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Potential Herbal Wound Healing Cream: Formulation and In-vitro Evaluation

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ABSTRACT
Multiple drug resistance has been developed in several bacteria in contemporary times as a result of the haphazard use of antimicrobial medications to treat infections. Hence a study was conducted to formulate wound healing cream containing methanolic extract of *Acalypha indica* and the sap of *Aloe barbadensis miller* showing effective antibacterial activities. This makes it possible to reevaluate traditional medicine. Several researches revealed the antibacterial, antioxidant, and anti-inflammatory properties of these plants. Varying concentrations of *A. indica* leaf extracts obtained from soxhlet extraction and the pulp extracted from *A. barbadesis miller* were used for the cream formulation. Numerous factors, including colour, consistency, irritancy, removability, spreadability, pH, and antibacterial activity, were evaluated. The prepared cream was found to be gelatinous and appeared honeydew. The formulations had pH values in the satisfactory range of 5.5-6.2. The extracts were uniformly distributed in all the formulations without causing edema, irritation, and redness.The formulations A1 and A2 showed an effective zone of inhibition using an antimicrobial assay against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Increasing the concentration of *A. barbadensis miller* and *A. indica,* increases the efficacy of the formulation. Thus, the formulated wound healing cream having a methanolic extract of *A. indica* and sap of *A. barbadensis miller* could be effectively used commercially.

KEYWORDS: *Acalypha indica, Aloe barbadensis miller,* Soxhlet, Antioxidant, Anti-inflammatory, Antimicrobial assay

INTRODUCTION

The essential components in medicinal plants are frequently employed in the pharmaceutical industry. Secondarymetabolites such as flavonoids, alkaloids, lipids, and polyphenols, are crucial in the creation of pharmaceuticals[1]. Medicinal plants constitute the richest bioresource in terms of ancient therapeutics, modern medications, nutraceuticals, chemical entities for synthesized compounds, food supplements, and traditional remedies[2].

One of the weed plants with significant medical benefits for use in human health is *A. indica* which is commonly found in India, Sri Lanka, Thailand, and Pakistan. Various illnesses like eye infections, respiratory issues, rheumatism, skin issues, and blood sugar levels were treated using plant parts which include the leaves, roots, and stems[3]. Since this plant is closely related to the Ayurveda, Sidda, and Unani medical treatments used by older Indian generations, many worldwide writings on *A. indica* have come from the Indian subcontinent[4]. Another essential medicinal plant from the Liliacea family is *A. barbadensis miller*[5]. Although the aloe vera plant thrives in hot, dry climates, it is widely grown regardless of the weather because of consumer desire for cosmetic products[6].It is sold in the market for pharmaceutical drugs for a wide range of therapeutic uses, such as the ability to cure wounds, lower blood sugar, soothe burns, ease intestinal issues, and lessen arthritis swelling. Aloin, also known as barbaloin, is a bitter-tasting yellow crystal found in aloe vera, and numerous studies have reported that its bioactive chemicals, particularly aloin, have protective and curative effects[7]. This is the most significant anthraquinone glycoside implicated in Aloe vera's health benefits.

Humans have experienced wounds ever since primitive times, and the art of treating and curing wounds predates civilization[8]. Chronic wounds affect a number of senior patients and significantly lower their quality of life. Modern biomedical sciences are seeing rapid development in the study of medications for wound healing. Even while there are new ways to speed up the healing process, wound care has gone back to its roots in medicine and is now adopting some of the treatments employed thousands of years ago[9]. Bioactive compounds from plants are important sources of therapeutics and models for developing new treatments[10]. In recent days scientists are looking forward to herbal therapeutics,as traditional folk remedies have high acceptability and good tolerance[11].

Researchers have developed herbal gels and assessed their pH, viscosity, medication content, spreadability, stability, investigation of skin irritation, and other factors in order to comprehend the antibacterial action against the pathogens *Staphylococcu. aureus, Bacillus subtilis, Aspergillus niger and Escherichia coli*[12]. *Azadirachta indica, Curcumalonga, Allium sativum, Ocimumsactum, Cinnamomum zeylanicum nees,* and *Tamarindus indica* ethanol extracts were used to create herbal gels. The dried leaves of *A. indica* were formulated into cream to cure bedsores and wounds[13].

