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Formulation and Evaluation of of Tridax procumbens Herbal Gel for its Antimicrobial and Wound healing Activity

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ABSTRACT: The objective of this study was to synthesize and characterize Tridax procumbens herbal gel for topical drug delivery system. The authentification of the plant was done from ASS, COLLEGE , Medha. The prepared herbal gel was evaluated for different evaluation parameters such as appearance, P^H, spreadability, viscosity etc. Also it was evaluated for its antimicrobial & wound healing activity. Antimicrobial activity was studied against different test mico-organisms viz E.coli, S.aureus and it was found that prepared gel exhibits god antibacterial properties against E.coli, S.aureus .Results of in-vivo wound healing activity reveals that prepared herbal gel can be successfully used in the treatment of wound healing.

I. INTRODUCTION

Tridax procumbens is a native of Central and South America. It is a perennial herbaceous plant of the Asteraceae family. Another name for it is coat buttons. This species has been employed in Indian Ayurveda since ancient times. Tridax angustifolia, tridax bicolor, tridax dubia, and tridax erecta are a few of the significant species for medicine. The plant has white blooms with golden centers, and its leaves resemble arrows. The fruit's bristles are stiff. Flavonoids, alkaloids, carotenoids, hydroxycinnamates, lignans, phytosterols, tannins, crude proteins, crude fiber, soluble carbohydrates, and calcium oxide are among its constituents. Benzoic acid derivatives are also present. Tridax procumbens is used in Indian traditional medicine for wound healing, antifungal, antibacterial, and insect repellent purposes, among other uses. Leaf extract is used or treated for a number of skin Infectious diseases. It also used in 'Bhringraj' which is well known medicine for liver disorders. Also, haxir growth activity has been found and antioxidant activity have been demonstrated (1).

Important details regarding the antibacterial properties of the chosen medicinal plants are revealed by the current study. Additional research on this plant's phytochemical analysis is crucial and might be highly helpful for the field's progress. Over other dosage forms, gel offers a number of advantages. gels are semisolid system consisting of dispersion of small or big molecule in an aqueous liquid vehicle made jelly like by the addition of gelling agent. Triethanolamine and carbopol 940 are two examples of synthetic macromolecules that are employed as gelling agents in gel formation. (2).

The gel dosage form has several advantages over other dosage forms, including softening the skin, less irritation, and ease of removal. Topical application of herbal gel involves using an ethanolic extract derived from Tridax procumbens leaves.

A wound is a physical trauma where the skin is torn, cut, or punctured.(3)

Wound healing is a complex multiphase process that involves a chain of well-orchestrated biochemical and cellular events. The process can be broadly classified in three stages- inflammation, proliferation and remodeling. The participation of various inflammatory cells is crucial for repair process. These cells promote migration and proliferation of endothelial cells, leading to neovascularization. Currently, the use of medicinal plants in pharmacotherapy is receiving a lot of attention.(4)

Herbs belonging to the Compositae family have been described in ancient Indian medicine system for the management of wounds. Traditionally in India, the fresh juice of Tridax procumbens leaves have been used as one of the most popular remedy for dermal wounds.(5,6)

In excision wound model, systemic administration (intraperitoneal) of juice from leaves of T. procumbens has been implicated with both pro and antihealing properties(7,8). In order to clearly establish its activity, the present study was

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undertaken to evaluate the effect, if any, of topical ointment formulation of juice from leaves of T. procumbens on paradigms of dermal wound healing.

Fresh leaves of T. procumbens were collected from the residential campus of College of Pharmacy, Medha ,India. All reagents were purchased from S. D. Fine Chemicals, Mumbai, India. The study protocol for using animals was approved by the standing Institutional Animal Ethics Committee; mice were procured from National institute of bioscience, Pune seven days before the commencement of the study. and all experiments were conducted in accordance with the guidelines laid down by the same. Male Swiss albino mice (20-35 g) were used in the study and maintained under standard laboratory conditions of housing, food and water.

