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Evaluation of Antimicrobial Cream Containing *Chromolaena odorata* Methanolic Leaves Extract for Wound Healing

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Abstract: **Plants are an essential source of potentially beneficial compounds for developing new medicinal drugs. *Chromolaena odorata* have been reported to have antimicrobial properties because they contain secondary metabolites flavonoid, terpenoid, tannin, terpenoid, alkaloid and phenolic compound. These compound affects antimicrobial activity and have the ability in the wound healing process. Therefore, this study was carried out to formulate and evaluate the antimicrobial cream from *C. odorata* methanolic extract. The leaves of *C. odorata* were collected, prepared, and extracted using a standard procedure with 95% methanol. Six formulations from two bases were used to develop an antimicrobial cream from *C. odorata* methanolic extract. The physical characteristics were evaluated on all cream formulations, such as organoleptic test, homogeneity, creaming properties, centrifugation and thermal cycle test. Determination of pH and antimicrobial activity assessment was observed after 24 hours, one week, one month, two months and three months of storage. All formulated creams were observed with dark green colour, homogenous and fragrant leaves smell. Their appearance was semi-solid, with F1 to F3 being very soft and oily while F4 to F6 being very soft and fatty. Formulation of F4, F5, and F6 was stable in the physicochemical and organoleptic tests. The pH formulated creams were between 4 to 5. Within three months of storage, the result that demonstrated no significant differences were F1 and F3 in pH measurement, F2, F3, and F5 in antibacterial activity, and F1, F3, and F5 in antifungal activity evaluation. In conclusion, out of the six formulations, the F3 formulation had the best stability during storage, antimicrobial activity, and minimal changes throughout the study. Thus, the F3 formulation is suitable as an antimicrobial cream for wound healing treatment.**

Keywords: ***Chromolaena odorata*****, antimicrobial cream, cream formulation, stability.**

I. INTRODUCTION

Recently, natural plants have become the main source in medicine production and becoming increasingly popular in treating various ailments due to the phytochemical content and capabilities of the plant. Numerous medicinal plants' leaves, stems, and roots have been used to treat various diseases, particularly in traditional medicine [1]. The ability of herbs to be utilized as wound healers is the subject of certain studies that have been conducted on herbal medicine. It was due to various natural compounds that confirmed has potential for wound healing, so it can be considered a potential natural medicine [2].

According to [3], wounds are defined as breaks in the continuity of cells that result from an injury to the skin caused by a physical, chemical, thermal, infectious, or immunological process. The successful restoration of functional tissue integrity is the defining characteristic of effective wound healing. The protection, repair and recovery processes may affect many cells, cytokines and growth factors [4]. The wound healing process can be influenced by medications used to speed up the healing process. There are plenty of herbs that are reported to be the potential to be used as wound healers. *Chromolaena odorata* is one of Malaysia's herbs traditionally used for wound healing [5].

Chromolaena odorata is a perennial weed of the Asteraceae (Compositae) family. This invasive alien plant species is known as 'Pokok Kapal Terbang' [5]. The fresh leaves have been used for many years to treat leech bites, soft tissue wounds, burn wounds, and skin infections. The squeezed water from its leaf is used to stop bleeding in the wound [6]. The pharmaceutical potential of this plant is possibly due to the presence of various compounds such as phenolics, terpenoid, flavonoids, tannins, and saponins [7]. In addition, a study of antimicrobial effects of *C. odorata* on pathogenic bacteria has been carried out, and the result showed a positive outcome in inhibiting bacterial growth [8].

Chromolaena odorata, widely available in Malaysia, is the best source for developing wound healing medicine. Although there is a lot of evidence of the effectiveness of *C. odorata* in wound healing, limited research has been conducted on the prospective synergism or formulation into conventional dosage forms for therapeutic consideration. As *C. odorata* has been demonstrated to have antimicrobial properties, this work aimed to formulate and evaluate an antimicrobial cream from *C. odorata* methanolic extract. The efficiency of *C. odorata* extracts as antimicrobial agents can be improved by transforming the extract into a cream.

