International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1215-1221 www.ijprajournal.com ISSN: 2456-4494

Formulation and Evaluation of Bryophyllum Pinnatum (Lam.) Kurz. Gel for Wound Healing Topical Application

Date of Submission: 05-08-2024 Date of Acceptance: 15-08-2024

ABSTRACT
Wound healing is a complex biological process that consists of haemeostasis, inflammation, proliferation and remodeling. Large number of cell types including neutrophils, macrophages, lymphocytes, keratinocytes, fibroblasts, and endothelial cells are involved in the process.
Bryophyllum pinnatum (crassulaceae) has been widely used traditional medicine for the treatment of various disease, including wound healing. The number of patients has affected by wound healing, the plant which we have selected for the treatment is Bryophyllum pinnatum.
The study aims to formulate and evaluate the wound healing extract of leaf part Bryophyllum pinnatum (crassulaceae) plant. The plant was choosen to detailed study about Pharmacognostical properties and extracted by maceration method.
Ethanol was taken and extraction was proceeded preliminary phytochemical analysis was conducted and tabulated further evaluated for In-vitro anti oxidant activity is performed using DPPH assay where ascorbic acid is taken as standard ethanol extract showed a high effect it was further subjected to In-vitro anti-microbial activity using Muller Hilton agar as medium the IC50 value was noted accordingly.
Ethanol was considered as a better solvent in each test, only ethanol showed comparatively high value than others. The extracted protein was checked by conducting antimicrobial studies on the following gram-positive bacteria and gram-negative bacteria using Muller Hinton agar as a medium and the extracted protein was found to be effective againstgram-positiveandgram-negative.EthanolicextractofBryophyllum pinnatum was found to possess high potentiality. Hence, it can be subjected to study further, and prominent anti cancer research can be done using it.
KEY WORDS : Anti-oxidant, anti-inflammatory, anti-microbial, Bryophyllum pinnatum

I. INTRODUCTION

The skin is the largest organ in the human body in terms of surface area. Internal tissues are protected by it from mechanical damage, microbial infection, UV light, and severe temperatures. This renders it extremely vulnerable to harm, with serious consequences for both individual patients and the healthcare system as a whole . Patients with diabetes, the elderly, and those with genetic abnormalities like sickle cell disease are all at risk for irregular wound healing, which can lead to long-term complications. Surprisingly, the existing interventions have had little influence on the issue. Although there are various wound healing methods available, they are only modestly effective. As a result, more effective wound healing therapies are required. The precise coordination of multiple different cell types in sequential phases is required for skin restoration. The epidermis is the outer, impermeable layer of the skin that protects it from the hostile external environment in healthy skin. Sebaceous glands and hair follicles are all found in the epidermis. Extracellular matrix (ECM), vasculature, and mechanoreceptors are abundant in the dermis, which serves as an energy reserve for the skin. It also provides the dermis with a steady supply of growth factors. Aside from these cell types, each layer has resident immune cells that constantly scan the skin for harm. When the skin is injured, multiple cell types in these three layers must work together at specific times to heal the wound. Hemostasis, inflammation, angiogenesis, growth, re-epithelialization, and re- modelling take place in a chronological order, but they also overlap .As a result, skin restoration is one of the most difficult processes in the human body. Constriction of the wounded blood arteries and activation of platelets to produce a fibrin clot are the first responses to a lesion .

PHARMACOLOGICAL ACTIVITIES

Antimicrobial activity

The researchers discovered that Bryophyllum pinnatum leaf extracts (aqueous, methanol, palm wine, Omidun, local gin, and fresh leaf juice) at dilutions of 256, 128, 64, 32, 16, 8, 4 mg/ml exhibited different antibacterial activity against the Gram-positive and Gram- negative microorganisms examined. With the control drug, methanol extract exhibited significant antibacterial

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1215-1221 www.ijprajournal.com ISSN: 2456-4494

activity against Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis, and Pseudomonas aeruginosa (Ciprofloxacin). Even the extract from Bryophyllum pinnatum's crushed leaves had a considerable effect on several Gram positive and Gram-negative bacteria. Other extracts had poor to moderate efficacy against the organisms tested.

Two isolated flavonoid compounds were found to have antibacterial action in another in-vitro research Compound 1(5I Methyl 4I, 5, 7 trihydroxy flavone) and compound 2(4I, 3, 5, 7 tetrahydroxy 5-methyl 5I -propenamine anthocyanidines) from Bryophyllum pinnatum leaf were tested in bacteria (three gram-negative organisms, including Escherichia coli, Pseudomonas aerugonosa, and Klebsiella pneumonia, and a gram- positive organism (Candidia albicans and Aspergillus Niger). Staphylococcus aureus, Pseudomonas aeroginosa, Klebsiella pneumoniae, Aspergillus niger, and Candida albicans were all successfully inhibited by the chemicals. However, compound 1 was unable to suppress E. coli.