The objective of the present work was to investigate the potential of Tridax procumbens Herbal Gel in the treatment of wound healing and for its antimicrobial activity.(9)

- **Scientific name** - Tridax procumbens
- **Kingdom** - Plantae
- **Order**- Asteroids
- **Family** -Asteraceae
- **Tribe** - Heliantheae
- **Genus** -Tridax
- **Species** -T. Procumbens

Preparation/extract		**Plant ailment uses**	**References**
Leaves	Juice Dried	Anemia, colds, inflammation, hepatopathies, vaginitis, stomach pain, diarrhea, mucosal inflammation, skin infections, bleeding. Reduce inflammation, gastrointestinal and respiratory infections, high blood pressure, diabetes	Caceres et al., 1998; Taddei and Rosas- Romero, 2000 Poll, 2005, Giovannini et al., 2016

Table no.1: Medicinal uses of tridax procumbens

Chemical Constituent	**Phytoconstituent**
Flavonoid	Kaempferol, catechin and its derivatives, Puerarin, Escluetin, Butein
Alkaloid	Akuammidine, , Ferulic acid, Tannins, Stigmasterol,
Other Phytochemicals	Caffeic acid, Ferulic acid, Tannins, Stigmasterol, Carotenoids,

II. MATERIALS AND METHODS

Harvesting of Plant Materials

We harvested fresh Tridax procumbens leaves. We ran some tap water over the leaves to clean them. The leaves were then shade-dried for two to three weeks. After being ground into a fine or coarse powder, the dry leaves were preserved.(10)

Authentification of the plant-
Autentification of the plant was done from ASS, COLLEGE , Medha.

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Preparation of an extract

Sun-dried plant materials including 10 g of powdered Tridax procumbens leaves were extracted with 100 ml of 100% ethanol and left to digest for a full day by using Soxhlet apparatus. A 250 ml iodine flask was used to separate and concentrate the resulting extract. (11,12)

Figure 1: Soxhlet extraction of Tridax procumbens

Procedure

1. After taking the necessary amount of carbopol and adding 20 milliliters of water, the mixture was homogenized for 15 minutes at 300 to 500 revolutions per minute.
2. Add triethanolamine and an additional 10 milliliters of water once a sticky consistency is reached. Over 500 RPM was swirled once again.
3. Tridax procumbens extract was added after another 20 minutes, and it was then agitated for an additional 10 minutes at a higher rpm. Propylene glycol, propyl paraben, and methyl paraben were then added in geometric proportions to produce a homogenous gel. To ensure good mixing, add glycerine to the formulation and whisk for ten minutes.

Figure 2: Prepared herbal gel

4. After 45 minutes, the entire mixture was finally stirred while a small amount of water was added at a time.

Phytochemical screening of tridax procumbens leaves

The phytoconstituents present in the Ethyl alcohol extracts of leaves Tridax procumbens were

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analyzed qualitatively by using standard procedures .

Test for Alkaloids

About 2 ml of extract was taken and added 2 ml of concentrated HCL and then Mayer's reagent was added drop wise. The formation of white precipitate indicates the presence of alkaloids(13,14,15)

Test for Flavonoids

The extract of 0.1 ml was taken and made up to 5 ml with distilled water, after which 0.3 ml of sodium nitrate was added and incubated for 5 mins at room temperature and then added 3 ml of 10% aluminium chloride which is incubated for 6mins at room temperature. Finally, 2ml of sodium hydroxide (NaOH) was added. The formation of yellow color indicates the presence of flavonoids (16)

Test for Saponins

About 2ml of filtrate was mixed with 1ml of distilled water and shaken vigorously for about 3 seconds and it was allowed to stand for few mins and then added 3 drops of olive oil and shaken vigorously. Formation of emulsion indicates the presence of saponins.(17)

Test for Terpenoids

About 1ml of the extract and 2ml of chloroform was taken and followed by the addition of 5ml of concentrated H2SO4 along the sides of the test tubes. Formation of a reddish-brown coloration in the interphase indicates the presence of terpenoids. (18)

Test for Phenolic Compounds

To 1ml of extract, 1ml of Iron (III) chloride was added and mixed well. A deep blue green color was formed which indicates the presence of phenolic compounds (19).

