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Formulation and Evaluation of A Topically Applied Herbal Wound Gel

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GRAPHICAL ABSTRACT

ABSTRACT
The goal of the current study was to create a herbal gel and evaluate its potential for antibacterial and wound-healing properties utilising Tinosporacardifolia and Tridax. Both the Tridax and Tinospora species, which have potent anti-inflammatory and therapeutic capabilities, are used in the mixture. The gelling agent used in this study was carbapol. Five formulations with different carbapol concentrations, as well as Tinosporacardifolia and Tridax extracts, were developed and optimised for the current study. For further investigation, the optimised formulation was chosen. To characterise the formulation, preliminary physiocochemical measurements such as surface pH, spreadability, viscosity, homogeneity, and antimicrobic tests can be used. The results showed that the surface pH was within the range of the skin. For optimised formulations in Trials 1and 2,thegel'sviscosityandspreadabilitywereappropriate, and the zone of inhibition was like wise accep table. Under typical storage settings, the preparation wasstable and did not cause skin irritation.

Keywords:Plantextract,Tinosporacardifolia,Tridex procumbens,Stepalococas,Ecoli

I. INTRODUCTION

1.1. Herbal mediciene[1]

Thepartsofnumerousplantsknowntohaveth erapeuticcharacteristics,suchastheirroots,stems,leav es,bark,fruits,seeds,orflowers,areusedtomakeherbal medicines.Additionally,alotofcommonlyusedmedic ationscomefrom plants. Theword "drug"actuallyderivesfrom theFrenchword "drogue,"which means"dryherb."

1.2. Raw material

Raw material means crude drugs. Unless otherwise stated, this phrase refers to primarily entire, fragmented, or cut plants, plants' components, algae, and fungus in an undisturbed state. Typically, herbs come in dried form. In some circumstances, exudates that have not undergone additional processing fall under the category of herbs.

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1.3. Herbal preparation

The term "herbal formulation" refers to a dosage form that contains one or more herbs or processed herbs in specific amounts to provide specific nutritional, cosmetic, and other benefits. These benefits may be used to diagnose, treat, or mitigate disease in humans or animals, or to change the physical characteristics of humans or animals.

1.4. Qualityin the context of herbal drugs[2]

Theeffectivenessandsafetyofherbalproductsarecrucialconsiderations.Adrug'sstatusasdeterminedbyitsidentity, purity, content, and other chemical, physical, or biological features, as well as by the manufacturingmethods, is referred to as its quality. Accepted Good Agricultural Practices (GAP) can regulate this. Thequality of a plant product is affected by the environmental conditions present during growing. These includepickingtherightseeds,fosteringhealthyplantgrowth,applyingfertiliser,andharvesting,drying,andstoring.Inactuality, GAP procedures areand always willbeacrucial component of qualityassurance.

1.4.1. Factorsaffecting its quality about herbs[3]:

1. DrugAdulteration
2. Faultycollection
3. Imperfectpreparation
4. Incorrect storage
5. Gross replacement of plant matter
6. Using previously used medications in place

1.4.2. Its need for quality evaluation of herbal drugs:

1. According to the WHO, "quality control" refers to the collection of actions done to guarantee the identification and purity of a certain medicine. An crucial function of the pharmaceutical sector is quality control[4].
2. Herbal medications are subject to quality control to guarantee their consistency, security, and effectiveness. Chemical fingerprinting has been shown to be an effective method for ensuring the quality of herbal medications[5].
3. Consider the hue, texture, flavour, and aroma. Herbs that have been dried need to resemble fresh herbs in terms of appearance, flavour, and aroma. The flavour and potency of the dried herbs have also been diminished along with their colour and fragrance[6].
4. Sensory examination (macroscopic and microscopic) is typically one of the procedures for quality control of herbal medicines[7].

1.4.3. Herbs have its following characteristics[7]:

- ❖ They are small plants with soft and delicatepetals.
- ❖ They have a tender, soft, and delicate green exterior.
- ❖ They have a short lifespan, which means they can only live one or two years.
- ❖ They are shorter in size, and they may grow between 2 and 3 metres.