II. REVIEW OFLITERATURE

PHARMACOGNOSY

1. Bryophyllum pinnatum belongs to the family Crassulaceae. It's used for Ethnomedical practices. Leaf gathered from south-eastern Nigeria in mice.
2. Bryophyllum pinnatum is generally known as Panphuti which belong to family Crassulaceae growing extensively in tropical Africa, tropical America, India, China, and Australia. It's a imperishable condiment grows 3-5 bases altitudinous, fleshy dark green leaves that are distinctively scalloped and trimmed in red, and bell-suchlike pendulous flowers .
3. The species Bryophyllum pinnatum (Lam) Pers. are native from Brazil and Madagascar, belonging to the Crassulaceae family and being extensively used by population as a naturalanti-inflammatory agent. These species haveanalogous splint morphology and for this reason, they're known by the same popular name as “saião” or “ coirama.”
4. Bryophyllum pinnatum (Lank.) Oken (Crassulaceae) is a imperishable succulent condiment extensively used in traditional drug to treat numerous affections. Its wide range of uses in folk drug justifies its being called “life factory “ or “ rejuvenation factory “, egging experimenters’ interest Bryophyllum pinnatum is a imperishable condiment, extensively used in the treatment of several conditions in myth drug .
5. Bryophyllum pinnatum is extensively used in ayurvedic system of drug as tangy, analgesic, carminative and also useful in diarrhea and vomiting. It's naturalized throughout the hot and wettish corridor of India. The leaves of Bryophyllum pinnatum have a variety of uses in the traditional system of drug in India. They're eaten for diabetes, diuresis, dissolving order monuments, respiratory tract infections, as well as applied to injuries, boils, and nonentity mouthfuls. It's useful for precluding alcoholic, viral and poisonous liver damages.
6. This study investigates the antioxidant exertion, carbohydrate digesting enzymes exertion and inhibitory exertion of cholinergic enzyme of waterless excerpt and fragments (n-hexane, ethyl acetate, n-butanol, residual waterless bit) of B. pinnatum leaves were delved .
7. It's extensively used in treatment of hemostatic and crack mending. It's also used in treatment of immunomodulatory, CNS depressant, analgesic, anti- inflammatory, antidiabetic, anticonvulsant, anticancer, antiallergic, nephroprotective, hepatoprotective, antileishmanial, antiulcer exertion. From this review of factory, it highlights the chemical element and medicinal uses of factory.
8. Antioxidant, anticancer, antidiabetic, anti-inflammatory, anesthetics, crack mending and hepatoprotective conduct which are incorporated.
9. Anti-inflammatory, antioxidant, anticancer, crack mending, antidiabetic.
10. The godly condiment contains a wide range of active composites, including alkaloids, triterpenes, glycosides, flavonoids, steroids, bufadienolides, lipidsand organic acids, have been insulated from this species .
11. Bryophyllum pinnatum contains precious phytochemicals similar as polyphenols, tannins, glycosaponins, flavonoids, steroidal glycosides and numerous other important chemical ingredients.
12. The qualitative phytochemical result showed that factory samples contains alkaloids, tannins, saponin, terpenoid, glycoside, phenols and flavonoid.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1215-1221 www.ijprajournal.com ISSN: 2456-4494

III. AIM AND SCOPE

AIM:

The present study was undertaken for the formulation and standardization of gelusing Bryophyllum pinnatum.

SCOPE:

Hemostasis, inflammation, proliferation, and remodeling are the four exact and highly planned phases of wound healing as a natural biological process in the human body. All four phases must occur in the correct order and time period for a wound to heal properly.

A wide range of plant-derived active principles representing a variety of phytochemicals have shown consistent wound healing activity and may be useful in wound treatment. With the foregoing facts in mind, the Bryophyllum pinnatum formulation was created, and it was chosen to test wound healing activity and the likely underlying mechanism of action.

IV. MATERIALS AND METHOD

PHARMACOGNOSTICAL STUDY

Fresh leaves are used for macroscopically and microscopically evaluation, while the coarse drug is used to determine the physiological parameters including loss on drying, extractive value, ash value and phytochemical analysis

Microscopy:

Sample was preserved in fixative FAA for more than 48 hr. The preserved specimens werecut into thin transverse section using a sharp blade and the sections were stained with safranin. Transverse sections were photographed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss Axiom Cam Erc5s digital camera under bright field light. Magnifications were indicated by scale bar.

