



**Biological activities of mouth gel containing 6 β -acetoxysandaracopimaradiene-
1 α , 9 α -diol (KG6) from *Kaempferia galanga* rhizomes**

Anupon Iadnut

**A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy in Pharmaceutical Sciences**

Prince of Songkla University

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ชื่อวิทยานิพนธ์	ฤทธิ์ทางชีวภาพของเจลป้ายปากที่มีส่วนผสมของสาร 6 β -acetoxysandaracopimaradiene-1 α ,9 α -diol จากเหง้าเปราะหอม
ผู้เขียน	นายอนุพนธ์ เอียดนุช
สาขาวิชา	เภสัชศาสตร์
ปีการศึกษา	2567

บทคัดย่อ

จากการสำรวจพบว่าประชากรโลกร้อยละ 25 เคยประสบปัญหาเป็นแผลในช่องปากมาแล้วจากการใช้ยาพื้นบ้านพบว่าเปราะหอมเป็นพืชสมุนไพรที่ใช้บรรเทาอาการปวดและช่วยเร่งการสมานของแผล การศึกษามากมายพบว่าเปราะหอมมีฤทธิ์ช่วยในการสมานแผลอย่างดี อย่างไรก็ตาม การศึกษาสารบริสุทธิ์ในกลุ่ม isopimarane diterpenes ที่สกัดมาจากเหง้าของเปราะหอมยังมีการศึกษาที่น้อย ดังนั้นในงานวิจัยครั้งนี้จึงสนใจที่จะตรวจวัดคุณสมบัติทางชีวภาพที่เกี่ยวข้องกับการสมานแผลของสาร 6 β -acetoxysandaracopimaradiene-1 α ,9 α -diol (KG6) ที่สกัดได้มาจากเหง้าของเปราะหอมและทั้งนี้ได้ทำการพัฒนาสูตรตำรับเจลป้ายปากที่มีส่วนผสมของสารดังกล่าว และทำการตรวจวัดคุณสมบัติทางกายภาพและชีวภาพของเจลป้ายปากอีกด้วย ในงานวิจัยนี้ได้ทำการพัฒนาสูตรตำรับเจลป้ายปากที่มีส่วนผสมของ KG6 โดยใช้สารก่อเจลที่เรียกว่า sodium carboxymethylcellulose เป็นสารก่อเจลหลัก จากนั้นทำการพัฒนาสูตรตำรับจำนวนสามสูตรด้วยกันได้แก่ 0.10, 0.25 และ 0.50% w/w และทำการตรวจวัดความคงตัวทางกายภาพและชีวภาพผ่านสภาวะเร่งโดยการใช้อุณหภูมิสูงสลับต่ำ (Heating-cooling) และทำการตรวจวัดปริมาณ KG6 ที่อยู่ในเจลป้ายปากที่มีส่วนผสมของ KG6 ด้วยเทคนิคแก๊สโครมาโทกราฟี จากนั้นทั้งสาร KG6 และเจลที่มีส่วนผสมของ KG6 ได้ถูกนำมาทำการตรวจประเมินคุณสมบัติทางชีวภาพที่เกี่ยวข้องกับการสมานแผลได้แก่ การเพิ่มจำนวนของเซลล์ การเคลื่อนที่ของเซลล์และการต้านอนุมูลอิสระ โดยใช้เซลล์ไฟโบรบลาสต์ในช่องปากของมนุษย์ (human gingival fibroblast, HGF-1- ATCC CRL-2014) และการต้านการอักเสบโดยใช้เซลล์เพาะเลี้ยงแมคโครฟาจ (RAW 264.7) จากผลการทดลองพบว่าหลังจากนำเจลป้ายปากที่มีส่วนผสมของ KG6 ไปบ่มในสภาวะเร่งแล้ว รูปลักษณะ สี กลิ่น pH ความหนืด การแยกชั้น และปริมาณ KG6 ภายในเจลมีความคงตัว ไม่เปลี่ยนแปลงไปจากเดิม และผลทางชีวภาพพบว่าฤทธิ์ของสาร KG6 มีประสิทธิภาพที่สูงในการช่วยสมานแผล ในส่วนของฤทธิ์

ทางชีวภาพของเจลป้ายปากที่มีส่วนผสมของ KG6 พบว่า ที่สูตรตำรับ 0.50% w/w ให้ผลการต้านการอักเสบได้ดีที่สุดโดยมีค่า IC_{50} ที่ 557.5 ไมโครกรัม/มิลลิลิตร ซึ่งมีฤทธิ์ที่ใกล้เคียงกับเจลป้ายปากขาวละออที่เป็นเจลทดสอบควบคุมเชิงบวก และผลการแบ่งตัวของเซลล์พบว่าสูตรตำรับ 0.25% w/w มีแนวโน้มในการเพิ่มจำนวนของเซลล์อยู่ที่ร้อยละ 101.7 ± 1.2 ถึง 103.5 ± 1.4 ในขณะที่ผลของเจลป้ายปากต่อการเคลื่อนที่ของเซลล์พบว่าทั้งสามสูตรตำรับเพิ่มการเคลื่อนที่ของเซลล์ร้อยละ 94.7 ถึง 98.9 ซึ่งสูงเป็น 1.29 – 1.35 เท่า เมื่อเทียบกับเจลป้ายปากขาวละอที่ออกฤทธิ์ทำให้เซลล์เคลื่อนที่เพียงร้อยละ 73.5 มากไปกว่านั้นสูตรตำรับ 0.50% w/w มีฤทธิ์ในการต้านอนุมูลอิสระที่อยู่ภายใต้สภาวะ H_2O_2 -induced oxidative stress ดีที่สุดเมื่อเทียบกับสูตรตำรับอื่นๆ จากการศึกษาครั้งนี้แสดงให้เห็นว่าเจลป้ายปากที่มีส่วนผสมของ KG6 มีฤทธิ์ทางชีวภาพที่ช่วยส่งเสริมให้การสมานแผล ซึ่งมีความน่าสนใจที่จะนำเจลป้ายปากดังกล่าวมารักษาแผลในช่องปากต่อไป

คำสำคัญ: เปราะหอม, สมานแผล, การต้านการอักเสบ, การต้านอนุมูลอิสระ, เจลป้ายปาก

Thesis Title	Biological activities of mouth gel containing 6 β -acetoxysandaracopimaradiene-1 α ,9 α -diol from <i>Kaempferia galanga</i> rhizomes
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Major Program	Pharmaceutical Sciences
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ABSTRACT

Oral ulcers have an impact on 25% of the global population. *Kaempferia galanga* L. has been traditionally used for pain alleviation and wound repair. The wound healing activity of *K. galanga* extract has been previously described in various studies. However, the research on isopimarane diterpenes isolated from *K. galanga* is still limited. This study is to evaluate the biological activities related to wound healing potential of 6 β -acetoxysandaracopimaradiene-1 α ,9 α -diol (KG6), isolated from *K. galanga* as well as to investigate the physicochemical and biological properties of the formulated KG6 mouth gels. The KG6 mouth gels at 0.10, 0.25 and 0.50% w/w were formulated using sodium carboxymethylcellulose as a gelling agent, and their physicochemical and biological stabilities were assessed through a heating-cooling acceleration test. The quantification of KG6 contents in KG6 mouth gels was determined using gas chromatography. Both KG6 and KG6 mouth gels were evaluated for their wound healing property including cell proliferation, cell migration, and antioxidant activity (H₂O₂-induced oxidative stress) in human gingival fibroblast (HGF-1 - ATCC CRL-2014) (HGF-1). In addition, the anti-inflammatory activity against nitric oxide (NO) production was investigated in macrophage cells (RAW 264.7). The results showed that after incubating the KG6 mouth gels under heating-cooling acceleration condition, the physicochemical properties of KG6 mouth gels remained stable across various parameters, including appearance, color, smell, texture, pH, viscosity, separation, and KG6 content. The biological studies indicated that KG6 compound possessed good wound healing potential. The 0.50% KG6 mouth gel exhibited marked anti-inflammatory activity by inhibiting NO production with an IC₅₀

of 557.7 $\mu\text{g/ml}$, comparable to that of Khaolaor mouth gel, a positive control. The 0.25% KG6 mouth gel increased HGF-1 cell proliferation at 101.7 ± 1.2 to $103.5 \pm 1.4\%$, whereas all formulars of KG6 mouth gel enhanced HGF-1 cell migration to 94.7-98.9% which was higher than 1.29 to 1.35 times of Khaolaor mouth gel (73.5%). Moreover, 0.50% KG6 mouth gel also showed good antioxidant effect under H_2O_2 -induced oxidative stress. This study substantiates the significant biological activities related to wound healing property of KG6 mouth gel, highlighting its potential for oral ulcer treatment.

Keywords: *Kaempferia galanga*, Wound healing, Anti-inflammation, Antioxidant, Mouth gel

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LIST OF ABBREVIATIONS AND SYMBOLS

cm ²	squarecentimeter
CO ₂	carbon dioxide
Conc.	concentration
cP	centipoise
DMEM	dulbecco's modified eagle medium
DMSO	dimethyl sulfoxide
et al.	et alibi
EtOH	ethanol
FBS	fetal bovine serum
g	gram
GC	gas chromatography
HGF-1	human gingival fibroblast
IC ₅₀	inhibitory concentration at 50% of tested subject
Kg	kilogram
KG	<i>Kaempferia galanga</i>
LPS	lipopolysaccharide
MeOH	methanol
nm	nanometer
mg	milligram
min	minute
min ⁻¹	revolutions per minute
ml	milliliter
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NO	nitric oxide

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

<i>P</i>	<i>p</i> -value
pA*s	peak area
PBS	phosphate buffered saline
pH	potential of hydrogen
qs.	quantity sufficient
rpm	round per minute
RPMI	Roswell Park Memorial Institute 1640 medium
S.M.E.	standard error of the mean
w/w	weight by weight
%	percentage
°C	degree of Celsius
μL	microliter
μg	microgram
μM	micromolar
μm	micrometer

CHAPTER 1

INTRODUCTION

1.1 Background and Rationales

Ulcers are very common in health care issues and can be classified into two groups: acute (short term) and chronic (long term). Acute ulcers persist for no longer than three weeks. However, chronic ulcers persist for weeks and months (Sivapathasundharam et al., 2018). There are many causes of ulcers such as traumatic ulcers, infectious disease, chemotherapy, and autoimmune disease. Wound healing is a necessary physiological process for healing wounds from ulcers. This process is composed of four stages including the hemostasis stage, inflammatory stage, proliferative stage, and remodeling stage. At the end of the wound healing process, the wound from ulcer will be cured (Waasdorp et al., 2021). It is well known that when wounding occurs, short-term process of inflammation caused by the excessive release of the inflammatory mediators and radical oxygen species by the macrophages mainly impair and delay the process of wound repair (Sampson et al., 2012). Thus, the inhibition of reactive radical production is an important consideration in recruitment of fibroblasts which are attracted into the site to initiate the proliferative phase of wound healing process. The prevalent pharmacological agent employed in the treatment of oral ulcers is triamcinolone acetonide (TA) oral paste. However, reported side effects associated with TA oral paste necessitate exploration for alternative therapeutic options (Coondoo et al., 2014). Consequently, there is significant interest in investigating medicinal plants as potential alternatives for the treatment of oral ulcers.

The medicinal plant called *Kaempferia galanga* L. (Zingiberaceae) is one of the interesting natural plants that can improve in the treatment of ulcers. In folk medicine, *K. galanga* has been used as herb that can treat many diseases such as dyspepsia,

inflammation, and analgesic. The decoction or powder of rhizomes can also applied for the treatment of many symptoms including cold, cough, sore throat, asthma, pectoral, headache, toothache, dyspepsia, abdominal pain, diarrhea, and postpartum care (Amalraj and Gopi, 2021). The leaves and rhizomes of *K. galanga* are edible as raw vegetables, cooked, or used as a spicy ingredient. In our laboratory, ten isopimarane diterpenes were isolated from the hexane fraction of *K. galanga* ethanol extract and tested for anti-inflammatory activity. The result showed that all ten isopimarane diterpenes compounds represent anti-inflammatory activity. The compound name 6 β -acetoxysandaracopimaradiene-1 α ,9 α -diol (KG6) are very interesting because KG6 presented the highest anti-inflammatory activity which showed 7.7 μ M of IC₅₀ on nitric oxide (NO) production (P. Tungcharoen et al., 2020). The objective of this study is to formulate gel containing KG6 compound and investigate the wound healing activity of KG6 gel and its pure compound as well as physicochemical and biological stabilities.

1.2 Review of Literature

1.2.1 Wound healing

The wound healing process can be characterized by four overlapping phases: hemostasis, inflammatory, proliferative and remodeling phases, each of which contributes to the repair and organization of the tissue structures, leading to increase tensile strength in the damaged tissue, either partially or completely, depending on the severity of the wound (Wild et al., 2010). Upon the occurrence of a lesion, only a few minutes, platelets migrate to the wound area and stop bleeding of the blood. Platelets release chemokines to attract immune cells to the affected area. After a few hours to 48 hours, the inflammatory stage was started by the immune cell consisting of neutrophils, monocytes, and macrophages. Consequently, the proliferation stage begins at 48 hours and can be persisted up to 14th days after the onset of the lesion. The fourth phase of healing process is remodeling, which begins at two to three weeks after the wound

occurred and this stage can continue for one year or more (Gonzalez et al., 2016). The stage of wound healing is shown in Figure 1.

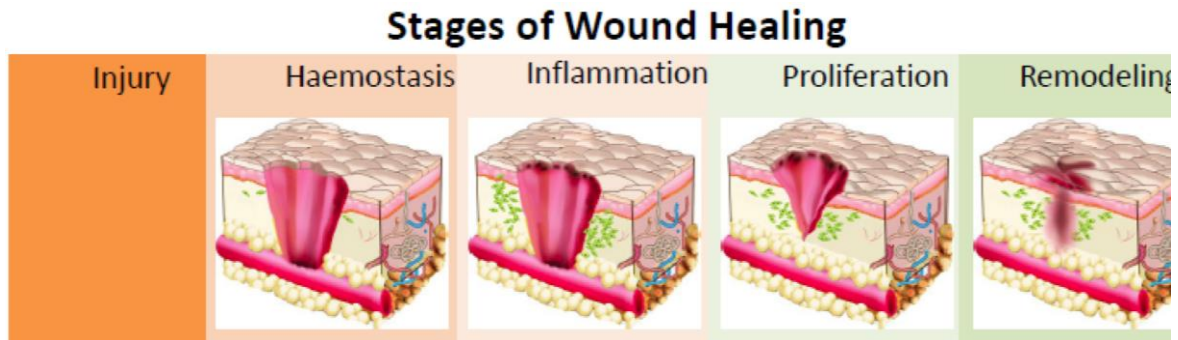


Figure 1. Steps of wound healing process (Lin et al., 2017)

1.2.1.1 Steps of wound healing

Wound healing requires cells to proliferate and migrate, the activities originated by growth factors (contusion hormones) that are discharged from the damaged cell. Healing starts in two important ways: reactivation and fascia. These arise ancillary on the sort of wound that is injured and the seriousness of the perniciousness. Reactivation is the displacement of deceased or destroyed cells with the identical cell genre as aforetime. Most skin prejudice (scratch and nominal burns) are treated by reactivation. Fibrosis is the displacements of the wound with scar tissue, mainly consisting of collagen procreate by fibroblasts (Kenneth, 2010). The healing of wounds occurs in four stages (Waasdorp et al., 2021).

1.2.1.2 Hemostasis and inflammation

First, upon a wound's occurrence, there is a release of histamine and other inflammatory chemicals by mast cells, damaged tissue cells, macrophages, and other cells, which leads to the dilation of capillaries. This dilation allows white blood cells (such as monocytes and neutrophils), abundant blood plasma containing antibodies, specialized clotting factors, and other substances to leak into the wound site. The clotting proteins then form clumps that effectively stop the loss of blood, seal the wound, bring the margins of the injured tissue together, and protect against the diffusion of toxins, bacteria, or other harmful substances into the surrounding area. The blood clots covering the wound dry and form a scab, which temporarily closes the wound and prevents infection. Beneath the scab, macrophages begin to phagocytose some pathogens and cell debris. (Elaine, 1995; Kenneth, 2010).

1.2.1.3 Proliferation

The purpose of the proliferation stage is to facilitate wound closure through three primary processes: angiogenesis, fibroplasia, and reepithelialization. These processes commence within the microenvironment of the wound within 48 hours to 14 days following its onset. Angiogenesis occurs within the extracellular matrix of the wound bed through the migration and mitogenic stimulation of endothelial cells. The formation of new blood vessels is crucial during this stage, as they facilitate the transportation of nutrients, oxygen, and immune cells to the wound area. Fibroplasia involves the formation of fibrous tissue, which initiates with the development of granulation tissue characterized by fibroblast proliferation. Fibroblasts play an important role in collagen production, contributing to the formation of intricate structures of fibrous tissue. During the initial stage, type III collagen is predominantly synthesized by fibroblasts within the granulated tissue. Reepithelialization is predominantly organized by keratinocytes, which undergo proliferation and migration towards the wound site. This process involves a combination of cell proliferation

during the proliferation stage and the migration of cells towards the wound surrounding area (Gonzalez et al., 2016).

1.2.1.4 Remodeling

The fourth stage of wound healing is the remodeling stage, which may span from two weeks to over 2 years. The objective of this stage is to enhance maximum tensile strength through three processes: reorganization, degradation, and resynthesis of the extracellular matrix. Reorganization occurs early in the remodeling stage, involving the restructuring of granulation tissue into scar tissue. Scar tissue exhibits reduced cellularity and vascularity but demonstrates a progressive increase in collagen fiber density. Degradation proceeds through the breakdown of collagen type III, leading to an increase in the synthesis of collagen type I. This process results in the thickening of collagen fibers and their alignment in parallel, thereby augmenting tissue tensile strength. In the final process, fibroblasts undergo a phenotypic change and begin expressing smooth muscle action, transitioning into myofibroblasts. Myofibroblasts initiate the resynthesis of the extracellular matrix, generating large quantities of various components including collagens, elastin, reticulin, fibronectin, laminin, and other extracellular matrix constituents (Gonzalez et al., 2016).

