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Comprehensive Pharmacognostical Standardization, Bioactive Phytochemical Analysis and Development of a Sterile Phytopharmaceutical Cream from Tridex Procumbens for Accelerated Healing Indiabetic Foot Ulcers

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ABSTRACT

Diabetic foot ulcers (DFUs) represent a major healthcare challenge affecting approximately 12-25% of diabetic patients worldwide. This study aimed to develop and evaluate a sterile topical herbal cream incorporating Tridax procumbens leaf extract for wound healing in diabetic foot ulcers. Fresh leaves of Tridax procumbens were collected, authenticated, and subjected to pharmacognostical and phytochemical evaluation. Soxhlet extraction using ethanol was performed to obtain the crude extract. The extract wasformulated into a water-in-oil (W/O) sterile cream under aseptic conditions. Pharmacognostical analysis confirmed the authenticity of the plant material through macroscopic and microscopic characterization. Phytochemical screening revealed the presence of flavonoids, tannins, alkaloids, saponins, phenolic compounds, and glycosides. The formulated creamexhibitedacceptablephysicochemicalparametersincludingpH5.62-7.1,goodspreadability(28.84-34.09 g·cm/sec), and excellent washability. Stability studies demonstrated no significant changes in color, odor, texture, or phase separation over 30 days. The results validate the traditional use of Tridax procumbens in wound management and support the development of this formulation as a cost-effective, safe, and biologically active alternative for diabetic foot ulcer treatment.

INTRODUCTION

Tridax procumbens Linn., belonging to the family Asteraceae, is a widely distributed medicinal herb commonlyknownascoatbuttons,JayanthiandGhamara.TheplantisnativetotropicalAmericaandhas become naturalised throughout tropical and subtropical regions including India, Africa, Asia and Australia.InIndia,itgrowsabundantlyasawildweedalongroadsides,agriculturalfields,wastelandsand disturbed habitats, yet it occupies an important place in traditional and folk systems of medicine.Morphologically, Tridaxprocumbens is acreeping, spreading perennialor annualherb bearing characteristicdaisy-likeflowers with yellow centraldiscs and whiteor yellow rayflorets. Theleaves are opposite, toothed and often arrow-shaped, while the fruits are achenes provided with a feathery pappus thatfacilitateswidedispersal.Theinvasiveandhighlyadaptivenatureoftheplantislargelyattributedto its

prolific seed production and effective wind dispersal mechanism, enabling its establishment across varied ecological conditions.

InIndiantraditionalmedicalpractice,Tridaxprocumbensiswidelyrecognisedasanimportantmedicinal plantandisoftenincludedasacomponentoftheAyurvedicformulation“Bhringraj”,whichisprescribed for promoting hair growth and maintaining scalp health. Ethnomedicinally, the plant isused for the treatment of wounds, cuts, skin infections, bleeding, inflammation, bronchial catarrh, dysentery, diarrhoea, malaria and high blood pressure.

SYNONYMS

CoatButtonsandTridaxDaisy(English),Ghamra(Hindi),JayantiVeda(Sanskrit),Dagdipala(Marathi), Gaddi Chemanthi (Telugu), Thata poodu (Tamil), Chiravanak (Malayalam), Cadilp Chisaca (Spanish), Herbe Caille (French), Kotobukigiku (Chinese)

BOTANICALCLASSIFICATION

Category	**Classification**
Kingdom	Plantae
Division	Angiosperm
Class	Dicotyledonae
Order	Asterales
Family	Asteraceae
Genus	Tridax
Species	TridaxprocumbensLinn.

2. METHODSMATERIALSAND

PlantMaterialCollectionand Authentication

Fresh leaves of Tridax procumbens Linn. were collected from the medicinal plant garden of K.G. College of PharmacyandResearchInstitute,Ashokapuri,duringtheappropriategrowingseason.Healthy,mature,disease-free leaves were manually collected during early morning hours to preserve bioactive phytoconstituents. Plant authentication was performed based on morphological, taxonomical, and organoleptic characteristics using standard herbarium references.

