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FORMULATION AND EVALUATION OF HERBAL GEL PREPARATION OF ANTIFUNGAL ACTIVITY CONTAINING *TRIDAX PROCUMBES* LEAVES EXTRACT.

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ABSTRACT:
This study concentrates on the creation and assessment of a herbal gel made from the powerful antifungal extract of *Tridaxprocumbens*leaves. Herbal remedies have a long history of being used to cure a variety of illnesses, and their current uses cover a broad spectrum of conditions. The tropical plant *Tridaxprocumbens* is used for its antifungal, anticoagulant, wound-healing, and insect-repelling qualities. The study focuses on the plant's antibacterial, wound-healing, antioxidant, and antifungal effects while examining its histological and pharmacological characteristics. Using ethanol, the active ingredients in dried *Tridax*procumbens leaves are extracted, and the existence of advantageous substances is then determined using phytochemical analysis. With potential uses in clinical and veterinary settings, the gel formulation seeks to offer a safe, non-toxic, and ecologically acceptable substitute for synthetic antifungal medicines. Along with discussing these issues, the evaluation offers suggestions for improving the final product's safety and efficacy. Other issues included in the review include stability and microbial susceptibility.
Keywords: Tridaxprocumbens, Herbal medicine, Antifungal activity, Phytochemical analysis, Pharmacological evaluation

INTRODUCTION

Introduction to herbal medicines:

Herbal medicines, derived from plants, For centuries, diverse cultures have utilized them to prevent and treat illnesses. These natural remedies, often known as phyto-medicines, utilize the

healing properties of plant parts such as leaves, roots, seeds, and flowers. As a cornerstone of traditional healing systems, such as ayurveda, Traditional Chinese Medicine (TCM) and traditional healing practices. Herbal medicines have gained increasing attention in modern healthcare for their potential therapeutic benefits and holistic approach to health. One of the main appeals of herbal medicines lies in their ability to provide a natural alternative to synthetic drugs. Many people turn to herbal remedies seeking treatments that are perceived as more natural, with fewer side effects. Scientific research has identified numerous active compounds in plants that exhibit medicinal properties, such as alkaloids, flavonoids, terpenoids, and polyphenols, which can exert various biological effects such as antioxidant, anti-inflammatory, and antimicrobial properties. The resurgence of interest in herbal medicines is also driven by a growing body of scientific evidence supporting their efficacy and safety. Advances in phytochemistry and pharmacology have led to a better understanding of how these plant compounds interact with the human body, offering insights into their mechanisms of action and potential therapeutic applications. This has facilitated the integration of herbal medicines into contemporary healthcare systems, often as complementary therapies alongside conventional treatments. Throughout human history, there has been a connection between plants, disease, and life. Rather of consuming artificial food, prehistoric humans relied on natural resources. Herbal medications are known to treat a wide range of illnesses, including cirrhosis, asthma, migraines, Alzheimer's, anti-aging, diabetes, arthritis, depression, anti-inflammatory, and anti-HIV. The World Health Organization defines herbal medicines as finalized and labeled compositions comprising plant material or active components. [(1)]

Tridaxprocumbens lin.

India uses the tropical American plant *Tridaxprocumbens* for its wound-healing, antifungal, anticoagulant, and insect-repelling qualities. Its potential for a variety of disorders is demonstrated by the fact that it is also utilized in traditional medicine to treat liver and skin diseases, including diabetes.*Tridaxprocumbens*is a widely recognized plant species in traditional medicine, recognized for its diverse therapeutic properties. Commonly referred to as "coat buttons" due to its distinctive flower appearance, It is a member of the Asteraceae family and is commonly found in tropical and subtropical regions worldwide. The plant is a perennial herb, typically seen growing as a weed along roadways and in fields.[(2)]

Research on *TridaxProcumbens L's* histology

The study looked at the transverse section of the *Tridaxprocumbens* plant and found that it had a spongy parenchyma with 2-4 layers, a single-layered top epidermis with cylindrical palisade cells, and polygonal tabular cells that measured 40–70 µm by 15–30µm.[(3)]

Pharmacological property

Antimicrobial activity: The goal of research is to find novel antimicrobial compounds from plants, microbes, animals, and soil that can be used as chemotherapeutic agents for infectious diseases. Phytochemicals from medicinal plants with antibacterial qualities, such the green perennial herb *Tridaxprocumbens Linn* in India, may satisfy this need. Traditional medicine provides a systematic screening methodology.