1.2.1.5 Overlapping stage of wound healing.

Wound healing is a complex process that follows four partially overlapping stages: hemostasis, inflammation, proliferation, and remodeling, with each stage involving different cell types and molecules. The process begins with the hemostasis stage, where platelets migrate to the wound site, stop bleeding, and release chemokines to recruit neutrophils. Subsequently, neutrophils eliminate bacteria and clear dying cells surrounding the wound. In the inflammation stage, neutrophils are the first cells to infiltrate the wound bed, releasing chemokines and proinflammatory

cytokines. These molecules attract and activate other immune cells, such as lymphocytes and M1 macrophages. Macrophages release proinflammatory cytokines and growth factors to stimulate tissue regeneration. During the proliferation stage, keratinocytes, endothelial cells, and fibroblasts migrate, proliferate, and differentiate in response to growth factors secreted by macrophages. In the remodeling stage, M2 macrophages and myofibroblasts play crucial roles. M2 macrophages secrete matrix proteins, while myofibroblasts produce collagen types I and III as well as matrix components to facilitate tissue renewal. The overview of all four stages and important cells is shown in Figure 2.

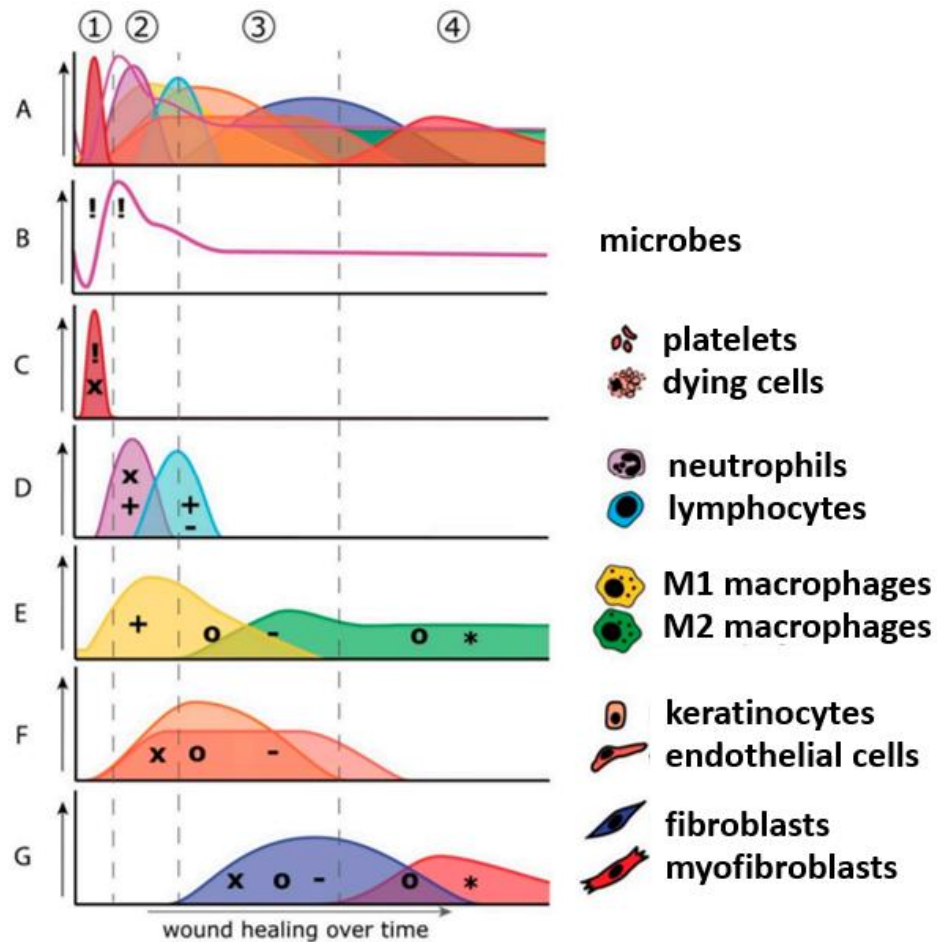


Figure 2. Overlapping phase of wound healing and relative contribution of cells
(Waasdorp et al., 2021)

1.2.2 *Kaempferia* plants

Kaempferia plants are distributed worldwide, composed of 60 species. Southeast Asian are the region that has highest diverse members of *Kaempferia* plants. Several *Kaempferia* species are used widely in folk medicine, including *K. parviflora*, *K. pulchra*, and *K. galanga* (Figure 3). In Laos and Thai, traditional medicines derived from *K. parviflora* rhizomes were reported for treatment of inflammation, hypertension, erectile dysfunction, abdominal ailments, and improvement of the vitality and blood flow (Akase et al., 2011; Yenjai et al., 2004). *K. pulchra* is used widely as a carminative, diuretic, deodorant, euglycemic, and can be treatment of urinary tract infection, fever, and coughs (Ekor, 2014). *K. galanga* is sold as an industrial plant in the market, and its rhizome has been used as a flavor spice of many food (Yao et al., 2018). The rhizome of *K. galanga* is used as a anti-cough, expectorant, anti-pyretic, diuretic, anabolic, carminative, as well as for curing gastrointestinal ailment, asthma, wounds, and skin disease (Sivarajan et al., 1994).

In this study, we would like to make the gel containing *Kaempferia* compound that improved wound healing process. *K. galanga* can be used in folk medicine treatment of wound. Hence, *K. galanga* is very interesting in studying in improving wound healing process.



Figure 3. The examples of *Kaempferia* species
(Elshamy et al., 2019)

1.2.2.1 Introduction of *Kaempferia galanga* L.

K. galanga is the plant in the Zingiberaceae family (Figure 4). Its' common name is aromatic ginger or sand ginger. The parts used of this plant are rhizome, root, and leave. It is widely cultivated throughout Southeast Asia. Moreover, it is a source of valuable bioactive compounds, *K. galanga* is famous for its medicinal as well as edible use. As folk medicine, the rhizome part of *K. galanga* is used for treatment of microbial infection, hypertension, asthma, rheumatism, indigestion, cold and headache, relief abdominal pain and toothache (Kanjapothi et al., 2004; Othman et al., 2002). Chemical constituents isolated from *K. galanga* represent different pharmacological activities such as antioxidant, antimicrobial, analgesic, anti-inflammatory, sedative, vasorelaxant, nematocidal, mosquito repellent, larvicidal, antiprotozoal and wound healing activities (Kanjapothi et al., 2004; Othman et al., 2002).



Figure 4. Botanical exemplification of *Kaempferia galanga*

1.2.2.2 Phytochemical of *Kaempferia galanga* L.

There are many studies on the identification and isolation of the chemical compounds of *K. galanga*. The important chemical compounds isolated from *K. galanga* extracts were 2-propeonic acid; pentadecane; ethyl paramethoxycinnamate; 3-carene; cadinenes; 3, 4-methoxyphenyl; borneol; 3H-3a, 7-methanoazulene; heptadecane; 1-methyl, 2-(1-methylethyl); 1,6-cyclodecadienen; 8-heptadecane; camphene; tetradecane; δ -limonene; α -pinene; germacrene; β -pinene; cyclooctene; 1-methyl-3-(1-methylethyl); γ -elemene; ethyl cinnamate; eucalyptol or 1, 8 cineole; cymene; α -terpineol; α -gurjunene; β -caryophyllene; kaempferide; cinnamaldehyde; and kaempferol (Koh et al., 2009; Othman et al., 2002; Sutthanont et al., 2010).

Our previous study by Tungcharoen and co-workers (P. Tungcharoen et al., 2020) reported that ten chemical compounds of *K. galanga* were isolated from the hexane fraction. Ten compounds of *K. galanga* were two new isopimarane diterpenes, 1 α -hydroxy-14 α -methoxyisopimara-8(9),15-diene (7) and 1 α ,14 α -dihydroxyisopimara-8(9),15-diene (9) and eight known isopimarane diterpenes including (-)-sandaracopimaradiene (1), 6 β -acetoxysandaracopimaradiene-9 α -ol (2), sandaracopimaradiene-7 β ,9 α -diol (3), sandaracopimaradiene-1 α ,9 α -diol (4), 6 β -acetoxysandaracopimaradiene-9 α -ol-1-one (5), 6 β -acetoxysandaracopimaradiene-1 α ,9 α -diol (6), 6 β ,14 α -dihydroxyisopimara-8(9),15-diene (8), and 6 β ,14 β -dihydroxyisopimara-8(9),15-diene (10) as shown in Figure 5.

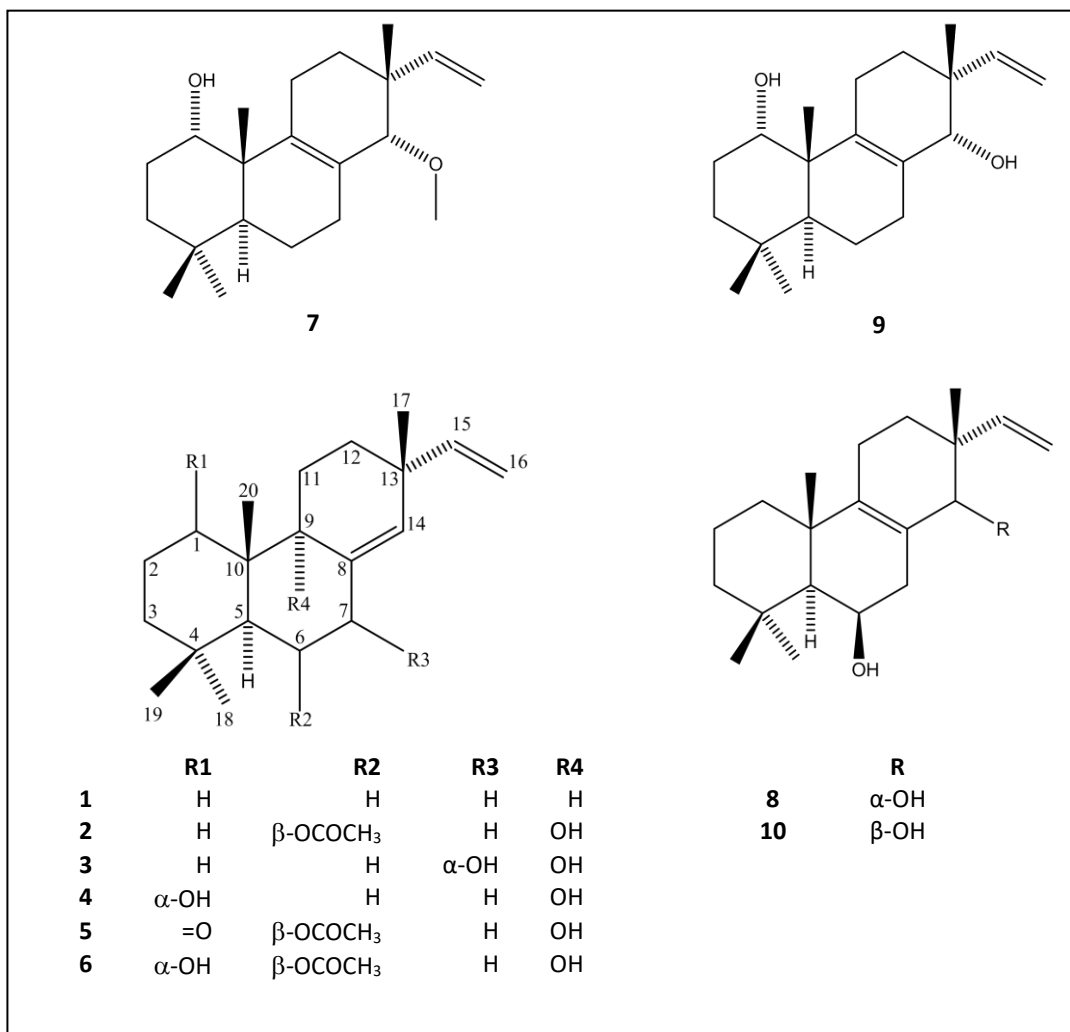


Figure 5. Isopimarane diterpenoids isolated from *K. galanga*.
(Tungcharoen et al., 2020)

1.2.2.3 Physical and chemical properties of KG6 and its related compounds

6 β -Acetoxysandaracopimaradiene-1 α ,9 α -diol (**KG6**) was isolated from rhizome of *K. galanga*. Its structure is diterpene. In general, diterpenes are less stable bearing more oxygen functional groups rendering them unsuitable for storage over longer periods. The solubility of diterpenes are insoluble in water but soluble in methanol, ethanol, chloroform, diethyl ether, as well as dimethyl sulfoxide (DMSO). DMSO was used as a solvent of diterpenes to perform experiments to determine their activity in living organisms or cell lines. The detection of diterpenes is often used by gas chromatography (GC) and normal-phase TLC. TLC can visualize diterpenes by spraying them with phosphomolybdic acid solution or cerium sulfate solution followed by heating (Arroo, 2007).

One of the well-known diterpene is sandaracopimaradiene, this compound is soluble in alcohol and insoluble in water. It has anthelmintic activity and antispasmodic activity (Van Puyvelde et al., 2018). Another compound is Kahweol, this compound was extracted from coffee beans. Kahweol is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF). The solubility of Kahweol in ethanol and DMF is approximately 5 mg/ml and approximately 3 mg/ml in DMSO. Kahweol presents many biological properties including anti-inflammation, anti-carcinogenesis, inducing apoptosis, anti-angiogenesis, anti-diabetic, and anti-osteoclastogenesis (Ren et al., 2019).

1.2.2.4 Biological studies of *Kaempferia galanga* L.

1) Anti-inflammatory activity

Study from Yao and co-workers demonstrated that diarylheptenoids isolated from *K. galanga* inhibited nitric oxide (NO) production in lipopolysaccharide (LPS)-induced macrophage RAW 264.7 cell line (Yao et al., 2018). Moreover, Tungcharoen and co-workers reported that compounds isolated from *K. galanga* inhibited the mRNA expression in gene-related inflammation including inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in LPS-induced macrophage RAW 264.7 cell line (P. Tungcharoen et al., 2020). There are numerous studies in animal models for testing anti-inflammatory activity. Ethyl-*p*-methoxycinnamate isolated from *K. galanga* inhibited inflammation in animal blood by reducing interleukin-1, tumor necrosis factor- α and angiogenesis (Umar et al., 2014). The methanol extract from *K. galanga* at concentration of 100 and 200 mg/kg exhibited anti-inflammatory activity and seemed to be dose- and time-dependent in experimental animals (Riditid et al., 2008). Sulaiman and co-workers reported that when injected the aqueous extracts of *K. galanga* in subcutaneous of rats at the concentration of 30, 100 and 300 mg/kg, the results showed the significant anti-inflammatory effect in rats in dose-dependent manner (Sulaiman et al., 2008).

2) Cytotoxic and antineoplastic activity

Swapana and coworkers have reported a new unusual seco-isopimarane, kaemgalangal A, and twelve known isopimara-8(14),15-diene compounds isolated from *K. galanga* chloroform fraction. They demonstrated the cytotoxic activity of these constituents against human cervical (HeLa) and oral squamous (HSC-2) cell carcinomas (Swapana et al., 2018). Methanolic extracts of *K. galanga* represented inhibitory impact on cell proliferation of human cardiac fibroblast and human T cell leukemia at doses more than 250 μ g/ml (Kirana et al., 2002). Ethyl-*p*-methoxycinnamate which was isolated from *K. galanga* exhibited the inhibitory effect on cell proliferation of human hepatocellular liver carcinoma (Hep G2 cell line) (Liu et

al., 2010). The bioactive compound ethyl *p*-methoxy cinnamate (EPMC) which is extracted from rhizome of *K. galanga* exhibited moderate cytotoxic activity against human CCA tumor (CL-6) cell line (Amuamuta et al., 2017).

3) Antimicrobial activity

Leaves and rhizomes of *K. galanga* were extracted with acetone and tested for anti-bacterial activity. The results showed that acetonic extract of *K. galanga* inhibited Gram positive bacteria such as *Staphylococcus aureus*, *Bacillus cereus* and Gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae* by using disc diffusion method (Dash et al., 2014). In the study from Kochuthressia and co-workers, they used various solvents for extraction of *K. galanga* including methanol, ethanol, chloroform, petroleum ether and water. The antimicrobial activity for all extracts were tested, the results showed that ten bacterial pathogenic species (*Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus cereus*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Vibrio cholerae*) and four fungal species (*Aspergillus niger*, *A. flavus*, *A. fumigatus* and *Candida albicans*) were susceptible to all extracts from *K. galanga* (K P et al., 2012).

1.2.3 Ulcers

1.2.3.1 Traumatic ulcers

Traumatic ulcers are very common and acute in nature. The cause of traumatic ulcers is usually physical, thermal, or chemical trauma to the area of skin causing tissue damage and resultant ulceration. Physical trauma can be caused by sharp objects, hot kitchen utensils, and local radiotherapy or chemotherapy (Sivapathasundharam et al., 2018). Traumatic ulcers usually heal within 7-10 days when the cause is removed. If the ulcers do not heal within two weeks, a biopsy should be performed to rule out a deep of fungal infection or malignancy. One of the most mucous areas that ulcers occur is oral.

1.2.3.2 Oral ulcers

Oral mucosa consists of three layers including oral epithelium layer, lamina propria, and submucosa (Figure 6). Normally, oral ulcers appear from the oral epithelium layer to lamina propria (Waasdorp et al., 2021). Oral ulcers can be acute or chronic. In acute oral ulcers can be caused by aphthous ulcers, herpetic ulcers, and chances. Moreover, chronic ulcers can be caused by odontogenic infection, malignant ulcers, gummas and some chronic diseases. Lips are a common site for hot burns and seen among young children. Burn injuries from hot food are small and localized to the hard plate, lips, and tongue. This area is present with tenderness that can be easily damaged and resulting in erythema which can develop into ulcers. The oral ulcers take several days to heal depending on the extent of injury. The popular oral ulcers that involve in non-infection ulcers are recurrent aphthous stomatitis (RAS) and chemotherapy and radioactive induced oral ulcers (Edgar et al., 2017; Naidu et al., 2004).