II. INTRODUCTION OF PLANTS

In both traditional and ayurvedic medical practises, tinospora cordifolia (also known as guduchi) is a common herb.Alternative names: Tinosporacardifolia, Its stem extract can be used to treat skin conditions and burning feelings. As a remedy for a snakebite or a scorpion sting, Tinospora cordifolia root and stem are used in combination with other medications. The aqueous extract of Tinospora cordifolia significantly reduced inflammation in both arthritis caused by formalin and cotton pellet granuloma.

Tridax procumbens The leaves of Mandoniawedd and PtilostephiumKunth (L.) are traditionally used by tribal people to treat wounds. The mature leaves are ground into a paste and applied to the wound's surface. Dermal wounds have historically been treated with the juice of Tridax procumbens leaves. A medical herb called Tridax procumbens has been used for a variety of ailments for centuries, most notably cuts, wounds, and burns. A wound may be an inevitable occurrence in an organism's life. The healing process starts in the early stages of inflammation but typically ends once the harmful impact has been removed[9]. There, both plants are demonstrating or testing. E. coliandStapalococcus bacteria and wound healing.

2.1. Morphology[10] :

2.1.1.TinosporaCardifolia:

Aerial Roots:

Fig 1: Aerial Root

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The young aerial roots are long and filiform, like a string, squarish. The mature roots are fleshy and resemble the structure of a young aerial stem. The dried aerial roots are 3-6 cm in diameter, light grey-brown or creamy white incolour, odourless and have a bitter taste

Stem:

Fig 2. Stem

The stem's morphology revealed a greyish green colour with smooth surfaces and node swelling. Older specimens have a light brown surface covered in round lenticels that resemble warts. The dried stem has a rough surface due to longitudinal fissures of fractures running along the rows of lenticels and is cylindrical, slender, somewhat twisted, and 6 to 12 cm in diameter. The outer bark is paper-thin and ranges in hue from brown to grey. Circular andnoticeable lenticels are present.

Leaves:

Fig 3. Leaves

The leaves are straightforward, according to morphology. The leaf blade measures 5 to 12 cm wide, ovate like a circle, cordate, and has smooth regions. Shiny and light brown in colour, the underside. When the leaves are seen in huge numbers, they appear to be very green, however the over-mature leaves are actually a yellowish-yellow tint.The leaves have an odd odour and a harsh flavour.

2.1.2. Tridax[11]:

Apperrance

A perennial herb known as Tridax procumbens has a creeping stem that can grow to be 8 to 30 inches (20 to 75 cm) long.

Foliage

The opposite, pinnate, oblong to ovate, 1-2 inches (2.55 cm) long leaves of Tridax procumbens have connate bases, coarsely serrated margins, and sharp apexes.

Flowers

White rays and yellow disc blooms are present in Tridax procumbens flowers. On a 4 to 12 inch (10 to 30 cm) long stalk, they measure about 0.4 to 0.6 inches (1.5-2 cm) in width. Spring is the time for flowering.

Fruits

Achenes, which are dark brown to black in colour, rectangular, and 0.08 inches (2 mm) long, are the fruits. Each one has a head of pappus bristles that varies in length from 0.12-0.24 inches (3-6 mm).

Fig 4: Tridax

The objectives of this study were:

1. To carry out extraction plant.
2. Evaluation of the prepared extract.
3. Formulation of gel by using green chemistry approach.
4. Physicochemical evaluation of formulated antimicrobial gel.
5. To studythe antimicrobial activity of developed formulation.
6. To carry out research on short-term stability in accordance with ICH recommendations

2.2. Extract and Excipient Profile

2.2.1. Mode of Action of TinosporaCardifolia [16]

The granulation tissue of the wound is predominantly made up of fibroblasts, collagen, edoema, and tiny new blood vessels when the

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extract is applied to an animal wound. The wound margin's undifferentiated mesenchymal cells change into fibroblasts and begin to migrate into the wound gap alongside the fibrin strands. Extracellular tissue's main building block is collagen, which provides stability and strength. Collagen is made of the amino acid hydroxyproline, which has been utilised as a biochemical indicator for tissue integrity.