II. LITERATURE REVIEW

A. Chromolaena odorata

Chromolaena odorata (Fig. 1) also known as *Eupatorium odoratum* originated from Africa, North and South America and Southeast Asia [9]. It is commonly called Siam weed, devil weed, French weed, hagonoy, co hoy, and kirinyuh [10]. The *C. odorata* leaves remove an intense aroma and are somewhat less palatable when squeezed. In Malaysia, it is known as *pokok kapal terbang* because of its serious invasive nature in the humid tropics [11]. *Chromolaena odorata* grows in pastures, marginal lands, open areas, dry deciduous forests, and interior shrub jungle and can threaten other plants because of its ability to inhibit plant growth [12]. *Chromolaena odorata* is famous for its traditional medicine, where many countries such as Vietnam, India, Africa, Thailand, Indonesia and Malaysia use its leaves to cure various diseases [13]. *Chromolaena odorata* is being used traditionally as medicinal properties, especially for external uses as in wound skin, skin infections, inflammation, a therapeutic agent for a variety of diseases such as wound healing, anti-inflammatory, analgesic, antipyretic, diuretic, and antimicrobial, anti-mycobacterial and many more [14]. Other than that, the leaves of *C. odorata* are antibacterial in some pathogenic bacteria in humans, for example *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* fungus [15].

Fig. 1 Chromolaena odorata

B. Wound Healing

After tissue damage, wound healing involves the protection, repair, and recovery processes phases [16]. The wound healing process can be divided into three phases. The initial phase is the inflammatory phase, which commences upon tissue damage. It is characterized by hemostasis and inflammation. The second proliferative phase includes granulation, contraction, and epithelialization. The final phase is wound maturation or remodeling. Infections, underlying diseases, and certain medications are some of the conditions that can contribute to slow wound healing [2]. Phytochemicals in medicinal plants

can modulate one or more phases of the wound healing process. According to scientific literature, plants possess phytochemicals that are not only capable of calming or smoothing the skin but also actively restoring, healing, and protecting it [17]. The phytochemicals are readily absorbed by the skin's outermost layers [18]. In addition, medicinal herbs aid in wound healing through multiple mechanisms, including the upregulation of VEGF and TGF-1, the activation of NF-kappa B and IL-8, the overexpression of iNOS and alpha-1 type 1 collagen production, and the activation of antioxidant enzymes [19].

C. Cream

A cream is a form of half-solid preparation containing one or more dissolved ingredients in the appropriate base material [20]. Ointments contain more oil than creams, whereas creams contain an equal amount of oil and water [21]. Creams are better at penetrating the skin than ointments [22]. The basic requirements of a good cream are stable, hamogen, easy to use, suitable active ingredients, and medicinal ingredients that can be divided smoothly and evenly in the base of the cream [23]. The cream must meet specific requirements, such as staying stable as long as it is still used to treat. According to [24], all the substances in the cream are in a smooth state and the whole product becomes soft and homogenous. Herbal oils, creams, and ointments have been used in wound healing with medicinal herbs [25]. A cream prepared with therapeutic components can substitute wound dressing to expedite recovery, decrease inflammatory reactions, and prevent bacterial infections [26]. Furthermore, the cream formulation can transfer therapeutic components to the outer layer of the skin.

III. METHODOLOGY

A. Plant Material and Extract Preparation

Chromolaena odorata leaves were collected around Teluk Intan, Perak. The leaves were rinsed with running water, dried, blended, weighed, and stored in bottles at room temperature. 250 grams of dried leaves were extracted with 2 L 95% methanol solvents. The mixture was extracted using a vertical shaker at 150 rpm for 48 hours. The extract was filtered through Whatman filter paper No. 1 and concentrated using a rotary evaporator at 50°C. After completing evaporation of the solvent, the filtrate was weighed and preserved at 4°C in airtight bottles until further used.

B. Formulation of Antimicrobial Cream

The cream production was made with two different bases: vegetable cream and emulsifying ointment.

