SILVERN: Kesum-Silver Nanoparticles as Antibacterial Wound Healing Agent

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ABSTRACT

When a wound becomes infected with germs, the treatment costs skyrocket, especially in terms of nursing and hospitalisation, which can be prohibitively expensive for low-income populations. As a result, silver nanoparticles can be utilised at the wound site to either kill or suppress bacterial growth. Silver nanoparticles are a frequently used antibacterial agent that is safe for humans at low concentrations. Physical and chemical synthesis approaches, on the other hand, had limitations, including hazardous waste creation and excessive energy consumption. As a result, biosynthesis of silver nanoparticles with kesum leaf extract could be a sustainable and safe process. Kesum leaf is also abundant, easy to grow in Malaysia, and rich in antioxidant activity. SILVERN applied to the wound site can assist to inhibit the spread of dangerous bacteria while also lowering treatment expenses, which benefits the community. According to in vitro skin cell and in vivo animal testing assays, SILVERN exhibits significant antibacterial activity against diverse bacteria, is safe for humans, and acts as an antibacterial wound healing agent. Copyrights for the biosynthesis process and its use as an antibacterial wound healing agent have been filed (LY2020004347 & LY2021E05078). Silver nanoparticles, antimicrobials, and wound healing have a big market, hence SILVERN's commercialization potential is enormous. A few local companies have been identified as potential industrial partners for our commercialization approach. SILVERN has the advantage of being able to generate silver nanoparticles in a sustainable manner without generating toxic waste, contributing to the circular economy manufacturing inventiveness.

Keywords: Silver nanoparticles; kesum; antibacterial; wound healing

INTRODUCTION

Bacterial infection of the wound bed is one of the most prevalent consequences following a skin injury. Because bacterial colonisation hampers natural regenerative and reparative processes, wound infections are the leading cause of delayed wound healing. Similarly, recurrent infections obstruct the wound's repair of structural and functional integrity, resulting in chronic wounds. Antibacterial medications, such as antibiotics, are used in modern medicine to either destroy or limit bacterial growth. Antimicrobial medicines are frequently used to prevent infection and excessive inflammatory response in wounds [1]. However, a significant

rise of antibiotic resistance in bacteria has resulted from insufficient research and development of novel medicines, as well as indiscriminate antibiotic use [2]. As a result of this condition, bacterial infection continues to be a leading cause of morbidity and mortality [2]. According to the Centers for Disease Control and Prevention (CDC) report on antimicrobial resistance (AMR), bacteria and fungi cause an estimated 2,868,700 illnesses per year [3]. Methicillin-resistant Staphylococcus aureus (MRSA) accounts for 40% of wound isolates and infects 14% to 17% of patients with burn wounds, according to the study [4].

Silver is an effective antimicrobial agent against bacteria, fungus, and yeast, as well as antibacterial resistant forms. The advancement of nanotechnology, which allows for the manufacture of silver nanoparticles, has ushered in a new possible therapeutic. Biogenic silver nanoparticles are a type of biocidal nanoparticle that is known to be harmful to bacteria through a variety of mechanisms and can help with wound healing by regulating the inflammation phase. The rate of wound closure was increased by modifying growth factors and cytokines via antibacterial and anti-inflammatory action in a study using silver nanoparticles produced by cellulose nanocrystals [5]. Another study concluded that biosynthesized silver nanoparticles have broad-spectrum antibacterial action and improve wound healing via anti-inflammatory activity [6]. Silver nanoparticle-mediated scaffolds have also been reported to be used in newborn wound care dressing materials that have a high wound healing capability [7]. These findings indicate silver nanoparticles' potential use in medicine, particularly as antibacterial and wound-healing agents.

Various forms of ointment, gel, or dressings, such as hydrogels, gauze, hydrocolloids, films, and polymer dressings, are being used in wound treatment. However, materials composed of synthetic chemicals and materials have non-biocompatibility difficulties, are difficult to remove after application, causing skin trauma, have poor water absorption, and have a high production cost [8]. Natural resources, such as plant extracts, have attracted a lot of study attention among the several modalities for manufacturing silver nanoparticles since they are safe and environmentally friendly [9]. The plant extract's biomolecules have a significant impact on the distribution and size of metal nanoparticles, which has a significant impact on the physical, chemical, and biological properties of the nanoparticles [10].