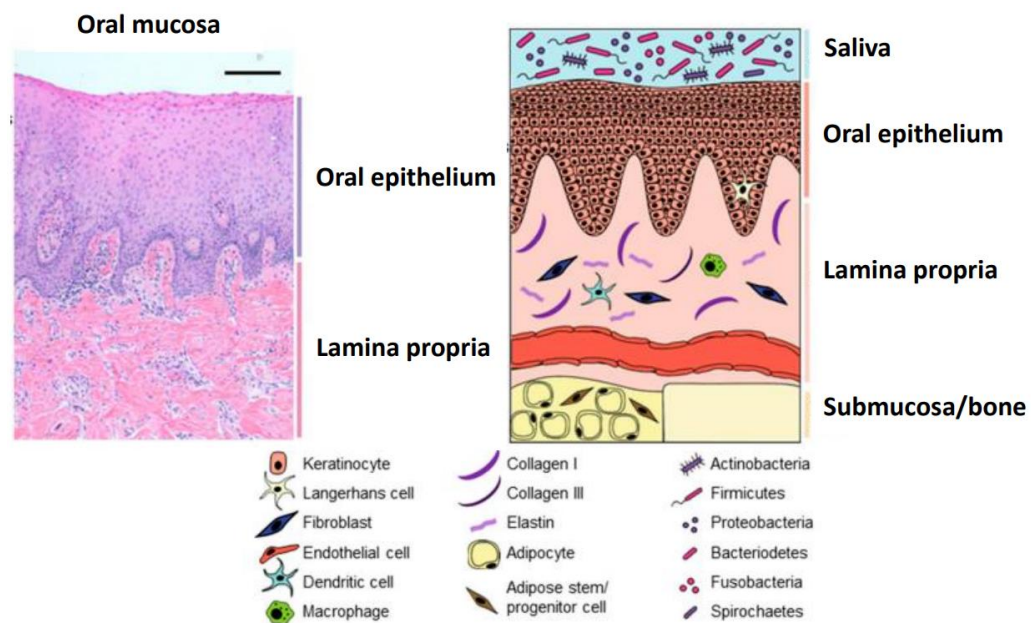


Figure 6. Histology of oral mucosa and its components (Waasdorp et al., 2021)

1) Recurrent aphthous stomatitis (RAS)

Recurrent aphthous stomatitis (RAS), also known as canker sores, is the most common disease of the oral membrane. Patients with RAS are unable to prevent, and the clinical appearance of RAS is characterized by occurred episodes of solitary or multiple painful ulceration without association with systemic diseases. The etiology and pathogenesis of RAS remain unclear. However, there are many factors that are involved in this disease including a positive family history, food hypersensitivity, smoking cessation, psychological stress and immune disturbance (Akintoye and Greenberg., 2014; Gallo et al., 2009). RAS can be classified into three forms: minor (>70% case), major (10%), and herpetiform (10%) (Edgar et al., 2017). These forms differ in morphology, distribution, severity, and prognosis (Table 1). Thus, all forms of RAS have a significant impact on quality of life and interfere the behavior in daily life.

Table 1. Clinical appearance of minor, major, and herpetiform recurrent aphthous stomatitis (RAS) (Edgar et al., 2017).

Gender predilection	Minor RAS	Major RAS	Herpetiform RAS
	Equal	Equal	Female
Morphology	Round or oval lesions Gray-white pseudomembranes Erythematous halo	Round or oval lesions Gray-white pseudomembranes Erythematous halo	Small, deep ulcers that commonly converge Irregular contour
Distribution	Lips, cheeks, tongue, floor of mouth	Lips, soft palate, pharynx	Lips, cheeks, tongue, floor of mouth, gingiva
Number of ulcers	1-5	1-10	10-100
Size of ulcers	<10mm	>10mm	2–3mm
Prognosis	Lesions resolve in 4-14 days without scarring	Lesions persist >6 weeks high risk of scarring	Lesions resolve in <30 days scarring uncommon

2) Chemotherapy and radioactive-induced oral ulcers

Chemotherapy and radiation therapy are the most widely used for the treatment of cancer. About 40% of patients that are treated with cancer with chemotherapy develop mucositis. The risk of developing mucosal injury increases with number of chemotherapy cycles and previous episodes of chemotherapy-induced mucositis. The pathogenesis of chemotherapy induces mucositis is not fully elucidated, but it is thought to have two mechanisms: direct mechanism and indirect mechanism, caused by chemotherapy and radioactive. Direct mucositis, the epithelial cells of the oral cavity are rapid turnover, usually 7 to 14 days. The chemotherapy and radioactive effect to the maturation and cellular growth of epithelial cells, causing changes to normal turnover and cell death. The indirect mucositis can cause by microbial infections because of the suppressed immune system by chemotherapy and radioactive treatment (Naidu et al., 2004).

1.2.4 Commercial products for the treatment of oral ulcers

1.2.4.1 Triamcinolone acetonide (TA)

Triamcinolone acetonide (TA), categorized as a medium to high potency corticosteroid, is a fluorinated prednisolone derivative and is regarded as an intermediate-acting glucocorticoid. It exhibits efficacy in the management of various dermatoses, asthma, and allergic rhinitis, and is employed in alleviating the signs and symptoms associated with numerous oral inflammatory conditions, including recurrent aphthous stomatitis (RAS). Triamcinolone acetonide dental paste is utilized as adjunctive therapy for the temporary relief of symptoms linked to oral inflammation and gingival disorders. However, TA is a member of steroid drug group which shows many side effects such as epidermal thinning, melanocyte inhibition, increases susceptible bacterial infection, allergic or irritant, perioral dermatitis, steroid addiction, and others (Coondoo et al., 2014).

1.2.4.2 Khaolaor mouth gel

Khaolaor mouth gel is the herbal product consisting of *Kaempferia galanga*, *Qurecus infectoria*, menthol, peppermint oil and other herbs which can treat oral ulcers by increasing wound healing process and decreasing inflammation condition. The recommended application method for Khaolaor mouth gel involves dispensing the gel onto an approximately 0.6 cm cotton swab or fingertip and subsequently applying a thin layer to the affected area. The cotton swab with Khaolaor mouth gel should be applied to the wound before bedtime to facilitate prolonged contact and healing throughout the night. If deemed necessary, additional applications of the medication can be administered during the day, preferably 2-3 times after meals (Khaolaor, 2021).

1.2.5 Oral semisolid dosage form and mucoadhesive dosage forms

Pharmaceutical semisolid forms may be defined as topical products that can apply onto the skin or accessible mucous membranes to provide localized or systemic effects for treatment of several diseases. In general, semisolid dosage forms are complex formulations having complex structural materials. They are usually composed of two phases (oil and water), one of which is a continuous (external) phase and the other a dispersed (internal) phase. The active compounds that are used in semisolid dosage form are often dissolved in one or both phases. The optimal properties of semisolid dosage forms are smooth texture, no gritty, no dehydrating, elegant in appearance, no irritating, no react with skin or membrane, miscible with skin secretion, easily applicable, and high aqueous wash ability (Gupta & Garg, 2002).

Mucoadhesive dosage forms are designed to increase retention time at the area of application, providing a controlled release of the drug for improved treatment outcomes. Mucoadhesive dosage forms offer several benefits over other drug-controlled release systems such as extend retention time, increase drug penetration, escaped first pass metabolism, delivery to local area, and patient acceptability.

Oral semisolid and mucoadhesive dosage forms have garnered significant interest in recent years. Oral semisolid dosage forms include ointments and cream. Additionally, mucoadhesive dosage forms consist of tablets, films, patches, and gels (Bhalerao & Shinde, 2013).

1.2.5.1 Types of conventional semisolid dosage forms and mucoadhesive dosage forms

1) Ointments

They are soft hydrocarbon based semisolid preparation, composed of fluid hydrocarbon dispersed in a matrix of higher melting solid hydrocarbon petrolatum being a tasteless, odorless, unctuous material with a melting range. The ointment base needs to be heated to above its melting temperature before adding the other ingredients. Mixing speeds and shear can be increased when the ointment base is liquid, to uniformly disperse the active ingredients. Ointment base is generally poor ability on drug delivery system (Lunter & Daniels, 2020).

2) Creams

They are viscous semisolid emulsion systems with opaque appearance as contrasted with translucent ointments. Consistency and rheological character depend on the cream is water in oil (w/o) or oil in water (o/w). The o/w creams are elegant drug delivery system, satisfying in both appearance and feel post application. O/W creams are non-greasy and are washable. They are good for most topical purposes and are considered particularly suited for application to oozing wounds and can be applied to the site of injury to speed up the healing process on infection wound (Lunter & Daniels, 2020).

3) Gels

Gels are semisolid systems in which a liquid phase is constrained within a 3-D polymeric matrix (consisting of natural or synthetic polymer) having a high degree of physical or chemical cross-linking. Gels are composed of two components which are gelling agent and its solvent. Gel can be classified based on gelling agents which are inorganic gels and organic gels. They can be used for medication, lubrication, and cosmetic application. The characteristics of gel are swelling, syneresis, ageing, structure, and rheology. Gel is widely used for oral semisolid dosage form to apply into the oral cavity (Lunter & Daniels, 2020).

4) Tablets

Mucoadhesive tablets adhere to the oral mucosa, where they remain until completely dissolved. The advantage of mucoadhesive tablets lies in their ability to facilitate efficient drug absorption and enhance drug bioavailability owing to their high surface area and direct contact with the mucosal layer. These tablets are often preferred due to their ability to sustain drug release over an extended period, resulting in reduced dosing frequency and improved patient compliance. However, a notable disadvantage of this formulation is the potential for unpleasant odor and taste (Bhalerao & Shinde, 2013).

5) Films

Mucoadhesive films are often preferred over tablets due to their flexibility and comfort. Additionally, they can be easily washed away and removed by saliva. In cases of oral disease, these films also serve to protect the wound surface and alleviate wound-associated pain. Another advantage of mucoadhesive films is the ability to tailor the duration of their active presence (Bhalerao & Shinde, 2013).

6) Patches

Patches are laminates composed of an impermeable backing layer, a drug-containing reservoir layer, from which the drug is released in a controlled manner, and a mucoadhesive surface for attachment to mucosal tissues. Patch systems are commonly employed in transdermal drug delivery. Two methods utilized in the preparation of adhesive patches are solvent casting and direct milling (Bhalerao & Shinde, 2013).

1.2.6 Evaluation of mucoadhesive dosage forms

1.2.6.1 pH measurement

The pH of various semisolid dosage forms are determined by using digital pH meter (Patil et al., 2019). Probe of digital pH meter is directly dipped into 30 grams freshly prepared gel that contain in a collected glass bottle. Each formulation was measured in triplicate and average values are calculated.

1.2.6.2 Viscosity measurement

Brookfield digital viscometer can be used to measure the viscosity of prepared semisolid dosage form (Saroja et al., 2013). The gel is carried out by rotating the gel with spindle number F at 10 rpm. The consistent dial readings were recorded, and the viscosity of gel was obtained by triplicate reading.

1.2.6.3 Spread ability

Spread ability refers to the extent of area to which gel readily spreads on application. It is determined by wooden block and glass slide apparatus (Patil et al., 2019). Spread ability is assessed using a wooden block and glass slide apparatus. The duration in seconds required for two slides to separate from the gel placed between them under a specific load represents. A shorter separation time indicates better spread ability.

1.2.6.4 Homogeneity

All developed semisolid dosage forms are tested for homogeneity by visual inspection after the gels have been set in the container. They are tested for their appearance and presence of any aggregates (Ganesh et al., 2013).

1.2.6.5 Grittiness

All the semisolid dosage forms are checked microscopically for the presence of any particulate matter (Patil et al., 2019). The incomplete dissolution of a drug or active compound can result in the presence of particles within the gel. Furthermore, these particles may contain impurities derived from other components such as solvents, gelling agents, and stabilizers.

1.2.6.6 Extrudability

The semisolid dosage forms are filled in collapsible tubes, after being set in the containers. The extrudability of gel formulations are determined in terms of weight required in grams to extrude 0.5 cm. ribbon of gel in 10 sec (Thorat & Rane, 2010).

1.2.6.7 Stability test

Stability study is carried out by heating-cooling cycling test (Patil et al., 2019). The gel is incubated at the temperature of 40-45°C for 24 h to one month, then cooling at 4°C for 24 h to one month. Finally, the changes of appearance, color, smell, and texture were observed.

1.3 Objectives of the research

1.3.1 To investigate wound healing activity including cell proliferation, cell migration, anti-NO production and anti-oxidation of compound KG6 in cell-based assay.

1.3.2 To develop the formulation of mouth gel containing compound KG6 (KG6 mouth gel) and evaluate the physicochemical and biological stabilities of KG6 mouth gels.

1.3.3 To investigate wound healing activity including cell proliferation, cell migration, anti-inflammation and antioxidation of KG6 mouth gels in cell-based assay.

CHAPTER 2

RESEARCH METHODOLOGY

2.1 Chemical reagents and Equipments

2.1.1. Chemical reagents

1) Dulbecco's modified eagle medium (DMEM)	Gibco, USA
2) Roswell Park Memorial Institute (RPMI) 1640 Medium	Gibco, USA
3) Fetal bovine serum (FBS)	Gibco, USA
4) Penicillin-streptomycin	Gibco, USA
5) Phosphate buffer saline (PBS)	Gibco, USA
6) Lipopolysaccharide (LPS)	Sigma, USA
7) 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)	Gibco, USA
8) 0.25% Trypsin-EDTA	Gibco, USA
9) Griess reagent	Invitrogen, USA
10) Trypan blue	Gibco, USA
11) Ethanol	Union Chemical
12) Glycerin	Sigma, USA
13) Sodium carboxymethyl cellulose (SCMC)	Sigma, USA
14) Dimethyl sulfoxide (DMSO)	Sigma, USA
15) Sodium benzoate	Sigma, USA
16) Indomethacin	Sigma, USA
17) Allantoin	Sigma, USA
18) Khaolaor mouth gel	Khaolaor laboratories Co., Ltd., Thailand
19) Triamcinolone acetone (TA) oral paste	Nida Pharma, Thailand

2.1.2. Equipments

1) 24 well-plate	SPL life sciences, Korea
2) 96 well-plate	SPL life sciences, Korea
3) Flask 25 ml	SPL life sciences, Korea
4) Flask 75 ml	SPL life sciences, Korea
5) Microplate reader	BMG LABTECH: SPECTROstar
6) Centrifuge	Kubota 5922
7) 25 ml Centrifuge tube	SPL life sciences, Korea
8) 50 ml Centrifuge tube	SPL life sciences, Korea
9) Auto pipette	Pipetman, France
10) Pipette tips	KIRGEN
11) Biosafety cabinet-ESCO	ISOCIDE
12) Biosafety cabinet-FASTER	ISOCIDE
13) Biosafety cabinet (AIRTECH)	SUZHOU ANTAI AIRTECH, China
14) CO ₂ incubator	Thermo scientific
15) pH meter	CyberScan pH510
16) Viscometer	Brookfield engineering laboratories, USA
17) Gas chromatography	Agilent Technologies 7890A GC system
18) Microscope	Nikon Eclipse TS100

2.2 Methods

2.2.1 Plant material and isolated KG6 compound

The rhizomes of *K. galanga* were provided from Prachinburi province, Thailand, in November 2011 and were subsequently identified by Prof. Dr. Chayan Picheansoonthon, from the Royal Society of Thailand, Bangkok, Thailand. The voucher specimen was SKP 201110701 and was kept at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. The isolation methodology and structure elucidation using the nuclear magnetic resonance (NMR) spectroscopy of 6 β -acetoxysandaracopimaradiene-1 α ,9 α -diol (KG6) from *K. galanga* was followed the previous literature (Pattreeya Tungcharoen et al., 2020). The identity of KG6 was further confirmed by gas chromatography (GC), with a retention time of 18.2 minutes, matching the retention time reported for KG6 in a study by Sudsai and colleagues (Sudsai et al., 2022). The yield of KG6 was 1.49 g (white solid, 0.87 %w/w) and the chemical structure is shown in Figure 7.

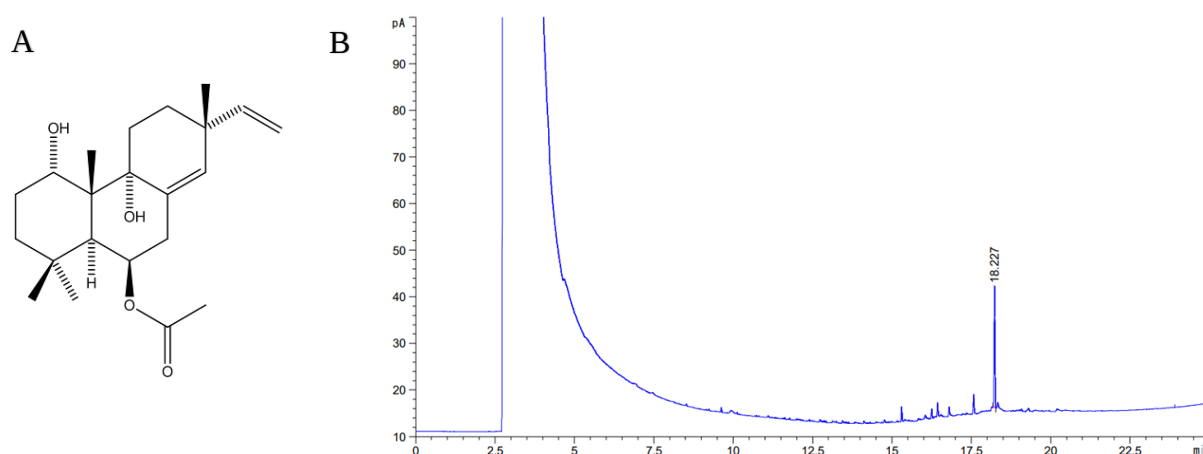


Figure 7. Chemical structure of 6 β -acetoxysandaracopimaradiene-1 α ,9 α -diol (KG6) (A) and GC chromatogram of KG6 compound (B)

2.2.2 Cell cultures and stock sample preparations

The human gingival fibroblasts (HGF-1 - ATCC CRL-2014) (HGF-1) were obtained from the American Type Culture Collection (ATCC) and cultured in a completed DMEM (DMEM supplemented with 10% FBS and 1% penicillin-streptomycin). RAW 264.7 cells (mouse macrophage-like cell line) were cultured in a completed RPMI 1640 (RPMI 1640 supplemented with 10% FBS and 1% penicillin-streptomycin). These cell lines were incubated at 37°C in a humidified atmosphere in a 5% CO₂ incubator. The stock solutions of pure compounds (i.e., KG6, allantoin, indomethacin and gallic acid) and gels (i.e., KG6 mouth gels and Khaolaor mouth gel) were dissolved in DMSO and adjusted to 100 mM and 100 mg/ml, respectively. All stock solutions were further diluted to various concentrations with the culture media before the assays.