Wound healing activity: Daily reductions in the area of the wound were observed in studies on the healing of excision wounds. The wound contraction rate of untreated animals was 83.53%, whereas the wound contraction rate of the formula-1 and formula-2 groups was 95.31% and 91.07%, respectively. Calculus 3 displayed similar rates of contraction. Therapies using formulas 1 and 2 might work. [(4, 5)]

Antioxidant activity: Using methods like the hydrogen peroxide method, radical scavenging, DPPH, ferric reducing antioxidant capacity, and hypoglycemic impact, this review investigates the antioxidant potential of *T. procumbens* and its preparations. While flavonoids and phenolic components in acetone, ethanol, and ethyl acetate extracts exhibit substantial action, ethanol extracts demonstrate high antioxidant activity. [6, 7]

Antifungal activity:On sabouraud dextrose agar medium, two fungal strains, Aspergillusflavus MTCC 277 and Aspergillusniger MTCC 282, were grown in culture.. The disc diffusion test 11 was used to measure antimicrobial activity. After testing three extracts, the activity index and zone of inhibition were determined.[8, 9]

Gels:

Topical gels are hydrophilic, semi-solid formulations made of both organic and inorganic macromolecules that are applied topically to treat and prevent skin diseases. Hydrophobic contacts, hydrogen bonds, and electrostatic interactions are used in physical gels.

Structure of Gels

When polymers are dispersed over a hydrophilic liquid, a gel is formed, which provides a three-dimensional matrix for the administration of drugs. The gel's interlocking agent particles control how flexible it is.(10)

MATERIAL AND METHOD

Collection of Plant Material

Before storage, fresh *Tridaxprocumbens* leaves were cleaned, dried in the shade for two to three weeks, and subsequently ground into a powder. [11]

Preparation of extract

The dried leaves of *Tridaxprocumbens* were ground into a powder and extracted using 100% ethanol (1:10). The plant material was then digested for 72 hours and concentrated into a 250 ml iodine flask. [12,13]

Thin layer chromatography (TLC): By conducting phytochemical screening and UV spectroscopy analysis, it has been confirmed that both the crude extract and fraction 4 contain flavonoids. Consequently, the crude extract and fraction 4 were selected for further examination through TLC study. Thin layer chromatography was carried out following established protocols using Quercetin as the reference standard on silica gel 60 F254 plates. The mobile phase consisted of Toluene: Ethyl acetate: Formic acid (7:3:0.5), with aluminum chloride reagent utilized as the detecting agent, as illustrated in Figure 23.[14, 15]

UV- Visible spectrophotometer: The plant extract ethyl acetate was subjected to UV-visible spectral analysis. Using the same solvent, the sample was diluted to a ratio of 1:10. Using a Jasco V-730 Spectrophotometer, the extract was scanned in the 200–800 nm wavelength range, and the distinctive peaks were found.

FTIR spectrophotometer: The potassium bromide (KBr) pellet (FTIR grade) technique was used to analyze the aqueous extract using Fourier transform infrared spectroscopy (FTIR) at 400–4000 cm-1. A Jasco FTIR-4600 Fourier transform infrared spectrometer was used to capture the spectra.[16, 17]

Table 4 Composition of the different formulation batches for TP gel

Ingredients (%w/w)	**TF1**	**TF2**	**TF3**	**TF4**	**TF5**	**TF6**
Extract (*TP Lin.)*	0.20gm	0.40gm	0.60gm	0.80gm	0.90gm	1gm
Carbapol 934	5gm	5 gm	5 gm	5 gm	5 gm	5 gm
Xantham Gum	1gm	1gm	1gm	1.5gm	1.5gm	1.5gm
Triethanolamine	Qs	Qs	Qs	Qs	Qs	Qs
Methyl Paraben	0.5gm	0.5gm	0.5gm	0.5gm	0.5gm	0.5gm
Tween 80	1ml	1ml	1ml	1ml	1ml	1ml
Light liquid paraffine	5ml	5ml	5ml	5ml	5ml	5mAl
Water	Qs	Qs	Qs	Qs	Qs	Qs

Preperation of *Tridaxprocumbens* lin. gel

1. The mixture was homogenized for 15 minutes at 300–500 rpm after 20 milliliters of water and the necessary quantity of carbopol 934 and xanthum gum were added.
2. After adding 10ml of water and triethanolamine, the liquid was agitated at more than 500 rpm until a sticky consistency was reached.
3. After agitating the *Tridaxprocumbens* extract for ten minutes in a gel basis, Tween 80, Light Liquid Paraffin, and methylparaben were added to ensure consistency.
4. After gradually adding water, the mixture was stirred for a further 45 minutes. [18]

Physical appearance: Scent and color were among the physical attributes that were assessed by the eye.

Measurement of pH: The pH of mixtures created from 100 CC of distilled water was measured three times using an electronic pH meter, with the average value being determined.