I. Vegetable Cream

The cream was produced according to the formulation using the double boiling method. All the ingredients were measured according to the formulation of *C. odorata* cream shown in TABLE I. The mixture was stirred until cold for the formation of cream. The prepared cream was stored in the glass container.

II. Emulsifying Ointment Cream

Thirty percent emulsifying wax, 20 percent liquid paraffin, and 50 percent petroleum jelly were used to make emulsifying ointment. In a container, the components were mixed with constant stirring until an oily combination was produced. Then, the aqueous cream was made by mixing 30 percent emulsifying ointment with 70 percent hot water. Both were melted together to form a homogeneous mixture that would be allowed to cool. The plant extracts were added to the produced cream base. TABLE I shows the composition of *C. odorata* cream. The cream will be stored in a glass container once had been produced.

TABLE I: FORMULATION OF ANTIMICROBIAL *C. ODORATA* CREAM

Formulation Code	Vegetable oil	Ointment	*C. odorata* extract	Glycerin	Propylene Glycol	Total
F1	37		10	0.5	2.5	50
F2	37		10	2.5	0.5	50
F3	37		10	1.5	1.5	50
F4		37	10	0.5	2.5	50
F5		37	10	2.5	0.5	50
F6		37	10	1.5	1.5	50

C. Physicochemical Properties of Cream

I. Homogeneity Test

Each sample was spread on a clean slide and observed using an optical microscope (×10 and ×40).

II. Creaming Properties

Each 10 g formulation sample was placed in a beaker and kept at room temperature for three months. After 24 hours, one week, one month, two month and three months of storage, their physical stability was verified.

III. Centrifugation

Each 1.0 g formulation sample was placed in a 1.5 mL centrifuge tube and centrifuged for 5, 15, 30, and 60 minutes at 2000 rpm. The sample phase separation and solid sedimentation were then assessed.

IV. Thermal Cycle Test

Each portion of the formulation were stored at 5°C for 48 hours and then 25°C for 48 hours. The procedure was repeated three times and then their stability and appearance were evaluated.

D. Stability of Cream

I. Determination of pH

Each portion of the formulation was suspended in 1% potassium nitrate solution to determine the pH value. A magnetic stirrer was used to produce homogeneity. The pH was determined after 24 hours, one week, one month, two month and three months after storage.

II. Organoleptic Observation

The sensory test was carried out by observing the color, smell and appearance. The organoleptic was observed after 24 hours, one-week, one-month, two-month and three-months after storage.

E. Antimicrobial Activity Assessment of Cream Formulation

Antimicrobial activity assessment of cream formulation was determined using the agar well diffusion method [27]. *Escherichia coli* was used in antibacterial activity, while *Candida albicans* was used in antifungal activity *Escherichia coli* and *Candida albicans* were platted in Muller Hilton Agar and incubated with incubation temperature at 37 °C for 24 hours. Then, the single colony of both microbes was diluted in sterile water and adjusted to a standard density corresponding to 0.5 McFarland standard. Both microbes were plated on sterile Mueller Hilton Agar (MHA) using the Kirby-Bauer swabbing technique by sterile cotton swabs to produce uniform microbe growth. Wells were bored into the set agar using a sterile cork borer. The sterile spatula was used to fill the holes with the formulated cream. The microbial plate was incubated at 30°C for 24 hours. Then, the inhibition zone's diameter for bacteria and fungi was measured. The antimicrobial activity assessment was repeated after 24 hours, one-week, one-month, two-months, and three-months after storage. Cream without extracted compound was used as a control.

IV. RESULTS AND DISCUSSION

Following the *Chromolaena odorata* leaves extraction, six antimicrobial creams are produced using the four primary ingredients: bases made of vegetable cream or emulsifying ointment, 20 percent of methanolic *C. odorata* extract, glycerin, and propylene glycol. F1 to F3 were formulated using the vegetable cream base, while F4 to F6 were formulated using emulsifying ointment base. Ointment and vegetable cream are used as oily materials, glycerin as a surfactant, propylene glycol as a humectant and *C. odorata* methanolic extract as a medicinal agent. The formulation in the form of a cream can assist in transferring the active components of the extract into the skin tissue, thereby increasing the local bioavailability of the extracts [23]. All the formulations were oil-water (O/W) type emulsion cream. The oil-in-water (O/W) cream compositions easily wash off the skin, improving customer compliance [28].

