FORMULATION OF ANTIMICROBIAL CREAM EXTRACTED FROM *Chromolaena odorata* (POKOK KAPAL TERBANG) FOR WOUND HEALING

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SULTAN IDRIS EDUCATION UNIVERSITY

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ABSTRACT

Chromolaena odorata has reported to have medicinal value due to the presence of active compounds which have antimicrobial and wound healing properties. Therefore, this study was carried out to formulate the antimicrobial cream from *C. odorata* extract used for wound healing treatment. The study aims to screen the secondary metabolites and determine *C. odorata* extract concentration for antimicrobial activity to be used in cream formulation. The leaves were extracted in 95% methanol and 95% ethanol for secondary metabolite screening and Gas Chromatography-Mass Spectrometry. *Chromolaena odorata* extract with a concentration of 5%, 10%, 15% and 20% was performed for antimicrobial activity tested on *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. The best of *C. odorata* extract concentration was used in six formulations of antimicrobial cream. The stability test was performed on all cream formulations. The methanolic extract was present with tannin, terpenoid, phenolic acids, alkaloid, and saponin while ethanolic extract was present with tannin and alkaloid. Both extracts are absent with flavonoid compounds. The GC-MS chromatogram identified as many as 26 and 15 compounds for ethanolic and methanolic extract, respectively. The *C. odorata* ethanolic extract consists mainly of Fatty acid (39.2%), Sesquiterpenoid (20.69%) and Phenol (0.51%). Meanwhile, *C. odorata* methanolic extract consists of Sesquiterpenoid (62.26%) and Fatty acid (17.11%). The best concentration was 20% *C. odorata* methanolic extract had the highest diameter of inhibition zone against *E. coli* and *C. albicans* at 20.67 mm and 13.3 mm, respectively. Formulation of F4, F5, and F6 was stable in physicochemical and organoleptic studies. No significant difference for F1 and F3 in pH measurement; F2, F3, F5 and F6 in antibacterial activity and F1, F3, and F5 in antifungal activity. In conclusion, out of the six formulations, the F3 formulation has optimal stability and is suitable as an antimicrobial cream for wound healing treatment.

FORMULASI KRIM ANTIMIKROB DARIPADA EKSTRAK *Chromolaena odorata* (POKOK KAPAL TERBANG) UNTUK PENYEMBUHAN LUKA

ABSTRAK

Chromolaena odorata telah dilaporkan mempunyai nilai perubatan disebabkan kehadiran bahan aktif yang mempunyai sifat antimikrob dan penyembuhan luka. Oleh itu, kajian ini bertujuan untuk menghasilkan formulasi krim antimikrob daripada ekstrak *C. odorata* bagi penyembuhan luka. Objektif kajian adalah untuk menyaring metabolit sekunder dan menentukan kepekatan *C. odorata* terbaik untuk aktiviti antimikrob yang akan digunakan dalam formulasi krim antimikrob. Daun *C. odorata* dikumpul, disediakan dan diekstrak dengan 95% metanol dan 95% etanol menggunakan prosedur standard. Kedua-dua ekstrak dijalankan penyaringan metabolit sekunder dan Kromatografi Gas-Spektrometri Jisim. Ekstrak *C. odorata* berkepekatan 5%, 10%, 15% dan 20% dilakukan ujian aktiviti antimikrob dengan menggunakan kaedah resapan telaga agar pada mikroorganisma *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* dan *Candida albicans*. Kepekatan ekstrak *C. odorata* terbaik digunakan dalam enam formulasi krim antimikrob. Ujian kestabilan dilakukan ke atas semua formulasi krim. Ekstrak metanol mengandungi tanin, terpenoid, fenol, alkaloid, dan saponin manakala ekstrak etanol mengandungi tanin dan alkaloid. Kedua-dua ekstrak tidak mempunyai sebatian flavonoid. Kromatogram GC-MS mengenal pasti masing-masing sebanyak 26 dan 15 bahan aktif pada ekstrak etanol dan ekstrak metanol. Sebatian utama dalam ekstrak etanol *C. odorata* ialah Asid Lemak (39.2%), Sesquiterpenoid (20.69%) and Phenol (0.51%). Manakala ekstrak metanol *C. odorata* pula terdiri daripada Sesquiterpenoid (62.26%) dan asid lemak (17.11%). Kepekatan 20% ekstrak methanol *C. odorata* mempunyai zon perencatan tertinggi terhadap *E. coli* dan *C. albicans* masing-masing 20.67 mm dan 13.3 mm. Formulasi F4, F5 dan F6 adalah stabil dalam kajian fizikokimia dan organoleptik. Tiada perbezaan yang signifikan untuk F1 dan F3 dalam pengukuran pH; F2, F3, F5 dan F6 dalam aktiviti antibakteria dan F1, F3, dan F5 dalam aktiviti antikulat. Kesimpulannya, daripada enam formulasi, formulasi F3 mempunyai kestabilan yang optimum dan sesuai sebagai krim antimikrob untuk rawatan penyembuhan luka.