ProcessingofPlantMaterial Cleaning:

Freshlycollectedleaveswerethoroughlywashedunderrunningtapwaterfollowedbydistilledwaterto remove soil, dust, and extraneous matter. Excess moisture was removed using clean blotting paper.

Drying:

Thecleaned leaveswereshade-dried atcontrolledroomtemperature(25-30°C)withadequateventilation to avoid photodegradation of heat-sensitive compounds such as flavonoids and phenolics.

Pulverization:

Driedleaveswerereduced tocoarsepowderusing amechanicalgrinderandpassedthroughasuitable mesh sieve to obtain uniform particle size, which is essential for reproducible extraction efficiency.

Storage:

Thepreparedleafpowderwasimmediatelytransferredintoairtight,moisture-free,and light-resistant containers to protect it from environmental factors such as humidity, oxidation, and light exposure.

SoxhletExtraction

Accurately weighed 30-50 g of shade-dried powdered leaf material was packed into a cellulose extraction thimble. Ethanol was selected as the extraction solvent due to its high polarity and proven efficiency in dissolving secondary metabolites. The extraction was performed using a Soxhlet apparatus attheboilingpointofethanolfor18-24hoursuntilthesiphoningsolvent appearedcolorless.Theextract was filtered, concentrated under reduced pressure using a rotary vacuum evaporator (40-50°C) to prevent thermal degradation, and stored in airtight containers under refrigeration.

PharmacognosticalEvaluation

MacroscopicEvaluation:

Externalmorphologicalcharacteristicsincludingleafarrangement,shape,size,margin,surfacetexture, Qualitative color, odor, and taste were examined and documented according to standard procedures.

MicroscopicEvaluation:

Thintransversesectionsofleaf,petiole, andstemwereprepared,stainedwithsafraninandfastgreen, and observed under compound microscope to identify diagnostic features including epidermis, trichomes, stomata, mesophyll, and vascular bundles.

QuantitativeMicroscopy:

Leaf constants including stomatal number, stomatal index, palisade ratio, vein islet number, and vein terminationnumberweredeterminedusingclearedleafpreparationsandcalibratedeyepiecegraticule.

PhysicochemicalParameters:

Standardashvalues(totalash,acid-insolubleash,water-solubleash,sulphatedash),extractivevalues (hexane, ethanol, water), loss on drying, and foreign organic matter were determined according to standard pharmacopoeial methods.

PhytochemicalScreening

phytochemical tests were performed to identify bioactive constituents: Mayer's test for alkaloids, Molisch'stestfor carbohydrates,Biurettestforproteins,Ninhydrintestforaminoacids,foamtestfor saponins, ferric chloride for tannins, Dragendorff's test for glycosides, ferric chloride for phenols, sodium hydroxide test for flavonoids, and histochemical studies for localization of phytochemicals.

3. FORMULATION

MethodofPreparation(Water-in-OilSterileHerbalCream)

The sterile herbal cream containing Tridax procumbens leaf extract was formulated using a sterile emulsification technique suitable for preparing water-in-oil (W/O) topical formulation intended for wound application.In W/Oemulsions,theaqueousphaseis dispersedwithinthecontinuousoilphase,whichprovides enhanced occlusiveness, improved skin hydration,

and prolonged drug retention at the wound site. All preparation steps were carried out under aseptic laboratory conditions, using sterilized glassware, instruments, and filtered ingredients.

PreparationandSterilizationofOilPhase(ContinuousPhase)

Accurately weighed quantities of beeswax, stearic acid, liquid paraffin, and suitable W/O emulsifying agents (e.g., Span 60) were transferred into a previously sterilized, dry beaker. The mixture was heated gently on a waterbath andmaintainedat70–75 °Cuntilcompletemeltingproducedaclearanduniformoilyphase.Theoil phase was subjected to dry heat sterilization to reduce microbial load. Continuous mild stirring using sterile rods ensured homogeneous mixing.