2.2.2.Tridax Procumbens Action Mode [17]
Lysyl oxidase activity, protein and nucleic acid content, and tensile strength were all significantly increased inanimals treated with extract.

2.2.3.Carbapol
Structure

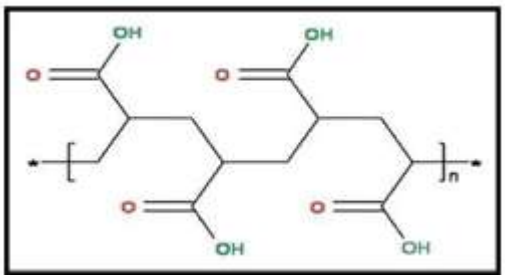

Fig 5. Structure of Carbapol

IUPAC Name: Poly(acrylic acid)
Other Names: PAA, PAAC, Acrysol, Acumer.
Chemical Formula: (C3H402)
Molar Mass: variable
Uses :

1) Ion exchange resins and adhesives are made with polyacrylic acid and its derivatives for use in disposablediapers.
2) They are frequently used in medicines as thickening, dispersing, suspending, and emulsifyingagents.

2.2.4. Propylene Glycol
Structure:

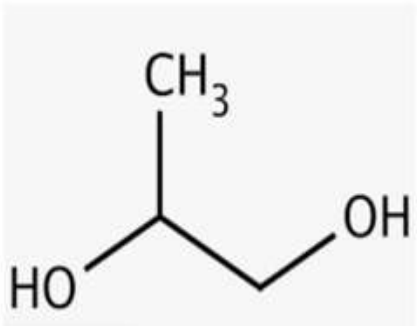

Fig 6: Structure of Propylene Glycol

IUPAC Name: Propane-1,2-diol
Other name: 1,2-dihydroxypropane,methylglycol
Chemical Formula : C3H8 O2 Molar Mass : 76.09 g/mol
Uses :

1. To make polyester compound and as a base for deicing solution.
2. It is used in gel formulation.
3. It helps presence moisture as well as dissolve colour and flavours.

2.2.5. Triethanolamine
Structure :

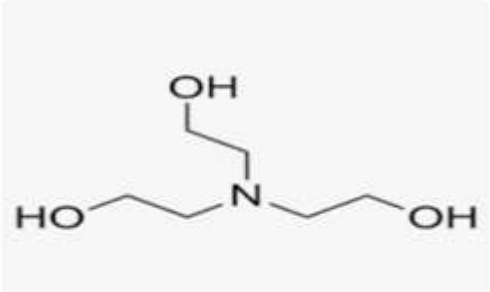

Fig 7 : Triethanolamine

IUPAC Name: 2-[bis(2-hydroxyethyl)amino]ethanol
Other Name : Trolamine Chemical Formula : C6H15NO3 Molar Mass : 149.19 g/mo
Uses :

1. It is used in making surfactant such as for emulsifier.
2. It is used in sunscreen lotion.

III. MATERIALS AND METHODS:

Materials: The Herbal Wound Healing Gel of TinosporaCardifolia and Tridax was prepared usingfollowingnaturalplant ,chemical, apparatus and instrument.
Natural Plant : TinosporaCardifolia,Tridax
Chemicals: Carbapol, Polyethylene glycol,Triethanolamine,
Apparatus: Apparatus such as beaker, glass slide, measuring cylinder, test tube, mortar volumetric flask,sonicator apparatus, Soxhlet apparatus.
Instruments: pH meter, Mechanical stirrer, Viscometer, Incubator,Auto-clave,Hot Air Oven.