A. Antimicrobial Activity Assessment of Cream Formulation

Physically, all formulations showed an attractive creamy appearance where the cream spread homogeneously and semi-solid, with F1 to F3 being very soft and oily while F4 to F6 being very soft and fatty (TABLE II). All formulated creams

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were dark green color due to chlorophyll's presence in the *C. odorata* extract (Fig. 2). The retention of the cream with a soft texture for each formulation at 5°C and 25°C also showed the physical stability of the cream. Microscopic observation showed a homogenous mixture between all ingredients in all formulations (Fig. 3). Unfortunately, the oily condition of the cream can be seen clearly when performing centrifugation where there is a separate oil on the cream, especially for F1-F3 formulations (Fig. N). Vegetable cream was made from hydrogenated and partially hydrogenated vegetable oils, such as corn, cottonseed or soybean. The oily composition of vegetable cream and adding glycerin may cause the cream to be oily.

TABLE II: PHYSICOCHEMICAL OF CREAM FORMULATION

Formulation Code	Property			
	Homogeneity test	Creaming properties	Centrifugation test	Thermal cycle test
Ointment base	Homogenous	stable	stable	stable
Vegetable oil base	Homogenous	stable	separated	stable
F1	Homogenous	stable	separated	stable
F2	Homogenous	stable	separated	stable
F3	Homogenous	stable	separated	stable
F4	Homogenous	stable	stable	stable
F5	Homogenous	stable	stable	stable
F6	Homogenous	stable	stable	stable

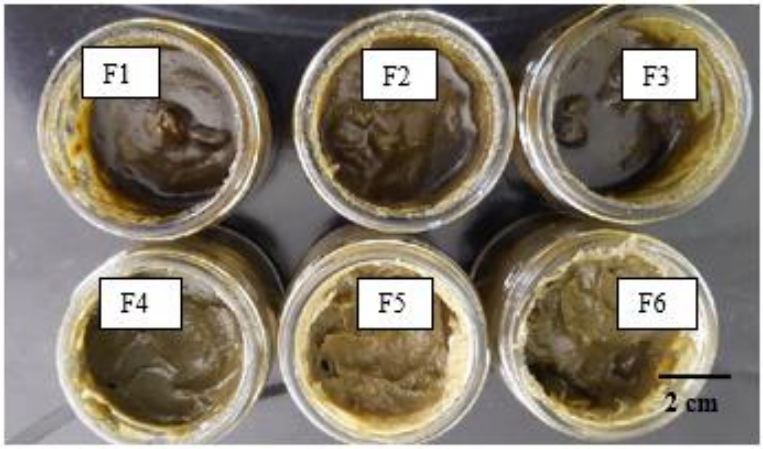

Fig. 2: Formulated antimicrobial cream, F1 to F6

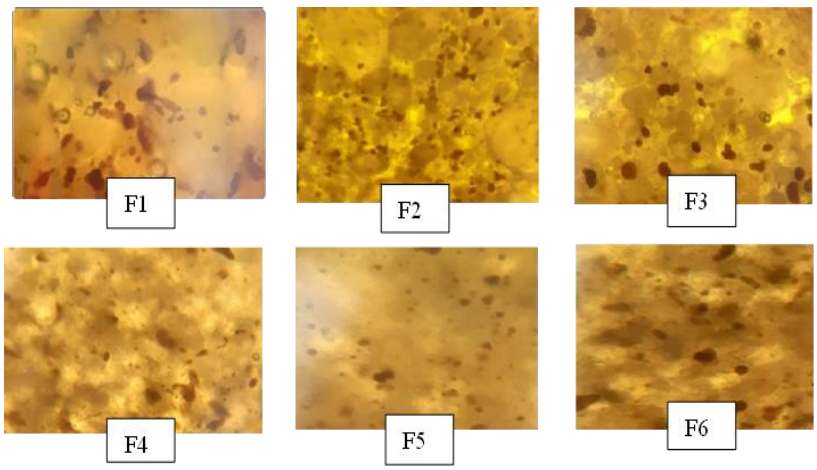

Fig. 3: Observation of formulated antimicrobial cream under light microscope (40x)

