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PREPARATION AND EVALUATION OF NEW POLYHERBAL FORMULATION THEIR FOR WOUND HEALING ACTIVITY

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ABSTRACT: Herbal medicine (also herbalism) is the study of pharmacognosy and the use of medicinal plants, which are the basis of traditional medicine. Any damage or break in the surface of the skin is a wound. In Soxhlet extraction, solvent extraction involves extraction with solvents of increasing polarity from a nonpolar to a more polar solvent to ensure that a wide polarity range of compounds can be extracted. 1 % of the extract was incorporated in the preparation of gel formulation using Carbomer 940 polymer as gelling agent. Three formulae 1, 2 and 3 of gel containing varying concentrations of Carbomer 940(0.5, 1 and 1.5%) were prepared. Carbomer 940 was soaked in distilled water overnight. Selected extracts were dissolved in ethanol. Standard methods and results examined different Physicochemical evaluation parameters of formulated ointments were found within the acceptable limits. *Cissampelos Pareira* Vein-islet number/mm^2 was 3.6 and it ranges from 3.5 to 3.9. the Palisade ratio was 3, and it ranges from 2.75 to 3.75. The Stomata length (µm) was found to be 20.5 and it ranges from 18.1 to 22.7. Hence we can conclude from our findings that the developed formulations containing bioactive Methanol extracts of *Cissampelos Pareira* and *Bergenia ciliate* formulation acted on each component of the wound healing process.

INTRODUCTION:

Herbal Medicine: Herbal medicine (also herbalism) is the study of pharmacognosy and the use of medicinal plants, which are the basis of traditional medicine [1]. There is limited scientific evidence for the safety and efficacy of plants used in 21st-century herbalism, which generally does not provide standards for purity or dosage [2]. The scope of herbal medicine commonly includes fungal and bee products, as well as minerals, shells and certain animal parts.

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Herbal medicine is also called phytomedicine or phytotherapy [3]. Paraherbalism describes alternative and pseudoscientific practices of using unrefined plant or animal extracts as unproven medicines or health-promoting agents [4]. Paraherbalism relies on the belief that preserving various substances from a given source with less processing is safer or more effective than manufactured products, a concept without evidence [5].

Skin: The human body has incredible systems with mechanisms to fight or kill pathogens. The internal defense system destroys the pathogens that have attacked the body. The external defense system prevents microorganisms from entering the body. Skin is a major part of the external defense system that covers the body's outside but has other functions besides the defense mechanism. It acts as

a mechanical barrier between the inner part of the body and the external world [6].

Anatomy of Skin: Skin is the largest organ in the body. It consists of three layers. The dermis follows the outermost epidermis, under which lies the hypodermis.

Epidermis: The epidermis, the outermost layer of the skin, forms a protective barrier over the body's surface. It is responsible for maintaining body water and protecting from pathogens entry. It consists of a stratified squamous epithelium, composed of proliferating basal and differentiated suprabasal keratinocytes. The epidermis also helps the skin to regulate body temperature [7].

Stratum Basale (Basal Cell Layer): The deepest single-layered sublayer of the epidermis is made of basal cells. It is like a boundary to the dermis. It produces keratinocytes. Also, melanocytes lie in this layer. It holds approximately 8% of the water in the epidermis. With aging, along with thinning, its ability to clutch water deteriorates [8].

Stratum Spinosum (Prickle Cell Layer): Unlike stratum basale, it has 10 to 20 layers. Basal cells, through the process of turn-over, make their shape somewhat flatter (multi-sided) and form these layers. These cells are known as prickle cells and have little spines on the outside of their membrane. The thickness of this sublayer is typically from 50 to 150 μm[9].

