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Development and Evaluation of Antimicrobial ointment of Clerodendrum Infortunatum

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ABSTRACT

IntheruralareasofNorthIndia,clerodin,anactivebitter substancefoundinClerodendruminfortunatumLinn. (Verbenaceae),isacommonandsignificantmedicinal plantthatisusedasananthelminticandtonic.Theplant requires carefulresearchtodetermine its specific medicinal activity, even though it isused inAyurveda, Unani medicine, and homeopathy in a variety of ways to treat conditions like diarrhea, skin disorders, venereal and scrofulouscomplaints,wounds,postpartumcomplicat ions,asavermifuge,laxative,andcholagogue,toremov e ascaridsfromtheanus,asexternalapplicationsontumor s,etc.Thereasonleavesandrootsareappliedexternallyt o tumors is because they contain clerodolone, clerodol, and a sterol that is now known as clerosterol. Extract from leavesandrootscanbeusedtonewlyopenedwounds.Be causeoftheleafextract,thepercentageofhemoglobin rose.

Patientswhoreceiveleafextracttreatmentexperiencesi gnificantrelief.Indiabeticpatients,thepercentageofsu gar decreased Within twelve hours, applying edible oil and slightly heated leaves to the sore area of the body provides significantalleviation.Therootextractwasalsousedtoi dentifysevensugars:fructose,galactose,lactose,malto se, sucrose, and raffinose.The hanging drop method was used to evaluate the vitality of the pollen. Fruit and seed set were used to measure reproductive success.

KEYWORDS: ClerodendruminfortunatumClerodi n,Antidiabetes,Unani,Homeopathy,Clerosterol.

I. INTRODUCTION

The terrestrial shrub Clerodendrum infortunatum Linn. (Family: Verbanaceae) is known by the Hindi names Bhat, Bengali names Ghentu, and Oriya names Bhania. It has a square, blackish stem and simple, opposite, decussate, petiolate,exstipulate, coriacious, hairyleaves with anunpleasant odor [(1,2)].The shrub's height ranges fromtwo to four feet [(3)].The terminal panicles are pyramid-shaped, with bluish-purple flowers that are frequently white.The plant has bluntquadrangularsternsandbranches,threeleavesata node,occasionallyoppositeoblongorelliptic,serratedl eaves, blueflowers,manyinlongcylindricalthyrsus,andsome whatsucculent,four-lobedpurpledurpefruitswithonepyrene in each lobe [(4)]. The plant is widespread in West Bengal and is found throughout India's plains [(5)]. Over the decades,variousspeciesoftheClerodendrumgenusha vebeenutilizedtraditionally,andtheirhepatoprotectiv eandantioxidant properties have already been demonstrated. Tribes use different portions of the plant to treat tumors, skin conditions, snake bites, scorpionstings, andcolic.The mildlybitter leaves helpwithsmallpox, inflammation, and skinconditions [(6)]. In Indian traditional medicine, the plant parts are also used to treat bronchitis, asthma, fever, blood disorders, inflammation,burningsensations,andepilepsy [(5)].Triterpenes,steroids,and flavonoidswere discoveredtobe present in the plant.The plant's analgesic, anthelmintic, antibacterial, anti-malaria, and antioxidant properties have also led to an increase in research on the plant [(6)].

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Fig1:Clerodendruminfortunatum Linn

II. MATERIALSANDMETHODS

PLANT COLLECTION,AUTHENTICATIONANDDRYING

FreshleavesofC.infortunatum werecollectedfromlocalareasinThrissurdistrict,Kerala,onNovember12,2024.Theplant was taxonomically identified and authenticated byDr. RanjushaA. P., HOD of the Department of Botany, NSS College, Ottapalam. ThespecimenisnowdepositedatNehruCollegeofPharmacy,Thrissur,forfuturereference.Aftercollection,theplantmaterial was shade-dried with proper aeration for 20-25 days, ground using a mixer grinder, and stored in an airtight container.