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Table 1- Different type of material and their functions

Sr.No	Material	Function
1	Carbopol934	GellingAgent
2	Propyleneglycol	Co-Solvent
3	Triethanolamine	AdjustpH
4	DistilledWater	Vehicle
5	ExtractionofTinosporaCardifolia	Activeingredients
6	ExtractionofTridax	Activeingredients

3.1. Extraction method of Tinospora Cardifolia[18]:

1. TinosporaCardifolia stems cleaned with 70% ethanol.
2. Then TinosporaCardifolia stem dried in shade and converts it into powder.
3. The powdered stem was ultrasonically extracted with chloroform for three hours.
4. Then it was filtered and filtrate was collected.
5. Collected filtrate was stored in well closed container and further used for preparation

Fig 8: Extraction of Tinospora Cardifolia

3.2. ExtractionTridax Procumbens[19]:

1. A Soxhlet extractor was used to pack the complete plant powder, and it was extracted at 50°C with 95% ethanol.
2. The extraction process lasted 72 hours.
3. The filtrate from the extract-filtering process was collected.
4. The recovered filtrate was placed in a tightly sealed container and used for further preparation.

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Fig 9: Extraction of Tridax procumbens

3.3.PreformulationStudy:

II)Phytochemical Screening of leaf and stem extracts ofTinosporacordifolia20,21,22

Preliminary Phytochemical Screening of the Tinospora Cordifolia extracts:

The Preliminary phytochemical analysis gives primary idea about presence ofphytochemical of the Extract.

Table 2- Different type of test and their observation of the Tinospora Cordifolia

Sr.No	Test	Observation
1	**Analyze for Alkaloids (Wagner;s reagent)**Wagner's reagent, whichcontains 1.27 grammes ofiodine and 2 grammes of potassium iodide in 100millilitresofwater,wasused totesttheextractforalkaloids.	rustybrownprecipitation(orcoloration).
2	**Analyzeforcarbohydrates(Benedict'sest):** A 2 ml amount of the variousextractswasmixedwithafewdrops of Benedict's reagent,heatedinawaterbathforfive minutes,andthencooled.	BrownishReddishPrecipitate

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3	**Analyzeforflavonoids(Alkalinereagent test)** To 2mL of extracts, a fewdrops of a 20% sodiumhydroxide solution wereapplied.formationofabrightyellowcolourthatfadesto colorlessness when dilutedhydrochloricacidisadded.	Flavonoids
4	**AnalyzeforAminoAcidandProteins (1% Ninhydrinsolution** 2 ml of filtrate were treatedwith 2–5 drops of 1%ninhydrin solution and thenplacedinaboilingwaterbath for1–2minutestotestforaminoacidsandproteins.	PurpleColour
5	**Analyzeforsaponin(foamtest):** In a test tube, 12ml of waterwasaddedto2mlofextract.The mixture was vigorouslyshaken.	creationofenduring
6	**AnalyzeforTannin(Braymer;stest)** 2 mls of the extract weretreated with a 10% ferricchloridesolutiontotestfortannins(Braymer'stest).	bluishorgreenSolutioninColor
7	**Analyzeforterpenoids(Salkowki'stest)** Involved treating 2 mL ofeach extract with 1 ml ofchloroformandafewdropsof strongsulfuricacid.	ReddishBrownppt

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3.4. Phytochemical Screening of Tridax Procumbens[23] :

Table 3- - Different type of test and their observation of Tridax

Sr.No	Test	Observation
1	**Analyze for Alkaloids (Wagner;s reagent)**Wagner's reagent was usedto test a little amount ofextract for the presence ofalkaloids (1.27g of iodineand2gofpotassiumiodide in100mlofwater)	ReddishBrownppt
2	**AnalyzeforCarbohydrate(Benedicttest)** To a 2 ml sample ofeachofthedifferentextracts, a few drops ofBenedict'sreagentwereadded.Themixturewasthenheated in a water bath for 5minutes, cooled, and lookedforareddish brown precipitate.	ReddishBrownppt
3	**AnalyzeforFlavonoids(Alkalinereagenttest)** To2mLofextracts,a few drops of a 20%sodium hydroxide solutionwere applied. formation of abrightyellowcolourthatfades to colorlessness whendilutedhydrochloricacidis added.	Flavonoids
4	**AnalyzeforPhenols(FerricChloridetest)** A portion of theextracts were given anaqueous5%ferricchloride treatment.	Deepblueorblackcolour
5	**Analyze for Saponin (Foamtest)** Inatesttube,12mlofwater was added to 2ml ofextract. The liquid wasforcefullyshaken before beingobserved.	ContinuousForm
6	**Analyze for Tannins (Braymer's test)** 10%ferricchloride solution was applied to 2 mloftheextract.	GreenishColour