Spreadability: Excess sample is compressed between two slides to measure spreadability; greater spreadability means less time is needed to separate the slides. [19]

$$S = M \times L T$$

Where,

S: Spreadability
M: Mass
L: Diameter
T: Time

Viscosity: With a spindle no. 3 at 10, 20, 30, 40, and 50 rpm, a Brook Field viscometer (RVDV-+Pro+)was used to measure the viscosity of gel compositions.

Wash ability: After applying the formulations to the skin, it was evaluated how simple it was to wash them off with water.

Skin irritancy testing: Human skin was treated with the test formulations, and the results were monitored.[20]

In – vitro Franz diffusion study (fish skin membrane):A 25 ml receptor compartment capacity Franz diffusion cell was used for in vitro skin penetration experiments. The membrane of removed fish skin was sandwiched between the donor and receptor chambers, and the skin was covered with a specially prepared cream. A pH 7.4 phosphate buffer was added to the receptor compartment, and the entire assembly was constantly shaken at 50 rpm. Samples were taken out at various times, and a UV-Visible spectrophotometer was used to determine the amount of drugs present. The configuration of the Franz diffusion cell assembly is seen in figure 28.[21]

FTIR Spectroscopy: Formulation of *tridaxprcoumbens*gel F1and F2 was analyzed by jasco FTIR_4600 for fuctional group analyzed.

Drug content determination: Using a UV-Visible spectrophotometer, the antifungal cream's content was estimated. The formulation was taken up to 0.5g in a 50 ml volumetric flask. Phosphate buffer pH 7.4 was used to build up the solution to the mark.The entire mixture was then agitated. After shaking the mixture, what man filter paper was used to filter it. A acceptable wavelength was determined by further diluting the filtrate (0.1 ml) to 10 ml using solvent.[22]

Drug content,

$$\frac{Concentration \times Dilution\ factor}{1000}$$

%Drug content,

$$\frac{Practical\ yield \times 100}{Theoretical\ yiel}$$

Stability Studies: Stability study was conducted as per ICH guidelines at (40±2°C/75±5% RH) for 90 days .The samples were placed in stability chamber (REMI) for 90 days and observed for colour, appearance, odour phase sepration and consistency. [23]

Antifungal activity: On sabouraud dextrose agar medium, two fungal strains-aspergillusflavus MTCC 277 and aspergillusniger MTCC 282—were cultured. The disc diffusion test 11 was used to measure antimicrobial activity. After testing three extracts, the activity index and zone of inhibition were determined. [24, 25, 26]

RESULTS AND DISCUSSION

1. Pre-formulation study

a) **Organoleptic properties:** The organoleptic characteristics of the drug like color, odour and appearance were studied as shows in table no.

Table 5 Organoleptic Properties

Sr No.	Properties	*Tridaxprocumbens lin.*
1	Color	Light greenish color
2	Odour	Pleasant odour
3	Appearance	Semi-solid

b) Solubility : Gel was soluble in boiling water, alcohol, ether, and chloroform etc.

Phytochemical Analysis:

Table 6 Phytochemical test for Tridaxprocumbens

Sr No.	Phytochemical test	Results
1	Test for Carbohydrates	-
2	Test for phenols and tannins	-
3	Test for Flavonoids	+
4	Test for Saponins	+
5	Test for Glycosides	-
6	Test for Terpenoids	+
7	Test for Quinines	+
8	Test for alkaloids	+

TLC-Thin layer chromatography: Three places with Rf values ranging from 0.66 to 0.72 in our TLC data, some employing different solvents, indicated polar and non-polar components in *T. procumbens*leaves show in figure 23.

Figure 23 TLC profile of *Tridaxprocumbens lin.*

UV- Visible spectrophotometer

The spectroscopy of photons in the UV-visible range is connected to UV-visible spectrophotometers. The visible spectrum or its nearby bands, from 200 nm to 800 nm, are used in UV-visible spectroscopy. The absorption in the visible ranges is directly impacted by the color of the substances involved. These electromagnetic spectrum regions are where molecules go through electronic transitions19. The UV-visible spectral profile in the current investigation displayed the peaks at 402 nm and 608 nm, as shown in figure 24.