PHARMACOGNOSTICALSTUDIES

ORGANOLEPTICEVALUATION

Organolepticevaluationwereperformedaccordingtocolour,size,odour,andtasteparameters.

MACROSCOPICEVALUATION

Themacroscopicstudyinvolvestheevaluationofcolour,smell,sizeandshape,flavour,otherhandle,textureandthetypeof leaves depending on margin, apex, base surface type and venation.

MICROSCOPICALEVALUATION

Asmall portion of midrib region is separated from the entire sample of leaf to take section. Thin transverse sections of leaf are taken, manual with sharp razor bladesand withthe help ofthermocolpith. Phloroglucnol wasused to stainthe sections.A little dropofphloroglucnolisdilutedindiluteHydrochloricacid.Add thethinsectionstothe stainandremoveitaftersomeseconds. Sections should now be washed in water to remove the excessive staining. Mount it on a clean glass slide using glycerin. Carefullyplaceacleancoverslipoverthesection.Withoutenteringanyairbubbles.Itisthenseenwiththe microscope,firstlow power and then high power. Clear pictures are taken and details noted.

Preparationofdriedpowder

ThecollectedleavesofClerodendruminfortunatumwaswashedwithrunningtapwatertoremoveanyassociatedmaterials.The leaveswerethenlefttodryundershadefor20-25days,thengroundedusingmechanical grinderandputinairtightcontainerfor future use

Fig2:powderformofClerodendrum infortunatum

POWDERANALYSIS

Pinchofleafpowderpreviouslysievedisputontheslide andmountedinglycerineandpowdercharactersare observed under compound microscope.

EXTRACTION

Fig3,4:MacerationandHeating

- Shadedriedleaveswerecoarselypowdered.
- 25gofcoarselypowderedleaveswaresoakedinethanolicextract
- Theextractionwascarriedoutatroomtemperature for7days.
- ExtractwascollectedfilteredthroughWhatnman No.1filterpaperandconcentrated.Thencalculate

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dthepercentage yield (%w/w).

Fig5:Driedformofextract Preliminary phytochemical screening of plant extract.

ifydifferentconstituentspresentinextracts

i. e,carbohydrates,alkaloids,glycosides,flavonoids,terpenoids,steroids,phenolsandtannins.Allthe weresubjectedtopreliminary phytochemical screening.

Preliminaryphytochemicalscreeningwasdonetoident

Sl.no.	**Test**		**Procedure**	**Positiveobservation**
1	Alkaloids	Mayer'stest	2mloftheextract+2 mlof Mayer'sreagent	Creamyprecipitate
		Hager'stest	2mloftheextract+1-2 mlof Hager'sreagent	Presenceofyellow precipitate
		Wagner'stest	2mloftheextract+1-2 mlof Wagner'sreagent	Reddish brown precipitate
2	Glycosides	Baljet'stest	Mixed2-3mlofsamplein2ml sodium picrate solution	Yellowto orange colour
		Borntrager'stest	To a little quantity of sample solutionaddedH2SO4andCCl4. Separated the organic layer and shaken with dilute ammonia	Pinktoredcolor
			To a little quantity of sample solutionaddedH2SO4andCCl4. Separated the organic layer and shaken with dilute ammonia	
3	Phenolic and	Ferricchloride test	Mixed2mlofthetestsolutionwith few ml of 5% Ferric chloride	Presence of blue color
	tannins		solution	
		Lead acetate test	Mixed2mltestsolutionwith1ml of lead acetate solution	Presenceof bulky whiteprecipitate
4	Flavonoids	Shinodatest	2 ml sample solution + Magnesiumpowderandfewdrops of Concentrated HCl	Pinkscarlet,crimson red or occasionally green to blue color
		Ferricchloride test	Add a few drops of ferric chloride solutionto2mlofthetestsolution	Theappeanceofan intense greencolor indicatesapositive result

