that uses Tridax procumbens L. leaf extract.
Material and Methods: To make the ethanolic extract, the Soxhlet device was used. Molecular docking studies and phytochemical analyses were done on the product. The oil-in-water emulsion method was used to make the cream. A microbial culture (MIC) was done with agar dilution, and the antifungal activity of the cream against Candida albicans was tested with agar diffusion.
Results: The current study shows that the leaf extract of T. procumbens has strong antifungal activity against the chosen fungus strain C. albicans. When compared to similar commercial topical treatments, the cream formulation that contained T. procumbens leaf extract was more effective at killing the test organism. Because it contains a lot of different chemicals that are thought to work in different ways against different targets, T. procumbens leaf extract and its cream form could be used instead of synthetic drugs to treat skin infections because they work just as well, are less harmful, and are less likely to lead to drug resistance.
Conclusion: This study concludes that the topical formulation of T. procumbens extract exhibits possible antifungal efficacy.

Keywords: Antifungal, topical formulation, extract, potential treatment

INTRODUCTION:

Herbal drugs are known to be an old-fashioned way to treat health problems. Linn's Tridax procumbens plant. It is in the family Compositae. It's also known as a "common button" or a "coat button." It is an uncontrolled plant that grows all over India. The plant may sometimes grow to be 60 cm tall [1-3]. Its roots can be found in ancient Greece around 1600 BC. Medicinal plants are now seen as an important part of treating wounds and the healthcare system. A lot of medicines are being made from these medical plants right now. Fungal diseases have become a major problem around the world. The skin is the first organ that bacteria attack [2-4].
A lot of skin problems, like rashes, eczema, and candidiasis, are caused by fungi. Fungal diseases are caused by fungi. It's also known as mycosis sometimes. Fungal infections can cause itching, redness, swelling, irritation, and other signs. To get rid of fungal diseases, different mixtures like ointments, creams, gels, and emulsions have been created [3-5]. Antifungal agents are medicines that are especially designed to kill fungus. Polyene-class drugs interact with ergosterol, which leads to the loss of cytoplasmic components and changes in how permeable the membrane is. Polyenes are things like nystatin and amphotericin B. Fungal conditions like cryptococcal meningitis and histoplasmosis are often treated with amphotericin B [6–8]. Nystatin is used to treat fungal infections on the skin that are caused by Candida albicans.

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Azole is the name of a different group of antifungal drugs. Some more are econazole, miconazole, and clotrimazole. People use these topical medicines to treat diseases on their skin, in their mouths, and in their vagina [7–9].
Two man-made antifungal drugs, terbinafine and naftifine, are put on the skin or taken by mouth to treat fungal diseases caused by dermatophytes. Flucytosine is used to treat Candida infections, chromoblastomycosis, and systemic cryptococcal infections. Glimeofulvin is taken by mouth to treat fungal diseases on the surface. Tridax procumbens can be used to treat wounds and also stops fungus, blood clotting, and bugs from coming into contact with it. When applied directly to wounds, herbal medicines made from leaves help them heal faster and are good for many skin diseases [8–10]. The goal of this work was to make a safe and effective antifungal cream with Tridax procumbens L. leaf extract that can be put on the skin.

MATERIALS AND METHODS:

Different chemicals, media, and solvents were used in the experiment. These included methyl paraben, propyl paraben, liquid paraffin, stearic acid, stearyl alcohol, triethanolamine, white beeswax, and Sabouraud dextrose agar. The company Loba Chemicals Pvt. Ltd. in Mumbai, India, sold these.

Plant Material Collection and Extraction:

The leaves of T. procumbens were gathered in Indian forests. After being picked up and identified, 100 g of fresh T. procumbens leaves were washed, left to dry in the air for two hours, and then put in a hot air oven for seven hours to remove all the water. The dried leaves were ground up into a coarse powder and put into a 1-liter Soxhlet device that was connected to a 1-liter round-bottom flask. Used 750 milliliters of pure ethanol to water the plant. Three days of work were spent on a device similar to a Soxhlet system to get the leaf extract. We took the extract and heated it up in a water bath at 50 °C until 75% of the solvent was gone. After that, it was dried out even more in desiccators [11-13].