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AKT	Protein kinase
Ang Tie	Angiopoietin
C	Celcius
COX-2	Cyclooxygenase-2
cm	centimeter
ECM	Extracellular matrix
g	Gram
GC-MS	Gas Chromatography Mass Spectrometry
GLC	Gas Liquid Chromatography
HO	Heme oxygenase
IL	Interleukin
iNOS	Inducible nitric oxide synthase
JNK	c-Jun N-terminal kinase
kg	Kilogram
MAPK	Mitogen-activated protein kinase
MHA	Muller Hilton Agar
MMP	Matrix Metalloproteinase
mL	mililiter
NIST	National Institute of Standard and Technology
PBMCs	Peripheral Blood Mononuclear cells
pH	potential Hydrogen
ROS	Reactive Oxygen Species

SD	Standard Deviation
SPSS	Statistical Package for the Social Science
TGF	Transforming Growth Factor
TIMP	Tissue Inhibitor of Metalloproteinases
TLC	Thin Layer Chromatography
TNF	Tumor Necrosis Factor
TXS	Thromboxane synthase
VEGF	Vascular Endothelial Growth Factor

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Plants material have been the source of many pharmaceuticals on the market for development into drug formulations. It is well known that plants have played a significant role in human history due to their fascinating phytocompound and pharmacological capabilities. Plants produce these molecules as a defence, but new studies show that many phytocompounds can offer health benefits to humans (Kanase & Shaikh, 2018). In addition, most of the drugs we use today come from herbal plant sources.

Recently, traditional medicine has become increasingly popular with various products that use herbs. Herbal products have provided several important life-saving drugs used in modern medicine. Herbal medicine is gaining prominence as a

treatment for various ailments. In traditional medicine, the extraction of different medicinal plants' leaves, stems, and roots has been used to cure various diseases (Pizzi, 2021; Ruckmani et al., 2015). One of the studies that had been done on herbal medicine focuses on the ability of herbs to be used is wound healing.

Wounds are disruptions to the continuity of cells due to a physical, chemical, thermal, infectious or immunological injury to the skin (Vadivel & Balasubramaniam, 2022b). According to Shah and Amini-nik (2017), effective wound healing is defined by the restoration of functional tissue integrity. Wound healing after tissue damage is a complex process. The protection, repair and recovery processes may affect many cells, cytokines and growth factors (Tottoli et al., 2020). Generally, three stages can be identified throughout the wound healing process (Srirod & Tewtrakul, 2019). When tissue is damaged, the first step of the process, known as the inflammatory phase. It includes both the process of homeostasis and inflammation. The proliferative phase, which consists of granulation, contraction, and epithelialization, is the second step of the process. The final phase of the healing process is called maturation, or known as wound remodelling (Srirod & Tewtrakul, 2019).

Accidents, surgeries, and burns are the three most common causes of damage to the skin (Ibrahim et al., 2018). In addition, the skin has the potential to get infected throughout the healing process, when microorganisms might enter the body and proliferate. Various agents, such as bacteria, viruses, fungi, or parasites, may bring on the infection. A bacterial infection of an open wound will remain until the wound is properly healed. The wound healing process can be influenced by medications used to speed up the healing process. There are plenty of herbs that reported to be potential to

be used to heal wound. *Chromolaena odorata* is one of herbs that been traditionally used as wound healing in Malaysia (Nur Zafirah Sabri & Hartini Yusof, 2021).