Test for Triterpenoids

A 10 mg of extract was dissolved in 1ml of acetic anhydride and then added 2ml of concentrated H2SO4. Formation of reddish violet color indicates the presence of triterpenoids. (20)

Test for Quinones

To 2ml of plant extract, 1ml of concentrated H2SO4 was added. Formation of red color indicates the presence of quinones.

Test for Steroids

To 10 mg of plant extract, 2ml of acetic anhydride and followed by 2ml of H2SO4 were added. Formation of violet or blue color indicates the presence of steroids.

Test for Tannins

To1ml of the extract added 0.1% of ferric chloride solution and observed brownish green or a blue-black coloration which indicates the presence of tannins (21) .

Test for Glycosides

About 1ml of extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicates deoxysugar which confirms the presence of cardenolides. A violet-green ring appearing below the brown ring in the acetic acid layer indicates the presence of glycosides.

Test for Coumarins

The extract was dissolved in methanol and then added alcoholic NaOH. A yellow color appears which later disappears on addition of drops of concentrated HCl indicates the presence of coumarins (22,23).

III. ANTIBACTERIAL ACTIVITY

1. LB broth was mixed in 100 ml water .
2. The petridishes and media were autoclaved for 30min.
3. Then media was spread over the petridish under the laminar airflow.
4. 100gm E.coli/S.aureus was spread over the media.
5. After the petridish were kept in the refrigerator for 10 min.
6. Under sterile condition drug was poured on plates (24,25,26).

IV. IN VIVO WOUND HEALING ACTIVITY OF PREPARED HERBAL GEL

After getting ethical clearance from Institutional Animal Ethics Committee; mice were procured from National institute of bioscience, Pune seven days before the commencement of the study. Animals were placed in polypropylene cages in a controlled room temperature 22±1°C and relative humidity of 60-70% in registered animal house (1915/PO/ReBi/CPCSEA). They were maintained with standard pellet diet (Amrut,

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Sangali, India) and water ad libitum.

Wound healing studies of prepared herbal wound dressings on rat model

Rats were anesthetized with diethyl ether, the surgical area will be shaved and a wound, approximately 1cm^2, will be created on the dorsal side of the rat, using surgical scissors.

Rats will be randomly divided into three groups:

a) Control group in which wound will be left to healspontaneously,
b) Group I in which wound will be treated with plain gel containing no antimicrobial agent.
c) Group II in which wound will be treated with prepared Tridax procumbens herbal gel.
d) Group III in which wound will be treated with standard marketed formulation of Tridax procumbens.(Puradine ointment)

Tissues of the wounded area will be taken on 4th, 8th and 12th day. Tissue sections 5mm thick will be cut using microtome, stained with haematoxylin-eosin, and photographed with Motic microscope, to study the changes in wounded skin. Wound healing of open wound, wound covered with drug loaded and plain gel containing no antimicrobial agent will be compared.(27)

V. RESULTS AND DISCUSSION

Table no. 1: Phytochemical screening of the extract

Name of the phytoconstituent	Ethanol Extract
	Leaves
Alkaloids	(+)ve
Flavonoids	(+)ve
Saponins	(-)ve
Terpenoids	(+)ve
Phenolic compounds	(+)ve
Triterpenoids	(-)ve
Quinones	(-)ve
Steroids	(+)ve
Tannins	(-)ve
Glycosides	(-)ve
Coumarins	(+)ve

Table 2: Formulation of Tridax procumbens herbal gel

Sr. no.	Ingredients	F1	F2	F3
1	Extract	0.20gm	0.40gm	0.80gm
2	Carbopol	1.0gm	1.0gm	1.0gm
3	Propylene glycol	10ml	10ml	10ml
4	Methyl paraben	0.2 ml	0.2ml	0.2ml
5	Propyl paraben	0.1ml	0.1ml	0.1ml
6	Glycerine	1.0ml	1.0ml	1.0ml
7	Triethanolamine	qs	qs	qs
8	Water	qs 100ml	qs 100ml	qs 100ml

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Table 3: Evaluation parameters of herbal gel