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5	Carbohydrate	Molisch'sTest.	1 ml of the test solution was mixed with 2 ml of Molisch's reagent,shakenthemixtureand added 1 ml of concentrated H2SO4alongthesidesofthetest tube	Presence of violet ringatthejunctionof two solutions
		Fehling'sTest	Boiled1mloftestsolutionwith1 ml Fehling's solutionAand 1 ml Fehling's solution B on a water bath	Presence of red residueatthebottom of test tube
6	Terpenoids	Salkowski's Test	Dissolved1-2mgofsamplein1 ml of CHCl3 and added 1 ml of Concentrated H2SO4	Appearance of red color in the chloroformlayerand greenish yellow fluorescence

Tab1:Preliminaryphytochemicalscreeningofplantextract.

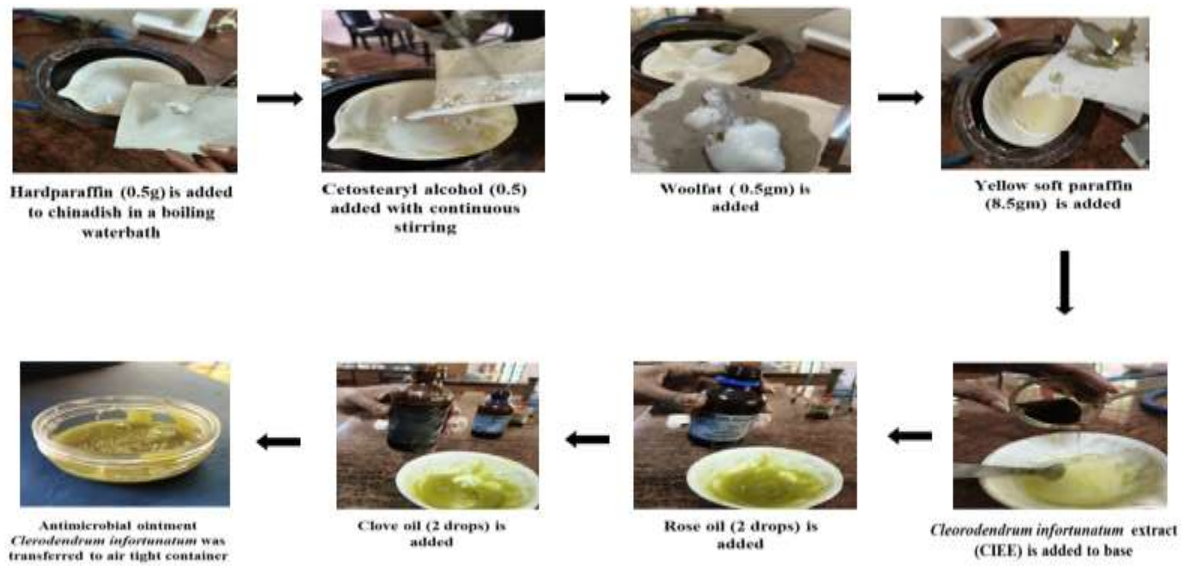
FORMULATIONSTUDIES

SL.NO	INGREDIENTS	QUANTITY
1	Wool fat	0.5g
2	Cetostearylalcohol	0.5g
3	Hard Paraffin	0.5g
4	Yellowsoftparaffin	8.5g
5	ClerodendrumInfortunatumextract	1g
6	Cloveoil	1ml

Tab2:Formulationstudiesofclerodendruminfortunatum

Procedure:

Take a chinadish in a boiling waterbath to which add hardparaffin (0.5g),with continuous stirring add the rest of ingredients(woolfat, cetostearyl alcohol,yellow soft paraffin). To the base prepared prepared add cleorodendrum infortunatum with continuousstirringto formauniformointment.Thecloveoilisaddedatlastan dtheointmentwastransferredtoointmenttube..