DETERMINATION OF PHYSICO-CHEMICAL STANDARDS

The ash composition of the synthetic drug is usually considered to be the residue that persists during incineration. It simply contains and adheres to inorganic salts that occur naturally in the product and may contain inorganic matter added for adulteration purposes. The amount of ash varies with limited limits for a specific drug that ranges greatly betweendifferent drugs.

PRELIMINARY PHYTOCHEMICAL SCREENING:

The leaf will be subjected to qualitative test for the identification of various active constituents' viz. flavonoids, glycosides, tannins, Saponin. According to standard procedure.

FORMULATION OF GELZ

CRBOPOL 934P GELS:

Weighed quantity of CARBOPOL 934P (for ease in discussion CARBOPOL 934P is considered as CARBOPOL 934P throughout the remaining text) was taken and added to the distilled water. Extract was solubilized in an appropriate amount of ethanol and this ethanolic dispersion of extract was transferred to aqueous dispersion of CARBOPOL 934. The mixture was stirred gradually by means of a stirrer and CARBOPOL 934 was allowed to soak for 2 h. Triethanolamine was added to neutralize the CARBPOL 934 solution and to form the gel. The pH was adjusted 6.8.

Table 1: Composition of topical gel formulations of Extract (% w/w)

Materials	C	C1	C2	C3	C4	C5
Extract	1.00	1.00	1.00	1.00	1.00	1.00
CRB	1.00	1.00	1.00	1.00	1.00	1.00
Triethanolamine	0.30	0.30	0.30	0.30	0.30	0.30
Menthol	-	1.25	2.5	3.75	5	6.25
Ethanol	15.00	15.00	15.00	15.00	15.00	15.00
Distilled water Q.S. to make	q.s	q.s	q.s	q.s	q.s	q.s
Total	100.00	100.00	100.00	100.00	100.00	100.00

Evaluation of Herbal Gel:

(A) Physical Appearance:

Physical parameters like coloured and appearance of gel were checked.

(B) Measurement of pH:

The pH of herbal gel formulations was determined by using digital pH meter. 1 gm of gel dispersed in 10 ml of water Keep aside for 2

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1215-1221 www.ijprajournal.com ISSN: 2456-4494

hours The measurement of pH of formulation was administered in 3 times.

INVITRO STUDIES:

Assay of Antibacterial Activity:

Antibacterial activity of Bryophyllum pinnatum extract was examined against several species of gram positive and gram-negative bacteria by using cup plate method. Late exponential phase of the test bacteria was prepared by inoculating 1% (v/v) of the cultures into the fresh Muller-Hinton broth and incubating on an orbital shaker at 370C and100rpm overnight. Before using the cultures, they were standardized with a final cell density of roughly 108 cfu ml-1. Muller Hinton agar were prepared and inoculated from the standardized cultures of the test organisms then spread as uniformly as possible throughout the whole media. Agar well was made with sterile borer, test proteins introduced into the well and incubated at 370C for 24hrs. Antibacterial activity was evaluated by measuring the diameter of inhibition zone(mm) on the surface of plates and the results were reported as Mean after three repeats.

Anti-Inflammatory Activity

(A) Membrane Stabilization

(a) Red blood cells (RBCs) suspension preparation

Fresh human blood was collected and taken in a centrifuge tube with heparin. The test tubes were centrifuged for 10 min at 3000 rpm. It was washed for 3 times with normal saline, and the volume of blood was measured. They are made up to 10% suspension using normal saline.

Antioxidant Activity

Preparation of DPPH: 0.024 g of DPPH was taken and 50ml of ethanol was added. Then transfer the solution in volumetric flask (100ml). Make up to 100ml using ethanol. After the DPPH Solution was prepared, the solution was kept in spectrometer at 517nm. if the spectra come between 1.08 – 1.12, the DPPH solution is ready for test. If it less than 1.08, add DPPHand if it is greater than 1.12, add ethanol.

Preparation of Standard:

Take 10mg of Ascorbic Acid and add 10ml of ethanol. Then five different concentrations were taken from the prepared standard (50µg, 100µg, 150µg, 200µg, 250µg) using ethanol make up the volume of 10ml.