Chromolaena odorata is a perennial weed of Asteraceae (Compositae) family. This invasive alien plant species is formerly known as *Eupatorium odoratum* or locally known as pokok kapal terbang (Matawali et al., 2019). *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 other plant growth (Zahara, 2019). *Chromolaena odorata* is being used 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 (Omokhua et al., 2017). *Chromolaena odorata* has been traditionally used as wound healer in local community. The fresh leaves have been used for many years for the treatment of leech bite, soft tissue wounds, burn wounds and skin infection (Sirinthipaporn & Jiraungkoorskul, 2017). The squeezed water from its leaf used to stop bleeding on the wound. The pharmaceutical potential of this plant is possibly due the presence of various compound such as phenolics, flavonoids, tannins and saponins (Akinmoladun & Ibukun, 2007). A number of studies also demonstrated that the extract of the leaves of *C. odorata* inhibited the growth of bacteria such as *Pseudomonas aeroginosa, Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumonia, Shigella sonnei* and *Psedomonas aeruginosa* (Bamigboye et al., 2020; Odutayo et al., 2017; Vijayaraghavan et al., 2018). A study of antimicrobial effects of *C. odorata* on

pathogenic bacteria have been carried out and the result showed positive outcome in inhibition of bacterial growth (Yutika et al., 2015).

According to the published research, several different plants have been identified, and their medicinal compounds have been formulated into topical treatments for wounds. According to Gwarzo et al. (2022), herbal oils, creams, and ointments have been used in wound healing with medicinal herbs. Therefore, the *C. odorata* extract that has antibacterial properties can be increased by formulating the extraction into a cream. Creams and ointments can remain on the wound surface longer than liquid formulations (Dandasi Jayachandra et al., 2020). Creams prepared with therapeutic components can replace wound dressings to speed up recovery, reduce inflammatory reactions, and prevent bacterial infections (Sarabahi, 2012). This is because the cream formulation has the ability to transmit therapeutic components to the top layer of the skin, where they can treat wounds. However, to formulate the cream, a number of factors need to be considered. Most importantly, the cream's stability and qualities need to be considered so that its efficacy can be ensured. In addition, it is essential that the cream's safety be highlighted to guarantee the users' safety.

1.2 Problem Statement of the study

Wound healing in the skin is essential for repairing and recovering tissue function after injury (Minutti et al., 2017). On the skin, both the epidermis and dermis typically protect or shield from any external physical and chemical environment. However, injuries such as burns, microbial infections, skin diseases, accidents or metabolic dysfunction can cause skin lesion (Rezaie et al., 2019). The failure of healing mechanism can lead to chronic wounds. The situation will worsen when there are diseases such as diabetes, venous or arterial disease and microbial infection on the wound (Maheswary et al., 2021). The inability of antibiotics or host clearance systems like antibodies and phagocytes to properly penetrate pathogenic biofilms makes eradicating the bacteria challenging (Mangoni et al., 2016). Furthermore, the toxins released from the bacteria will produce an excessive and damaging inflammatory response to the skin (Dorantes & Ayala, 2019). Apart from that, antibiotic resistance can also arise in circumstances where the bacteria in interest are not affected by the antibiotics being given (Dadgostar, 2019). As a result, some individuals have developed resistance to the currently available treatments for their skin infections. Nowadays, wound infections have become an increasing cause and can cause death if the infection worsens and is not treated appropriately. Expensive treatment and care systems have also placed a tremendous burden on patients (Kayir et al., 2018).

Medicinal plants have a significant role in the treatment of a wide variety of infections and are also the basis for the production of natural medications. In modern science, herbal plants traditionally used to cure diseases have been extensively studied

to identify their bioactive compound in developing new drugs. Studies on plant compounds' mechanism of action and efficacy have shown that many are pharmacologically safe but still require further testing in preclinical studies and clinical trials (Bhusnure et al., 2019). Many developing countries still rely on medicinal plants as effective, safe and inexpensive alternative wound healing agents (Vadivel & Balasubramaniam, 2022). Wounds caused by infection can be mild when treated with natural remedies. In addition, using plants with antimicrobial agents is an excellent option to avoid antibiotic resistance. Therefore, medicinal plants that naturally have antibacterial properties can serve as drugs to replace antibiotics in treating infectious diseases (Okwu et al., 2019). Considering how important the effects of plants and their parts are on skin tissue, *Chromolaenana odorata* that has many active compounds is a good choice for a wound healing medicine because it has a therapeutic effect.

Chromolaena odorata is one of underutilized plant that been categorized as weeds. The plant is abundantly found at distributed area, farm, near paddy field and small shrub (Olawale et al., 2022). Traditionally in Malay culture, this plant being used to treat small wound, where it believes to has ability to stop the bleeding. The leaves were crushed and applied on the wound and the healing process monitored by assessing the rate of contraction or closure of the wound (Aziz et al., 2020). Previous results in the literature have shown that secondary metabolites from *C. odorata* facilitate wound healing and have been tested in various animal models. *Chromolaena odorata* leaf extract has been shown to have a hemostatic effect by reducing bleeding and clotting times in a dose-dependent way in experimental Winstar rats, as reported

by Okoroiwu et al. (2016). Review by Vijayaraghavan et al. (2017a), strongly suggested that the bioactive compounds found in the leaf extract play a significant role in treating various ailments in veterinary medicine and alternative medicine in humans in the future. In addition, a supporting study from Budi et al. (2021) showed that a concentration of 20% *C. odorata* extract increases the healing value of incision wounds in mice infected with *Staphylococcus aureus.* Therefore, I hypothesized that the numerous secondary metabolites found in *C. odorata* leaf extract would have potentially beneficial synergistic effects on the healing of wounds.