Phytochemical Analysis of the Extract:

Standard chemical tests were used to qualitatively examine the phytochemical components of the ethanolic extract. Standard tests were used to prove the presence of different parts, such as alkaloids, carbohydrates, fixed oils and fats, flavonoids, glycosides, phenolic substances, proteins, steroids, tannins, terpenes, and terpenoids [14, 15].

Topical Formulation Preparation:

The oil-in-water emulsion method, shown in Table 1, was used to make the formulation base. Incorporating leaf extract into the soft mass of the formulation base led to the creation of different amounts of topical formulas. Instead of the extract, the base was used as the negative control in the test [16-18].

Table 1: Leaf extract topical formulation composition

Oil Phase		Aqueous Phase	
Components	**Qty (% w/v)**	**Components**	**Qty (% w/v)**
White Bees Wax	2	Methyl Paraben	0.020
Stearyl Alcohol	5	Triethanolamine	2.0
Liquid Paraffin	3	Propylene Glycol	5.5
Stearic Acid	1	Propyl Paraben	0.05
Cetyl Alcohol	5	Water	100

Topical Formulation characterization:

Organoleptic study:

Visual tests were used to check the formulated goods' color, texture, phase separation, and homogeneity, among other things. To check for uniformity and structure, a small amount of the finished product had to be pressed together between the thumb and index finger. The consistency and appearance of coarse particles were used to test the texture and homogeneity of the formulation [19, 20].

Viscosity:

Utilizing a Brookfield Viscometer with a Spindle S-04 and operating at 20 revolutions per minute, the viscosity of the formulations that were created was determined [21].

Stability study:

The stability was evaluated by storing the formulations in an environmental stability chamber at a temperature range of 25 to 27 degrees Celsius for a period of thirty days [22-26].

pH of the formulation:

A digital pH meter was used to determine the pH of the formulation after it had been suspended in a solution of potassium nitrate at a concentration of one percent. Therefore, in order to establish homogeneity, a magnetic stirrer was

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utilized [27-30].

Spreadability study:
After one gram of each mixture was put on the first glass plate, it was covered with a second one to see how well it spread. Between two plates, the stuff was squished. It took five minutes for the 100-gram weight to rest on the plate. As you can see, the circumference grew because the formulation spread out [31-34].

Antifungal Activity of prepared Formulation:
The antifungal effectiveness of external medicines was tested using the method we use in our lab. For growing fungi, 30 ml of Sabouraud dextrose agar was put into three clean Petri dishes and left to harden. After the mixture had hardened, a clean cork borer was used to make three holes in each plate, each 10 mm in diameter. This was done to make sure that the agar plugs were fairly spaced. A cotton swab was used to put the usual C. albicans culture on the plates. Two of the wells on each plate held about 1 milliliter of the defined topical formulations, and the third well held 1 milliliter of the approved commercial topical formulation. The plate was turned over and left to rest at room temperature for an hour. It was then heated to 25 °C and kept there for seven days after hardening for two to three minutes. After the cells were incubated, the inhibitory zones' widths were determined [35-37].

RESULTS AND DISCUSSION:
Phytochemical Analysis:
According to the results of the usual chemical tests that are provided in Table 2, the ethanolic extract of the leaves of T. procumbens included a variety of phytoconstituents.

Table 2: Tridax procumbens L. ethanolic extract phytoconstituents

Sr. No.	Phytoconstituents	Plant Extract
1	Tannins	+
2	Carbohydrates	+
3	Terpenes and Terpenoids	+
4	Steroids	+
5	Glycosides	-
6	Fixed Oils and Fats	+
7	Phenolic Compounds	+
8	Flavonoids	+
9	Proteins	-
10	Alkaloids	+

Topical Formulation development:
The drug was made in a uniform cream form, which was tested and found to meet all the requirements. The extract in amounts of 5 mg/g and 10 mg/g were added to the mixture. When the antifungal effectiveness of the formulation was compared to that of widely available products, good results were seen.