Hence finding novel antimicrobial compounds from alternative sources, such as plants, has become necessary due to the emergence of drug resistance in human infections against regularly used antibiotics. Making antibacterial therapy inexpensive, safe, and successful has been a recent area of attention[14]. Therefore, steps must be taken to overcome this problem such as limiting the usage of antibiotics, conducting

research to better understand the genetic pathways underlying resistance, and continuing efforts to produce medicines from natural sources.

MATERIALS AND METHODS

Materials

The leaf samples of *A.indica* and *A.barbadensis miller* required for the study were collected from VELTECH HIGH TECH DR.RANGARAJAN DR.SAKUNTHALA ENGINEERING COLLEGE garden, Chennai.All the chemicals and reagents were purchased from M/s.HiMedia Laboratories, India.All the glass wares were purchased from M/s. Borosil Limited.

Extraction of *A. indica* leaves

Soxhlet extraction was used to obtain plant extract from *A. indica*. The leaves of the plant were cleaned with water and air-dried. The dried leaves were ground into a fine powder using a household grinder. These powdered sampleswere stored in anairtight container for further studies. 250 grams of the sample was first defatted with hexane and 1000ml of methanol was used for extraction using soxhlet apparatus. The extraction process was carried out for 72 hours at the rate of Three cycles per hour and the extract was then concentrated. The sample was heated to between 45° to 50 °Celsius in order to evaporate any remaining methanol[16].

Aloe vera gel preparation

Fully expanded *A.barbadensis miller* leaves were chosen and cleaned with distilled water. Peeling the parenchymatous layer off the leaves allowed the gel inside to leak out. A mechanical crusher was used to further crush the acquired pulp. The pulp was filtered to eliminate the connected fibers after being crushed. The harvested sap was gathered and kept in storage at 40°C for later use[17].

Cream made using the emulsification process

A cream with an oil-in-water (O/W) emulsion base was created. The oil phase was heated to 75°C while lecithin, the emulsifier and coconut oil, the oil-soluble component were dissolved in it. While the aqueous phase was being heated to 75°C, preservatives and other water-soluble compounds (Glycerol, methanolic extract of *A. indica*, and pulp of *A. barbadensis miller*) were added. After heating, the aqueous phase was progressively added while the oil phase was continuously mixed. The efficiency of similar high-shear, forceful mixing procedures is the same[18]. The mixture was continuously swirled with an electrical stirrer to produce a homogeneous cream. The various formulations are shown in Table 1

Table 1: Cream Formulations

INGREDIENTS	FORMULA%w/w					
	A1	A2	A3	A4	A5	A6
A. indica Methanolic extract	0.80	0.75	0.70	0.65	0.60	0.55
A. barbadensis miller extract	0.55	0.50	0.45	0.40	0.35	0.30
Lecithin	8	8	8	8	8	8
Coconut oil	3	3	3	3	3	3
Gellan gum	0.1	0.1	0.1	0.1	0.1	0.1
Glycerol	2	2	2	2	2	2
Essential oil	qs	qs	qs	qs	qs	qs

Cream evaluation

Physical characteristics:

The prepared cream's colour and appearance were assessed. Further pH, homogeneity, spreadability, removability, and irritancy were tested for effective application. 0.5 g of the formulations were diluted in 50 ml of distilled water and the pH was measured using EC PH Tutor-S Eutech pH meter[19]. The homogeneity of the formulation was studied by visual examination and touch[20]. The cream was applied and the type of film or smear produced on the skin was observed and the removability of the cream applied was analyzed by washing the applied part with tap water[18]. For performing the irritancy test one square cm area was marked on the dorsal surface of the skin and the cream was applied to the specified area. Irritancy, erythema, and edema were examined at regular intervals up to 24 hrs and reported[21].