Silver nanoparticles have been used in a variety of biomedical applications in recent years. Plant-derived drugs have risen in popularity in recent years as a result of their potential efficacy and absence of side effects [9]. Furthermore, they are said to be more beneficial to the human body than modern synthetic drugs [11]. In reality, there is a pressing need to better understand and investigate the effects of plant-derived medications. Kesum is a common plant from the Polygonaceae family that is frequently used in Asian cookery, particularly in Malaysia. Although the phytochemical components of this plant have been identified, the chemicals' lowering potential for the production of silver nanoparticles has yet to be investigated. As a result, this invention is concerned with the biogenic manufacture of silver nanoparticles utilising kesum leaves extract as a reducing agent, and it might be utilised to treat antibacterial wound healing.

INNOVATION DEVELOPMENT

Silver nanoparticles were created via a green biosynthesis approach using kesum leaf extract in the SILVERN cream. In the creation of silver nanoparticles, kesum leaf extract acts as a green bioreducing agent. The procedure is environmentally friendly since kesum leaf extract, a plant extract, replaces the usage of a harmful chemical reducing agent. SILVERN cream has been demonstrated to destroy a variety of pathogenic bacteria as well as heal wounds.

Experimental results from in vitro antibacterial assays, human skin cell culture, and in vivo animal testing [12] support this efficiency. As a result, SILVERN cream can be utilised as a topical antibacterial wound healing agent. Figure 1 depicts a SILVERN product and how the cream should be applied to the skin.

Figure 1: Picture of SILVERN and how it can be applied on our skin

The claims in the IPR support the product's originality, novelty, and uniqueness. This product is protected by two copyrights. First, the copyright (LY2020004347) is for an enhanced AgNP biosynthesis process using kesum (Persicaria odorata) leaf extract. Another copyright application has been filed for the antibacterial wound healing activity of biosynthesized silver nanoparticles utilising kesum extract (LY2021E05078). Due to the sustainable and green synthesis technique, SILVERN is significantly cheaper and safer for humans than other silver nanoparticles. SILVERN also performs two roles at the same time: antimicrobial and wound healing.

Because it was built based on multiple research projects, it has a high level of originality. The results of the experiment have been published in our publication [12]. SILVERN as an antibacterial wound healing agent is founded on a strong scientific principle in which the synthesis method of silver nanoparticles has been modified in the laboratory to obtain the best synthesis conditions, including a shorter synthesis time but a higher yield. The physicochemical properties of the biosynthesized silver nanoparticles are subsequently validated using numerous high-end instruments. Because the silver particles are so tiny, characterisation is critical to avoid particle aggregation or agglomeration (Figure 2). Figure 2 shows how the manufacturing of silver nanoparticles has been optimised in the lab (a). The yield of the optimised silver nanoparticles was then determined using a spectrophotometer, as shown in Figure 2. (b). Figure 2(c) depicts the morphology of silver nanoparticles, which are spherical in shape and range in size from 5 to 20 nm. Figure 2(e) indicates that silver nanoparticles have a significant antibacterial activity against two harmful microorganisms. The biosynthesized silver nanoparticles' in vitro cytotoxicity and in vivo wound healing activities were tested to ensure the product's usefulness. In terms of human cell toxicity, SILVERN has a low toxicity, as seen in Figure 3. (f). In vitro and in vivo wound healing activities demonstrated that SILVERN can heal the wound faster, as shown in Figures 3(g) and 3(h). As a result of these findings, it may be concluded that the SILVERN product can be employed as an effective antibacterial wound healing agent.