Evaluation of Physical parameters of prepared Formulations:
In Table 3, the physical properties of both versions were shown. During creaming, coalescence, and centrifugation tests, the data showed that the formulations were stable, looked good, and were uniform. When compared to the 5% formulation, the 10% formulation of T. procumbens leaf extract is easier to spread. The math showed that there was no weight loss.

Table 3: T. procumbens leaf extract topical formulation physicochemical evaluation

Sr. No.	Evaluation parameters	Trail Batch	Batch 1 (10% w/w)	Batch 2 (5% w/w)
1	Color	White	Light Green	Dark Green
2	Physical state	Semi-solid	Semi-solid	Semi-solid
3	Homogeneity	Homogenous	Homogenous	Homogenous
4	Texture	Smooth	Smooth	Smooth
5	Fluidity	Viscous	Viscous	Viscous
6	Appearance	Soft	Soft	Soft
7	Stability	Stable	Stable	Stable
8	pH	7.9	8.12	8.5
9	Spreadability (cm)	7.2	7.5	8.3
10	Viscosity (cP)	360.1	390.0	540.8

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Antifungal Activity study:
Both the antifungal properties of two creams containing 10% and 5% leaf extract are outlined in Table 4, along with the weights of the creams. Both between 16 and 20 mm and between 20 and 24 mm, a blocking zone was observed for the 10% cream throughout the examination. By using the 10% cream, the zone of suppression was significantly larger than when using the 5% cream, and the effectiveness of the 10% cream was quite similar to that of prescription medications. There was a 20 mm zone of inhibition in the 10% cream, which was larger than the 17 mm zone of inhibition in the Amorolfine standard.

Table 4: Antifungal effectiveness of T. procumbens leaf extract formulation

Strain Used	**Zone of Inhibition (in Diameter)**								
	AB 1			**AB 2**			**AB 3**		
Candida albicans	**B1 10%**	**B2 5%**	**Ketoconazole**	**B1 10%**	**B2 5%**	**Clotrimazole**	**B1 10%**	**B2 5%**	**Amorolfine**
	20	**22**	**26**	**21**	**25**	**29**	**18**	**21**	**18**

CONCLUSION:
The research demonstrated that the selected type of fungus, Candida albicans, was effectively killed by the T. procumbens leaf extract. Cream formulations containing T. procumbens leaf extract had greater antifungal efficacy against the test organism compared to topical treatments of a similar kind available on the market. As an alternative to synthetic drugs for treating skin infections, T. procumbens leaf extract and cream contain a wide range of chemicals that are believed to work in different ways against different targets. They are more effective, less harmful, and less likely to lead to drug resistance.