2.2.3 Formulation and characterization of mouth gel containing KG6 (KG6 mouth gel)

2.2.3.1 Formulation of KG6 mouth gels and heating-cooling acceleration test

SCMC was used as a gelling agent in this experiment. Six formulas of gel base (5.5-8.0% w/w SCMC) were formulated and characterized for appropriate physical properties (Table 2). Consequently, the optimal concentration of SCMC in the formulas was selected to create three different formulations of KG6 mouth gel (0.10%, 0.25%, and 0.50% w/w). The dispersion method was used to prepare the gel, whereas SCMC and sodium benzoate were dispersed in purified water and subsequently mixed gently using a mortar and pestle to form the gel base. After that, the active compound (KG6) was dissolved in glycerin and added to the gel base to prepare KG6 mouth gels. The components of both the gel base and KG6 mouth gels

are recorded in Table 2. Once the KG6 mouth gels were successfully formulated, 30 grams of KG6 mouth gels were weighed and tested for physicochemical and biological stabilities. The heating-cooling acceleration test was performed under specific conditions; the gel underwent six cycles for 12 days (each cycle consisted of heating at 45°C for 24 hours and cooling at 4°C for 24 hours) (Srirod & Tewtrakul, 2019).

Table 2. The components of gel base and KG6 mouth gel

Chemical	Gel base (g)						Gel containing active compound (100g)		
							0.10%	0.25%	0.50%
1. SCMC	5.50	6.00	6.50	7.00	7.50	8.00	8.00	8.00	8.00
2. Sodium benzoate	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
3. Glycerin	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
4. Active compound (KG6)	-	-	-	-	-	-	0.10	0.25	0.50
5. Purified water qs. to	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

2.2.3.2 Physical characterization of KG6 mouth gels

Macroscopic organoleptic characteristics of KG6 mouth gels were inspected for their appearance, color, smell, and texture. The pH of KG6 mouth gels was measured using a digital pH meter (Thermo Scientific Co., Ltd., USA) at room temperature. The recorded pH values represented the average of three determinations. After that, viscosity measurements of KG6 mouth gels were conducted using a Brookfield Dial Reading Viscometer (Brookfield Engineering Laboratories Inc., USA) at room temperature with a spindle number F (W&J Instrument Co., Ltd., China) at a rotational speed of 10 rotations per minute (min). The measurement was performed in triplicate.

2.2.3.3 Chemical characterization of KG6 mouth gels

The content of KG6 in KG6 mouth gels was evaluated using gas chromatography (GC). The GC analysis of diterpenoids was previously described by Thanasakdecha and Tewtrakul (2021) (Thanasakdecha and Tewtrakul, 2021). In brief, gels were dissolved in methanol, adjusted to a concentration of 10 mg/ml, and filtered through 0.45 μ m nylon filters. Subsequently, 1 μ l of the filtrated gel solutions were injected into an Agilent Technologies 7890 (Agilent Technologies Co., Ltd., USA) equipped with a HP-5 capillary column (30 m $\times$ 0.32 mm $\times$ 0.25 μ m) in splitless mode. The analytical system was carried out under a helium flux of 1 ml/min. The column temperature was initially at 60°C for 2 min, increased to 250°C at a rate of 15°C/min and finally increased at 5°C/min until 305°C. To quantify the content of KG6 in KG6 mouth gels, the standard calibration curve of KG6 was constructed. KG6 powder was weighted and dissolved in methanol, and the resulting solutions were filtered through 0.45 μ m nylon filters. One microliter of the filtrated KG6 solution, at concentrations of 6.25, 12.50, 25.00, 50.00 and 100.00 μ g/ml, was injected into the GC system. The amounts of KG6 in KG6 gels were calculated using the linear regression equation obtained from the standard calibration curve of KG6.

2.2.4 Biological tests of KG6 compound and mouth gel containing KG6 (KG6 mouth gels)

2.2.4.1 Evaluation of anti-inflammatory property using RAW 264.7 cells

The inhibitory effect of KG6 and KG6 mouth gels on nitric oxide (NO) production in mouse macrophage-like cell line (RAW 264.7 cells) was evaluated by using the Griess reagent method (Sudsai et al., 2022). RAW 264.7 cells (1×10^5 cells/well) were maintained in complete RPMI-1640 medium and incubated at 37°C with 5% CO₂ for 1 hour. After incubation, RAW 264.7 cells were induced by fresh RPMI-1640 medium containing 100 ng/ml of lipopolysaccharide (LPS). The LPS-induced RAW 264.7 cells were co-incubated with various concentrations of KG6, KG6

mouth gels, and positive controls (indomethacin was the positive control for KG6, while Khaolaor mouth gel was a positive control for KG6 mouth gels) at 37°C with 5% CO₂ for 24 hours. One hundred microliters of supernatant were aspirated from each well and reacted with 100 µl of Griess reagent. The optical density (OD) was measured with a microplate reader at 570 nanometer (nm) and the percentage of inhibition on NO production was calculated using the equation below. The half-maximal inhibitory concentration (IC₅₀) was determined through the application of a logarithmic equation graph.

$$\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

$$A_{\text{control}} = \text{Absorbance of control} - \text{Absorbance of control blank}$$

$$A_{\text{sample}} = \text{Absorbance of sample} - \text{Absorbance of sample blank}$$

2.2.4.2 Evaluation of cell proliferation using HGF-1 cells

The measurement of cell proliferation was performed by using HGF-1 cells. HGF-1 cells (1×10⁴ cells/well) were maintained in complete DMEM for 24 hours. After incubation, HGF-1 cells were exposed to various concentrations of KG6, KG6 mouth gel, and positive controls. Allantoin was served as a positive control for KG6, and Khaolaor mouth gel was a positive control for KG6 mouth gels. The exposure took place at 37°C with 5% CO₂ for 48 hours. Subsequently, 100 µl of supernatant were removed and 10 µl of 5 mg/ml MTT solution was added directly to the medium in each well, and the plate was then incubated at 37°C for 2 hours. After the incubation, all medium was removed and replaced with DMSO to dissolve the formazan crystal and the OD at 570 nm was recorded. The percentage of cell proliferation was calculated and compared to the non-treatment group (Sudsai et al., 2022).

$$\% \text{ Cell viability} = (\text{Absorbance of treatment group} / \text{Absorbance of non-treatment group}) \times 100$$

2.2.4.3 Evaluation of cell migration using HGF-1 cells

The migration of HGF-1 cells was examined by using a tip scratch assay. Briefly, HGF-1 cells (5×10^4 cells/well) were seeded into a 24-well plate and incubated at 37°C with 5% CO₂. After the confluent monolayer of HGF-1 cells was formed, cells were vertically scratched by using a 1,000 µl sterile pipette tip. Subsequently, cell debris was removed by washing with incomplete DMEM and 1 ml of fresh DMEM containing 2% FBS with or without samples and positive controls was added (allantoin and Khaolaor mouth gel were positive controls for KG6 and KG6 mouth gels, respectively). Photographs were taken immediately and noted as day 0 by using an Eclipse TS100 inverted microscope (Nikon Co., Ltd., Japan). The HGF-1 cells were incubated at 37°C, and photographs were taken 3 times every 24 hours, noted as days 1, 2, and 3, respectively. The migration of HGF-1 cells was assessed by measuring the length between the scratched cells (LBSC), and the analysis was performed using NIS-element D4 software. The percentage of cell migration was calculated and compared to the length obtained from day 0 (Sudsai et al., 2013).

$$\% \text{ Cell migration of day } x = [(\text{LBSC of day 0} - \text{LBSC of day } x) / \text{LBSC of day 0}] \times 100$$

Where x = 1, 2, and 3

2.2.4.4 Evaluation of antioxidant activity using HGF-1 cells

H₂O₂-induced oxidative stress was used as a model to evaluate the antioxidant activity of KG6 and KG6 mouth gels. The methodology outlined by Zgorzynska and co-workers (2015) (Zgorzynska et al., 2015) was utilized with a slight modification. In brief, HGF-1 cells (5×10^3 cells/well) were seeded in a 96-well plate and incubated at 37°C with 5% CO₂ for 24 hours. After the time of incubation, the cells were treated with various concentrations of KG6, KG6 mouth gels, and a positive control for 24 hours. Gallic acid was a positive control for KG6, while Khaolaor mouth gel was used as a positive control for KG6 mouth gels. Subsequently, after pre-treatment with compounds or gels, the test wells were exposed to 3.75 mM H₂O₂ and

incubated at 37°C with 5% CO₂ for 1 hour. At the end of incubation, cell viability was assessed using the MTT assay.

2.2.5 Statistical analysis

Statistical analyses were conducted using the statistical package for the social sciences (SPSS) software. All data values were expressed as the mean $\pm$ S.E.M. based on three determinations. Statistical differences between groups were evaluated using a paired *t*-test and a one-way ANOVA, followed by Dunnett's test. A significant level of *p*-values < 0.05 was statistically significant.

CHAPTER 3

RESULTS

3.1 Formulation of KG6 mouth gels

3.1.1 Physical stability of KG6 mouth gels

Before adding KG6 to KG6 mouth gels, it is essential to determine the optimal concentration of SCMC. Six different concentrations of SCMC were utilized to formulate six gel bases (5.5-8.0% w/w SCMC), which process evaluation for physical stability via a heating-cooling acceleration test (Table 2). The results revealed that the appearance, color, smell, and texture of the all SCMC gel bases remained unchanged both before and after heating-cooling test. The pH values at all concentrations of SCMC in each formulation ranged from approximately 5.8 to 6.0, which is an appropriate pH range for oral gel application. In addition, the viscosity of 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0% w/w SCMC gel bases were $0.50-0.80 \times 10^4$, 1.50×10^4 , $2.30-3.00 \times 10^4$, $3.30-3.50 \times 10^4$, $3.90-4.00 \times 10^4$, and $5.00-6.50 \times 10^4$, respectively (Table 3). The optimal formulation was determined to be the 8.0% w/w SCMC gel base, exhibiting suitable physical stability with a pH of 5.86 and a viscosity of $5.00-6.50 \times 10^4$ centipoise (cP). Consequently, the 8.0% w/w SCMC gel base was then selected to prepare three different formulations of KG6 mouth gels at 0.10%, 0.25%, and 0.50% w/w. The appearance of the gel base, 0.10%, 0.25%, and 0.50% KG6 mouth gels are shown in Figure 8. Subsequently, KG6 gels were successfully formulated, and a heating-cooling test was conducted to assess their physical, chemical, and biological stability properties. The results indicated that the appearance, color, smell, and texture remained unchanged before and after incubating the gel under heating-cooling

conditions. The pH and viscosity of the gel base and KG6 mouth gels were 5.86-5.89 and $5.00-6.67 \times 10^4$ cP, respectively. Notably, a significant increase in viscosity was observed in the gel base, 0.25%, and 0.50% KG6 mouth gels. The data results of the physical stability test are presented in Table 4.

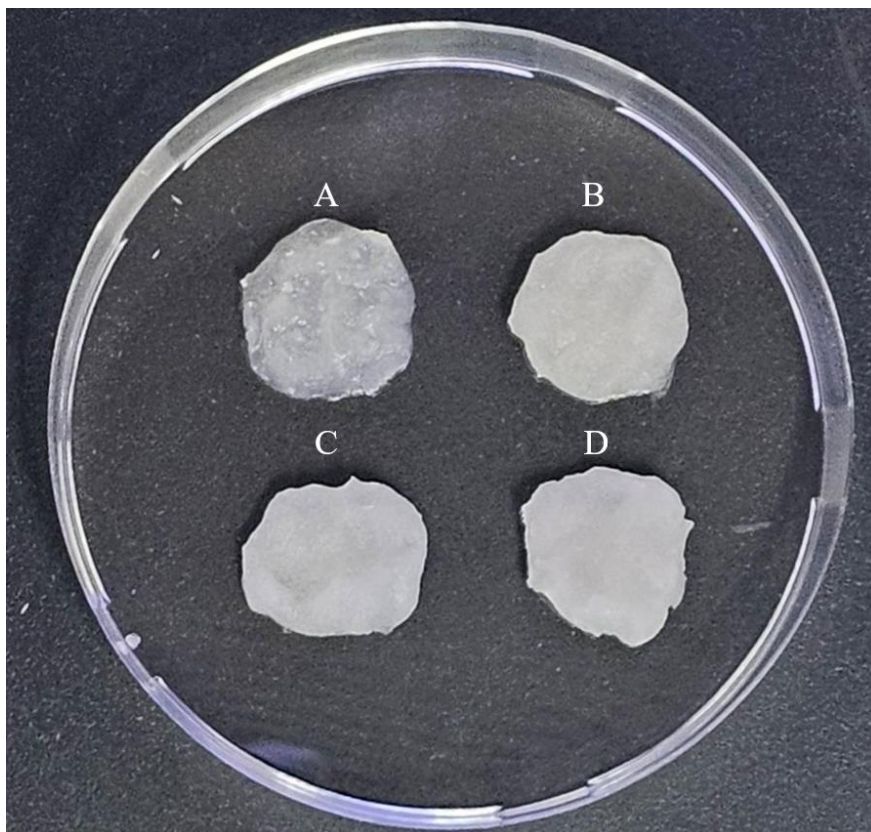


Figure 8. Appearance of mouth gels. Gel base (A), 0.10% KG6 mouth gel (B), 0.25% KG6 mouth gel (C), and 0.50% KG6 mouth gel (D).

Table 3. Physical stability of SCMC gel base before and after heating-cooling test

Parameters	Gel base (5.5% SCMC)		Gel base (6.0% SCMC)		Gel base (6.5% SCMC)		Gel base (7.0% SCMC)		Gel base (7.5% SCMC)		Gel base (8.0% SCMC)	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Appearance	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid
Color	Milky white	Milky white	Milky white	Milky white	Milky white	Milky white	Milky white	Milky white	Milky white	Milky white	Milky white	Milky white
Smell	Non scent	Non scent	Non scent	Non scent	Non scent	Non scent	Non scent	Non scent	Non scent	Non scent	Non scent	Non scent
Texture	Homogeneous and smooth	Homogeneous and smooth	Homogeneous and smooth	Homogeneous and smooth	Homogeneous and smooth	Homogeneous and smooth	Homogeneous and smooth	Homogeneous and smooth	Homogeneous and smooth	Homogeneous and smooth	Homogeneous and smooth	Homogeneous and smooth
pH values	5.92 ± 0.00	5.98 ± 0.01*	5.91 ± 0.00	5.97 ± 0.01*	5.86 ± 0.00	5.92 ± 0.00*	5.89 ± 0.00	5.92 ± 0.00*	5.88 ± 0.00	5.90 ± 0.00	5.86 ± 0.00	5.86 ± 0.00
Viscosity (x10 ⁴ cP)	0.50 ± 0.00	0.80 ± 0.08*	1.50 ± 0.00	1.50 ± 0.08	2.30 ± 0.16	3.00 ± 0.00*	3.50 ± 0.00	3.30 ± 0.17	4.00 ± 0.00	3.90 ± 0.08	5.00 ± 0.00	6.50 ± 0.29*
Separation	No	No	No	No	No	No	No	No	No	No	No	No

Each value represents the mean ± S.E.M. of three determinations.

*Statistically significant difference between before and after heating-cooling test of samples, $p < 0.05$ (paired t -test).

Table 4. Physical stability of gel base and KG6 mouth gel before and after heating-cooling test

Parameters	Gel base		0.10% KG6 mouth gel		0.25% KG6 mouth gel		0.50% KG6 mouth gel	
	Before	After	Before	After	Before	After	Before	After
Appearance	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid
Color	Colorless	Colorless	Milky white	Milky white	Milky white	Milky white	Milky white	Milky white
Smell	Non scent	Non scent	Non scent	Non scent	Non scent	Non scent	Non scent	Non scent
Texture	Homogeneous and smooth	Homogeneous and smooth	Homogeneous and smooth	Homogeneous and smooth	Homogeneous and smooth	Homogeneous and smooth	Homogeneous and smooth	Homogeneous and smooth
pH values	5.86 ± 0.01	5.87 ± 0.00	5.88 ± 0.01	5.87 ± 0.01	5.85 ± 0.01	5.88 ± 0.01	5.89 ± 0.01	5.82 ± 0.01
Viscosity (×10 ⁴ cP)	5.00 ± 0.00	6.50 ± 0.29*	6.33 ± 0.30	6.67 ± 0.44	5.16 ± 0.60	6.00 ± 0.28*	5.03 ± 0.20	5.83 ± 0.16*
Separation	No	No	No	No	No	No	No	No

Each value represents the mean ± S.E.M. of three determinations.

*Statistically significant difference between before and after heating-cooling test of samples, $p < 0.05$ (paired t -test).

3.1.2 Chemical stability of KG6 mouth gels

Analysis of gas chromatography (GC) was conducted before and after heating-cooling accelerated conditions. The quantification of KG6 content in KG6 mouth gels was performed using GC by comparing the retention time (min) and peak area (pA) against the KG6 standard calibration curve. The GC chromatograms revealed an average retention time of 18.1 min for KG6 (Figure 9). The KG6 content in 0.10% and 0.25% KG6 mouth gels exhibited no significant alteration before and after the heating-cooling test. However, for the 0.50% KG6 mouth gel, the KG6 content before the heating-cooling test (159.93 mg) was lower compared to that observed after the test (167.61 mg) (Table 5).