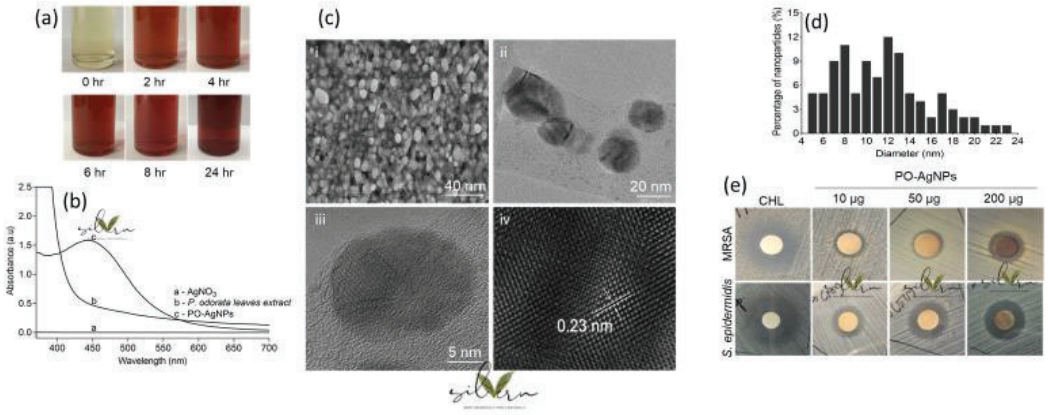

Figure 2: Characterization results of the biosynthesized silver nanoparticles. (a) colloidal silver nanoparticles formation based on different periods, (b) UV-Vis spectra, (c) scanning electron microscope (SEM) and transmission electron microscope (TEM) micrographs of the silver nanoparticles, (d) particle size, and (e) antibacterial activity of silver nanoparticles [12].

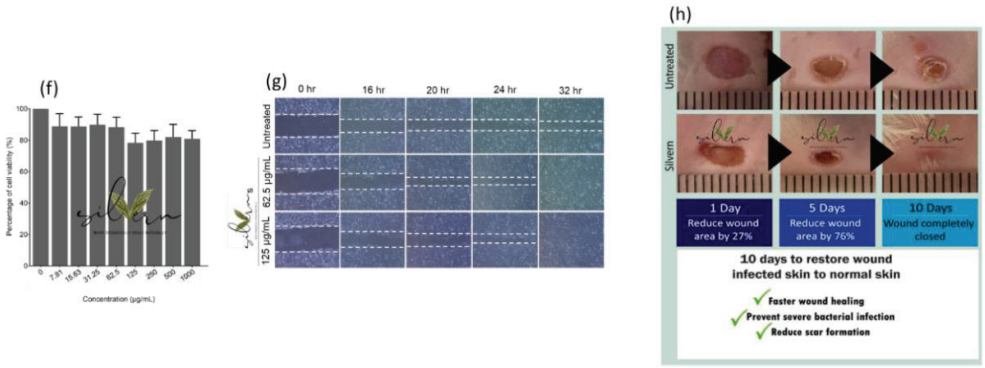

Figure 3: In vitro and in vivo analyses of silver nanoparticles: (f) cell viability, (g) scratch assay, and (h) in vivo wound healing assay on rat.

Because Malaysia is a tropical country with high humidity and temperatures, germs can grow easily on a variety of surfaces, including human skin. According to the Malaysian Ministry of Health (MOH), 15 percent of Malaysia's 3 million diabetic residents would suffer lower limb ulcers and wounds [13]. This number is expected to rise gradually, with an alarming 7 million adults in Malaysia impacted by diabetes, both diagnosed and undiagnosed, by 2025 [14]. The utilisation of kesum extract to biosynthesize silver nanoparticles will unlock new knowledge and technologies about other native plant herbs that may be used to synthesise AgNP and employed as an antibacterial wound healing agent.

In the forecast period, 2021-2028, the global wound care market is expected to increase at a CAGR of 6.1 percent, from $15.84 billion in 2021 to $24.01 billion in 2028. (www.fortunebusinessinsights.com). From 2020 to 2027, the global silver nanoparticles market is expected to increase at a CAGR of 15.7 percent, from $1.8 billion in 2019 to $4.1 billion in 2027. (www.alliedmarketresearch.com). The global antimicrobial additives market was estimated to be worth $4.7 billion in 2020, rising to $9.3 billion by 2030, with a CAGR of 7.1 percent from 2021 to 2030. (www.alliedmarketresearch.com). Silver nanoparticles, wound and antimicrobial agents have a favourable trend and a large market, indicating that SILVERN as

an antibacterial wound healing agent will be in high demand in the near future. Because of the COVID-19 pandemic, everyone is looking for an antimicrobial agent that can inhibit microbial growth and promote a healthy lifestyle.

Because wound care and antimicrobial products are intertwined, the market for both is enormous. As a result, combining the antibacterial and wound-healing properties of biosynthesized silver nanoparticles with plant extract is appropriate for this market. Furthermore, the biosynthetic approach to silver nanoparticles can cover a portion of the global silver nanoparticles market while also being more environmentally friendly and sustainable. Due of the COVID-19 pandemic, biosynthesized silver nanoparticles are also a solution to fulfil the rising demand for antimicrobial agents. To attain Technology Readiness Levels (TRL) 6 to TRL 9, the next step in the commercialization process is to seek precommercialization funding.