PreparationandSterilizationofAqueousPhase(Dispersed Phase)

Inaseparatesterilizedbeaker,requiredquantitiesof glycerin,borax,preservatives(methylparabenandpropyl paraben), and sterile distilled water were taken. The aqueous phase was heated to the same temperature range (70–75 °C) to ensure proper emulsification. Distilled water was previously sterilized via autoclaving. Gentle stirring ensured complete dissolution of water-soluble components.

SterileEmulsificationProcess(W/OType)

Under aseptic conditions, the hot aqueous phase was slowly added to the hot oil phase with continuous sterile mechanicalstirring. Thismethodensuredformation ofastablesterilewater-in-oilemulsion,wherefinedroplets of aqueous phase are uniformly dispersed within the oil phase. Continuous stirring was maintained to achieve proper globule size distribution, smooth consistency, and prevention of phase separation.

SterileIncorporationofTridaxprocumbens Extract

ThepreviouslypreparedsterileTridaxprocumbensextractwasincorporatedgraduallyintotheemulsionunder aseptic conditions. Gentle and uniform stirring ensured even distribution of phytoconstituents throughout the cream. Care was taken to avoid excessive temperature exposure to prevent degradation of thermolabile compounds such as flavonoids and phenolics.

Cooling,Filling,andStorageofSterile Cream

Theformulation was allowed to coolgradually to roomtemperaturewith continuous sterilestirring. Controlled coolingensuredpropersemisolidconsistency,smoothtexture,andstabilityofW/Oemulsion.Thesterilecream was filled into pre-sterilized airtight and light-resistant containers under aseptic conditions. The final product obtained was a smooth, homogeneous, sterile, and stable water-in-oil herbal cream, suitable for topical wound application.

FormulationComposition

Ingredient	Concentration	Role
Tridaxprocumbensextract	3 g	Activewound-healingagent
Beeswax	5 g	Stiffeningagent
Span60(Sorbitan monostearate)	2 g	PrimaryW/Oemulsifier
Stearicacid	3 g	Emulsifier
Liquidparaffin	20 g	Emollient
Glycerin	5 g	Humectant
Borax	0.5 g	Emulsifying aid
Methylparaben	0.18 g	Preservative

Propylparaben	0.03 g	Preservative
Distilledwater	q.s.	Vehicle

Table1:FormulationCompositionTable

EVALUATION

Theevaluationwasdesignedtoconfirmtheformulation'sphysicalintegrity,safetyforwoundedskin,and microbiological purity through rigorous physicochemical and organoleptic analysis.

PhysicochemicalAnalysis

pHDetermination:

Purpose: To verify skin compatibility. A pH significantly different from the skin (approx. 4.5–6.5) can cause irritation.Procedure:1gramof thecream isdispersedin100mLofdistilledwater. Themixtureisstirred fora specified time to ensure uniform dispersion. A calibrated Digital pH Meter is then used to measure the pH of this solution at room temperature 25°C ± 2°C.

SpreadabilityTest:

Purpose: Essential for wound healing to ensure the cream spreads easily over sensitive, painful areas without excessive pressure. Procedure: A specific amount of cream (e.g., 2g) is placed between two glass slides. A weight (e.g., 100g) is applied to the top slide for 5 minutes to create a uniform film. The time (seconds) it takes forthetopslidetoseparatefrom thebottomslideunderafixedpullisrecorded.Spreadability(S)=(M×L)/T, where M = weight tied to upper slide (g), L = length of glass slide (cm), T = time taken (sec).

Washability:

Purpose:Toensurethecreamcanbeeasilyremoved fromtheskinorclothes.Procedure:Asmallamountofthe cream is applied to a1-inch areaof theskin. After 10 minutes, theareais rinsed under moderatetap water. The ease of removal is qualitatively assessed (e.g., Very Good, Good, Poor).