Dermis: Under the epidermis, lies dermis that comprises connective tissue and it protects the body from stress and strain. It gives tensile strength and elasticity to the skin through an extracellular matrix comprising collagen fibrils, micro fibrils, and elastic fibers, implanted in proteoglycans [10]. Hair follicles, sweat glands, sebaceous glands, apocrine glands, lymphatic vessels and blood vessels are also present in dermis. Major role of the dermis is to regulate temperature. The encapsulated blood vessels of this layer provide nutrients to epidermis also. Thickness of dermis is 3-5 mm [11].

Wound: Any damage or break in the surface of the skin is wound. It may be defined as a loss or breaking of cellular, anatomical and functional continuity of living tissue [12]. Amplitude of damage can be from a simple crack in the epithelial integrity of the skin or extending deeper into subcutaneous tissue with damage to other internal structures such as tendons, muscles, vessels, nerves and even bone [13]. It may be caused by physical, chemical, thermal, microbial, or immunological assault to the tissue. When skin is torn, cut, or punctured, it is termed as an open wound and when blunt force trauma causes a contusion, it is called closed wound, whereas the burn wounds are produced by fire, heat, radiation, chemicals, electricity, or sunlight [14, 15]. A wound is a break in the skin (the outer layer of the skin is called the epidermis). Cuts or scalps usually cause wound; symptoms at wound or injury include swelling, stiffness, tenderness, discoloration skin tightness, scabbing, itching and scar formation, two types of tissue injury [16].

Classification of Wounds: Time is important in injury management and wound repair. Wounds can be clinically categorized as acute and chronic according to their healing time frame.

Acute Wounds: Healing proceeds in an orderly and timely fashion through predictable stages of wound healing is known as an acute wound. The period of healing is usually 5 to 10 days or within 30 days. Acute wounds result from traumatic loss of tissue or a surgical procedure [17].

Chronic Wounds: A chronic wound is one in which the normal process of healing is disrupted at one or more points in the phases of haemostatic, inflammation, proliferation or remodelling, resulting in a delay in healing beyond the anticipated time. The time period of healing might be a couple of weeks or up to six weeks in some cases [18, 19].

Diabetic Wounds: Foot ulcers are usually observed in people with other medical conditions, such as diabetes or peripheral vascular disease. In diabetic patients, foot ulcers may be categorized into [20]:

Neuropathic: It leads to loss of protective sensation. However, foot pulses may still be noticed.

Neuro-ischaemic: Both loss of protective sensation and ischemia or lack of blood supply occurs in such condition. In foot ulcers, wounds

can be superficial, including only the top layer of skin, or deep, extending into the foot involving tendons, bones and other structures.

Basic Principles of Wound Healing: The process by which tissue repair takes place is called wound healing and is comprised of a continuous sequence of inflammation and repair, in which epithelial, endothelial, inflammatory cells, platelets, and fibroblasts briefly come together outside their normal domains and interact to restore resemblances of their used discipline and having done to resume their normal function. Wound healing is a complex dynamic process. The wound environment changes with the changing health status of the individual. Knowledge of the physiology of the normal wound healing trajectory through the phases of hemostasis, inflammation, granulation and maturation provides a framework for understanding the basic principles of wound healing [21, 22].

MATERIAL & METHOD:

Identification, Collection and Authentication of the Plant Material: The different parts of *Cissampelos Pareira* and *Bergenia ciliate* were collected from the Botanical garden of Prayagraj in May 2022. The material was authenticated and identified by Dr. Arti Garg, Principal Scientist, Botanical Survey of India Central Regional Centre, 10 Chatham Line, Prayagraj-211002. The plant parts were washed thoroughly in tap water, shade dried at room temperature for 10 days, coarsely powdered, and the powder was passed through sieve No.60 and used for extraction. The Botanical Survey of India has received a specimen with the voucher number of *Bergenia ciliate* SIP/2022-23/163 & *Cissampelos Pareira* SIP/2022-23/164.