Sr.No.	Paramters	Observation		
		F1	F2	F3
1	Appearance	Green	Green	Green
2	Colour	Light green	Green	Dark green
3	PH	6.8	6.9	7.0
4	Spreadability	16.25mm	15.47mm	14.13mm
5	Viscosity	18600cp	7890cp	4820cp

Table 4: Zone of inhibition antibacterial activity

Sr.No	Organisms	Zone of inhibition for F1	Zone of inhibition for F2	Zone of inhibition for F3
1	E. coli	13mm	15mm	16mm
2	S.aureus	2mm	4mm	6mm

1.1 In vivo wound healing activity of prepared herbal gel

Control group-no treatment, **Group 1**- plain gel, **Group 2**- Tridax procumbens herbal gel **Group 3**- Standard marketed formulation (Puradine ointment)

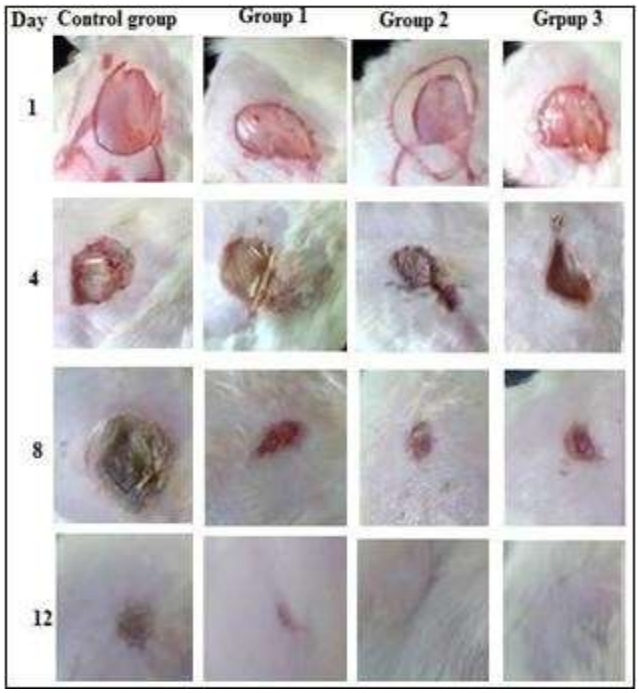

Figure 3: In vivo wound healing activity of prepared herbal gel

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Day	Control group	Wound size (mm)		
		Group 1	Group 2	Group 3
1	2.5	2.5	2.5	2.5
4	1.5	1.4	1.0	1.2
8	1.1	1.1	0.3	0.9
12	0.6	0.9	0.1	0.6

Table 5: wound size (mm) of control group, group 1, Group 2- , Group 3

Figure 3 shows in vivo wound healing activity of prepared herbal gel and table 5 illustrates wound size (mm) of control group, group 1, Group 2- , Group 3, in Rats. The wound contraction was faster in case of Group 2 (Tridax procumbens herbal gel) than control group. Results revealed that the wound size of all groups of rats was decreased with time interval of 4^{th},8^{th} and 12^{th} day. The healing was significantly high in case of prepared herbal gel as compared to other groups. This indicates that prepared Tridax procumbens herbal gel exhibited good wound healing activity (28)

VI .CONCLUSION

In the present study, Tridax procumbens herbal gel was successfully prepared. herbal gel for topical drug delivery system. The authentification of the plant was done from ASS, COLLEGE , Medha. The prepared herbal gel was evaluated for different evaluation parameters such as appearance, P^H, spreadability, viscosity etc. Also it was evaluated for its antimicrobial & wound healing activity. Antimicrobial activity was studied against different test mico-organisms viz E.coli, S.aureus and it was found that prepared gel exhibits god antibacterial properties against E.coli, S.aureus .Results of in-vivo wound healing activity reveals that prepared herbal gel can be successfully used in the treatment of wound healing.

In vivo wound healing study showed that prepared herbal gel has significant higher wound healing rate than control group, group 1, and group 3.This indicated that prepared hydrogel films can be successfully used as dressings for wound healing.

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