Table 5. KG6 contents obtained from GC analysis of mouth gel containing 0.10, 0.25, and 0.50% w/w of KG6 before and after heating-cooling test.

Sample	Amount of KG6 compound (mean $\pm$ S.E.M.) (mg/30g)	
	Before	After
Gel base	Not detectable	Not detectable
0.10% KG6 mouth gel	36.24 $\pm$ 1.11	35.62 $\pm$ 1.10
0.25% KG6 mouth gel	77.86 $\pm$ 2.23	86.10 $\pm$ 2.08
0.50% KG6 mouth gel	159.93 $\pm$ 1.59	167.61 $\pm$ 0.05*

Each value represents the mean $\pm$ S.E.M. of three determinations.

*Statistically significant difference between before and after heating-cooling test of samples, $p < 0.05$ (paired t -test).

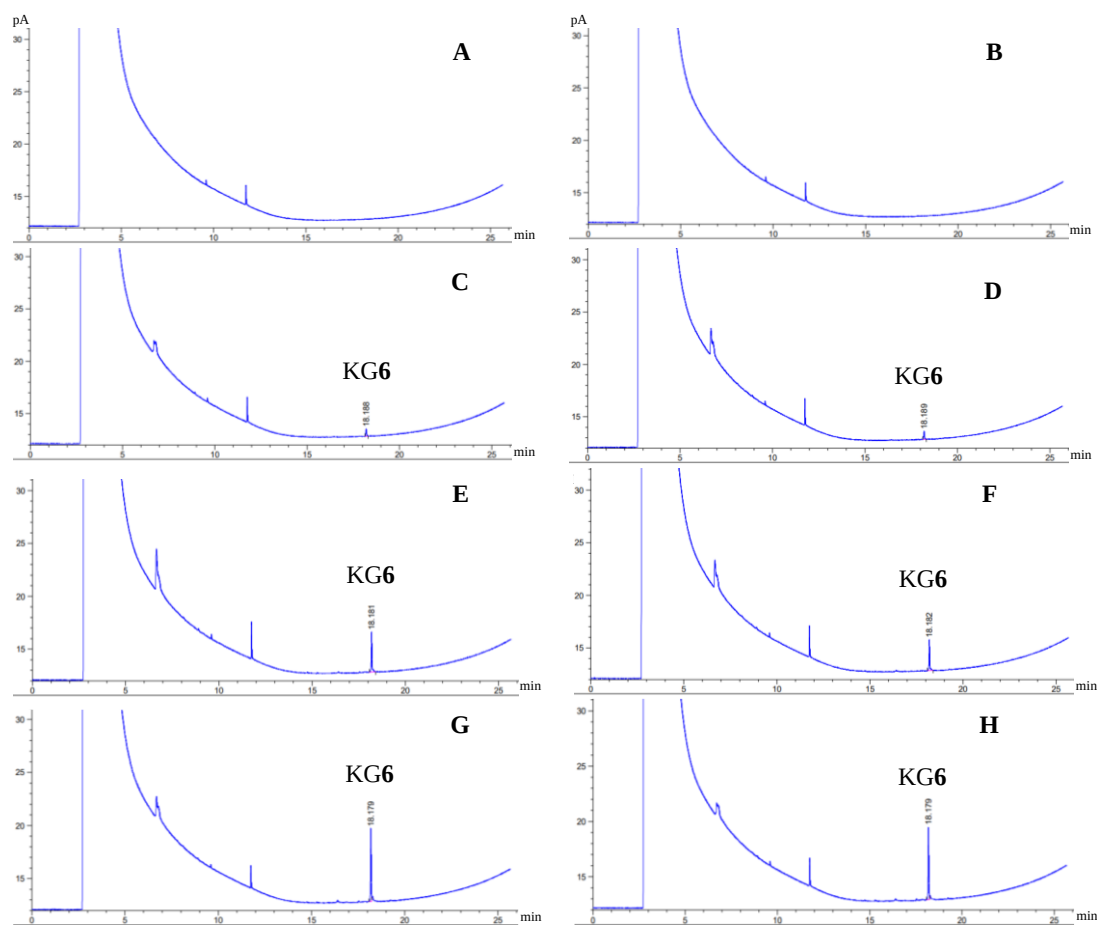


Figure 9. GC chromatograms of mouth gels before and after heating-cooling test. Gel base before heating-cooling (A), gel base after heating-cooling (B), 0.10% KG6 mouth gel before heating-cooling (C), 0.10% KG6 mouth gel after heating-cooling (D), 0.25% KG6 mouth gel before heating-cooling (E), 0.25% KG6 mouth gel after heating-cooling (F), 0.50% KG6 mouth gel before heating-cooling (G) and 0.50% KG6 mouth gel after heating-cooling (H).

3.2 Biological properties of KG6 and KG6 mouth gels

3.2.1 Anti-inflammatory activity

The anti-inflammatory activity of both KG6 compound and KG6 mouth gels underwent assessment using RAW264.7 cells. KG6 compound exhibited inhibition of nitric oxide (NO) production, with an IC_{50} value of 34.5 μ M, comparable to the positive control indomethacin ($IC_{50} = 40.3 \mu$ M), a nonsteroidal anti-inflammatory drug (Table 6). Additionally, 0.10%, 0.25%, and 0.50% KG6 mouth gels, both before and after heating-cooling test, demonstrated effective inhibition of NO production, with IC_{50} values ranging from 617.3-744.2, 599.6-679.9, and 557.7-575.6 μ g/ml, respectively. Conversely, the gel base inhibited NO production with IC_{50} range of 947.2-1015.5 μ g/ml (Table 7). The positive control employed was Khaolaor mouth gel, a commercial product designed to alleviate symptoms associated with oral inflammation and aphthous ulcers. Among its active compounds, peppermint essential oil has demonstrated anti-inflammatory and wound-healing properties by reducing inflammatory cytokines and promoting processes such as cell migration, collagen synthesis, and fibroblast cell re-epithelialization (Modarresi et al., 2019). The present investigation revealed that Khaolaor mouth gel exhibited inhibitory effects on NO production, with an IC_{50} value of 500.3 μ g/ml. Notably, the IC_{50} value against NO production of 0.50% KG6 mouth gel ($557.7 \pm 48.6 \mu$ g/ml) was comparable to that of Khaolaor mouth gel ($500.3 \pm 50.3 \mu$ g/ml). Furthermore, no significant difference was observed in the anti-NO production activity of both gel base and KG6 mouth gels, before and after the heating-cooling test. This finding suggests that these mouth gels maintain good stability in terms of their anti-inflammatory activity (Table 7).

Table 6. Anti-NO production in RAW264.7 cells of KG6 from *K. galanga*

Sample	% inhibition at various concentrations (μM)						IC ₅₀ (μM)
	0	1	3	10	30	100	
KG6	0.0 $\pm$ 0.0	10.0 $\pm$ 3.8	11.3 $\pm$ 2.8	19.2 $\pm$ 3.3	40.9 $\pm$ 2.6	77.9 $\pm$ 1.4	34.5 $\pm$ 2.3
Indomethacin	0.0 $\pm$ 0.0	14.6 $\pm$ 4.6	15.2 $\pm$ 3.7	20.1 $\pm$ 5.0	43.3 $\pm$ 2.4	70.6 $\pm$ 10.0	40.3 $\pm$ 4.0

Each value represents the mean $\pm$ S.E.M. of three determinations.

*Statistically significant difference between the IC₅₀ of indomethacin and KG6 at $p < 0.05$.

Table 7. Anti-NO production of gel base and mouth gel containing KG6 before and after accelerating conditions

Sample		% Inhibition of various concentrations ($\mu\text{g/ml}$)						IC ₅₀ values ($\mu\text{g/ml}$)
		0	10	30	100	300	1,000	
Before	Gel base	0.0 $\pm$ 0.0	6.6 $\pm$ 2.1	9.5 $\pm$ 0.8	7.5 $\pm$ 4.1	14.5 $\pm$ 2.6	54.6 $\pm$ 6.5	1015.5 $\pm$ 167.7
	0.10% KG6 mouth gel	0.0 $\pm$ 0.0	7.2 $\pm$ 6.2	8.1 $\pm$ 4.9	9.5 $\pm$ 4.1	14.4 $\pm$ 5.1	63.7 $\pm$ 9.5	744.2 $\pm$ 128.6
	0.25% KG6 mouth gel	0.0 $\pm$ 0.0	6.3 $\pm$ 0.3	7.3 $\pm$ 2.2	7.4 $\pm$ 2.7	9.5 $\pm$ 1.0	69.2 $\pm$ 8.4	679.9 $\pm$ 71.8
	0.50% KG6 mouth gel	0.0 $\pm$ 0.0	3.2 $\pm$ 1.8	7.6 $\pm$ 3.8	6.2 $\pm$ 3.5	14.2 $\pm$ 5.2	75.9 $\pm$ 3.7	557.7 $\pm$ 48.6
After	Gel base	0.0 $\pm$ 0.0	1.5 $\pm$ 2.1	19.8 $\pm$ 10.4	25.9 $\pm$ 8.6	28.3 $\pm$ 9.9	54.2 $\pm$ 2.6	947.2 $\pm$ 245.8
	0.10% KG6 mouth gel	0.0 $\pm$ 0.0	20.3 $\pm$ 8.7	24.9 $\pm$ 9.3	23.0 $\pm$ 7.9	25.1 $\pm$ 10.2	64.4 $\pm$ 4.7	617.3 $\pm$ 74.3
	0.25% KG6 mouth gel	0.0 $\pm$ 0.0	9.6 $\pm$ 7.8	17.9 $\pm$ 10.6	22.2 $\pm$ 12.1	25.5 $\pm$ 11.8	65.1 $\pm$ 7.4	599.6 $\pm$ 36.2
	0.50% KG6 mouth gel	0.0 $\pm$ 0.0	11.7 $\pm$ 9.1	17.9 $\pm$ 7.6	20.7 $\pm$ 8.5	20.2 $\pm$ 8.1	69.4 $\pm$ 4.2	575.6 $\pm$ 86.0
Positive control	Khaolaor mouth gel	0.0 $\pm$ 0.0	-3.2 $\pm$ 2.7	-5.1 $\pm$ 1.2	-1.1 $\pm$ 0.4	13.8 $\pm$ 1.6	84.0 $\pm$ 4.5	500.3 $\pm$ 50.3

Each value represents the mean $\pm$ S.E.M. of three determinations.

*Statistically significant difference between before and after the accelerating conditions, $p < 0.05$ (paired t -test).

^aStatistically significant difference between IC₅₀ of sample and a positive control (Khaolaor mouth gel) at $p < 0.05$ (Dunnett's test).

3.2.2 Evaluation of HGF cell proliferation

The proliferation of treated HGF-1 cells following exposure to KG6 and a positive control (allantoin) was assessed using the MTT assay. Results indicated that KG6, at a concentration of 30 μM , significantly increased cell proliferation (114.9%) compared to both the control group (100%) and allantoin (99.4%), a well-established compound known for its wound-healing properties (Kim et al., 2018) (Table 8). Furthermore, all concentrations of KG6 mouth gels, before and after heating-cooling test, exhibited no cytotoxicity towards HGF-1 cells, with cell viability exceeding 90%. Additionally, the 0.25% KG6 mouth gel, both before and after heating-cooling test, demonstrated increased HGF-1 cell proliferation at concentrations of 10, 30, and 100 $\mu\text{g/ml}$, with proliferation percentages ranging from 100.4-101.3, 100.4-102.1, and 101.7-103.5, respectively (Table 9). These findings suggest that the 0.25% KG6 mouth gel formulation represents an optimal choice for promoting HGF-1 cell proliferation. Moreover, no significant difference result was observed in both before and after the heating-cooling of gel base and KG6 mouth gels. This observation emphasizes the suitable stability of cell proliferative activity of KG6 mouth gels.

Table 8. Effect of KG6 and allantoin on HGF-1 cell proliferation

Sample	% Cell proliferation at various concentrations (μM)					
	0	1	3	10	30	100
Control	100.0 $\pm$ 0.0	-	-	-	-	-
KG6	-	96.8 $\pm$ 1.3	98.8 $\pm$ 2.0	106.2 $\pm$ 1.9	114.9 $\pm$ 1.8 ^{a*}	104.1 $\pm$ 2.5
Allantoin	-	96.6 $\pm$ 5.1	99.7 $\pm$ 3.2	101.6 $\pm$ 2.1	99.4 $\pm$ 2.9	92.3 $\pm$ 3.1

Each value represents the mean $\pm$ S.E.M. of three determinations.

*Statistically significant difference between control and various concentrations of sample (KG6 and allantoin) at $p < 0.05$. ^a; >allantoin at $p < 0.05$.

Table 9. Effect of gel base and mouth gel containing KG6 before and after the accelerating conditions on HGF-1 cells proliferation

Sample		% Viability of HGF-1 cells at various concentrations (µg/ml)					
		0	1	3	10	30	100
Before	Control	100.0 ± 0.0					
	Gel base	-	97.1 ± 0.9	98.8 ± 0.4	98.7 ± 0.6	98.9 ± 0.7	99.4 ± 0.4
	0.10% KG6 mouth gel	-	91.9 ± 4.4	93.8 ± 4.9	95.9 ± 2.5	96.5 ± 1.8	96.6 ± 1.7
	0.25% KG6 mouth gel	-	99.1 ± 1.1	99.8 ± 1.3	101.3 ± 1.1	100.4 ± 1.8	101.7 ± 1.2
	0.50% KG6 mouth gel	-	94.1 ± 6.0	94.6 ± 5.2	96.5 ± 3.3	98.9 ± 1.2	99.6 ± 1.1
After	Control	100.0 ± 0.0					
	Gel base	-	96.0 ± 0.6	97.3 ± 0.6	98.6 ± 0.3	98.8 ± 0.8	98.7 ± 0.5
	0.10% KG6 mouth gel	-	97.2 ± 0.1	96.8 ± 0.4	96.0 ± 1.0	95.9 ± 1.0	96.0 ± 0.8
	0.25% KG6 mouth gel	-	98.8 ± 1.0	99.1 ± 0.3	100.4 ± 0.9	102.1 ± 2.1	103.5 ± 1.4
	0.50% KG6 mouth gel	-	99.4 ± 1.4	98.5 ± 0.3	99.6 ± 1.1	98.1 ± 0.9	97.2 ± 1.4
Positive control	Khaolaor mouth gel	-	104.3 ± 3.6	99.9 ± 3.6	89.0 ± 4.2	97.6 ± 8.5	90.2 ± 5.4

Each value represents the mean ± S.E.M. of three determinations.

*Statistically significant difference between before and after the accelerating conditions, $p < 0.05$ (paired t -test).

Statistically significant difference between control and various concentrations of sample at $p < 0.05$. Dunnett's test treated one group as a control and compared it with all other groups (^a; >control, ^b; >Khaolaor mouth gel).

3.2.3 Evaluation of HGF-1 cell migration

Cell migration was evaluated using a cell scratch assay, a sterile tip was vertically scratched across the monolayer of HGF-1 cells. Subsequently, the treated HGF-1 cells were observed, and the length between the scratched cells was measured. Results presented in Figure 10 and Table 10 indicated that KG6, across all concentrations tested (10, 30, and 100 μ M), significantly increased the percentage of cell migration to 89.5%, 88.8%, and 91.8%, respectively, compared to the control group (77.7%). Like the effects observed with allantoin (94.4%). These findings suggest that KG6 at a concentration of 100 μ M exhibited wound healing effects comparable to that of allantoin. Furthermore, KG6 was incorporated into SCMC to formulate KG6 mouth gels at concentrations of 0.10%, 0.25%, and 0.50% w/w. Results showed in Table 11 revealed that all formulations of KG6 mouth gels, including 0.10%, 0.25%, and 0.50%, exhibited a significant increase in the percentage of cell migration, ranging from 96.3-98.9%, 95.4-97.8%, and 94.7-96.6%, respectively, compared to both the control group (69.9%) and Khaolaor mouth gel (73.5%). Khaolaor mouth gel, utilized as a positive control in the study, demonstrated a modest increase in the percentage of cell migration (73.5%) when compared to the control group (69.9%) (Figures 11 and 12). These results suggest that KG6 mouth gels promote HGF-1 cell migration to a greater extent than Khaolaor mouth gel, thereby presenting a promising candidate for the treatment of oral ulcers. Additionally, the data demonstrated that the percentage of cell migration induced by gel base and KG6 mouth gels did not exhibit significant differences between before and after heating-cooling experiments, except for 0.10% KG6 mouth gel at 10 μ g/ml. However, this difference did not impact the overall findings (96.3% before and 98.9% after) (Table 11).

Table 10. HGF-1 cells migration of compound KG6 and allantoin

Sample	Dose (μM)	Length between the scratch (μm)				% Cell migration		
		Day 0	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Control	-	992.2 $\pm$ 17.9	744.0 $\pm$ 11.9	302.3 $\pm$ 20.4	220.4 $\pm$ 9.5	24.9 $\pm$ 2.5	69.6 $\pm$ 1.8	77.7 $\pm$ 1.4
KG6	10	1000.7 $\pm$ 29.5	640.2 $\pm$ 6.9	355.2 $\pm$ 9.9	105.5 $\pm$ 16.2	35.9 $\pm$ 1.6	64.4 $\pm$ 2.1	89.5 $\pm$ 1.4*
	30	979.9 $\pm$ 12.6	684.5 $\pm$ 13.9	277.4 $\pm$ 8.9	109.2 $\pm$ 15.3	30.1 $\pm$ 2.0	71.7 $\pm$ 1.0	88.8 $\pm$ 1.6*
	100	992.2 $\pm$ 3.1	624.7 $\pm$ 2.9	334.9 $\pm$ 7.7	81.6 $\pm$ 14.2	37.0 $\pm$ 0.1	66.2 $\pm$ 0.9	91.8 $\pm$ 1.5*
Allantoin	10	963.0 $\pm$ 3.7	584.7 $\pm$ 6.9	272.8 $\pm$ 40.9	80.8 $\pm$ 31.5	39.3 $\pm$ 0.5	71.6 $\pm$ 4.4	94.4 $\pm$ 3.4*

Each value represents the mean $\pm$ S.E.M. of three determinations.