OrganolepticStability:

Purpose: To evaluate the physical stability and acceptability of the herbal cream during storage. Changes in color,odor,texture,orphaseseparation mayindicate instabilityordegradation.Procedure:Asuitablequantity of the herbal cream is stored in well-closed containers at room temperature (25°C ± 2°C). The sample is observedatregularintervals(0,7,15,and30days).Theformulation isvisually inspectedforchangesin color, odor, texture, and the presence of phase separation.

4. RESULTS

Macroscopic(Morphological)Evaluation

Feature	**Description**	**Significance**
Leaf type	Simple,opposite,petiolate	Easyfield identification
Shape	Ovatetolanceolatewithserrated margins	Characteristicsofthespecies
Surface	Rough,hairy(pubescent)	Trichomesassociatedwithprotective phytochemicals
Colour	Darkgreen(uppersurface),palegreen(lower surface)	Indicateschlorophyllrichness
Taste	Slightlybitter	Suggestspresenceofflavonoidsand alkaloids

Table2:MacroscopicEvaluation

Theleavesshowedoppositeanddecussatearrangement,simpleexstipulatestructure,ovatetolanceolateshape (3–7 cm in length and 1–3 cm in width), irregularly toothed or serrated margins, and rough hairy pubescent surface. The reticulate venation pattern was clearly visible. Petioles were short, hairy, and easily breakable.

Freshleavesexhibitedbrightgreen colorationturningdullgreenupondryingwithcharacteristicslightodorand slightly bitter taste.

MicroscopicEvaluation

Tissue	Observation	PharmacognosticImportance
Epidermis	Single-layeredwiththickcuticle	Protectsleafandpreventsmoisture loss
Trichomes	Multicellular,covering type	Defensiverole;storebioactivecompounds
Stomata	Anomocytictype	Importanttaxonomicmarker
Mesophyll	Differentiatedintopalisadeandspongy layers	Palisadecellsrichinchloroplastsfor flavonoid synthesis
Vascular bundle	Well-developed,collateraltype	Efficienttransportofmetabolites

Table3:MicroscopicEvaluation

Transversesection of leaf showed dorsiventralstructurecharacteristicof dicotyledonous plants. Single-layered upper and lower epidermis with well-developed cuticle. Abundant multicellular uniseriate covering trichomes (3–6 cells) present on both surfaces. Anomocytic stomata more numerous on lower epidermis. Mesophyll differentiatedintosingle-layeredpalisadeand5–7layersofspongyparenchymawithlargeintercellularspaces. Prominentmidribregionwithcollateralvascular bundles.

QuantitativeMicroscopy(LeafConstants)

Parameter	UpperEpidermis	LowerEpidermis
Epidermalnumber	140	145
Stomatalnumber	28	46

Table4:QuantitativeMicroscopy

Parameter	Value
Stomatalindex	15–16%(upper),25–30% (lower)
Palisaderatio	2–3
Veinisletnumber	5–6

Table5:OtherLeafConstants

PhysicochemicalParameters

AshValues

Parameter	Value(% w/w)	Significance
Totalash	11.65	Indicatestotalinorganicmatterpresentinthe drug
Acid-insolubleash	3.25	Reflectscontaminationwithsiliceousmattersuchassandandsoil

Water-insolubleash	2.56	Indicatesamountofwater-insolubleinorganiccomponents
Sulphatedash	20.24	Representstotalinorganicresidueaftersulphuricacid treatment

Table6:Ash Values

ExtractiveValues

Solvent	ExtractiveValue (% w/w)	Interpretation
Hexane	8.75	Indicatespresenceofnon-polarconstituentssuchaslipids
Ethanol	7.65	Suggestspresenceofmoderatelypolarcompoundslikeflavonoidsand alkaloids
Water	28.34	Indicateshighcontentofpolar constituentssuchasglycosides, tannins, and phenolics