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7	**AnalyzeforTerpenoids(Salkowki'stest)** Two millilitres ofeach extract were treatedwith one millilitre ofchloroformandafewdrops ofstrongsulfuricacid.	Areddishbrownppt

IV. METHOD OF PREPARATION:

4.1.Formulation Gel:

Procedure:

1. Carbopol 940 was measured and distributed in half the amount of water. It was permitted to enlarge.
2. Accurately weighed plant extract was added to Propylene Glycol & Sonicated.
3. Sonicated solution added in carbopol base.
4. The PH was changed by adding triethanolamine to make it 7. The remaining water was added to the mixture to correct the weight.

Table 4- Formulation of gel

Chemicals	(Trial)1	2	3	4	5
Carbopol	0.5%	1.0%	1.0%	1.0%	1.0%
Propyleneglycol	10%	10%	10%	10%	10%
Triethalamine	ToCalibrate pH7	ToCalibrate pH7	ToCalibratepH7	To Calibrate pH7	To Calibrate pH7
Water	QS	QS	QS	QS	QS
Extract ofTinosporaCardifolia	0.5%	0.5%	0.75%	1%	1.25%
ExtractofTridax	0.5%	0.5%	0.75%	1%	1.25%

4.2. Evaluation of Gel[24,25] :

Different factors, including appearance, colour, pH, viscosity, homogeneity, Spreadability, extrudability, extract content, and extract content uniformity, were assessed for formulations.

Appearance:

The Appearance of formulation is lustrous

Colour: The colour of formulation was pale yellow.

Homogeneity:

Visual inspection was used to check the homogeneity of the gel formulations for the presence of any aggregates.

Spreadability:

When two slides are placed in between them and a specific force is applied, spreadability is measured in terms of the number of seconds it takes for the slides to separate from the gel. In order to compress the glass slides into uniform thickness, the extra sample was sandwiched between the two glass slides and a set amount of weight was applied to them. The process of separating the two slides was timed while a weight of 70 g was added. The formula S = ML/T, where M is the weight attached to the top slide, L is the length of the glass slides, and T is the time it takes to separate the slides, was used to calculate spreadability.

pH:

With the aid of a digital pH metre, gel compositions' pH levels were determined. A precise 2.5gm of gel was used. 25ml of pure water was used to weigh, dissolve, and store the mixture

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for two hours. The formulation's pH was measured in triplicate, and the average results range from 6.8 to 7.2.

Microbial growth:

Agar media with nutrients was used to research microbial growth. This approach involved using blank and sample petriplates, aseptically transferring the gel sample onto the sample plates in a cross pattern, and then observing the microbial growth. Staphylococcus aurous strain antimicrobial activity was evaluated and found to display strong antibacterial activity. No signs of microbial development were seen.

II) Anti-Microbial Studies:

The formulation was evaluated for its anti-microbial properties by the zone of inhibition method; the micro- organisms used in the study were Staphylococcus aurous,E coli

II) Stability:

A stability protocol was created, and in accordance with it, stability tests of the gel formulation were done for three months at 25°C and 60% relative humidity and 40°C and 75% relative humidity. To determine the stability of the produced formulation, the impact of temperature and time on the physiochemical properties of the gel was examined. The several physical characteristics that were investigated include pH, colour, odour, viscosity, homogeneity, and grittine.