Animals Housing and Feeding Conditions: Weighting 160-180 gm, Wistar albino rats were purchased from Saha Enterprises in Kolkata, India. The animals were kept in propylene cages with rice husk bedding at 24-degree temperature with 30-40 % relative humidity. A 12:12 light-dark cycle was maintained using the typical commercial pellet (M/S. Hindustan Lever Ltd., Mumbai, Maharashtra, India) & an endless supply of purified water. Following CPCSEA guidelines, the Animal Ethical Committee (1632/PO/Re/S/12/CPCSEA) approved all experimental protocols & procedures.

Determination of Physico-Chemical Parameters:

Ash Values: Ash values help determine the quality and purity of the crude drug, especially in powder form. The crude drug's ash content is generally taken as the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include inorganic matter added for adulteration.

Hence, an ash determination furnishes a basis for judging the identity and cleanliness of a drug and gives information relative to its adulteration with inorganic matter. The total ash of a crude drug reflects the care taken in its preparation. The acid-insoluble ash is a part of the total ash which is insoluble in dilute hydrochloric acid. A higher limit of acid-insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high. The total ash usually consists of carbonates, phosphates, silicates, and silica. Sulphate present in the drug on long storage gets converted in to carbonates and oxide, on the treatment of drug with conc. H2SO4 the carbonates and oxides get reconverted to sulphate which is stable at high temperatures. The procedures given in Indian Pharmacopoeia were used to determine the different ash values [23].

Total Ash Value: Accurately weight of about 3 gm of the air-dried drug was taken in a tared silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for a constant value. Then the percentage of total ash was calculated concerning the air-dried drug [24].

Weight accurately a cantered crucible after ignition and cooling = a gram.

Weight of crucible + 03 gram of powder = b gram.

Weight of powder (b-a) = X gram.

After incineration, weight of crucible + powder remaining = c gram.

Weight of ash = c-a gram.

% ash content = c-a / X × 100

Acid Insoluble Ash Value: The ash obtained as directed under total ash was boiled with 25 ml of HCl for 5 min. The insoluble matter was collected

on an ashless filter paper, washed with hot water, ignited and weighed, then the percentage of acid insoluble ash was calculated with reference to the air-dried drug [25].

Water Soluble Ash Value: The total ash obtained was boiled with 25 ml of water for 5 min. The insoluble matter was collected on an ashless filter paper and washed with hot water ignited for 15 minutes. The weight of insoluble matter was subtracted from the weight of the total ash. The differences in weight represent the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug [26].

Solvent Extraction: Extraction is the separation of medicinally active portions from plant tissue using selective solvents through standard procedure. In this step, solvent extraction involves extraction with solvents of increasing polarity from a nonpolar to a more polar solvent to ensure that a wide polarity range of compound can be extracted [27]. On the basis of the literature survey methanol was selected for *Cissampelos Pareira* extraction while methanol was selected for the extraction of and *Bergenia ciliate* plants constituents.

Mature fresh parts of *Cissampelos pareira*, *Bergenia ciliate* were washed first in sterilized distilled water, followed by washing in mercuric chloride solution (0.1 %) and again washed in sterilized distilled water. Leaf material was weighed and transferred to a sterile mortar and pestle to make a crude crushing of the material, which was then transferred to a sterile homogenizer and finely crushed. About 100 grams of coarsely powdered plant material was exhaustively extracted for 2 h with 200 ml of different solvents individually at their boiling point temperature in Soxhlet apparatus [28]. The extracts obtained were filtered and evaporated under reduced pressure using rotary evaporator (Jyoti Scintific, India). The extracts were dissolved in dimethyl sulphoxide to make the final concentrations kept in refrigerator until used. While *Cissampelos Pareira* and *Bergenia ciliate* extract was prepared by placing 100 grams bark powder with different solvents (Methanol) in soxhlet apparatus for 8 hours.

The % Yield in different solvents plant extracts were calculated by using the following formula

% Yield = Net weight of powder in gram after extraction / Total weight of powder in gram taken for extraction x 100

Extractive Values: Extractive values of the crude drug are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug [29].