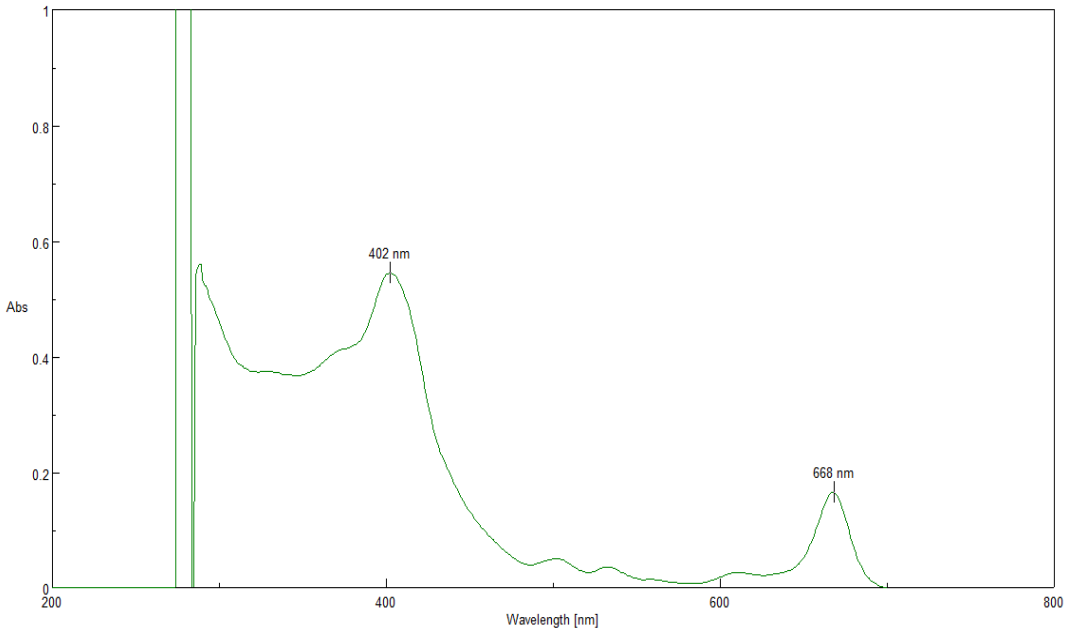

Figure 24 UV analysis of *Tridaxprocumbens*

FTIR Spectroscopy *Tridaxprocumbens*Extract sample was analyzed by jasco FTIR-4600 for functional group analysis and spectrum was analyzed for functional groups shows in fig no 25.

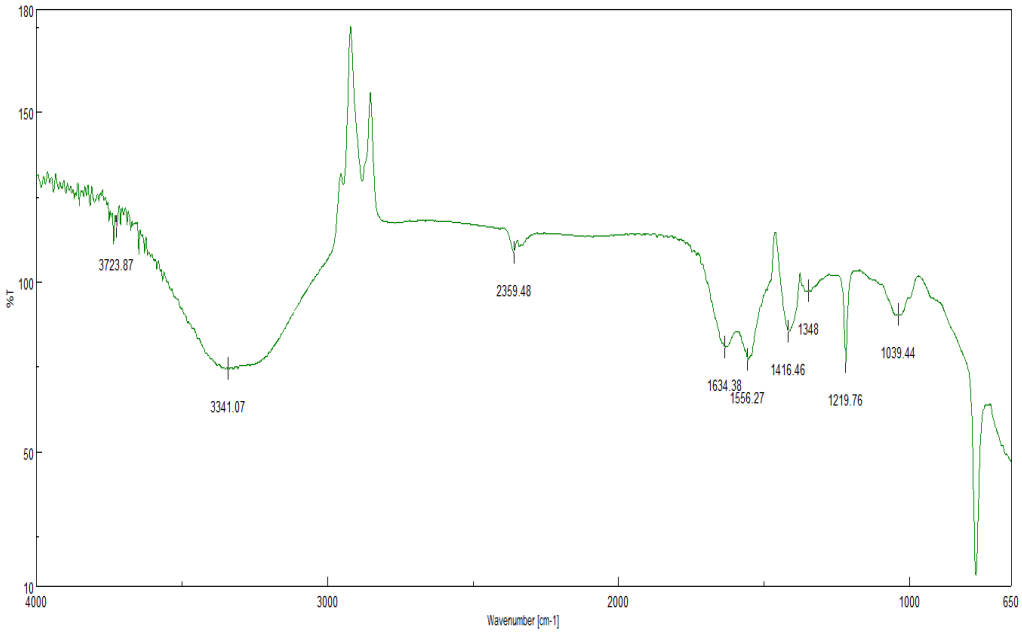

Figure 25 FTIR spectra of *Tridaxprocumbens* extract.

Table 7 FTIR spectra of *Tridaxprocumbens* extract.

Functional group	Reported Frequency (cm^{-1})	Observed Frequency (cm^{-1})	Inference
C-C	1300-800	1039.44	Complied
C=C-O-C	1270-1220	1219.76	Complied
-C-H	1485-1340	1348	Complied
$-CH_3$	1450-1375	1416	Complied
N=O	1550-1350	1556.27	Complied
N-H	1640-1550	1634.36	Complied
C-O	2400-2300	2359.48	Complied
O-H	3500-3200	3341.07	Complied
O-H	3800-3700	3723.87	Complied

Physical appearance: Formulation prepared was evaluated for the color, odour and consistency. The color of the gel was observed by visual examination which is Light greenish color. The odour of gel was found to be pleasant. The State was gel was examined visually. The gel was semisolid in nature. The formulation was examined by rubbing gel on hand manually. Gelwas smooth consistency. Results are show in table 8.