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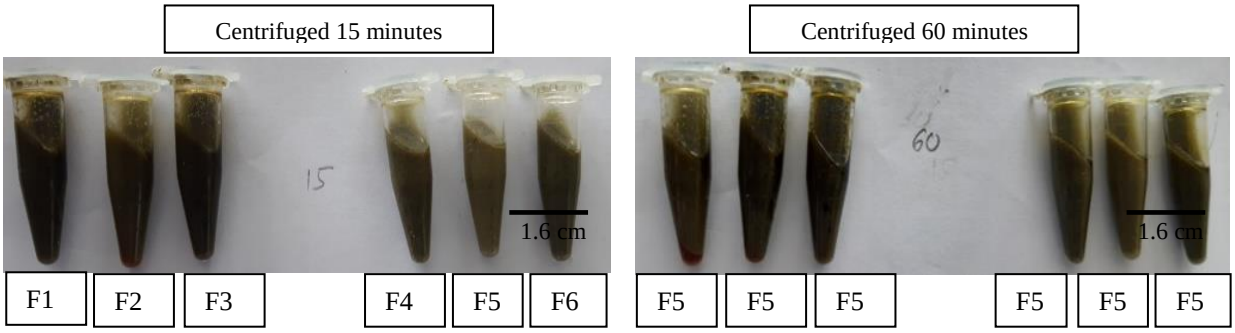

Fig. 4: Observation for centrifugation test of formulated antimicrobial cream

TABLE III: ORGANOLEPTIC OBSERVATION OF FORMULATED CREAM

Storage	Color	Smell	Appearance
		Formulation F1	
Day 1	dark green	fragrant leaves	very soft and oily
One week	dark green	fragrant leaves	very soft and oily
One-month	dark green	fragrant leaves	soft but little sticky
Two-month	dark green	fragrant leaves	little rough and sticky
Three-month	dark green	fragrant leaves	rough and sticky
		Formulation F2	
Day 1	dark green	fragrant leaves	very soft and oily
One week	dark green	fragrant leaves	very soft and oily
One-month	dark green	fragrant leaves	soft but little sticky
Two-month	dark green	fragrant leaves	little rough and sticky
Three-month	dark green	fragrant leaves	rough and sticky
		Formulation F3	
Day 1	dark green	fragrant leaves	very soft and oily
One week	dark green	fragrant leaves	very soft and fatty
One-month	dark green	fragrant leaves	soft but little sticky
Two-month	dark green	fragrant leaves	soft but little sticky
Three-month	dark green	fragrant leaves	little soft and sticky
		Formulation F4	
Day 1	soft green	fragrant leaves	very soft and fatty
One week	soft green	fragrant leaves	very soft and fatty
One-month	soft green	fragrant leaves	soft but little sticky
Two-month	soft green	fragrant leaves	soft but little sticky
Three-month	soft green	fragrant leaves	soft but little sticky
		Formulation F5	
Day 1	soft green	fragrant leaves	very soft and fatty
One week	soft green	fragrant leaves	very soft and fatty
One-month	soft green	fragrant leaves	soft but little sticky
Two-month	soft green	fragrant leaves	soft but little sticky
Three-month	soft green	fragrant leaves	soft but little sticky
		Formulation F6	
Day 1	soft green	fragrant leaves	very soft and fatty
One week	soft green	fragrant leaves	very soft and fatty
One-month	soft green	fragrant leaves	soft but little sticky
Two-month	soft green	fragrant leaves	soft but little sticky
Three-month	soft green	fragrant leaves	soft but little sticky