V. RESULT AND DISCUSSION:

5.1.Preformulation Study[20,21,22] :

Table 5-Phytochemical Screening of Extraction:

Sr.No	Test	Inference
1	Analyze the Alkaloid(**Wagner'sreagent)**	Present
2	Analyze the Carbohydrate(**BenedictTest**)	Present
3	Analyze the Flavonoids(**AlkalineReagent Test**)	Present
4	AnalyzetheAminoacidandProtein(**1%ninhydrinsolution**)	Present
5	AnalyzetheSaponin(**FoamTest**)	Present
6	Analyze the Tannins (**Braymer'stest**)	Present
7	Analyze theTerpenoids(**Salkowki'sTest**)	Present
8	Analyze the CardiacGlycosides(**KellerKelliani's Test)**	Present

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Table 6-Phytochemical Screening OfTridax Procumbens[23]

Sr. No	Test	Observation
1	Analise the Alkaloid (**Wagner's reagent**)	Present
2	Analise the Cardiac glycosides(**Keller Kelliani'sTest**)	Present
3	Analise the Flavonoids (**AlkalineReagent**Test)	Present
4	AnalisethePhenols(**Ferricchloride test**)	Present
5	Analisethe Saponin (**FoamTest**)	Present
6	Analisethe Tannins(**Braymer's test**)	Present
7	Analisethe Terpenoids (**Salkowki's Test**)	Present
8	Analisethe Carbohydrate (**Benedict' s Test**)	Present

Fig10:LeafExtractionTest

Fig11:StemExtractionTest

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5.2. Extraction Test of Tridax:

Fig 12: Tridax Extraction Test

5.3. FormulationTrial:

Fig 13: Wound Healing Gel

5.4. Antimicrobial Studies

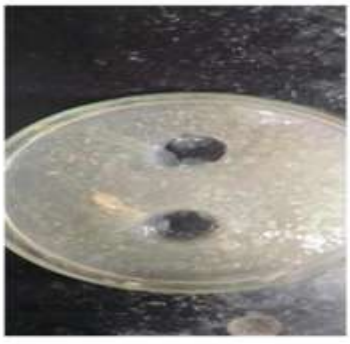

Fig 14, 15-E. coli Culture

Antimicrobial Study was carried out by using Staphylococcus aureusand, E.coli. The presence ofa clear zone around the formulation suggests that the formulation contains antimicrobial activity capable of inhibiting the growth of micro-organisms.

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5.5. Stability:

Table 7-: StabilityStudy

TEST	STATION						
	INITIAL	**ONEMONTH**		**TWOMONTH**		**THREEMONTH**	
		25°C/60 %RH	40°C/75 %RH	25°C/60 %RH	40°C/75 %RH	25°C/75 %RH	40°C/75 %RH
Appearance	Lustrous	Lustrous	Lustrous	Lustrous	Lustrous	LessLustrous	LessLustrous
pH	7	7	7	7	7	7	7
Colour	PaleYellow	PaleYellow	PaleYellow	PaleYellow	PaleYellow	LightYellow	LightYellow
Odour	Attribute	Attribute	Attribute	Attribute	Attribute	Attribute	Attribute
Viscosity	**Viscous**	Viscous	Viscous	Viscous	Viscous	Visous	Viscous
Spreadability	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent

VI. CONCLUSION:

There is a tonne of evidence supporting the importance of plant extracts in wound healing. Regarding Tridax and Tinosporacardfolia identification, the current study offers useful information. In accordance with the Green Chemist method, we created a polyherbal gel. Fresh extract from TinosporaCardfolia and Tridax plants was gathered and evaluated in that study, demonstrating the phytochemical content of the extract. Additionally to the creation of polyherbal gel with enhanced formulation and prepared antimicrobial activity The formulation's performance against E. coli and Steplalococcus clearly demonstrated that it had an impact on a number of infections.Therefore, additional research could be done to isolate, purify, and standardise the plant. The scientific community will be able to use the plantina herbal formulation after the wound healing study is done using an animal investigation that reveals the pharmacological activity of the formulation. Thus, this offers a wide range of inquiry potential into potential future developments.

CONSENT OF PUBLICATION:
The author give consent for publication.

CONFLICT OF INTEREST:
The author declares no conflict of interest.

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