Table 8 Evaluation of physical characteristics

Sr No.	Properties	*Tridaxprocumbens lin.*
1	Color	pale greenish
2	Odour	Pleasant
3	Appearance	Mostly solid
4	Consistency	Smooth

Formulation was found smooth and consistent semisolids.

Measurement of pH: All formulations had pH values between 6.5 and 6.7, which is compatible with skin and corresponds to skin pH.

Table 9 pH of *tridaxprocumbens* gel

Sr No.	Formulation batch	pH value
1	TF1	6.5
2	TF2	6.7

pH values were shown neutral pH of gel indicated less chances of skin irritation on use

Spreadability: Gel formulation TF2 produced good Spreadability than the TF1 Formulation. Spread ability of gel were found to have in the range of 15 to 35 gm .cm/s indicating good Spread ability the result in were shown in table 10.

Table 10 Spreadability test

Sr No.	Weight	TF1	TF2
1	25	14.1	13.3
2	50	16. 3	15.1
3	75	19.2	17.7

The less viscous formulation F1 was shown better spreadability than F2.

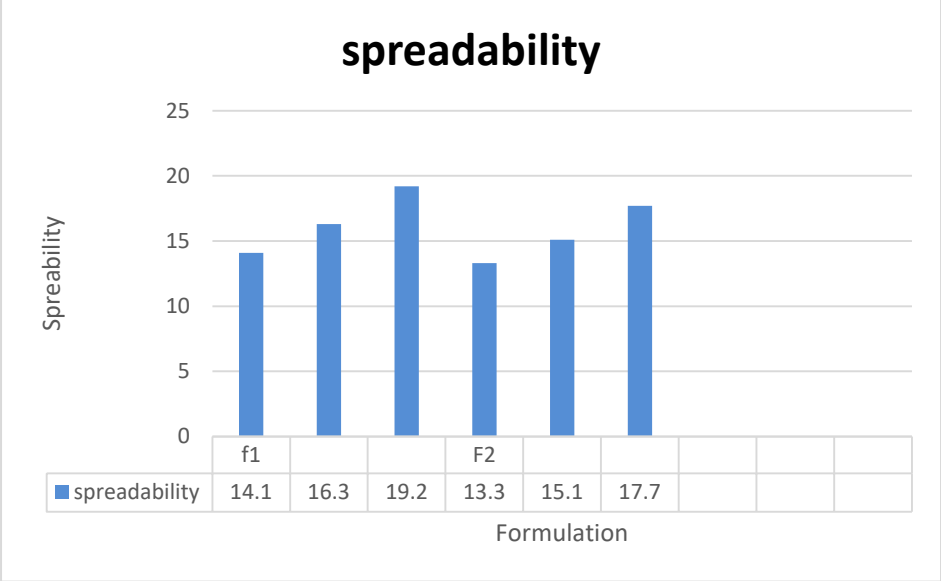

Figure 26 Graph representing spreadability tests of gel

Viscosity: Using spindle number 07, the Brookfield viscometer was used to measure the viscosity of the tridaxprcoumbens gel.

Table 11Viscosity of *tridaxprcoumbens*gel

Rpm	**Viscosity**	
	TF1	**TF2**
10	32140	28585
20	19530	15600
30	7870	7250
40	5780	4250
50	4830	28585