Spreadability test

Spreadability of the formulations were studied using the apparatus made with wooden board, scale and two glass slides having two pans on both sides mounted on a pulley called spreadability apparatus. The formulations were placed between the two glass slides and 100g weight was placed on the glass slide for 5 min to compress the sample to a uniform thickness. Then 250 g Weight was added to the pan. The time in seconds required to separate the two slides was taken as a measure of spreadability[22].

$S = m \times l/t$

m – Weight tied on the upper glass slide

l – Length of the glass slide

t – Time in seconds

Assay for growth of microorganism in the formulation:

The sample formulations were streaked on the plates of agar media and incubated at 37°C for 24 hours to check for signs of microbial growth.

Wound Sample collection

Samples were collected from students using sterile cotton swabs. A swab was taken after the wound was meticulously cleaned with sterile water to prevent surface contamination. Within an hour the swabs were inoculated on MacConkey agar, Blood agar, Chocolate agar, and Cystine lactose electrolyte-deficient agar (CLED) to avoid drying of the swabs and were aerobically incubated at 37°C for 24-48 hours. Biochemical assays were performed for the identification and classification of bacterial isolates.

Antimicrobial activity

Inoculum preparation

A loopful of colony was taken from the agar plate and inoculated into a sterile nutrient broth and were incubated for 24 h at 37°C in an orbital shaking incubator (M/S. Remi Instruments Limited, Mumbai, India) at 200 rpm. One ml of this 24 hour culture containing *Pseudomonas aeruginosa* and *Staphylococcus aureus* was used as inoculum in further studies.

Agar well diffusion method

The antimicrobial activities of various plants or microbial extracts were evaluated using this method. The agar plates were

inoculated by spreading the microbial inoculums of *P. aeruginosa* and *S. aureus* over the entire agar surface. A hole of 6 to 8 mm diameter is punched aseptically with a sterile tip, and volumes of 50µl of various cream formulations (A1 to A6) were introduced into the well. Then, agar plates were incubated at 37°C for 24 hours under suitable conditions depending on the test microorganism. The antimicrobial compounds in the plant extract diffuse in the agar medium and inhibits the growth of the microbial strain tested and the zone of inhibition was measured.

RESULTS

Physical characteristics

The colour of the prepared formulations was honeydew and appeared gelatinous. The homogeneity of the formulations was confirmed by visual appearance and touch and they produced a uniform distribution of both plant extracts. The type of smear was observed to be non-greasy and the removability was easy. The formulations were assayed for irritancy and found safe to be used on the skin as no redness, edema,inflammation, or irritation was observed during the studyas shown in Table 2.

Table 2: Physical characteristics of the formulations

Formulation	Parameters					
	Colour	Appearance	Homogeneity	Irritancy (Irritant, edema, erythema)	Type of smear	Removal
A1	Honeydew	Gelatinous	Good	NIL	Non-greasy	Easy
A2	Honeydew	Gelatinous	Good	NIL	Non-greasy	Easy
A3	Honeydew	Gelatinous	Good	NIL	Non-greasy	Easy
A4	Honeydew	Gelatinous	Good	NIL	Non-greasy	Easy
A5	Honeydew	Gelatinous	Good	NIL	Non-greasy	Easy
A6	Honeydew	Gelatinous	Good	NIL	Non-greasy	Easy

pH of the cream

The pH of the formulations was in the range 5.5 to 6.2 and appeared to be satisfactory (Figure 1).