The SILVERN product for topical antibacterial application has reached TRL (Technology Readiness Level) stage 5 because the prototype was verified in the lab utilising in vivo investigations. As a result, in order to attain TRL 6 to 9, qualifying for a precommercialization fund is a high priority (development and deployment). The precommercialization fund will cover large-scale manufacturing in order to cut costs and gain Ministry of Health approval (MoH). The SILVERN product for wound healing, on the other hand, is presently at TRL3, and more preclinical and clinical testing is needed. To reach TRL4 to TRL9, precommercialization funding is also required to ensure that the product can be approved by the Ministry of Health.

SILVERN has a minimal manufacturing cost due to easy preparation processes that do not require chemicals, significant energy, or waste. Plant extract is used in the biosynthesis process, which is abundant in Malaysia and neighbouring countries such as Indonesia and Thailand. Experimental in vitro and in vivo research have revealed that the substance has immediate effects with excellent antibacterial and wound healing activity. Based on in vitro cytotoxicity and in vivo animal tests, the SILVERN is likewise safe for human skin, with no signs of skin irritation. The inclusion of silver nanoparticles, an inorganic antibacterial agent that prevents bacteria and other microbes from growing in the product, keeps the product stable for longer.

SILVERN inhibits bacterial growth in the wound region, preventing the wound from worsening. The preventative technique can help to keep bacteria from spreading throughout the community. Aside from that, the prevention technique can help the low-income community avoid greater care costs for infected wounds. By using kesum extract as a bioreducing agent for silver nanoparticles, water pollution caused by chemical reduction agents can be avoided. The use of biosynthesized silver nanoparticles is a green and long-term solution that can benefit our community.

Competitor of SILVERN is the antimicrobial or wound healing products at the clinics or pharmacies such as Betadine cream, guardian antiseptic cream and Bepanthen cream. Table 1 compares SILVERN with its competitors in the market. Betadine cream contains water soluble iodine, which can stain our cloth, as opposed to SILVERN cream, which is safe to use on skin. Because of its chemical composition, chloroxylenol and chlorhexidine in the most antiseptic cream have side effects such as skin irritation, itching, and other unpleasant skin consequences. SILVERN contains silver nanoparticles along with bioactive substances from plants that are safe for humans, as opposed to normal commercial silver nanoparticles. The SILVERN can be employed as an antibacterial agent in the future, as well as antifungus, antiviral, antibiofilm, and other applications.

Table 1: Comparison with competitor

Product image	Product name	Active ingredient	Product feature	Issue
	SILVERN Cream	Biosynthesized silver nanoparticles	Proven to kill bacteria, safe to human and can treat wound, and it can rejuvenate the skin	No skin irritation, no color stain, safe to the cells
	Betadine Cream	Povidone-iodine water soluble-ointment	Proven to kill bacteria and other microbes	Not proven to heal the wound. Yellowish stain that hard to be removed.
	Guardian Antiseptic Cream	Chloroxylenol 0.3%w/w, Triclosan 0.3%w/w	Guardian antiseptic cream is a dual protection against infection. It is suitable for cuts, abrasions, minor burns & wounds	Chloroxylenol has side effects such as skin irritant, and highly toxic to fish and cats.
	Bepanthen First Aid Kit Cream	Chlorhexidine hydrochloride	Specially formulated to help protect wounds and fight infection whilst soothing damaged skin	Chlorhexidine topical side effects include severe burning, itching, or redness; blistering or peeling

CONCLUSION

SILVERN is an alternative treatment for treating bacteria-infected wounds or preventing bacterial growth in the wound region. The invention's originality derives from the use of kesum leaf extract in the manufacture of silver nanoparticles. The invention is also founded on a strong scientific methodology that has been validated using

characterisation techniques, in vitro antibacterial and cell assays, and in vivo animal testing. The product has a large market, and it is believed that it will be able to replace several commercial wound healing and antibacterial products.

ACKNOWLEDGEMENT

The inventor would like to that Ministry of Higher Education Malaysia and Universiti Teknologi Malaysia for several research grants specifically UTM-Transdisciplinary Research Grant (UTM-TDR) Vot No. 06G72 and 06G86. Thanks to Platinum Coatings (M) Sdn Bhd for Research Grant Contract Vot No. 4C535.

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