Loss on Drying: Weight about 1.5 g of the powdered drug into a weighed flat and thin porcelain dish. Dry in the oven at 1000 c or 1050 c. Cool in a desiccator and watch. The loss in weight is recorded as moisture [30].

Formulation Development and Characterization of Gel Formulation:

Extracts used: Methanol extract of *Cissampelos pareira* and Bergenia ciliate were used individually in preparation of formulation [31].

Concentration used: 1% of the extract was incorporated in the preparation of gel formulation using Carbomer 940polymer as gelling agent. Three formulae 1, 2 and 3 of gel containing varying concentrations of Carbomer 940(0.5, 1 and 1.5%) were prepared. Carbomer 940 was soaked in distilled water overnight. Selected extracts were dissolved in ethanol. Extracts were then added to the soaked polymer. Propylene glycol was then added to the above mixture and stirred till a uniform suspension was obtained. After the addition was complete, gels were spontaneously formed with triethanolamine addition and pH adjustment to 7.

TABLE 1: FORMULATION OF BATCHES OF GEL FORMULATIONS

S. no.	Ingredients	F 1	F2	F3
1	Carbomer 940	1.5 %	2%	2.5%
2	Extract /Fraction	1.5%	1.5%	1.5%
3	Methanol	5ml	5ml	5ml
4	Propylene Glycol	5gm	5gm	5gm
5	Purified water	40gm	40ml	50ml

Physicochemical Evaluation of Formulation: Colour and Odour Physical parameters like colour and odour were examined by visual examination. Consistency Smooth and no greediness is observed.

Homogeneity: All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and the presence of any aggregates.

pH: pH of prepared herbal ointment was measured by using digital pH meter. The solution of ointment was prepared by using 100 ml of distilled water and set aside for 2 h. pH was determined in triplicate for the solution and average value was calculated.

Spreadability: Spreadability represents the magnitude of the space where gel easily scatters when applied to the epidermis. The remedial competence of the emulgel formulation also relies upon its spreadability. All the formulations exhibited reasonably good spreadability. Spreadability of values of *Pothos scandens* emulgels extended from 11.97 to 26.67g.cm/sec and that of *Cissampelos pareira* ranges from 10.23 to 22.10 g.cm/sec. All the Bergenia ciliate formulations exhibited significant ($P < 0.01$) spreadability; in contrast with formulations FM1 and FM2.

All the *Cissampelos pareira* formulations exhibited significant ($P < 0.01$) spreadability, in contrast with formulation FM2. Bergenia ciliate formulation PGF1 exhibited significant ($P < 0.01$) spreadability in comparison with all other Pothos scandens and *Basella alba emulgels* formulations. Spreadability of *Cissampelos pareira* and *Bergenia ciliate* were depicted. The spreadability was determined by placing an excess sample between two slides compressed to uniform thickness by placing a definite weight for a definite time. The time required to separate the two slides was measured as spreadability. Lesser the time taken for the separation of two slides results better spreadability. Spreadability was calculated by following formula

$$S = M \times L / T$$

Where, S = Spreadability, M = Weight tide to the upper slide, L = Length of glass slide, T = Time taken to separate the slides.

Extrudability: Extrudability indicates the ease with which the *Cissampelos pareira* and *Bergenia ciliate* can be ejected from its container. It is the power required to push or force the contents out of its container. As the force required to squeeze out from its container increases, its extrudability also increases. Greater the extrudability value, lesser the spreadability of the formulation. Extrudability values of *Cissampelos pareira* ranges from 11.13 to 16.10 g/cm2 and that of *Bergenia ciliate* ranges from 10.07 to 15.43 g/cm^2. The extrudability of all the formulations was found to be satisfactory. The extrudability of *Cissampelos pareira* and *Bergenia ciliate* were illustrated in Table.