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Conflict of Interest
None

REFERENCES:
1. Patil KS, Samant SS. Formulation and Evaluation of Topical Polyherbal Gel Containing Cocculus Hirsutus and Tridax Procumbens. International Journal of Pharmaceutical Sciences and Research. 2023;14(9):4560-66.
2. Kesharwani RK, Keservani RK, Sharma AK, editors. Enhancing the therapeutic efficacy of herbal formulations. IGI Global; 2021 Mar 19.
3. Ahmed SS, Prakash KC, Tabassum S, Salma N, Devi KH. Pharmacognostical and Pharmacological Review on Tridax procumbens Linn. Research Journal of Pharmacology and Pharmacodynamics. 2019;11(1):11-6.
4. Sutar PB. Chitosan-Polyvinyl alcohol Blend Transdermal Patches Consist of Curcuma longa L., Tridax procumbens L., Aloe vera (L.) Ethanol-water Extract for Healing of Wounds. Journal of Current Pharma Research. 2019 Apr 1;9(3):2894-903.
5. Sharma AK, Keservani RK, Gautam SP, editors. Herbal Product Development: Formulation and Applications. CRC Press; 2020 Nov 24.
6. Ahmad, Mohd F., Ahmad, S. M., Keservani, Raj K., Sharma, Anil K. A Study on Anti-Inflammatory Activity of Tuber Extracts of Solanum Tuberosum (Solanaceae) in Male Albino Rats, Nat. Aca. Sci. Lett. India. 2016, 39 (6): 421-425.
7. Keservani, Raj K., Sharma, Anil K., Jarouliya, U., Singh, A.K. Ebola Virus Disease and its Complications Universal Journal of Pharmaceutical Research, 2016c, 1 (1), 54-58.
8. Keservani, Raj K., Kesharwani, Rajesh K., Sharma, Anil K., Naturally Occurring Toxicants as Etiologic Agents of Food-borne Disease, In: Food Toxicology, Edited by Bagchi, Debasis., Swaroop, Anand, CRC Press, Taylor and Francis. Chapter 12, 2016d, 245-262. ISBN: 9781498708746
9. Ahmad, Mohd F., Ahmad, S. M., Keservani, Raj K., Pradhan, A. "Anti-Ulcer Activity of Tuber Extracts of Solanum Tuberosum (Solanaceae) In Rats", Acta Fac. Pharm. Univ. Comen. LXII, 2015a (2): 32-37.
10. Jarouliya U, Zacharia A, Keservani RK, Prasad GB. Spirulina maxima and its effect on antioxidant activity in fructose induced oxidative stress with histopathological observations. European Pharmaceutical Journal. 2015;62(2):13-9.
11. Ahmad, Mohd F., Keservani, Raj K., Babu, DJM., Interaction study between Ocimum sanctum and Glimepiride (sulfonylurea derivative) in diabetic rats, J. Chin. Pharm. Sci. 2015b, 24 (3), 156–163.
12. Keservani Raj K., Shelke Suvarna Jagannath, Gawali Vikas, Gaviraj E.N., Binorkar Sandeep V., Rane Sachin
13. Shrikrushna, Sarvadnya Anagha Amit and Patil Sharangouda J.: Anti-Alzheimer effect of Ammannia baccifera whole plants ethanolic extract. Intern. J. Zool. Invest. 2024, 10(2): 671-678.