Chromolaena odorata, widely available in Malaysia, is the best source for developing wound healing medicine. Although there is a lot of evidence on the effectiveness of *C. odorata* in wound healing, more research on formulation into conventional dosage forms for therapeutic considerations is needed. The efficiency of *C. odorata* extract as an antimicrobial agent can be improved by turning the extract into a cream. A deeper study studies should be conducted to suggest *C. odorata* cream as one of the alternative medicines that can heal wounds. Therefore, this study was designed to evaluate the antimicrobial activity and wound healing potential of *C. odorata* cream formulation from methanol leaf extract. Phytocompounds that responsible for wound healing will be identified to evaluate the characteristics and properties of these compounds. Furthermore, the cream containing *C. odorata* extract needs to be analyzed with wound microorganisms. Then, the stability of the cream should be determined to ensure the safety of consumer use.

1.3 Significant of the studies

Pharmaceutical drugs based on herbal plants can be potential agents, especially in wound healing. Secondary metabolites from the herbal plant can be one of the natural sources for replacing antibiotics that play an important role in human health. *Chromolaena odora* is an easy-to-find herb, including in Malaysia, where it is also traditionally used in wound healing. *Chromolaena odorata* is known for various secondary metabolites such as flavonoids, terpenoids, tannins, saponins, and alkaloids that have various properties such as antimicrobial, anti-inflammatory and antioxidant properties. Therefore, the characteristics of these secondary metabolites can be used as a source for wound healing drugs. Thus, developing these natural products is a source of new drug development that provide better effectiveness against drug-resistant organisms.

Formulating the extract of *C. odorata* in the form of cream will bring the active ingredients of the extract to the wound area on the skin for healing purposes. The cream will also prevent infections that may occur if using the extract plant directly. The crude extract can act as a potential source for the growth of microorganisms that will cause the wound to worsen and interfere wound healing process. Therefore, the antimicrobial formulation of *C. odorata* is one of the alternative methods other than commercial medicine in wound healing. In addition, the antimicrobial cream produced is easily in high demand due to their wide availability, easy production and cost-effectiveness.

This study is essential for developing research by expanding the information and knowledge about *C. odorata*, especially regarding its chemical properties, bioactivity and antimicrobial properties. In addition, developing antimicrobial creams and investigating their stability can guarantee safety for users. The results of this study will be a future reference and a source of knowledge about therapeutic discoveries from *C. odorata* that can lead to the development of potential and safe medicines in the medical field. The results also can contribute to the availability of natural bioactive cream formulations in the market for wound healing for treating microorganism infections. From traditional use, products based on *C. odorata* can be produced for medicinal purposes and can then be commercialized for patient use.

1.4 Objectives of the study

This study generally aimed at formulating the antimicrobial cream from *C. odorata* extract used for wound healing treatment. The specific objectives of this studies are:

1) To screen secondary metabolites responsible for wound healing properties of *C. odorata* extract.
2) To identify the best crude percentage of *C. odorata* extract on antimicrobial activity using agar well diffusion method.
3) To select the best antimicrobial cream based on different types of cream base.

1.5 Scope and Limitation of The Study

In this study, an antimicrobial cream was developed with the leaves of *C. odorata*, serving as the primary source of raw material. The area near Teluk Intan, Perak, served as the sampling location for *C. odorata*. The only solvents used for the extraction process were ethanol or methanol at a concentration of 95%. *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis,* and *Candida albicans* were used in this study to test the antimicrobial activity of the *C. odorata* extract. All the microorganisms were tested directly on 5%, 10%, 15%, and 20% concentrations of *C. odorata* extract. Following the completion of the formulation of the cream, an organoleptic evaluation was carried out to evaluate the product's homogeneity, creaming qualities, centrifugation, and thermal change. In the meantime, the cream's appearance, color, odor, pH, and antimicrobial activity were assessed at twenty-four hours, one week, one month, two months, and three months after production of the cream.