The formulation was filled in a collapsible tube container. The extrudability was determined in terms of weight of the ointment required to extrude 0.5cm of ribbon of ointment in 10 seconds.

Diffusion Study: The diffusion study was carried out by preparing agar nutrient medium. A hole board at the center of the medium and ointment was by placed in it. The time taken by ointment to get diffused through was noted. (After 60 minutes)

Loss on Drying (LOD): LOD was determined by placing the formulation in petri-dish on water bath and dried for a temperature 105°C.

Washability: Formulation was applied on the skin and then ease extend of washing with water was checked.

Non-Irritancy: Test Herbal ointment prepared was applied to the skin of human being and observed for the effect.

Stability Study: Physical stability test of the herbal ointment was carried out for four weeks at various temperature conditions like 2°C, 25°C and 37°C. The herbal ointment was found to be physically stable at different temperatures *i.e.* 2°C, 25°C, 37°C within four weeks

RESULT:

Quantitative Microscopy: Quantitative Microscopy evaluation of the dried drugs were performed to determine various parameters such as color, odour, taste, shape, size, texture, fracture, *etc.* by visual inspection and by optical microscopy. Behavior Pattern and fluorescence analysis of

powdered *Cissampelos Pareira and Bergenia ciliate* bark on treatment with different chemical reagents was also performed. These results give general as well as broad insight of content of the drug.

Physicochemical Evaluation of Formulation: Different Physicochemical evaluation parameters of formulated ointments were examined by standard methods and results were shown in table below: **Table 2** physicochemical evaluation of formulation.

TABLE 2: PHYSICOCHEMICAL EVALUATION OF FORMULATION OF *CISSAMPELOS PAREIRA*

S. no.	Parameters	Description
1	Vein-islet number/mm^2	3.6 [3.5-3.9]
2	Palisade ratio	3 [2.75-3.75]
3	Stomata length (µm)	20.5 [18.1-22.7]
4	Stomata width (µm)	11.4 [7.7-13.7]

TABLE 3: PHYSICOCHEMICAL EVALUATION OF FORMULATION OF *BERGENIA CILIATE*

S. no.	Parameters	Description
1	Vein-islet number/mm^2	20.6 [10-25]
2	Palisade ratio	12.4 [8-21]
3	Stomata length (µm)	24.2 [13-31]
4	Stomata width (µm)	19-34 [15.8]

Determination of Physico-Chemical Parameters: In this step various Physico-chemical parameter of selected plants were determined as per standard procedures.

TABLE 4: PHYSICO-CHEMICAL CHARACTERS OF *CISSAMPELOS PAREIRA* LEAVES

S. no.	Parameters	% Yield (w/w)
1	Total ash	12.7 %
2	Acid insoluble ash	5.12 %
3	Water insoluble ash	8.7 %
4	Foreign matter	1.45 %
7	Loss on Drying	3.42 %

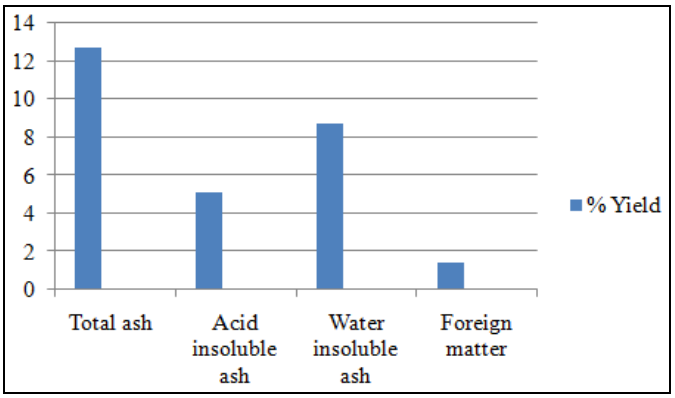

FIG. 1: GRAPH SHOWING PHYSICO-CHEMICAL CHARACTERS OF *CISSAMPELOS PAREIRA* LEAVES

TABLE 5: PHYSICO-CHEMICAL CHARACTERS OF *BERGENIA CILIATE*

S. no.	Parameters	% Yield (w/w)
1	Total ash	11.7 %
2	Acid insoluble ash	1.12 %
3	Water insoluble ash	11.6 %
4	Foreign matter	3.12 %
5	Loss on Drying	2.12 %