Table7:ExtractiveValues

PhytochemicalAnalysis

NameofTests	Inference	Observation
Alkaloids	+	Formationofprecipitateobserved
Carbohydrate	–	Nocharacteristiccolourchange
Protein	–	Nobluish-violetcolour
Saponin	+	Persistentfoamformation
Phenols	+	Colourchangewithprecipitate
Flavonoids	+	Yellowcolourturningcolourlesswithacid
Steroids	+	Characteristiccolourformation
Glycosides	–	Noreddish-brownring

Table8:PhytochemicalAnalysis

FormulationEvaluationResults

pHEvaluation:

S.No	Observation	F1	F2
1	Ph	5.62	5.76

Table9:pH Determination

ThepH oftheformulatedherbalcreamwas foundtobewithin theacceptableskinrange(5.62-7.1). This indicatesthattheformulationiscompatiblewithskin andwillnot causeirritationwhenappliedonwoundedor sensitive areas. The result confirms the suitability of the cream for topical application.

SpreadabilityTest:

Trial	M(g)	L (cm)	Time(sec)	Spreadability(g·cm/sec)
F1	50	7.5	12	31.25
F2	50	7.5	11	34.09
F3	50	7.5	13	28.84

Table10:SpreadabilityTestObservation

The spreadability value of the cream was found to be satisfactory (28.84-34.09 g·cm/sec). This shows that the formulationcanbeeasilyspreadontheskinwithminimumeffort.Goodspreadability isimportantforuniform application over the wound surface and improves patient compliance.

WashabilityTest:

S.No	Observation	F1	F2
1	Washability	Washable	Notwashable

Table11:Washability Evaluation

The formulation showed good washability. This indicates that the cream can be easily removed from the skin
usingwaterwithoutleavingexcessiveresidue.Thispropertyisbeneficialformaintainghygieneandrepeated application on wounds.

OrganolepticStabilityStudy:

Parameter	Initial	15 Days	30 Days	ReferenceLimit
Color	Lightgreen	Nochange	Nochange	Nosignificantchange
Odor	Herbal	Nochange	Nochange	Norancidity
Texture	Smooth	Smooth	Smooth	Nogrittiness
PhaseSeparation	Absent	Absent	Absent	Shouldbe absent

Table12:OrganolepticStability Study

Nosignificantchangesin color,odor,texture,orphaseseparationwereobservedduringthestudyperiod. This indicates that the formulation is physically stable under storage conditions. The stability ensures consistent performanceandshelflifeofthe product.

5. DISCUSSION

Medicinal plants continue to play a crucial role in traditional and modern healthcare systems, serving as valuable sources of bioactive compounds with therapeutic potential. Tridax procumbens Linn., a widely distributed herb belonging to the family Asteraceae, has long been recognized in traditional medicine for the treatment of wounds, inflammation, infections, and metabolic disorders. The present study focused on the pharmacognosticalevaluationandphytochemicalinvestigationofTridaxprocumbens leaves, alongwiththeir relevance to wound-healing activity.

Pharmacognosticalanalysisisessentialfortheauthentication,standardization,andqualitycontrolofcrudeplant materials. In this study, the macroscopic and microscopic characteristics of the leaves confirmed diagnostic features consistent with reported descriptions of Tridax procumbens. Such identification ensures the purity and genuineness of the plant material used for further phytochemical and pharmacological investigations, thereby strengthening the scientific reliability of herbal drug development.

Preliminaryphytochemicalscreeningoftheleafextractrevealed thepresenceofflavonoids,tannins,alkaloids,

saponins,glycosides,terpenoids,andphenoliccompounds.Thesesecondarymetabolitesarewidelyreportedto contribute to antioxidant, antimicrobial, anti-inflammatory, and tissue-repair mechanisms, all of which are fundamental processes in wound healing. Flavonoids and phenolics, in particular, are known to neutralize free radicals, reduce oxidative stress at the wound site, and promote collagen synthesis and epithelial regeneration. Tannins aid in wound contraction and protein precipitation, forming a protective layer that prevents microbial invasion, while saponins and alkaloids contribute to antimicrobial and anti-inflammatory effects.