B. Organoleptic observation

Organoleptic observations were adopted to characterize the cream by evaluating its color, smell, and appearance. These also assessed their stability by monitoring their physical characteristics at different storage periods. Organoleptic observation of cream formulation was presented in TABLE III. The freshly made cream from F1 to F6 was green and smelled like fragrant leaves extracted from *C. odorata*. No changes of color show their stability when stored at room temperature until three months of storage. Their appearance was semi-solid, with F1 to F3 being very soft and oily while F4 to F6 being very soft and fatty. However, the placebo, vegetable cream, and ointment base were semi-solid with white and light cream-colored, respectively. Even after three months of storage for all formulated cream, there were no changes in the colors and smell. After one month of storage, F1 to F3 formulated cream, the appearance changes when the cream is rougher and stickier. The F4 to F6 were more stable, with slightly sticky appearances. These results indicate that an ointment base, glycerin, and propylene glycol mixture was more physically stable than a vegetable cream.

C. Determination of pH

The pH of the formulated cream is presented in TABLE IV. The results showed that adding the glycerol and propylene glycol to the formulated cream lowers the pH. The pH of formulated cream is more acidic compared to placebo, ointment and vegetable cream. However, the formulation of F1 and F3 showed not significant difference within three-month of storage. Besides that, the pH obtained from the base and formulated cream showed within the optimal pH range for the skin. The human skin's pH ranges from 4.0 to 7.0 [29]. A higher pH generates increased consequences of dehydration, irritation, and a rise in the number of bacteria. In contrast, a higher acidity enables the skin to fight free radicals and microbes more effectively [30]. All cream formulations are compatible with the skin's pH and have resistance pH until three months of storage.

TABLE IV: THREE-MONTH PH TEST RESULTS FOR THE FORMULATION OF *C. odorata* CREAM

Formulation Code	pH				
	Day 1	One week	One-month	Two-month	Three-month
Ointment base	6.14 ± 0.10	5.45 ± 0.14	5.4 ± 0.27	5.2 ± 0.81	6.38 ± 0.40
Vegetable oil base	5.42 ± 0.13	5.74 ± 0.53	5.66 ± 0.12	5.24 ± 0.10	6.16 ± 0.05
F1	4.31 ± 0.19	4.3 ± 0.05	4.34 ± 0.06	4.25 ± 0.06	4.35 ± 0.06
F2*	4.53 ± 0.10	4.31 ± 0.36	4.46 ± 0.17	4.27 ± 0.85	4.33 ± 0.58
F3	4.25 ± 0.07	4.38 ± 0.15	4.45 ± 0.12	4.31 ± 0.15	4.24 ± 0.09
F4*	4.71 ± 0.17	4.33 ± 0.05	4.45 ± 0.04	4.32 ± 0.66	4.28 ± 0.07
F5*	5.01 ± 0.17	4.47 ± 0.32	4.21 ± 0.12	4.34 ± 0.10	4.24 ± 0.90
F6*	4.54 ± 0.04	4.36 ± 0.51	4.28 ± 0.11	4.35 ± 0.07	4.25 ± 0.05

Figures are mean ± SD; Significant* ($p<0.05$). Confidence interval level at 95%

D. Assessment of Antimicrobial Cream Formulation

Escherichia coli and *Candida albicans* were employed in the current study to test the antimicrobial activity since they are pathogens that can be detected in infected wounds [31]. The diameter of the zone of inhibition that formed on the plate was measured to evaluate the antimicrobial activity. The results obtained in antibacterial and antifungal activity are shown in TABLE V and TABLE VI, respectively. The addition of 20% *C. odorata* extract in each cream formulation caused microbial activity to be disrupted by forming an inhibition zone on the tested plate. F1 formulation has the highest effect of antibacterial activity within three-month of storage. While the formulation of F4 to F6 only shows antibacterial activity up to one month of storage. No antibacterial activity in the second and third months of storage because the cream is no longer effective on *E. coli* after a month of storage. F2, F3 showed not significantly difference within three months of storage.

The antifungal activity of *C. albicans* was slightly varied, most formulations showed an increasing inhibition zone diameter over the storage period. Formulations of F1, F2 and F6 showed maximum antifungal activity during three months of storage with 15.33mm, 14.67mm and 13.00mm, respectively. While F1 showed not significant different within three months of storage, The vegetable cream base exhibited strong antimicrobial properties against both pathogens compared to the ointment base.