*Statistically significant difference between control and various concentrations of each sample (KG6 and allantoin) at $p < 0.05$.

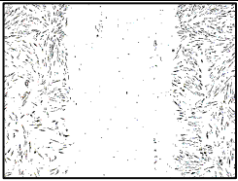
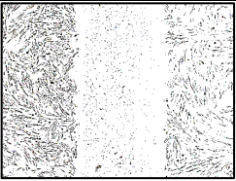
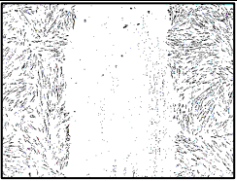
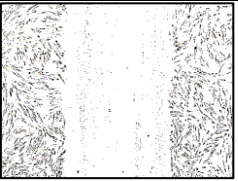
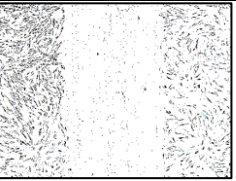
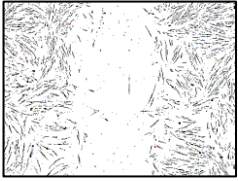
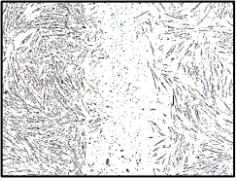
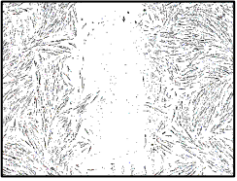
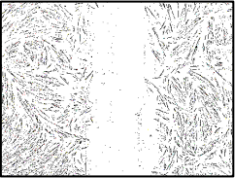
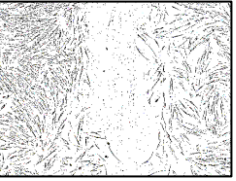
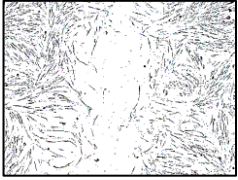
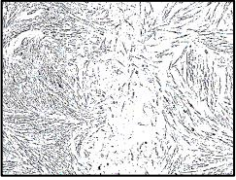
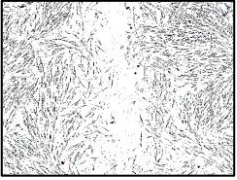
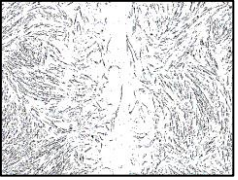
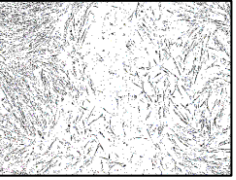
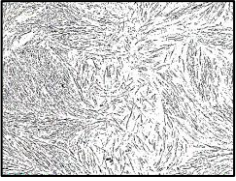
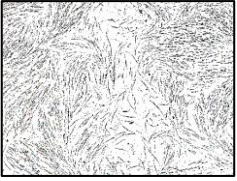
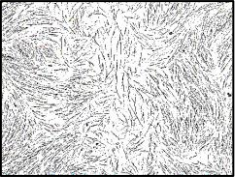
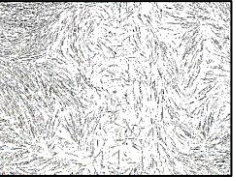
Day	Control	KG6 compound			Allantoin
		10 μ M	30 μ M	100 μ M	10 μ M
Day 0					
Day 1					
Day 2					
Day 3					

Figure 10. The migration behavior of HGF-1 cells under the influence of the KG6 treatment.

Table 11. HGF-1 cell migration of gel base and mouth gel containing KG6 before and after the accelerating conditions.

Sample		Dose ($\mu\text{g/ml}$)	Length between the scratch (μm)				% Cell migration		
			Day 0	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
	Control	-	1034.7 $\pm$ 35.5	709.0 $\pm$ 38.0	403.5 $\pm$ 59.7	309.4 $\pm$ 17.8	31.5 $\pm$ 1.8	61.0 $\pm$ 5.5	69.9 $\pm$ 2.6
Before	Gel base	10	1089.2 $\pm$ 18.5	668.8 $\pm$ 51.2	413.2 $\pm$ 13.3	363.9 $\pm$ 33.3	38.7 $\pm$ 4.0	62.1 $\pm$ 1.2	66.7 $\pm$ 2.6
		100	1063.2 $\pm$ 22.5	795.3 $\pm$ 37.6	443.1 $\pm$ 46.4	345.8 $\pm$ 68.2	25.2 $\pm$ 3.0	58.3 $\pm$ 4.4	67.2 $\pm$ 7.1
	0.10% KG6 mouth gel	10	1172.6 $\pm$ 15.4	424.6 $\pm$ 25.7	237.5 $\pm$ 18.8	43.2 $\pm$ 2.6	63.8 $\pm$ 1.9	79.8 $\pm$ 1.4	96.3 $\pm$ 0.3 ^{ab*}
		100	1127.8 $\pm$ 13.1	432.6 $\pm$ 20.2	322.9 $\pm$ 15.8	30.3 $\pm$ 10.4	61.7 $\pm$ 1.5	71.3 $\pm$ 1.6	97.3 $\pm$ 0.9 ^{ab}
	0.25% KG6 mouth gel	10	1041.7 $\pm$ 39.5	342.2 $\pm$ 12.3	154.0 $\pm$ 11.8	48.2 $\pm$ 2.3	67.0 $\pm$ 2.2	85.2 $\pm$ 1.3	95.4 $\pm$ 0.3 ^{ab}
		100	1148.1 $\pm$ 57.5	439.3 $\pm$ 24.2	192.7 $\pm$ 26.4	37.9 $\pm$ 13.7	61.4 $\pm$ 4.0	83.2 $\pm$ 2.0	96.6 $\pm$ 1.2 ^{ab}
	0.50% KG6 mouth gel	10	1123.7 $\pm$ 10.1	394.2 $\pm$ 6.3	206.8 $\pm$ 6.5	60.0 $\pm$ 4.4	64.9 $\pm$ 0.5	81.6 $\pm$ 0.5	94.7 $\pm$ 0.4 ^{ab}
		100	1112.0 $\pm$ 30.5	366.1 $\pm$ 10.6	197.9 $\pm$ 5.0	37.4 $\pm$ 4.5	67.0 $\pm$ 1.2	82.2 $\pm$ 0.1	96.6 $\pm$ 0.5 ^{ab}
After	Gel base	10	1088.5 $\pm$ 19.2	774.6 $\pm$ 12.7	546.2 $\pm$ 7.5	331.5 $\pm$ 24.5	28.8 $\pm$ 2.1	49.8 $\pm$ 1.4	69.5 $\pm$ 2.4
		100	933.5 $\pm$ 33.2	676.0 $\pm$ 15.3	453.4 $\pm$ 25.3	188.8 $\pm$ 11.2	27.5 $\pm$ 1.7	51.3 $\pm$ 3.4	79.7 $\pm$ 1.7
	0.10% KG6 mouth gel	10	1239.5 $\pm$ 19.3	353.9 $\pm$ 4.7	162.9 $\pm$ 4.3	13.6 $\pm$ 2.0	71.4 $\pm$ 0.1	86.8 $\pm$ 0.5	98.9 $\pm$ 0.2 ^{ab*}
		100	1145.3 $\pm$ 27.0	422.4 $\pm$ 12.2	265.8 $\pm$ 4.7	28.6 $\pm$ 6.1	63.1 $\pm$ 1.0	76.8 $\pm$ 0.5	97.5 $\pm$ 0.5 ^{ab}
	0.25% KG6 mouth gel	10	1093.3 $\pm$ 25.8	387.8 $\pm$ 15.1	178.6 $\pm$ 12.0	49.5 $\pm$ 6.2	64.5 $\pm$ 2.0	83.6 $\pm$ 1.4	95.5 $\pm$ 0.5 ^{ab}
		100	1193.3 $\pm$ 18.0	386.7 $\pm$ 4.0	152.1 $\pm$ 12.9	25.7 $\pm$ 12.2	67.6 $\pm$ 0.8	87.2 $\pm$ 1.2	97.8 $\pm$ 1.0 ^{ab}
	0.50% KG6 mouth gel	10	1167.5 $\pm$ 42.1	423.6 $\pm$ 34.5	145.0 $\pm$ 11.2	46.0 $\pm$ 4.7	63.8 $\pm$ 1.8	87.6 $\pm$ 0.9	96.0 $\pm$ 0.5 ^{ab}
		100	1122.8 $\pm$ 40.9	316.4 $\pm$ 8.3	151.3 $\pm$ 10.5	40.6 $\pm$ 1.1	71.7 $\pm$ 1.6	86.5 $\pm$ 1.1	96.4 $\pm$ 0.1 ^{ab}
Positive control	Khaolaor mouth gel	10	985.4 $\pm$ 13.5	637.0 $\pm$ 61.4	451.5 $\pm$ 70.3	261.4 $\pm$ 16.6	35.2 $\pm$ 6.7	54.1 $\pm$ 7.4	73.5 $\pm$ 1.7

Each value represents the mean $\pm$ S.E.M. of three determinations.

*Statistically significant difference between before and after the accelerating conditions, $p < 0.05$ (paired t -test).

Statistically significant difference between control and various concentrations of sample at $p < 0.05$. Dunnett's test treated one group as a control and compared it with all other groups (^a; > control, ^b; > Khaolaor mouth gel).

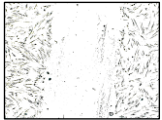
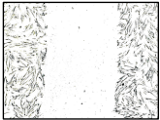
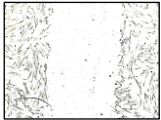
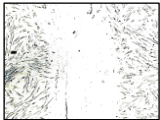
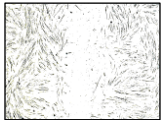
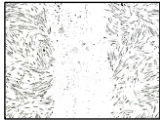
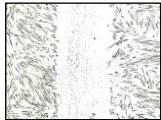
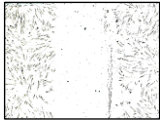
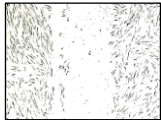
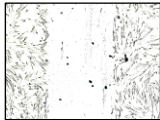
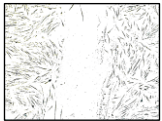
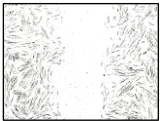
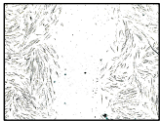
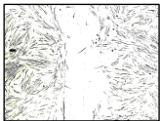
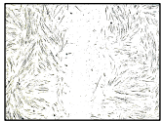
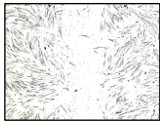
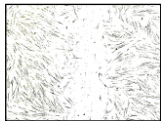
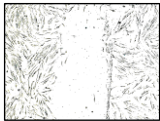
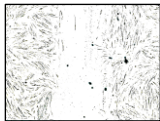
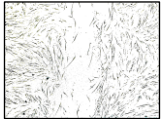
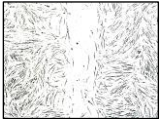
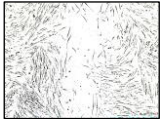
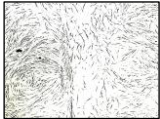
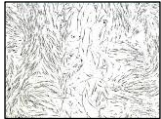
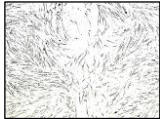
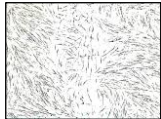
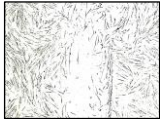
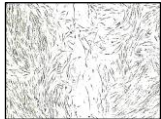
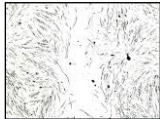
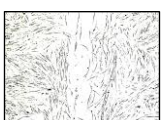
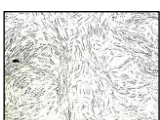
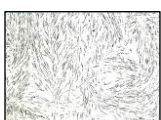
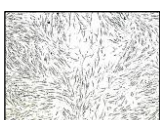
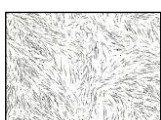
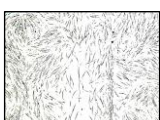
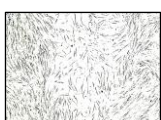
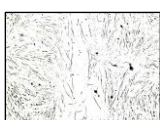
Day	Control	Gel base		0.10% KG6 mouth gel		0.25% KG6 mouth gel		0.50% KG6 mouth gel		Khaolaor mouth gel
		10 µg/ml	100 µg/ml	10 µg/ml	100 µg/ml	10 µg/ml	100 µg/ml	10 µg/ml	100 µg/ml	10 µg/ml
0										
1										
2										
3										

Figure 11. The migration behavior of HGF-1 cells was examined in response to the before heating-cooling test of gel base and KG6 mouth gel treatment.

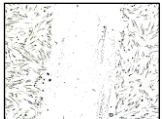
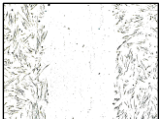
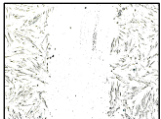
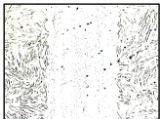
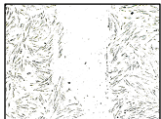
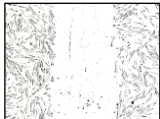
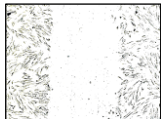
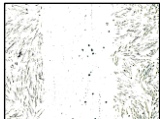
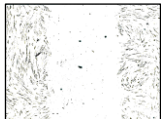
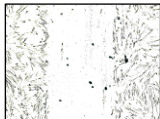
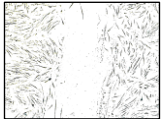
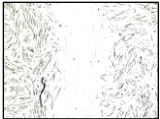
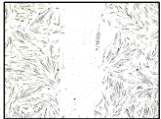
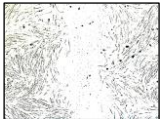
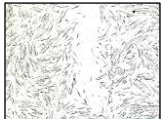
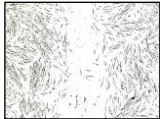
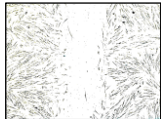
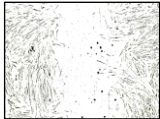
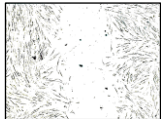
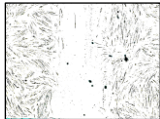
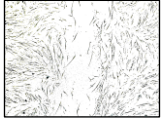
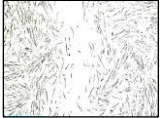
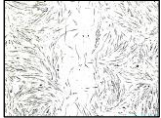
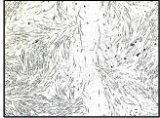
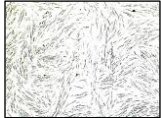
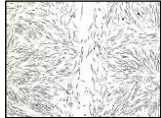
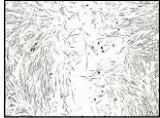
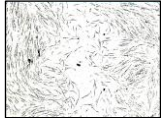
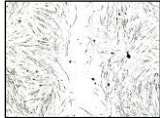
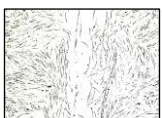
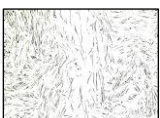
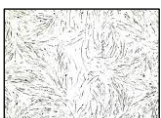
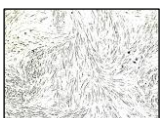
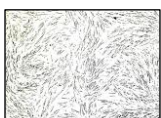
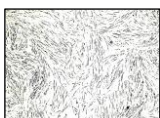
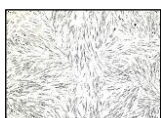
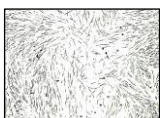
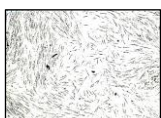
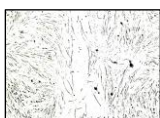
Day	Control	Gel base		0.10% KG6 mouth gel		0.25% KG6 mouth gel		0.50% KG6 mouth gel		Khaolaor mouth gel
		10 µg/ml	100 µg/ml	10 µg/ml	100 µg/ml	10 µg/ml	100 µg/ml	10 µg/ml	100 µg/ml	10 µg/ml
0										
1										
2										
3										

Figure 12. The migration behavior of HGF-1 cells was examined in response to the after heating-cooling test of gel base and KG6 mouth gel treatment.

3.2.4 Antioxidant activity

The assessment of antioxidant activity of KG6 and its formulated mouth gels was conducted utilizing an H₂O₂-induced oxidative stress assay, with cell viability evaluated via the MTT assay. Findings indicated that KG6, across all concentrations tested (1, 3, 10, 30, and 100 µM), demonstrated a light increase in the percentage of survival of HGF-1 cells, ranging from 32.6% to 33.5%, compared to the H₂O₂-treated group (30.3%), which received alone with H₂O₂ treatment. Moreover, gallic acid, employed as a positive control at a concentration of 10 µM, exhibited a significant increase in HGF-1 cell survival, reaching 39.0%, compared to the H₂O₂-treated group (30.3%) (Table 12). Subsequently, the formulation of KG6 mouth gels, their antioxidant activity was assessed. Results revealed that the 0.50% KG6 mouth gel, both before and after heating-cooling test, at concentrations of 30 and 100 µg/ml, significantly enhanced HGF-1 cell survival to percentages ranging from 50.2-53.2 and 50.4-53.8, respectively, compared to the H₂O₂-treated group (35.8%) (Table 13). Additionally, it was observed that the 0.50% KG6 mouth gel (50.2-53.8%) exhibited a higher antioxidant effect compared to that of Khaolaor mouth gel (42.1-44.7%) when compared at equivalent concentrations (30 and 100 µg/ml). Furthermore, no significant difference was observed in the antioxidant activity of gel base and KG6 mouth gels in both before and after the heating-cooling test (Table 13). These findings suggest that KG6 mouth gels demonstrate strong stability in their antioxidant activity under conditions of H₂O₂-induced oxidative stress.