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III. EVALUATION

Organoleptictest: Thepreparedsamplewereinspectedvisuallytocheckthetexture,odour,andthecolourofthe ointmentasper Indian Pharmacopoeiaprocedure.

pHevaluation: ThepHmeasurementoftheformulatedointmentwasmeasuredusingdigitalpHmeter.

Skinirritationtest: Itiscarriedoutbyapplyingtheointmentonskinandtestedforanyrednessoritchingaftertwohours.

Washability: Formulationwasappliedontheskinandtheneaseextendofwashingwithwaterwaschecked.

Solubility: Solubleinboilingwater,misciblewithalcoholandsparinglysolubleinether.

Spreadability: Thespreadabilitywasdeterminedbyplacingexcessofsampleinbetweentwoslideswhichwascompressedto uniform thickness by placing a definite weight for definite time.The required to separate the two slides was measured as spreadability.

IV. RESULTANDDISCUSSION

CollectionandAuthenticationofleavesofclerodendrum infortunatum

TheleavesofClerodendruminfortunatum werecollectedfromlocalareasofThrissurdistrict,Kerala,onNovember12,2024. The plant's authenticity was confirmed by Dr. RanjushaA.P, Head of the Department of Botany, NSS College Ottapalam.

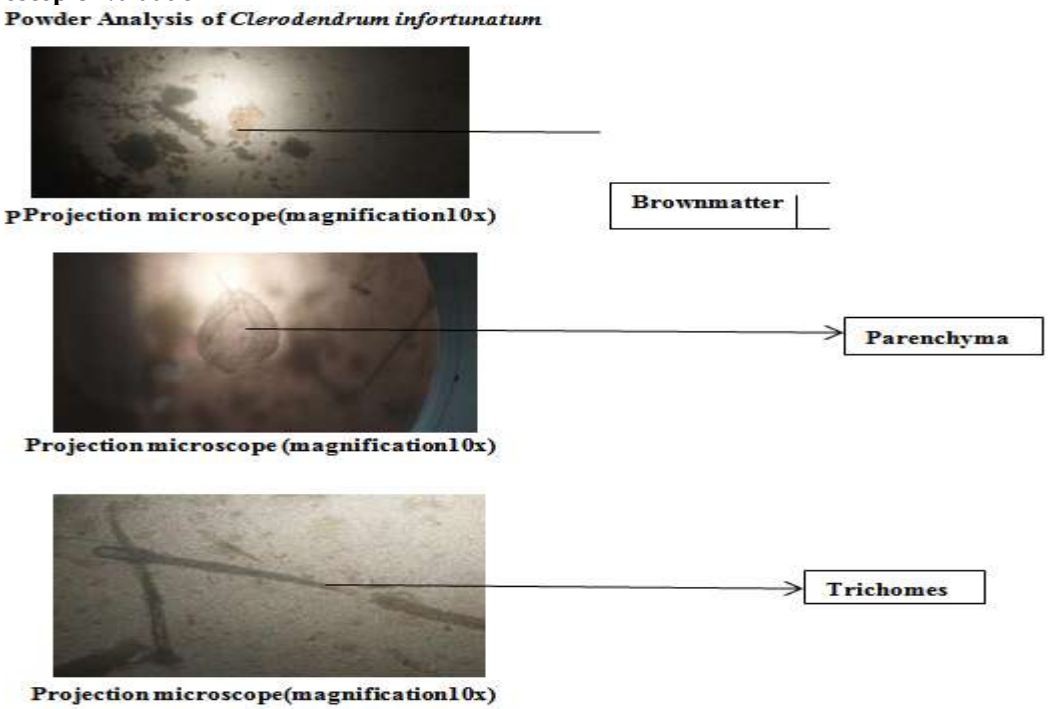
MacroscopicEvaluation

Themacroscopicevaluationofclerodendruminfortunatumleafwasperformed.