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TABLE V: ANTIBACTERIAL ACTIVITY OF THE CREAM FORMULATION AGAINST *Escherichia coli* FOR THREE- MONTH STORAGE

	Escherichia coli WDCM 0012				
Formulation Code	Day 1	One week	One-month	Two-month	Three-month
Ointment base	0	0	0	0	0
Vegetable oil base	0	0	0	0	0
F1*	12.00 ± 1.73	9.67 ± 1.52	13.33 ± 0.57	7.33 ± 0.57	9.67 ± 0.57
F2	9.67 ± 2.51	11.67 ± 1.52	10.67 ± 2.51	7.00 ± 1.00	10.00 ± 1.00
F3	12.67 ± 1.52	12.67 ± 0.50	11.00 ± 3.00	11.00 ± 1.00	11.00 ± 1.00
F4*	8.33 ± 1.52	10.33 ± 0.50	10.00 ± 1.00	0	0
F5	7.33 ± 0.57	8.33 ± 1.52	12.00 ± 1.00	0	0
F6	9.33 ± 1.15	10.33 ± 0.57	11.00 ± 1.00	0	0

Figures are mean ± SD; Significant* ($p<0.05$). Confidence interval level at 95%

TABLE VI: ANTIFUNGAL ACTIVITY OF THE CREAM FORMULATION AGAINST *Candida albicans* FOR THREE- MONTH STORAGE

	Candida albicans WDCM 00054				
Formulation Code	Day 1	One week	One-month	Two-month	Three-month
Ointment base	0	0	0	0	0
Vegetable oil base	0	0	0	0	0
F1	10.00 ± 1.00	12.00 ± 1.00	11.00 ± 1.73	13.67 ± 1.15	15.33 ± 0.57
F2*	11.67 ± 0.57	9.67 ± 0.57	13.67 ± 1.15	13.67 ± 1.15	14.67 ± 0.57
F3	12.67 ± 0.57	12.00	13.00 ± 1.73	15.00	14.67 ± 0.57
F4*	7.63 ± 0.57	7.00	11.33 ± 1.52	12.67 ± 0.57	12.67 ± 0.57
F5	7.33 ± 0.57	7.00	10.67 ± 0.57	12.00± 1.00	10.33 ± 0.57
F6*	9.00 ± 1.00	7.33 ± 0.57	12.67 ± 1.15	11.33 ± 1.52	13.00 ± 0.57

Figures are mean ± SD; Significant* ($p<0.05$). Confidence interval level at 95%

In addition, the phytochemical elements found in *C. odorata* promote wound healing. The ability of the alkaloidal components to react with amino, carboxyl, sulfhydryl, and hydroxyl groups in bacterial protein and nucleic acids is thought to be responsible for their antibacterial effect [29]. It has been suggested that tannins inhibit microbial growth by precipitating microbial protein, rendering nutritional proteins inaccessible to the microbes [32]. One of the most important classes of phenolic compounds found in plants, flavonoids serve an antioxidant function by forming a complex with extracellular soluble proteins [31]. Based on all finding incorporating physicochemical, organoleptic studies, pH and the antimicrobial activity evaluation, the F3 was the best formulation because there were changes at a minimum level from the beginning of the cream preparation up to three months of storage. The most effective antimicrobial cream for treating wounds is the F3 formulation since it has optimum stability.

V. CONCLUSION

Formulating antimicrobial cream incorporating 20 % methanolic *C. odorata* leaf extracts could be effectively used in wound treatment as they inhibited the growth of *Escherichia coli* and *Candida albicans*. The formulation cream of F3 showed high stability during storage, antimicrobial activity, and minimal changes throughout the study compared to other formulations. Besides that, the antimicrobial cream using a vegetable cream base was elegant and sturdy during the three-month storage period. Therefore, it is possible to develop creams containing *C. odorata* extracts for wound healing treatment. Nevertheless, additional research on the cream's pharmacological evaluation and biosafety assay needs to be done to ensure consumer safety.

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