Table 12. Effect of KG6 and gallic acid on H₂O₂-induced HGF-1 cell viability

Sample	% Cell viability of HGF-1 cells at various concentrations (μM)					
	0	1	3	10	30	100
Control	100.0 ± 0.0					
H ₂ O ₂	30.3 ± 0.9					
KG6		33.2 ± 4.3	33.3 ± 0.7	33.2 ± 2.5	32.6 ± 0.2	33.5 ± 0.4
Gallic acid		33.5 ± 3.8	36.1 ± 2.8	39.0 ± 2.7*	37.9 ± 2.5	37.6 ± 2.7

Each value represents the mean ± S.E.M. of three determinations.

*Statistically significant difference between the H₂O₂-treated group (H₂O₂) and various concentrations of sample (KG6 and gallic acid) at $p < 0.05$.

Table 13. Effect of gel base and mouth gel containing KG6 on H₂O₂-induced HGF-1 cell viability

Sample		% Cell viability of HGF-1 cells at various concentrations (µg/ml)					
		0	1	3	10	30	100
Control		100.0 ± 0.0					
H ₂ O ₂		35.8 ± 1.6					
Before							
	Gel base	-	35.3 ± 2.1	36.0 ± 1.2	35.7 ± 1.3	34.6 ± 0.4	35.9 ± 0.4
	0.10% KG6 mouth gel	-	37.0 ± 0.4	37.0 ± 4.9	35.3 ± 1.2	33.8 ± 2.4	40.1 ± 1.3
	0.25% KG6 mouth gel	-	39.5 ± 4.2	39.9 ± 40.9	40.9 ± 2.0	38.4 ± 0.2	42.1 ± 0.4
	0.50% KG6 mouth gel	-	40.9 ± 1.8	39.1 ± 3.4	44.4 ± 4.7	53.2 ± 1.8 ^a	53.8 ± 2.1 ^a
After							
	Gel base	-	36.0 ± 1.5	35.7 ± 1.0	36.7 ± 1.3	35.8 ± 0.5	35.1 ± 0.9
	0.10% KG6 mouth gel	-	36.0 ± 1.6	37.5 ± 0.3	34.7 ± 1.0	33.8 ± 0.4	38.8 ± 0.3
	0.25% KG6 mouth gel	-	38.5 ± 1.1	38.6 ± 1.7	40.4 ± 2.3	39.9 ± 2.6	44.5 ± 2.8
	0.50% KG6 mouth gel	-	40.5 ± 0.5	39.5 ± 2.1	44.9 ± 1.9	50.2 ± 2.8 ^a	50.4 ± 7.9 ^a
Positive control	Khaolaor mouth gel	-	43.3 ± 2.3	45.2 ± 1.5	43.0 ± 2.0	44.7 ± 1.2	42.1 ± 2.4

Each value represents the mean ± S.E.M. of three determinations.

*Statistically significant difference between before and after heating-cooling test of each sample at the 0.05 level (paired *t*-test).

Statistically significant difference between control and various concentrations of sample at *p* < 0.05. Dunnett's test treated one group as a control and compared it with all other groups (^a; > H₂O₂, ^b; > Khaolaor mouth gel).

CHAPTER 4

DISCUSSION

Oral ulcers are among the most prevalent medical conditions, affecting around 25% of the global population. Recurrent aphthous stomatitis (RAS) is a particularly common condition that affects individuals across all age groups, with a notable prevalence among adolescents and young adults (Tarakji et al., 2015). RAS is characterized by the recurrent occurrence of painful, oval, or round-shaped ulcers, either singularly or in multiple clusters, within the oral mucosa, often targeting the non-keratinized mucosa (Bilodeau & Lalla, 2019). The severity of pain and discomfort associated with RAS can be considerable, significantly impacting patients' quality of life by disrupting routine oral functions such as eating, swallowing, and speaking (Rivera et al., 2022).

The drug delivery system designed for oral application is known as mucoadhesive dosage forms, which enable close adhesion between the oral mucous membranes and the dosage forms. This intimate adherence prolongs the residence time, facilitates targeted drug release, and consequently improves bioavailability. Mucoadhesive dosage forms encompass four main types: tablets, films, patches, and gels (Bhalerao & Shinde, 2013). Of these, mucoadhesive gel is remarkable due to its utilization of mucoadhesive polymers like SCMC, carbopol, hyaluronic acid, and xanthan gum. These polymers amplify viscosity, leading to prolonged retention time, sustained, and controlled release of the drug.

SCMC has been selected for the formulation of mucoadhesive gel due to its favorable characteristics, including non-toxicity, non-irritation to mucous membranes, high bioadhesion strength, lack of odor and taste, and cost-effectiveness (Al-Tayyar et al., 2020; Biswal & Singh, 2004). Moreover, the formulation with SCMC did not require pH adjustment during the formulation process, whereas some gelling agents

such as carbopol 934 required pH adjustment to a range of 5.5 to 7.0 using triethanolamine to form a gel. To avoid pH conditions that might interfere with the activity of KG6, we therefore did not use carbopol 934 as the gelling agent for this experiment. In addition, research by Singh and colleagues demonstrated the successful development of ganglioside-coated nanoparticles incorporated into an SCMC gel base. This formulation exhibited prolonged retention time, controlled localized infection, easy application due to its smooth texture, prevention of new lesion formation, and absence of observed side effects in patients with periodontitis (Singh et al., 2015). Additionally, it has been documented that ten formulations of oral gel containing *Punica granatum* flower extract were assessed for their physical properties. The findings revealed that formulation code F₄ outperformed other formulations, demonstrating an optimal release profile, suitable appearance, stability, and superior mucoadhesive properties. The enhanced mucoadhesion was attributed to the high content of mucoadhesive gelling agents, particularly SCMC (Aslani et al., 2016).

In this investigation, various concentrations of SCMC ranging from 5.5% to 8.0% w/w were incorporated into six gel base formulations and evaluated for their physical properties. The formulation containing SCMC at 8.0% w/w was chosen for the development of KG6 mouth gels due to its optimal viscosity falling within the range of $5.0\text{--}6.6 \times 10^4$ cP. This viscosity range facilitates strong adhesion between the gel and mucous membranes. Ideally, oral drug delivery formulations should maintain a pH range of 5.5 to 8.0, aligning with the pH of saliva. The incorporation of SCMC into KG6 mouth gels resulted in a pH of approximately 5.8, which helps preserve the acid-base equilibrium and oral cavity homeostasis (Maslii et al., 2020). The three concentrations of KG6 in the formulations 0.1%, 0.25%, and 0.5% w/w were selected for this study based on their reported wound healing activity, as documented by Sudsai and colleagues (Sudsai et al., 2022). Additionally, these concentrations have been used in studies of 2 α -acetoxysandaracopimaradien-1 α -ol (KM1), a compound isolated from *Kaempferia marginata*, which has also demonstrated wound healing activity (Thanasakdech and Tewtrakul, 2021). The viscosity of the 0.50% KG6 mouth gel notably increased after the heating-cooling test, and there was also an increase in KG6 content in this gel after the heating-cooling test. These results may be due to the water evaporation from the formula. Nonetheless, these values remained within acceptable

limits. Moreover, the biological activities of KG6 mouth gels remained unchanged before and after the heating-cooling test, indicating that their biological activities were stable.

The anti-inflammatory properties of crude extracts derived from *K. galanga* have been previously documented. In a study by Lallo and coworkers, a screening test was conducted on 35 ethanolic extracts from medicinal plants, revealing that the *K. galanga* extract exhibited the most potent anti-inflammatory activity among all plant extracts by inhibiting nuclear factor-kappa B (NF- κ B) activity (Lallo et al., 2022). Furthermore, the anti-inflammatory activity of the essential oil extracted from *K. galanga* rhizome was evaluated. Results showed that the rhizome oil of *K. galanga* dose-dependently reduced NO production in lipopolysaccharide (LPS)-induced macrophages (Chittasupho et al., 2022). Previous studies on isolated compounds from *K. galanga*, including diarylheptenoids and ethyl-*p*-methoxycinnamate, have also reported anti-inflammatory effects by inhibiting NO production and reducing leukotriene B4 production, respectively (Dwita et al., 2021; Yao et al., 2018). The present study focuses on investigating the anti-inflammatory activity of an isopimarane diterpene, specifically 6 β -acetoxysandaracopimaradiene-1 α ,9 α -diol (KG6), isolated from *K. galanga*. Tungcharoen and coworkers demonstrated the mechanism of action of KG6 in anti-inflammatory activity, showing its down-regulation of inflammation-related genes, including inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2), and tumor necrosis factor-alpha (TNF- α), in the LPS-induced macrophages cell line (RAW 264.7 cell) (Pattreeya Tungcharoen et al., 2020). Consequently, KG6 emerges as a promising candidate for treating inflammatory-related diseases and may be formulated into a mucoadhesive dosage form for targeted application. Given the interest in mucoadhesive gel for oral drug delivery, KG6 was incorporated into an SCMC gel base. The anti-inflammatory activity of these mucoadhesive gels was evaluated, demonstrating that all formulations of KG6 mouth gels exhibited significant anti-inflammatory activity. Thus, KG6 mouth gels hold promise as a potential treatment option for oral ulcers by delivering the active compound (KG6) directly to the ulcer site in the oral cavity. Additionally, various extracts from *K. galanga*, such as aqueous, alcoholic, chloroform, hexane, and petroleum ether extracts, have exhibited anti-inflammatory activity in animal studies (Jagadish et al., 2016; Ridditid

et al., 2009; Sulaiman et al., 2008; Vittalrao et al., 2011). Since KG6 is an interesting compound in *K. galanga* rhizomes, these previous findings may support the use of KG6 mouth gels as anti-inflammatory agents for wound treatment.

Cellular proliferation and migration play crucial roles in the secondary phase of wound healing, typically occurring between the initial 48 hours and the 14th day after the wound onset (Gonzalez et al., 2016). These processes involve the interactions of cells, extracellular matrix molecules, and the release of mediators and cytokines into the wound site to facilitate tissue regeneration (Grada et al., 2017). Previous studies have investigated the wound healing potential of *K. galanga*. Wahyuni and colleagues reported that the ethanol extract of *K. galanga* L. rhizome promoted wound healing by accelerating the percentage recovery of the wound area in rat oral mucosa (Wahyuni et al., 2022). Another study by Shanbhag and coworkers demonstrated the beneficial effects of the ethanolic extract of *K. galanga* on various wound models, including incision, dead space, and excision wound models. Their findings indicated increased wound breaking strength, collagen maturation, and epithelialization in these models (Tara Shanbhag et al., 2006). However, further investigation into the wound healing properties of isolated compounds from *K. galanga* is warranted. Hence, our study focuses on evaluating the wound healing potential of KG6, a compound isolated from *K. galanga* rhizome, as well as KG6 mouth gels. Our results revealed that KG6 promoted both proliferation and migration of HGF-1 cells. Notably, 0.25% KG6 mouth gel enhanced HGF-1 cell proliferation, while all formulations of KG6 mouth gels significantly improved HGF-1 cell migration, surpassing the performance of Khaolaor mouth gel, a commercial product. Similarly, Sudsai and coworkers evaluated KG6 gels for wound healing activity using human dermal fibroblasts (HDF), the predominant cell type in skin connective tissue. They found that KG6 gels moderately increased HDF cell proliferation and markedly enhanced HDF cell migration across all formulations (Sudsai et al., 2022). Therefore, our findings suggest that the primary mechanism of action of KG6 mouth gels in the secondary phase of wound healing may predominantly involve enhancing cell migration.

Recent insights have highlighted the role of oxidative stress in regulating wound healing processes, where an optimal level of ROS is crucial. ROS at appropriate levels is beneficial, as they help prevent wound infection and promote

wound healing (DeCoursey, 2016; Rezende et al., 2018). It has been demonstrated that low concentrations of H_2O_2 at the wound site exhibit a chemotactic effect on neutrophils (Klyubin et al., 1996). As a member of ROS, H_2O_2 plays a vital role in wound healing by disinfecting the wound, activating keratinocyte regeneration, recruiting neutrophils, and facilitating angiogenesis (van der Vliet & Janssen-Heininger, 2014). However, excessive ROS levels at the wound site can impair wound healing by reducing fibroblasts, keratinocytes, and endothelial cells (Sampson et al., 2012). Furthermore, elevated ROS levels can decrease platelet adhesion capacity and aggregation, thereby potentially delaying the wound healing process (Hosseini et al., 2020). Hence, exploring the antioxidant activity of natural products becomes essential to regulate ROS levels within a non-toxic range, particularly in cases of excessive ROS accumulation at the wound site (Wang et al., 2023). Our investigation showed the antioxidant activity of KG6, an isopimarane diterpene, which holds promise for controlling ROS levels and enhancing the wound healing process. The findings indicate that KG6 exhibits mild antioxidant activity but increasing the concentration of KG6 in KG6 mouth gels, particularly in 0.50% KG6 mouth gel at a concentration of 100 $\mu\text{g/ml}$ (50.2-53.8%), significantly enhances the antioxidant effect compared to the experimental control (35.8%, H_2O_2 -treated group). This suggests that KG6 mouth gel possesses antioxidant activity, offering a potential alternative for controlling ROS levels in the wound area and thereby enhancing the wound healing process.

In industrial-scale production, the extraction of a KG6-rich extract should be considered for the preparation of KG6 mouth gel, with the potential for selling as a marketable product.

CHAPTER 5

CONCLUSION

Kaempferia galanga L., is widely recognized as one of the most interesting plants within the Zingiberaceae family. Numerous studies have elucidated its diverse biological activities, often echoing its traditional uses over the past decade. The rhizomes, a longstanding component of this plant, exhibited many properties beneficial for treating various diseases. Consequently, isolating compounds from the rhizomes has considerable interest, offering insights into potentially therapeutic agents. This study elucidates the wound healing activity within the oral cavity of a compound isolated from the rhizomes of *K. galanga*, named 6 β -acetoxysandaracopimaradiene-1 α ,9 α -diol or codenamed KG6, and investigates its gel formulations. Initially, the KG6 mouth gel was formulated utilizing an 8.0% w/w SCMC gel. These KG6 mouth gels were successfully formulated and divided into three formulations: 0.10%, 0.25%, and 0.50% w/w. These formulations exhibited appropriate physical properties and stability, even when incubated under heating-cooling acceleration conditions. Various parameters, including appearance, color, odor, texture, pH values, viscosity, and phase separation, were assessed to determine the physical properties of the KG6 mouth gels. The results revealed that all formulations exhibited a semisolid appearance, milky-white color, absence of odor, homogeneous and smooth texture, a pH value of 5.86, viscosity ranging from 5.00 to 6.67 $\times 10^4$ cP, and no phase separation observed. Furthermore, chemical stability tests were conducted to ensure the stability of KG6 when incorporated into the SCMC gel. The findings, assessed via GC analysis, confirmed the stability of KG6 within the KG6 mouth gels. Subsequently, KG6 and KG6 mouth gels were evaluated for their wound healing properties, including anti-inflammatory, cell proliferation, cell migration, and antioxidant activities. The compound KG6 demonstrates anti-inflammatory activity comparable to the positive control (indomethacin, IC₅₀ = 40.3

μM) by inhibiting NO production, with an IC_{50} value of $34.5 \mu\text{M}$. Moreover, it exhibits a significant potential to enhance cell proliferation at concentrations of $30 \mu\text{M}$, resulting in a proliferation rate of up to 114.9%, surpassing that of allantoin (99.4%), the positive control. Regarding cell migration properties, KG6 demonstrates an increase in cell migration across all concentrations compared to the control group, which received only culture media. Notably, at a concentration of $100 \mu\text{M}$, KG6 enhanced cell migration by 91.8% on day 3, a result comparable to allantoin (94.4%). The antioxidant activity of KG6 was assessed using the H_2O_2 oxidative stress assay, revealing a slight antioxidant activity. KG6 enhances cell survival from H_2O_2 -induced oxidative stress, with rates ranging from 32.6% to 33.5%, compared to the experimental control (30.3%). Biological activities of three formulations of KG6 mouth gels were evaluated. Among these formulations, the 0.50% KG6 mouth gel exhibited superlative anti-inflammatory and antioxidant activities, inhibiting NO production with an IC_{50} ranging from 557.7-575.6 $\mu\text{g/ml}$ that comparable effect to Khaolaor mouth gel ($\text{IC}_{50} = 500.3 \mu\text{g/ml}$) and increased cell survival from H_2O_2 -induced oxidative stress condition in the percentage ranging from 50.2-53.8 which was higher than Khaolaor mouth gel (42.1-44.7%). The KG6 mouth gel was found to be non-toxic to HGF-1 cells, with cell viability maintained above 90%. Moreover, 0.25% KG6 mouth gel at the concentration of $100 \mu\text{g/ml}$ presented trend to increase HGF-1 cell proliferation with the percentage ranging from 101.7-103.5. In terms of cell migration, all formulation, including 0.10%, 0.25%, and 0.50% w/w at both 10 and $100 \mu\text{g/ml}$ concentrations, exhibited significantly enhanced HGF-1 cell migration above 95% when compared to control and Khaolaor mouth gel. These findings highlight that KG6 is one of the interesting compounds that have high potential to increase cell migration. The present study demonstrated that KG6, an isopimarane diterpene derived from *K. galanga*, exhibits significant wound healing activity and when incorporated into mouth gel, the KG6 mouth gel presents good physical, chemical, and biological stabilities which make it a promising candidate for the treatment of oral ulcers resulting from recurrent aphthous stomatitis, chemotherapy, and radiotherapy.