ORGANOLEPTICCHARACTERS	NATURE
COLOUR	Green
SIZE	5-15cm(2-6inches)longand3-7cm(1.2-2.8inches)wide
ODOUR	Distinctive,somewhatpungentsmellwhencrushed
TASTE	Bitter

Tab3:Macroscopicevaluationofclerodendruminfortunatum

MicroscopicEvaluation



FigNo6,7,8:Powdermicroscopyofclerodendruminfortunatum

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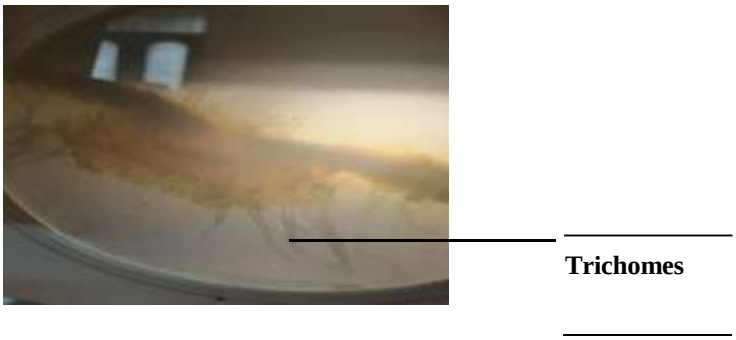
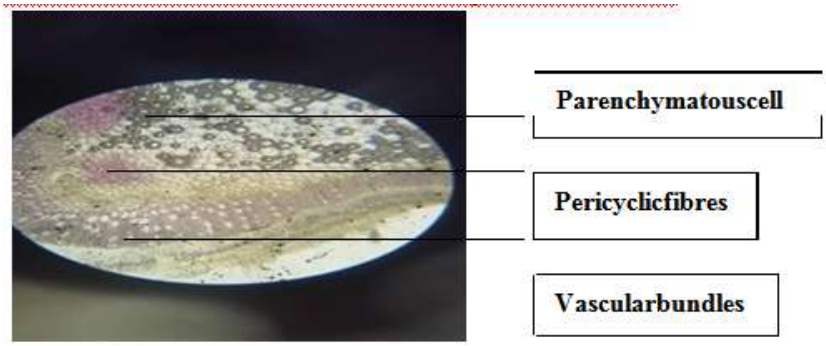
TRANSVERSESECTIONOFClerodendruminfortunatumLEAF



FigNo9:Transversesectionofclerodendendruminfortunatum

TRANSVERSESECTIONOFClerodendruminfortunatumSTEM



FigNo10:Transversesectionofclerodendruminfortunatumstem

PhytochemicalScreening

PHYTOCONSTITUENTS	RESULTS
Alkaloids	+
Glycosides	-
Flavonoids	+
Terpenoids	+
Steroids	+
Carbohydrates	+
PhenolicandTannins	+

Tab4:Phytochemicalscreeningofclerodendruminfortunatum

EVALUATIONTESTFORANTIMICROBIALOINTMENT

Stabilityofformulation

Parameter	**ointment**			
	initial	10 days	20 days	30 days
color	Greenishyellow	Greenishyellow	Greenishyellow	Greenishyellow
odour	Characteristic	Characteristic	Characteristic	Characteristic
pH	6.3	6.3	6.3	6.3
Skinirritaion	No	No	No	No

Tab5:Stabilityofherbalointmentindifferentparameters

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PhysicalAppearance
Thephysicalappearance,colourandfeelofthedevelop edointmentwerevisuallytest.Therewasnopresenceof anyforegin particles.The color observed was greenish yellow.

pHtest
pHofthepreparedointmentwasobserved6.3

Skinirritation
Itiscarriedoutbyapplyingointmentonskinandtestedfo ranyrednessoritchingafter2hours.Thereisnorednesso ritching.