14. Muthukumar B, Nandini MS, Elumalai P, Balakrishnan M, Satheeshkumar A, AlSalhi MS, Devanesan S, Parthipan P, Rajasekar A, Malik T. Enhancement of cell migration and wound healing by nano-herb ointment formulated with biosurfactant, silver nanoparticles and Tridax procumbens. Frontiers in Microbiology. 2023 Aug 2;14:1225769.
15. Bera A, Banerjee N. Physicochemical and antistaphylococcal evaluation of two herbal ointments from Mikania micrantha Kunth and Tridax procumbens Linn. Pharmacognosy Journal. 2023;15(6).
16. Kethamakka SR, Deogade MS. Jayanti veda (Tridax procumbens)-unnoticed medicinal plant by Ayurveda. Journal of Indian System of Medicine. 2014 Jan 1;2(1):6-22.
17. Gunawardana S, Dias B. Methodological advances in formulation and assay of herbal resources-based topical drug delivery systems. Journal of Complementary and Integrative Medicine. 2024 Sep 19(0).
18. Wilkie P, Poulsen AD, Harris D, Forrest LL. The collection and storage of plant material for DNA extraction: the teabag method. Gardens' Bulletin Singapore. 2013;65(2):231-4.
19. Vyas N, Keservani RK, Gavatia NP, Jain S, Argal A. Effect of Tamarindus indica and its Polyherbal Formulation on Radiation induced Alopecia. Int J Pharma Tech Res. 2010;2:1543-46..
20. Aliaño-González MJ, Richard T, Cantos-Villar E. Grapevine cane extracts: Raw plant material, extraction methods, quantification, and applications. Biomolecules. 2020 Aug 17;10(8):1195.
21. Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies. 2020 Mar 1;8(2):603-8.
22. Igbinosa OO, Igbinosa EO, Aiyegoro OA. Antimicrobial activity and phytochemical screening of stem bark extracts from Jatropha curcas (Linn). African journal of pharmacy and pharmacology. 2009 Feb 28;3(2):058-62.
23. Simoes A, Veiga F, Vitorino C, Figueiras A. A tutorial for developing a topical cream formulation based on the quality by design approach. Journal of pharmaceutical sciences. 2018 Oct 1;107(10):2653-62.
24. Chauhan L, Gupta S. Creams: a review on classification, preparation methods, evaluation and its applications. Journal of drug delivery and therapeutics. 2020 Oct 15;10(5-s):281-9.
25. Gwarzo ID, Mohd Bohari SP, Abdul Wahab R, Zia A. Recent advances and future prospects in topical creams from medicinal plants to expedite wound healing: a review. Biotechnology & Biotechnological Equipment. 2022 Dec 31;36(1):82-94.
26. Kikwai L, Babu RJ, Prado R, Kolot A, Armstrong CA, Ansel JC, Singh M. In vitro and in vivo evaluation of topical formulations of spantide II. Aaps Pharmscitech. 2005 Dec;6:E565-72.
27. Gupta A, Mishra AK, Singh AK, Gupta V, Bansal P. Formulation and evaluation of topical gel of diclofenac sodium using different polymers. Drug invention today. 2010 May 1;2(5):250-3.
28. Thombre NA, Laddha UD, Ahire ED, Tajanpure AB, Surse SN, Kakad SP, Kakad AV, Anumone D, Sonawani R. Formulation Development and Evaluation of Herbal Antifungal Cream by using Psidium guajava Leaf Extract. International Journal of Drug Delivery Technology. 2023;13(4):1229-44.
29. Aiyalu R, Govindarjan A, Ramasamy A. Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model. Brazilian Journal of Pharmaceutical Sciences. 2016 Sep;52(03):493-507.
30. Rao M, Kadam M, Rao S. Formulation and evaluation of topical formulation for cutaneous tuberculosis. Journal of Drug Delivery and Therapeutics. 2018 Jul 14;8(4):102-16.
31. Deshmukh MD, Patil MP, Ahire ED, Gosavi SB. Shatdhauta Ghrita: A Promising agent in the development of herbal creams. Journal of Pharmaceutical Negative Results. 2022 Oct 3:1332-43.
32. Mitkari BV, Korde SA, Mahadik KR, Kokare CR. Formulation and evaluation of topical liposomal gel for fluconazole. Indian J Pharm Educ Res. 2010 Oct 1;44(4):324-33.
33. Sable RR, Thite DG, Udavant PB, Ahire ED, Khairnar SJ. Isolation and identification of flavonoids compounds and formulation of tecoma undulata (Sm.) seem. Herbal ointment. Materials Today: Proceedings. 2023 Dec 12.
34. Helal DA, El-Rhman DA, Abdel-Halim SA, El-Nabarawi MA. Formulation and evaluation of fluconazole topical gel. Int J Pharm Pharm Sci. 2012;4(5):176-83.
35. Bseiso EA, Nasr M, Sammour O, Abd El Gawad NA. Recent advances in topical formulation carriers of antifungal agents. Indian journal of dermatology, venereology and leprology. 2015 Sep 1;81:457.
36. Zafar A, Imam SS, Alruwaili NK, Yasir M, Alsaidan OA, Alshehri S, Ghoneim MM, Khalid M, Alquraini A, Alharthi SS. Formulation and evaluation of topical nano-lipid-based delivery of butenafine: in vitro characterization and antifungal activity. Gels. 2022 Feb 18;8(2):133.
37. Wavikar P, Vavia P. Nanolipidgel for enhanced skin deposition and improved antifungal activity. AAPS pharmscitech. 2013 Mar;14:222-33.