Concentration of Antioxidant Activity

Concentration	**50µg**	**100µg**	**150µg**	**200µg**	**250µg**
100ml ofDPPH Solution	3ml	3ml	3ml	3ml	3ml
Ascorbic Acid (10mg/ml)	5ml	5ml	5ml	5ml	5ml
Extract (10mg/ml)	5ml	5ml	5ml	5ml	5ml

V. RESULTS AND DISCUSSION

A gel dosage form for the plant Bryophyllum pinnata was formulated using its ethanolic leaf extract. An antibacterial assay was performed on agar plates and other mediausing the formulated gel. Further studies to be done include gel evaluation and anti- inflammatory activity and anti-oxidant activity is done.

GEL PARAMETER

Evaluation Parameter of Gel

Concentration(µg/ml)	**% Inhibition Of heat induced hemolysis**

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1215-1221 www.ijprajournal.com ISSN: 2456-4494

IN VITRO STUDIES

Anti-Inflammatory Activity

Concentration	pH	viscosity	Spread ability	Net content	Extrude ability	Physical Appearance
C	6.8	0.3850	32.19	67 gm	Good	Dark green, homogenous
C1	6.8	0.3862	45.05	68 gm	Excellent	Greenish, homogenous
C2	6.8	0.3873	56.39	69 gm	Excellent	Greenish, homogenous
C3	6.8	0.3882	64.00	70 gm	Excellent	Greenish, homogenous
C4	6.8	0.3891	71.38	75 gm	Excellent	Light green, homogenous
C5	6.8	0.3906	75.74	76 gm	Excellent	Light green, homogenous

One-way Anova for Anti-inflammatory Activity

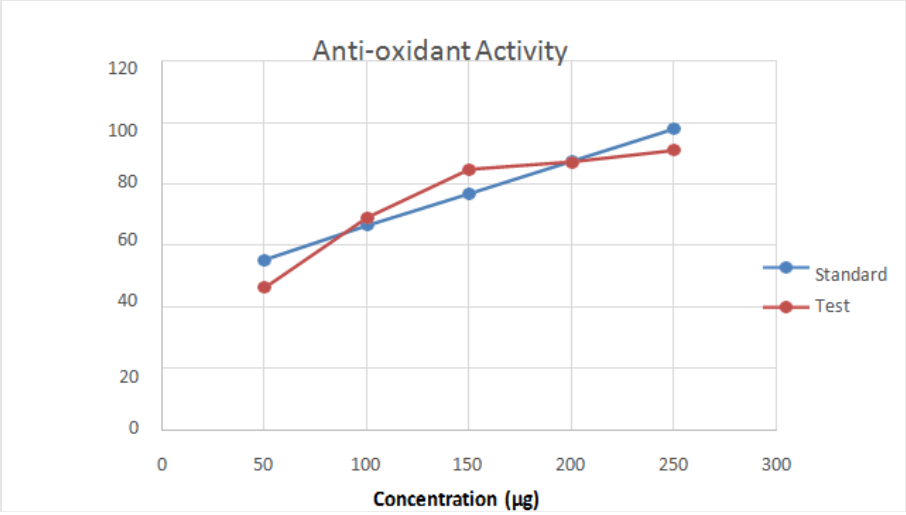
SUMMARY				
Groups	**Count**	**Sum**	**Average**	**Variance**
Column 1	5	247.6045	49.52089	221.5067
Column 2	5	233.6769	46.73538	32.92186

ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-Value**	**F crit**
Between Groups	19.397	1	19.397	0.1524	0.7063	5.3176
Within Groups	1017.7	8	127.21			
Total	1037.1	9				

Antioxidant Activity

Concentration (µg)	**DPPH ASSAY**	
	Standard	**Test**
50	55.32164	46.19883
100	66.49123	69.00585
150	76.78363	84.79532
200	87.36842	87.1345
250	97.95322	90.93567
IC50	23.8247	30.9804

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1215-1221 www.ijprajournal.com ISSN: 2456-4494



SUMMARY				
Groups	**Count**	**Sum**	**Average**	**Variance**
Column 1	5	383.9181	76.78363	281.685
Column 2	5	378.0702	75.61403	340.173

ANOVA

Source ofVariation	**SS**	**df**	**MS**	**F**	**P-value**	**Fcrit**
BetweenGroups	3.419875	1	3.419875	0.010999	0.919056	5.317655
WithinGroups	2487.432	8	310.929			
Total	2490.852	9				

Antibacterial activity

From the crude sample characterization of Protein by SDS-Page was carried out and the mixture of protein was observed. The effectiveness of the extracted protein was checked by conducting antimicrobial studies on the following gram-positive bacteria and gram negative bacteria using Mueller Hinton agar as medium and the extracted protein was found to be effective against gram positive and gram negative Bacteria.