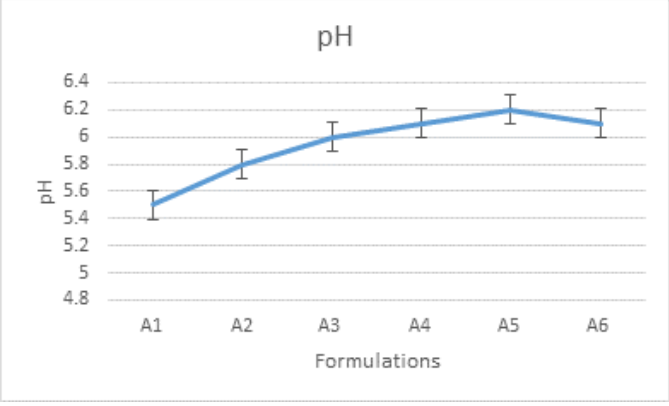

Figure 1: pH of the formulations

Spreadability of the cream

The formulations were tested for the spreadabilty by comparing them with commercially available creams and all had better spreadabilty. The values of prepared wound healing creams were in the range of 10–12which is the satisfactory range as shown in Table 3.

Table 3: Spreadabilty of formulations

Formulation	Time	Spreadability
A1	12	11.6
A2	15	12
A3	15	11
A4	12	10
A5	13	10.54
A6	14	10.22
Marketed cream	13	11.43

Wound sample analysis

The majority of pathogens found in the swabs were *P. aeruginosa*, while *Escherichia coli* was the strain that was least frequently found. Only one gram-positive bacterium*S. aureus*was found. The most common microorganism representing nearly half of the isolates was *P. aeruginosa* which was taken from wound swabs.

Antimicrobial activity

Agar well diffusionmethod was used for carrying out an antimicrobial activity.Testorganismusedforthiswas*P. aeruginosa and S. aureus*.Invitro evaluation of the developed formulations exhibited a clear zone of inhibition.A1 and A2 showed effective zone of inhibition when compared to other formulationsas shown in Table 4.

Table 4: Zone of Inhibition of each formulation against *P. aeruginosa and S.aureus*

Formulation	Diameter of zone of inhibition (cm) against *P. aeruginosa*	Diameter of zone of inhibition (cm)against *S. aureus*
A1	1.4	1.3
A2	1.2	1.4
A3	1	1.1
A4	1.1	0.9
A5	0.7	0.8
A6	1	0.9

DISCUSSION

A. barbadensis miller and *A. indica* is widely known for their medicinal uses and also possess antiseptic, tissue penetrating, and anti-inflammatory qualities[23]. They include several beneficial ingredients, including vitamins, lipids, and amino acids[24]. Due to the therapeutic values of *A. indica* and *A. barbadensis miller,* they as a combination could be used in wound healing. There isn't already a commercial wound healing creamon the market that contains both *A. indica* and *A. barbadensis miller*. Therefore, in this study, various

formulations containing varying concentrations of *A. indica* and *A. barbadensis miller* plant extracts were prepared and characterized using standard protocols.The homogeneity test of creams revealed that their composition was uniform[21]. The formulations appeared to be gelatinous and honeydew in color. The smear test demonstrated that all formulations had good moisturizing properties and were non-greasy.The fact that prepared creams could be easily removed with tap water suggested that prepared herbal creams might be utilized, which supports the earlier conclusion. The irritancy testdemonstrated that formulated creams did not result in an allergic reaction or skin irritation, proving their safety for use. The literature supported the findings. Further according to earlier research, oil/water emulsions are stable for the formulation of creams. The pH of skin creams is a crucial factor in determining how effective they are. Human skin has a somewhat acidic pH. Hence the prepared formulations have a pH between 5.5 and 6.1.Formulations A1 and A2 produced an effective zone of inhibition against*P. aeruginosa and S. aureus*. Hence, they can be used as an effective wound-healing cream.

CONCLUSION

Our study concludes that inexpensive wound healing cream formulated with *A. indica* and *A. barbadensis miller* may be made following simple procedures. Fewer ingredients were used to make the creams and they are effective in treating wounds. Different ratios of extracts were used to make the formulations. All formulations were homogenous and gelatinous. The produced formulations were safe for human skin and had no negative side effects. The pH of all formulations was within a satisfactory range. A1 and A2 formulations showed better antimicrobial activities. Increasing the *A. barbadensis miller and A. indica* concentration increases the effectiveness of the formulation. By altering the base composition, the current formula can be used to create a variety of wound-healing creams.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this work**.**

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