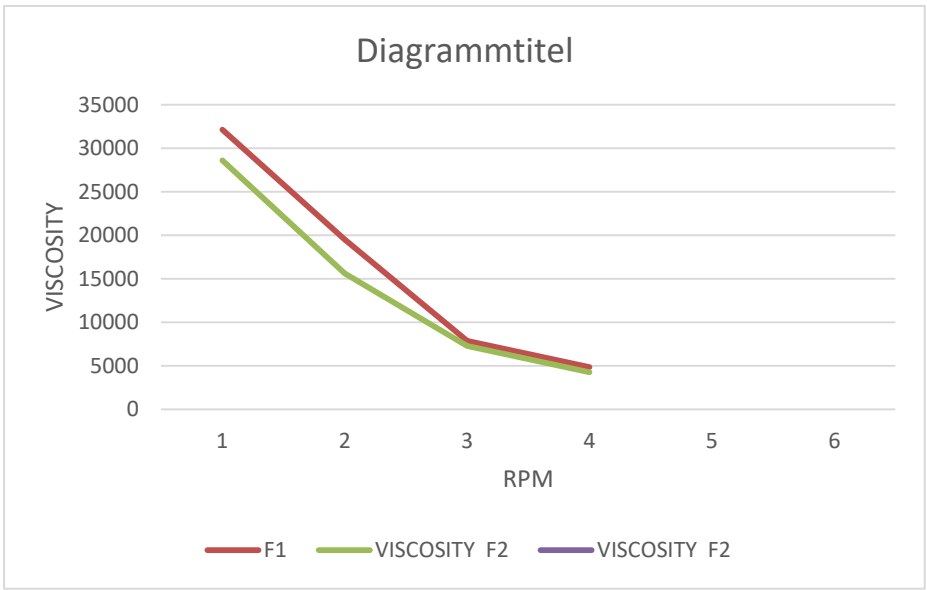

Figure 27Graph of viscosity for *tridaxprcoumbens*gel formulations

The gel formulation TF1, and TF2 were shown decrease in viscosity with application of force (rpm) indicate shear thinning system.

Wash ability: After applying the formulation to the skin, the ease of washing with water was examined. Table 12 displayed the results.

Table 12Washability test

Sr No.	Formulation batch	Wash ability
1	TF1	Washable with ease
2	TF2	Washable with ease

The gel formulation was easily washable.

Skin irritancy testing: The no irritancy test was used to assess the formulation of *Tridaxprocumbens*gel. There was no redness, edema, inflammation, or irritancy in the preparation. The condition was observed for a full day. Table 13 displayed the results.

Table 13Skin irritancy test

Sr No.	Formulation batch	Result
1	TF1	Non-irritancy
2	TF2	Non-irritancy

Franz diffusion study (Fish skin membrane): The In-vitro diffusion studies of all formulations F1 and F2 were conducted and the results are shown in table 14. As the amount of drug released from different formulations (TF2 & TF1) at the end of 3.5 hours.

Table 14Franz diffusion study (%CDR)

Time (min)	%CDR	
	TF1	**TF2**
10	9.24	11.3
30	15.27	21.75
60	26.67	32.12
90	40.11	43.15
120	55.25	58.55
150	63.75	70.05
180	73.24	84.11
210	85.21	94.35

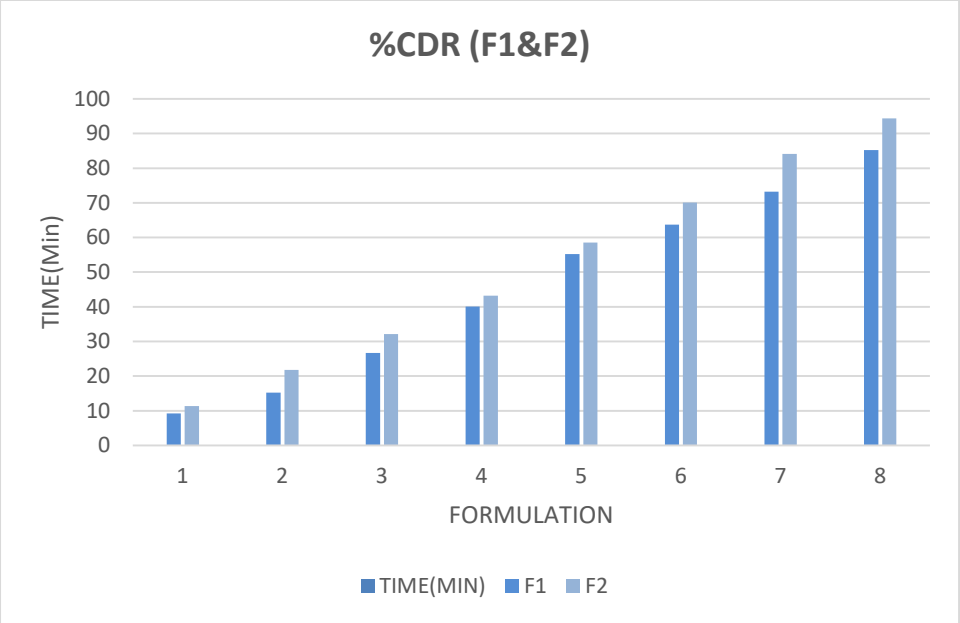

Figure 28 Graph representing In-vitro study: Franz diffusion study of gel

Formulations TF2 and TF1 was shown more than 98% release in 3.5 hours.

FTIR Spectroscopy: Formulation of *tridaxprcoumbens*gel TF1 and TF2 was analyzed by jasco FTIR-4600 for functional group analyzed.

Table 15 FTIR spectra of *Tridaxprocumbens* gel (TF1).