REFERENCES

- Akase T, Shimada T, Terabayashi S, Ikeya Y, Sanada H, Aburada M (2011). Antiobesity effects of *Kaempferia parviflora* in spontaneously obese type II diabetic mice. *Journal of Natural Medicines*. 65: 73-80.
- Al-Tayyar NA, Youssef AM, Al-hindi R (2020). Antimicrobial food packaging based on sustainable Bio-based materials for reducing foodborne Pathogens: A review. *Food Chemistry*. 310: 125915.
- Amalraj A, Gopi S (2021). 15 - Values of natural products to future antiinflammatory pharmaceutical discovery. *Inflammation and Natural Products*. pp. 333-361. Academic Press, Cambridge, England.
- Amuamuta A, Plengsuriyakarn T, Na-Bangchang K (2017). Anticholangiocarcinoma activity and toxicity of the *Kaempferia galanga* Linn. rhizome ethanolic extract. *BMC Complementary Medicine and Therapies*. 17(1): 213.
- Arroo R (2007). Eberhard breitmaier. terpenes - flavors, fragrances, pharmaca, pheromones. Wiley-VCH, 2006, 214 pp ISBN 3-527-31786-4. *Applied Organometallic Chemistry*. 21(5): 377-377.
- Akintoye SO, Greenberg MS (2011). Recurrent aphthous stomatitis. *Dental Clinics of North America*. 58 (2): 1-22.
- Aslani A, Zolfaghari B, Davoodvandi F (2016). Design, formulation and evaluation of an oral gel from *Punica granatum* flower extract for the treatment of recurrent aphthous stomatitis. *Advanced Pharmaceutical Bulletin*. 6(3): 391-398.
- Bhalerao M, Shinde M (2013). Novel approaches for mucoadhesive gel—a review. *Journal of Pharmaceutical Research and Development*. 1(6) : 156-170.
- Bilodeau EA, Lalla RV (2019). Recurrent oral ulceration: etiology, classification, management, and diagnostic algorithm. *Periodontology*. 80(1): 49-60.
- Biswal DR, Singh RP (2004). Characterisation of carboxymethyl cellulose and polyacrylamide graft copolymer. *Carbohydrate Polymers*. 57(4): 379-387.

- Chittasupho C, Ditsri S, Singh S, Kanlayavattanakul M, Duangnin N, Ruksiriwanich W, Athikomkulchai S (2022). Ultraviolet radiation protective and anti-inflammatory effects of *Kaempferia galanga* L. rhizome oil and microemulsion: formulation, characterization, and hydrogel preparation. *Gels*. 8(10).
- Coondoo A, Phiske M, Verma S, Lahiri K (2014). Side-effects of topical steroids: a long overdue revisit. *Indian Dermatology Online Journal*. 5(4): 416-425.
- Dash P, Nasrin M, Ali M (2014). *In vivo* cytotoxic and *in vitro* antibacterial activities of *Kaempferia galanga*. *Journal of Pharmacognosy and Phytochemistry*. 3: 172-177.
- DeCoursey TE (2016). The intimate and controversial relationship between voltage-gated proton channels and the phagocyte NADPH oxidase. *Immunological Reviews*. 273(1): 194-218.
- Dwita LP, Hikmawanti NPE, Yeni, Supandi (2021). Extract, fractions, and ethyl-*p*-methoxycinnamate isolate from *Kaempferia galanga* elicit anti-inflammatory activity by limiting leukotriene B4 (LTB4) production. *Journal of Traditional and Complementary Medicine*. 11(6): 563-569.
- Edgar NR, Saleh D, Miller RA (2017). Recurrent Aphthous Stomatitis: A Review. *The Journal of Clinical and Aesthetic Dermatology*. 10(3): 26-36.
- Ekor M (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*. 4: 177.
- Elshamy A I, Mohamed TA, Essa AF, Abd-ElGawad AM, Alqahtani AS, Shahat AA, Yoneyama T, Farrag ARH, Noji M, El-Seedi HR, Umeyama A, Paré PW, Hegazy MF (2019). Recent advances in *Kaempferia* phytochemistry and biological activity: a comprehensive review. *Nutrients*. 11(10): 1-33.
- Elaine NM (1995). *Human anatomy and physiology* 3rd ed. The Benjamin-Cummings Publishing Company: New York. pp. 127-129.
- Gallo CdeB, Mimura MA, Sugaya NN. (2009). Psychological stress and recurrent aphthous stomatitis. *Clinics (Sao Paulo, Brazil)*, 64(7), 645–648.
- Gonzalez AC, Costa TF, Andrade ZA, Medrado AR (2016). Wound healing - a literature review. *Anais Brasileiros de Dermatologia*. 91(5): 614-620.

- Grada A, Otero-Vinas M, Prieto-Castrillo F, Obagi Z, Falanga V (2017). Research techniques made simple: analysis of collective cell migration using the wound healing assay. *Journal of Investigative Dermatology*. 137(2): e11-e16.
- Gupta P, Garg S (2002). Recent advances in semisolid dosage forms for dermatological application. *Pharmaceutical Technology*. 26: 144-162.
- Hosseini E, Solouki A, Roudsari Z O, Kargar F, Ghasemzadeh M (2020). Reducing state attenuates ectodomain shedding of GPVI while restoring adhesion capacities of stored platelets: evidence addressing the controversy around the effects of redox condition on thrombosis. *Journal of Thrombosis and Thrombolysis*. 50(1): 123-134.
- Jagadish PC, Latha KP, Mudgal J, Nampurath GK (2016). Extraction, characterization and evaluation of *Kaempferia galanga* L. (Zingiberaceae) rhizome extracts against acute and chronic inflammation in rats. *Journal of Ethnopharmacology*. 194: 434-439.
- K P K, Britto S J, Raphael R. (2012). *In vitro* antimicrobial evaluation of *Kaempferia galanga* L. rhizome extract. *American Journal of Biotechnology and Molecular Sciences*. 2(1): 1-5.
- Kenneth S (2010). *Anatomy and Physiology: the unity of form and function* 5th Ed. McGraw Hill: New York. pp. 189-194.
- Kanjanapothi D, Panthong A, Lertprasertsuke N, Taesotikul T, Rujjanawate C, Kaewpinit D, Sudthayakorn R, Choochote W, Chaithong U, Jitpakdi A, Pitasawat B (2004). Toxicity of crude rhizome extract of *Kaempferia galanga* L. (Proh Hom). *Journal of Ethnopharmacology*. 90(2-3): 359-365.
- Khaolaor (2021). Khaolaor mouth gel. Accessed from <https://www.khaolaor.com/product/5966-6625/khaolaor> (Accessed on 11 September 2021).
- Kim J, Shin YK, Kim KY (2018). Promotion of keratinocyte proliferation by tracheloside through ERK1/2 Stimulation. *Evidence-Based Complementary and Alternative Medicine*. 2018: 4580627.
- Kirana C, Record IR, McIntosh G, Jones GP (2002). Screening for antitumor activity of 11 species of Indonesian Zingiberaceae using MCF-7 and HT-29 cancer cells. *Pharmaceutical Biology*. 41: 271-276.

- Klyubin IV, Kirpichnikova KM, Gamaley IA (1996). Hydrogen peroxide-induced chemotaxis of mouse peritoneal neutrophils. *European Journal of Cell Biology*. 70(4): 347-351.
- Koh HL, Chua TK, Tan CH (2009). *A guide to medicinal plants*. World Scientific. p. 312.
- Lallo S, Hardianti B, Hayakawa Y (2022). Anti-inflammatory and cytoprotective effect of *Kaempferia galanga* extracts by targeting NF- κ B activity. *Asian Journal of Plant Sciences*. 21(2): 183-191.
- Liu B, Liu F, Chen C, Gao H (2010). Supercritical carbondioxide extraction of ethyl p-methoxycinnamate from *Kaempferia galanga* L. rhizome and its apoptotic induction in human HepG2 cells. *Natural Product Research*. 24: 1927-1932.
- Lin PH, Sermersheim M, Li H, Lee PHU, Steinberg SM, Ma J (2017). Zinc in wound healing modulation. *Nutrients*. 10(1): 1-20.
- Lunter DJ, Daniels R (2020). Semisolid Dosage. *Pharmaceutics*. 12(4): 315.
- Maslii Y, Ruban O, Kasparaviciene G, Kalveniene Z, Materiienko A, Ivanauskas L, Mazurkeviciute A, Kopustinskiene DM, Bernatoniene J (2020). The Influence of pH values on the rheological, textural and release properties of carbomer polacril® 40P-based dental gel formulation with plant-derived and synthetic active components. *Molecules*. 25(21): 5018.
- Modarresi M, Farahpour MR, Baradaran B (2019). Topical application of *Mentha piperita* essential oil accelerates wound healing in infected mice model. *Inflammopharmacology*. 27(3): 531-537.
- Naidu MU, Ramana GV, Rani PU, Mohan IK, Suman A, Roy P (2004). Chemotherapy-induced and/or radiation therapy-induced oral mucositis-complicating the treatment of cancer. *Neoplasia*. 6(5): 423-431.
- Othman R, Ibrahim H, Mohd M A, Awang K, Gilani AU, Mustafa MR (2002). Vasorelaxant effects of ethyl cinnamate isolated from *Kaempferia galanga* on smooth muscles of the rat aorta. *Planta Medica*. 68(7): 655-657.
- Patil PB, Datir SK, Saudagar RB (2019). A review on topical gels as drug delivery system. *Journal of Drug Delivery and Therapeutics*. 2019 (3): 989-994.

- Ren Y, Wang C, Xu J, Wang S (2019). Cafestol and Kahweol: a review on their bioactivities and pharmacological properties. *International Journal of Molecular Sciences*. 20(17): 4238.
- Rezende F, Moll F, Walter M, Helfinger V, Hahner F, Janetzko P, Ringel C, Weigert A, Fleming I, Weissmann N, Kuenne C, Looso M, Rieger MA, Nawroth P, Fleming T, Brandes RP, Schröder K (2018). The NADPH oxidizers NoxO1 and p47phox are both mediators of diabetes-induced vascular dysfunction in mice. *Redox Biology*. 15: 12-21.
- Ridditid W, Sae-Wong C, Reanmongkol W, Wongnawa M (2008). Antinociceptive activity of the methanolic extract of *Kaempferia galanga* Linn. in experimental animals. *Journal of Ethnopharmacology*. 118(2): 225-230.
- Rivera C, Muñoz-Pastén M, Núñez-Muñoz E, Hernández-Olivos R (2022). Recurrent aphthous stomatitis affects quality of life. a case-control study. *Clinical, Cosmetic and Investigational Dentistry*. 14: 217-223.
- Sivapathasundharam B, Sundararaman P, Kannan K (2018). Oral ulcers - a review. *Journal of Dentistry and Oral Disorders*. 4 (4): 1-9.
- Sivarajan V and Balachandran I (1994). *Ayurvedic drugs and their plant sources* Oxford and IBH publishing Co; Oxford & IBH Pub. The New Delhi Company. pp. 570.
- Sampson N, Berger P, Zenzmaier C (2012). Therapeutic targeting of redox signaling in myofibroblast differentiation and age-related fibrotic disease. *Oxidative Medicine and Cellular Longevity*. 2012: 458276.
- Saroja K, Singh S, Aggarwal A, Nanda S (2013). Transdermal gels - an alternative vehicle for drug delivery. *International Journal Pharmaceutical Chemistry and Biological Science*. 3: 495-503.
- Singh Y, Vuddanda PR, Jain A, Parihar S, Chaturvedi TP, Singh S (2015). Mucoadhesive gel containing immunotherapeutic nanoparticulate satranidazole for treatment of periodontitis: development and its clinical implications. *RSC Advances*. 5(59): 47659-47670.
- Srirod S, Tewtrakul S (2019). Anti-inflammatory and wound healing effects of cream containing *Curcuma mangga* extract. *Journal of Ethnopharmacology*. 238: 111828.

- Sudsai T, Tungcharoen P, Tewtrakul S (2022). Wound healing properties of pharmaceutical gel containing isopimarane diterpene isolated from *Kaempferia galanga* L. *Journal of Ethnopharmacology*. 289: 115052.
- Sudsai T, Wattanapiromsakul C, Nakpheng T, Tewtrakul S (2013). Evaluation of the wound healing property of *Boesenbergia longiflora* rhizomes. *Journal of Ethnopharmacology*. 150(1): 223-231.
- Sulaiman MR, Zakaria ZA, Daud IA, Ng FN, Ng YC, Hidayat MT (2008). Antinociceptive and anti-inflammatory activities of the aqueous extract of *Kaempferia galanga* leaves in animal models. *Journal of Natural Medicines*. 62(2): 221-227.
- Sutthanont N, Choochote W, Tuetun B, Junkum A, Jitpakdi A, Chaithong U, Riyong D, Pitasawat B (2010). Chemical composition and larvicidal activity of edible plant-derived essential oils against the pyrethroid-susceptible and -resistant strains of *Aedes aegypti* (Diptera: Culicidae). *Journal of Vector Ecology*. 35(1): 106-115.
- Swapana N, Tominaga T, Elshamy AI, Ibrahim MAA, Hegazy MF, Brajakishor Singh C, Suenaga M, Imagawa H, Noji M, Umeyama A (2018). Kaemgalangol a: unusual seco-isopimarane diterpenoid from aromatic ginger *Kaempferia galanga*. *Fitoterapia*. 129: 47-53.
- Tara Shanbhag V, Chandrakala S, Sachidananda A, Kurady B L, Smita S, Ganesh S (2006). Wound healing activity of alcoholic extract of *Kaempferia galanga* in Wistar rats. *Indian Journal of Physiology and Pharmacology*. 50(4): 384-390.
- Tarakji B, Gazal G, Al-Maweri SA, Azzeghaiby SN, Alaizari N (2015). Guideline for the diagnosis and treatment of recurrent aphthous stomatitis for dental practitioners. *Journal of International Oral Health*. 7(5): 74-80.
- Thanasakdecha S, Tewtrakul S (2021). Wound healing gel containing compound 2 α -acetoxysandaracopimaradien-1 α -ol from *Kaempferia marginata* rhizomes. *Journal of Herbal Medicine*. 28: 100437.
- Thorat S P, Rane S (2010). Formulation and *in vitro* evaluation of lecithin (soya and egg) based Aceclofenac organogels. *Journal of Pharmacy Research*. 3: 1438-1441.

- Tungcharoen P, Wattanapiromsakul C, Tansakul P, Nakamura S, Matsuda H, Tewtrakul S (2020). Anti-inflammatory effect of isopimarane diterpenoids from *Kaempferia galanga*. *Phytotherapy Research*. 34(3): 612-623.
- Umar MI, Asmawi MZ, Sadikun A, Majid AM, Al-Suede FS, Hassan LE, Altaf R, Ahamed MB (2014). Ethyl-*p*-methoxycinnamate isolated from *Kaempferia galanga* inhibits inflammation by suppressing interleukin-1, tumor necrosis factor- α , and angiogenesis by blocking endothelial functions. *Clinics (Sao Paulo)*. 69(2), 134-144.
- van der Vliet A, Janssen-Heininger YM (2014). Hydrogen peroxide as a damage signal in tissue injury and inflammation: murderer, mediator, or messenger? *Journal of Cellular Biochemistry*. 115(3): 427-435.
- Van Puyvelde L, Liu M, Veryser C, De Borggraeve WM, Mungarulire J, Mukazayire MJ, Luyten W (2018). Active principles of tetradenia riparia. IV. anthelmintic activity of 8(14),15-sandaracopimaradiene-7 α ,18-diol. *Journal of Ethnopharmacology*. 216: 229-232.
- Vittalrao AM, Shanbhag T, Kumari M, Bairy KL, Shenoy S (2011). Evaluation of antiinflammatory and analgesic activities of alcoholic extract of *Kaempferia galanga* in rats. *Indian Journal of Physiology and Pharmacology*. 55(1): 13-24.
- Waasdorp M, Krom BP, Bikker FJ, van Zuijlen PPM, Niessen FB, Gibbs S (2021). The bigger picture: why oral mucosa heals better than skin. *Biomolecules*. 11(8): 1165.
- Wahyuni IS, Sufiawati I, Nittayananta W, Levita J (2022). Anti-inflammatory activity and wound healing effect of *Kaempferia galanga* L. rhizome on the chemical-induced oral mucosal ulcer in Wistar rats. *Journal of Inflammation Research*. 15: 2281-2294.
- Wang G, Yang F, Zhou W, Xiao N, Luo M, Tang Z (2023). The initiation of oxidative stress and therapeutic strategies in wound healing. *Biomedicine and Pharmacotherapy*. 157: 114004.
- Wild T, Rahbarnia A, Kellner M, Sobotka L, Eberlein T (2010). Basics in nutrition and wound healing. *Nutrition*. 26(9): 862-866.

- Yao F, Huang Y, Wang Y, He X (2018). Anti-inflammatory diarylheptanoids and phenolics from the rhizomes of kencur (*Kaempferia galanga* L.). *Industrial Crops and Products*. 125: 454-461.
- Yenjai C, Prasanphen K, Daodee S, Wongpanich V, Kittakoo P (2004). Bioactive flavonoids from *Kaempferia parviflora*. *Fitoterapia*. 75(1): 89-92.
- Zgorzynska E, Wierzbicka-Ferszt A, Dziedzic B, Witusik-Perkowska M, Zwolinska A, Janas A, Walczewska A (2015). Docosahexaenoic acid attenuates oxidative stress and protects human gingival fibroblasts against cytotoxicity induced by hydrogen peroxide and butyric acid. *Archives of Oral Biology*. 60(1): 144-153.

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List of Publication and Proceedings

1. Pathan N, Iadnut A, Tewtrakul S (2024). Anti-inflammatory and wound healing effects of mouth gel containing kaempulchraol K from *Kaempferia galanga* rhizomes. Journal of Ethnopharmacology. 324. 117762.
2. Iadnut A, Sae-lee T, Tewtrakul S (2024). Wound healing potential of mouth gel containing isopimarane dierpene from *Kaempferia galanga* rhizomes for treatment of oral stomatitis. Journal of Ethnopharmacology. Submitted
3. อนุกิตติบัตร เรื่อง สูตรตำรับเจลด้านการอักเสบในช่องปากที่มีส่วนผสมของสารจากเหง้าเปราะหอม เลขที่คำขอ: 2303001173 วันที่ยื่นขอ 27 เมษายน พ.ศ.2566