Table : Antibacterial activity of isolated proteins

Sample	**Staphylococcus aureus-5021**	**Bacillus substillis-2717**	**Pseudomonas aeruginosa-2492**	**Klebsiella pneumonia-2957**
8 hrs	3mm	2mm	5mm	4mm
12 hrs	10mm	10mm	15mm	8mm
16 hrs	15mm	13mm	17mm	16mm
24 hrs	19mm	18mm	23mm	21mm

VI. SUMMARY AND CONCLUSION

The dissertation entitled "Formulation and Evaluation of gel using Bryophyllum pinnatum for wound healing'' deals with macroscopical and microscopical character including phytochemical and pharmacological aspects of Bryophyllum pinnatum. Therapeutically Bryophyllum pinnatum beneficial plant in traditional claim and the in Indian medicine system, has been selected in the present work. The research survey shows that no investigation on the aspects of wound healing activity was performed on herbal extract containing Bryophyllum pinnatum.

The Pharmacognostical part of the research can be widely used for the determination of the crude drug from the plant. The qualitative

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1215-1221 www.ijprajournal.com ISSN: 2456-4494

study or physiochemical analysis was performed, and potentiality of the drug was noted.The antioxidant activity is carried out by free radical scavenging activity by DPPH and total antioxidant phomolybdenum assay methods. The IC50 value of the ethanol extract of leaves in each method were found to be nearly equal to the standard Ascorbic acid drug and demonstrated the strongest antioxidant activity. As the ethanolic extract showed highest antioxidant activity hence it is chosen for In-vitro wound healing activity.

Similarly, the wound healing activity is performed by anti-inflammatory activity and anti-microbial activity. The gel was prepared and evaluated by several methods. The extract was prepared by maceration and introduced for the study. In all the in-vitro assays the ethanol extract was found to be more effective. Having said that, Bryophyllum pinnatum can be widely used against wound healing, it highly full fills the healing property. Hence it can be suggested in the field of Wound medicaments.The future work of this study presents the structural elucidation of protein components by using NMR, Mass spectroscopy, etc. Then this study undergoes animal study in future.

REFERENCE

[1]. Fife CE, Carter MJ (2012) Wound Care Outcomes and Associated Cost Among Patients Treated in US Outpatient Wound Centers: Data From the US Wound Registry. Wounds a Compend Clin Res Pract 24:10–17

[2]. Leavitt T, Hu MS, Marshall CD, Barnes LA, Lorenz HP, Longaker MT (2016) Scarless wound healing: finding the right cells and signals. Cell Tissue Res 365:483–493

[3]. Gurtner GC, Werner S, Barrandon Y, Longaker MT (2008) Wound repair and regeneration. Nat 2008 4537193 453:314–321

[4]. Clark RAF (2003) Fibrin is a many splendored thing. J Invest Dermatol. https://doi.org/10.1046/J.1523-1747.2003.12575.X

[5]. Wilgus TA, Roy S, McDaniel JC (2013) Neutrophils and Wound Repair: Positive Actions and Negative Reactions. Adv wound care 2:379–388

[6]. Park JE, Barbul A (2004) Understanding the role of immune regulation in wound healing. Am J Surg 187:S11–S16

[7]. Davies LC, Rosas M, Jenkins SJ, Liao C Te, Scurr MJ, Brombacher F, Fraser DJ, Allen JE, Jones SA, Taylor PR (2013) Distinct bone marrow-derived and tissue-resident macrophage lineages proliferate at key stages during inflammation. Nat Commun. https://doi.org/10.1038/NCOMMS2877

[8]. Davies LC, Jenkins SJ, Allen JE, Taylor PR (2013) Tissue-resident macrophages. Nat Immunol 14:986–995

[9]. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM (1997) Isolation of putative progenitor endothelial cells for angiogenesis. Science 275:964–967

[10]. Tepper OM, Capla JM, Galiano RD, Ceradini DJ, Callaghan MJ, Kleinman ME, Gurtner GC (2005) Adult vasculogenesis occurs through in situ recruitment, proliferation, and tubulization of circulating bone marrow-derived cells. Blood 105:1068–1077

[11]. Martin P (1997) Wound healing--aiming for perfect skin regeneration. Science 276:75–81

[12]. Werner S, Krieg T, Smola H (2007) Keratinocyte-fibroblast interactions in wound healing. J Invest Dermatol 127:998–1008