Functional group	**Reported Frequency (cm^{-1})**	**Observed Frequency (cm^{-1})**	**Inference**
O-H	3500-3200	3420.14	Complied
O-H	3500-3200	3349.75	Complied
O-H	3500-3200	3317.93	Complied
-C-H	1485-1340	1373.07	Complied
C-C	1300-800	1029.8	Complied

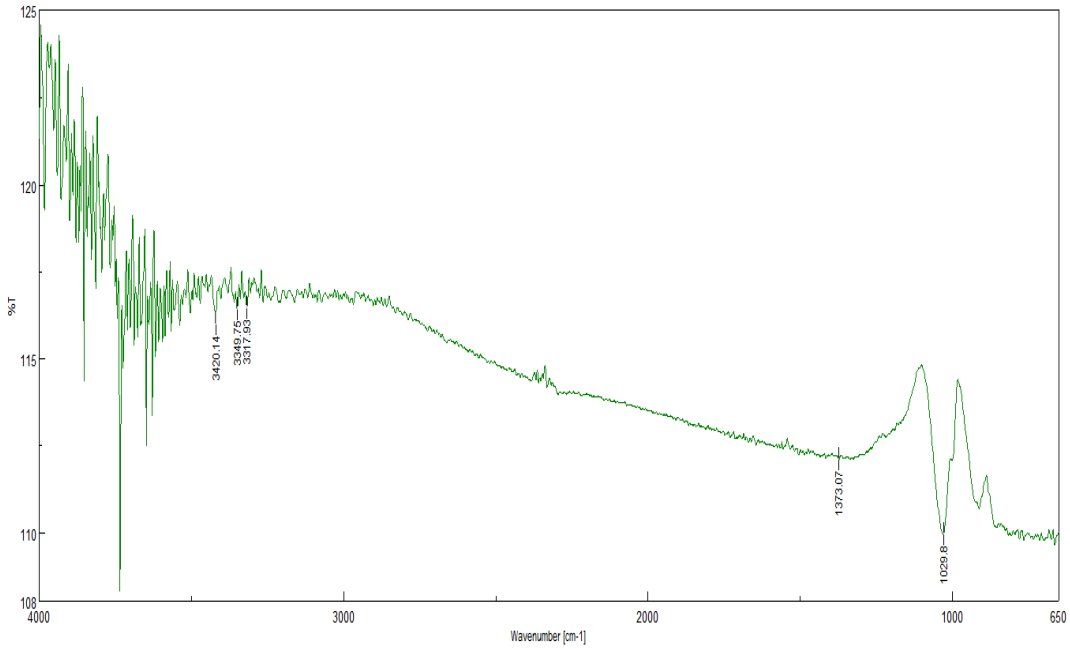

Figure 29FTIR spectra of *Tridaxprocumbens* gel (TF1).

Table 16 FTIR spectra of *Tridaxprocumbens* gel (TF2).

Functional group	**Reported Frequency (cm^{-1})**	**Observed Frequency (cm^{-1})**	**Inference**
N-H	1640-1550	1635.34	Complied
-CH_3	1450-1375	1417.42	Complied
-C-H	1485-1340	1338.36	Complied
C-C	1300-800	1028.84	Complied
-C=O	550-800	772.351	Complied