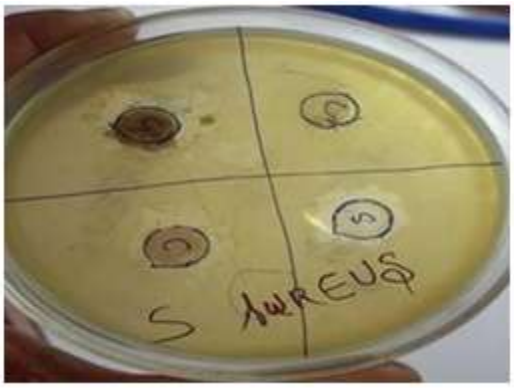
Spreadability
Fromtheresultofevaluation,itisshowedthattheformul ationshowevenlyspreading.

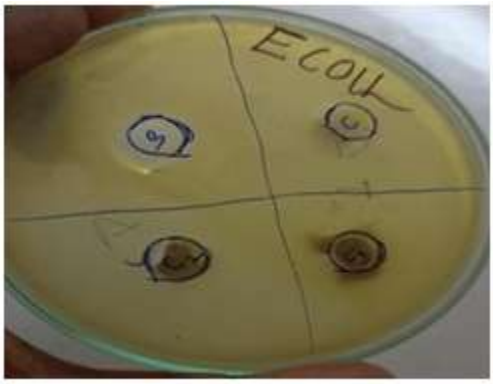
Washability
Theproductwillbeappliedbyhandandwasobservedun derrunningwater.Theseformulationexhibitexcellent washability characteristics,ensuring that it can be conveniently removed without leaving any residue.

INVITROANTIMICROBIALACTIVITYTESTFOROINTMENT
Agarwell diffusion

Nameof microorganism	Zoneofinhibition			
	Standard clindamycin ointmentH&H	Negative Control(c2)	C (100mg)	C1(400mg)
Staphylococcus Aureus	+ve 8mm	-ve	+ve 6mm	+ve 7mm
E.coli	-ve	-ve	-ve	-ve

Tab6:Zoneofinhibitionofointment





FigNo11,12:PositiveresponseofstaphylococcusaureusandNegativeresponseofE.coli

Discussion
Theleaveswerecollectedfrommedicinalgar denofNehruCollegeOfPharmacyThrissurdistrict,Ke ralaregionand authenticated.TheleavesweresubjectedtoPharmacog nosticalinvestigation.Macroscopicandmicroscopicc haracteristicsofthe leaf were studied. The microscopic study shows that it contains Trichomes, brown matter, pericyclic fibers The leaves were subjected to extractionbyusingethanoland the extract wassubjected toPharmacognostical investigation.The alcoholic extract containsalkaloids,steroids,terpenoids,carbohydrates etc.Theanti-microbialactivitiesoftheplantwerestudiedbyusingAg ar- Well Diffusion method.

V. CONCLUSION

The study was conducted to get the information regarding macroscopy, microscopy, and antimicrobial activity of Clerodendrum infortunatum. Presence of brown matter, parenchyma, trichomes, pericyclic fibres, vascular bundles, parenchymatous cell were observedinmicroscopicalevaluationofClerodendru minfortunatum.Phytochemicalscreeningwasperfor medandthepresenceof chemicalconstituentssuchasflavonoids,phenols,tann ins,alkaloids,terpenoidsandsteroidsexceptGlycoside

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wasobserved.Anti- microbial ointment of Clerodendrum infortunatum was prepared using trituration method. Evaluation tests such as PH, skin irritation, spreadabiliy ,washability, solubility for ointment were performed and quality of ointment was confirmed. The antimicrobial activity of the ointment was estimated by comparing with using Agar well diffusion test method against staphylococcus aureus (in-vitro test). Clerodendrum infortunatum has shown good antimicrobial activity. The main chemical constituent has to be isolated and its mechanism of action has to be explored in future studies.

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