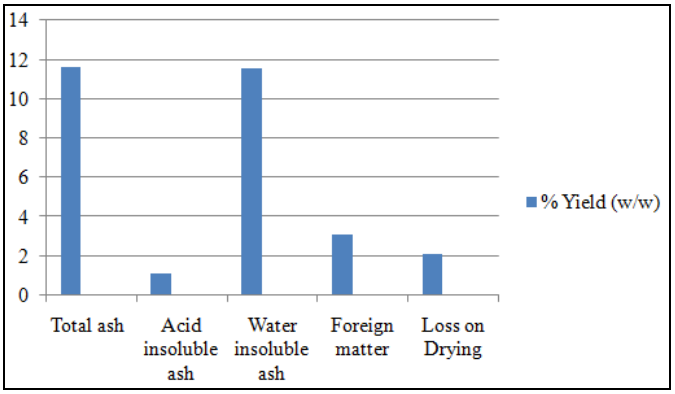

FIG. 2 GRAPH SHOWING PHYSICO-CHEMICAL CHARACTERS OF *BERGENIA CILIATE*

Two herbal Formulations, one with *Cissampelos pareira* and *Bergenia ciliate* extract were successfully prepared and evaluated for assessing their safety and wound healing efficacy and pharmaceutical quality. The development of wounds is a serious complication for patients. Numerous factors related to wound can impair wound healing, nutrition deficiencies, and the disease itself. Very slow and non-healing wounds occur due to various connective tissue abnormalities. These abnormalities are caused by reduced biosynthesis and accelerated degradation of the collagen content. The decrease of collagen content in skin leads to the impairment of wound healing. Poor wound healing may be due to deficiency in cell proliferation, proneness to infection, decrease in cell survival, and reduction of rates of wound contraction. The exploration of wound repairing potential of the extracts using excision, sutured and incision models revealed that aqueous extract *Cissampelos pareira* and *Bergenia ciliate* Methanolic extract exhibited maximum wound healing activity and hence these concentrations were chosen for the development of formulation. Safety evaluations of the formulation were proved to be free from skin irritation and a topical product's desirability.

CONCLUSION: Two herbal Formulations, one with *Cissampelos pareira* and *Bergenia ciliate*

extract, were successfully prepared and evaluated to assess their safety, wound healing efficacy, and pharmaceutical quality. *Cissampelos pareira* Vein-islet number/mm^2 was 3.6 and it ranges from 3.5 to 3.9. The Palisade ratio was 3 and it ranges from 2.75 to 3.75. The Stomata length (µm) was found to be 20.5 and it ranges from 18.1 to 22.7. The Stomata width (µm) was found to be 11.4 and it ranges from 7.7 to 13.7.

Bergenia ciliate Vein-islet number/mm2 was found to be 20.6 and it ranges from 10 to 25. The Palisade ratio was 12.4 and it ranges from 8 to 21. The Stomata length (µm) was found to be 24.2 and it ranges from 13 to 31. The Stomata width (µm) was found to be 15.8 and it ranges from 19 to 34.

Future Scope: The developed formulations containing bioactive Methanol extracts of *Cissampelos pareira* and *Bergenia ciliate* formulation were found to be acting on each component of wound healing process. Though the physicochemical evaluations, safety evaluations and wound healing activity studies proved that emulgels formulated using the extracts of *Cissampelos pareira* and *Bergenia ciliate* are promising products for wound healing, for both burn and surgical wound healing.

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