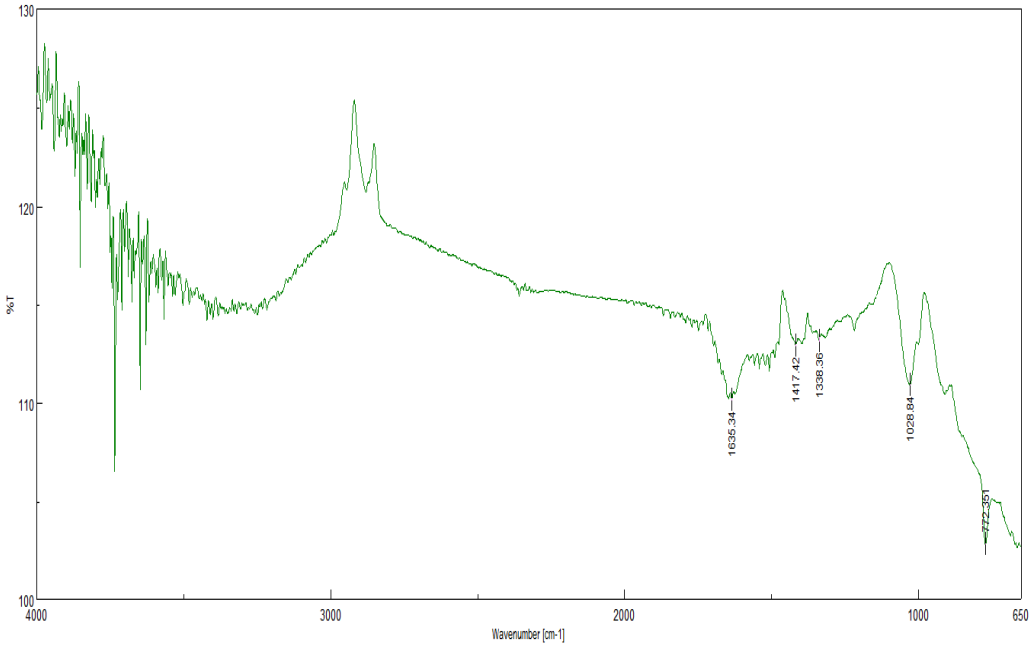

Figure 30FTIR spectra of *Tridaxprocumbens* gel (TF2).

Drug content

Table 17% Drug content of *Tridaxprocumbens* gel

Sr no	Formulation	Drug content
1	TF1	98.27%
2	TF2	98.75%

The gel was show more than 98% content in all (TF1) and (TF2) formulation. The formulation show homogenous distribution of gel.

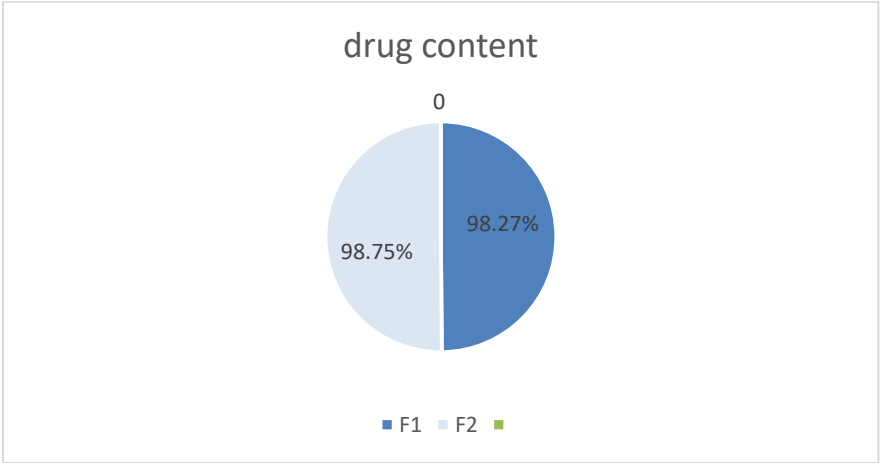

Figure 31% drug content

Stability study: Batch TF1 and TF2 was Light greenish color and viscous with a smooth consistency .There was no major changes in appearance, color, odor which indicated the stability of gel.

Table 18 Stability data of *Tridaxprcoumbens*gel.

Sr No	Parameter	Formulation	Storage condition (40±2°C/75±5% RH)		
			30 days	**60 days**	**90 days**
1	Appearance	TF1	Mostly solid	Mostly solid	Mostly solid
		TF2	Mostly solid	Mostly solid	Mostly solid
2	Color	TF1	pale greenish	pale greenish	pale greenish
		TF2	pale greenish	pale greenish	pale greenish
3	Odour	TF1	Pleasant	Pleasant	Pleasant
		TF2	Pleasant	Pleasant	Pleasant
4	Phase Separation	TF1	No separation	No separation	No separation
		TF2	No separation	No separation	No separation

In-vitro Antifungal activity

Figure 32 A&B sample for after and before 6 days antifungal test activity

Table 19Antifungal test activity

Sr no.	Test	Organism tasted	Result (zone of inhabitation in mm)	Reference Std.
A	Test sample (*Tridaxprocumbesn*)	Candida spp.	11mm	18mm(nystatin)
B	Commercial gel (Clotrimazole)	Candida spp.	10mm	18mm(nystatin)

Formulation TF2 used concentration was shown best antifungal effect than its lower concentrations against Candida spp.

CONCLUSION

A study has developed and tested a gel containing Tridaxprocumbens leaf extract using natural polymers. The gel was prepared by homogenizing the mixture with Carbopol 934 and xanthan gum, followed by adding water, triethanolamine, tween 80, light liquid paraffin, and methylparaben. The gel showed a light greenish color, pleasant odor, and semisolid consistency, with a pH range of 6.5 to 6.7, indicating skin compatibility. The gel showed good spreadability, particularly in formulation F1, and was easily washable and did not cause skin irritancy. In vitro release studies showed that formulations TF1 and TF2 released more than 98% of the active components within 3.5 hours. Stability studies confirmed the gel's effectiveness over time. Formulation TF2, with a higher concentration, showed the best antifungal effect against Candida spp. The gel's effectiveness makes it a promising candidate for topical applications in wound care and dermatological uses.

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