Traditional usage of Tridax procumbens in treating cuts and wounds is strongly supported by experimental findings.Previousstudieshavedemonstratedenhancedwoundcontraction,increasedhydroxyprolinecontent, improved collagen deposition, and faster epithelialization in treated models. The antimicrobial activity of ethanolic extracts, especially against pathogenic and multidrug-resistant organisms, further supports its therapeutic relevance in preventing wound infection, a critical factor that delays healing.

Theformulatedsterilecreamdemonstratedacceptablephysicochemicalparametersandexcellentstability, confirming its suitability for pharmaceutical use. The integration of traditional botanical knowledge with modern pharmaceutical engineering has resulted in a standardized, safe, and potentially effective herbal formulation addressing critical challenges in diabetic foot ulcer management.

6. CONCLUSION

The present study on pharmacognostical and phytochemical evaluation of Tridax procumbens leaf extract confirmsthattheplantpossessessignificantmedicinalvaluerelevanttowound-healing applications.Detailed morphological and microscopic characterization established the authenticity and quality of the crude drug, ensuring its suitability for therapeutic use and further research.

Phytochemical investigation revealed the presence of diverse bioactive constituents, particularly flavonoids, tannins, phenolics, alkaloids, and saponins, which are known to contribute to antioxidant, antimicrobial, anti-inflammatory,andtissue-regenerativeactivities.Thesepropertiescollectivelysupportthetraditionalclaimthat Tridax procumbens promotes faster wound contraction, collagen formation, epithelialization, and protection against microbial infection.

Theformulatedsterileherbalcreamexhibitedexcellentphysicochemicalcharacteristicsandstability,makingit a promising candidate for diabetic foot ulcer management. Further studies involving isolation of active compounds, detailed mechanism-of-action analysis, toxicity evaluation, and clinical validation in human diabetic populations are necessary to transform this traditional remedy into a standardized herbal therapeutic agent.

In conclusion, the integration of pharmacognostical standardization and phytochemical evidence strongly justifiesthemedicinalpotentialofTridaxprocumbensandsupportsitsfuturedevelopmentinherbalwound-care therapy and pharmaceutical research.

7. LIMITATIONSOFTHESTUDY

Every scientific study, no matter how carefully designed, carries certain boundaries—and this work is no exception.WhiletheexplorationofTridaxprocumbenshasofferedpromising insights,afewlimitations shape the scope of the conclusions drawn.

Tobeginwith, thestudyprimarilyreliesonpreliminaryphytochemicalscreeningandbasicpharmacognostical evaluation. Although these methods confirm the presence of bioactive constituents, they do not precisely identifyorquantify theindividualcompoundsresponsiblefortheobservedtherapeuticeffects.Theplant, inits complexity, still holds secrets that require deeper analytical exploration using advanced chromatographic and spectroscopic techniques.

Anotherconstraintliesinthelimitedexperimentalscale.Theformulationand evaluationof thewound-healing cream were conducted under controlled laboratory conditions, which, while reliable, cannot fully mirror the dynamic and often unpredictable environment of living biological systems. The absence of extensive in vivo studies or clinical validation means that the transition from bench to bedside remains incomplete.

In addition, factors such as geographical variation, seasonal changes, and differences in plant harvesting conditions may influence the phytochemical composition of Tridax procumbens. These variations can subtly

affectthereproducibilityandconsistencyoftheresults,posingchallengesforstandardizationacrossdifferent batches and sources.

Thestudyalsodoesnot extensivelyaddresslong-termstabilityandshelf-lifeoftheformulatedcreambeyond 30days.Withoutsuch extendeddata, itisdifficultto predicthowtheformulationwillbehaveoverprolonged periods under varying storage conditions.

Lastly, while the antimicrobial and wound-healing potential is encouraging, the study does not explore its effectivenessagainstresistantmicrobialstrainsorchronicwoundconditionsindepth. Thisleavesanimportant clinical dimension yet to be uncovered. These limitations, rather than diminishing the value of the work, highlight the natural progression of research—where each answer opens the door to new questions.

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