STUDY OF PHYTOCHEMICALS AND BIOLOGICAL PROPERTIES OF GALANGAL AND BITTER GINGER EXTRACTS FOR COSMETIC AND BEAUTY APPLICATIONS

TANAT NA NONGKHAI

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IN

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THIS THESIS IS A PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CREATIVE INNOVATION IN COSMETIC SCIENCE

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Author: Tanat Na Nongkhai

Examination Committee:

Associate Professor Mayuree Kanlayavattanakul, Ph. D. Chairperson

Assistant Professor Tinnakorn Theansungnoen, Ph. D. Member

Sarah Elizabeth Maddocks, Ph. D. Member

Assistant Professor Nont Thitilertdecha, Ph. D. Member

Assistant Professor Santi Phosri, Ph. D. Member

Advisors:

.....Advisor

(Assistant Professor Tinnakorn Theansungnoen, Ph. D.)

.....Co-Advisor

(Sarah Elizabeth Maddocks, Ph. D.)

Dean:

.....

(Associate Professor Wandee Rungseevijitprapa, Ph. D.)

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Tanat Na Nongkhai

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Advisor	Assistant Professor Tinnakorn Theansungnoen, Ph. D.
Co-Advisor	Sarah Elizabeth Maddocks, Ph. D.

ABSTRACT

Acne vulgaris, a common inflammatory skin disorder, is often exacerbated by bacterial colonization, particularly *Cutibacterium acnes*. Conventional treatments, including antibiotics and topical agents, are increasingly challenged by antibiotic resistance and side effects, prompting the exploration of plant-based alternatives. This study investigates the therapeutic potential of galangal (*Alpinia galanga*) and bitter ginger (*Zingiber zerumbet*) extracts for cosmetic applications, focusing on their phytochemical composition, antioxidant properties, and antimicrobial efficacy against acne-associated bacteria.

Extracts were obtained from rhizomes, stems, and leaves using methanol and ethanol as solvents and analyzed via and LC-MS/MS to identify bioactive compounds. Antioxidant capacity was evaluated using DPPH assay, while broth microdilution assays assessed antimicrobial activity against *C. acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. To enhance applicability, hydrogel formulations incorporating the extracts were developed and evaluated for pH, viscosity, color stability, and long-term durability under cyclic temperature stress conditions.

The findings revealed that rhizome extracts exhibited superior antioxidant activity, with IC50 values comparable to standard antioxidants. Antimicrobial assays identified significant inhibitory effects, particularly from rhizome extracts, with low MIC and MBC values against acne-associated bacteria. Hydrogel formulations exhibited

excellent stability, maintaining homogeneity, viscosity, and pH throughout testing, confirming their suitability as carriers for bioactive compounds.

This research highlights the potential of galangal and bitter ginger as plant-based alternatives for acne treatment, offering natural, effective, and stable skincare solutions.

Keywords: *Acne vulgaris, Alpinia galanga,* Antimicrobial, Hydrogels, Phytochemicals, *Zingiber zerumbet*

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ABBREVIATIONS AND SYMBOLS

BLE	Bitter ginger leaf ethanolic extract
BLM	Bitter ginger leaf methanolic extract
BRE	Bitter ginger rhizome ethanolic extract
BRM	Bitter ginger rhizome methanolic extract
BSE	Bitter ginger stem ethanolic extract
BSM	Bitter ginger stem methanolic extract
^{o}C	Degree Celsius
DPPH	2,2-Diphenyl-1-picrylhydrazyl
LC MS/MS	Liquid Chromatography Tandem Mass Spectrometry
GLE	Galangal leaf ethanolic extract
GLM	Galangal leaf methanolic extract
GRE	Galangal rhizome ethanolic extract
GRM	Galangal rhizome methanolic extract
GSE	Galangal stem ethanolic extract
GSM	Galangal stem methanolic extract
ROS	Reactive oxygen species

CHAPTER 1

INTRODUCTION

1.1 Background

Pathogenic bacteria are responsible for various infectious and chronic health conditions that significantly impact the quality of life. The infection process relies on bacterial adaptation to physical barriers, such as the skin, and the immune components of the human body. Infections occur when bacteria penetrate damaged skin, leading to both mild and severe outcomes, such as erythema, edema, and localized inflammation (Aly, 1996). *Acne vulgaris* is one such inflammatory skin disorder that results from the colonization of pilosebaceous follicles by the anaerobic Gram-positive bacterium *Cutibacterium acnes*. This bacterial colonization can cause inflammation and blockage of the follicles, typically affecting adolescents (>85%), but often persisting into adulthood (Tan et al., 2017). Acne has both physiological and psychosocial impacts. Physiologically, it leads to abnormal skin conditions, including lesions and scars. Psychosocially, it adversely affects self-esteem, contributing to feelings of social isolation and distress (Gieler et al., 2015).

The pathogenesis of acne involves multiple factors, including the overproduction of sebum, hyperkeratinization of follicles, hyperproliferation of *Cutibacterium acnes* (C. *acnes*), and inflammation (Williams et al., 2012). Beyond being a causative factor for acne, *C. acnes* plays a pivotal role in its progression and severity (Dréno et al., 2018). In severe cases, *C. acnes* and *Staphylococcus epidermidis* (S. *epidermidis*) promote pus formation and lead to inflammatory lesions (Shalita & Lee, 1983). Furthermore, *S. epidermidis* and *Staphylococcus aureus* (*S. aureus*) are frequently co-isolated from acne patients, with their prevalence being independent of gender (Muhammed & Dabbagh, 2016).

Recent studies have also identified oxidative stress as a key contributor to acne pathogenesis. Elevated levels of reactive oxygen species (ROS) and lipid peroxidation markers, such as malondialdehyde and nitric oxide, have been reported in acne patients,

while antioxidant defense mechanisms, including superoxide dismutase and catalase, are significantly impaired (Sarici et al., 2010). This imbalance suggests that antioxidants could mitigate acne severity by neutralizing free radicals, reducing inflammatory responses, and improving skin barrier function.

The relationship between antimicrobial and antioxidant properties of phytochemicals has been widely studied, demonstrating that these activities are often interconnected due to their structural characteristics and biological effects. Liu et al. (2020) reported that antimicrobial efficacy tends to decrease as the number of hydroxyl and methoxy groups increases, as these functional groups enhance antioxidant activity by stabilizing free radicals but may simultaneously reduce antimicrobial potential by limiting the compound's ability to disrupt microbial membranes or interfere with bacterial metabolism. However, this inverse relationship is not absolute, as several phytochemicals, including polyphenols and flavonoids, have been shown to exhibit both antioxidant and antimicrobial activities depending on their concentration, molecular interactions, and target organisms.

In the context of acne pathogenesis, oxidative stress plays a crucial role in bacterial proliferation, inflammation, and skin damage, making the antioxidant capacity of plant extracts highly relevant in combination with their antimicrobial properties. Reactive oxygen species (ROS) generated during acne inflammation can weaken the skin's defense, providing a favorable environment for *C. acnes*, *S. aureus*, and *S. epidermidis* to thrive. Bagde et al. (2024) highlighted that oxidative stress contributes to acne severity by promoting bacterial colonization and triggering inflammatory responses, reinforcing the importance of antioxidant-rich plant extracts in acne treatment formulations. By incorporating plant extracts with both antimicrobial and antioxidant properties, this study aims to not only directly inhibit bacterial growth but also mitigate oxidative stress, creating an unfavorable environment for bacterial survival and promoting skin recovery. This dual-action approach supports the therapeutic potential of bitter ginger and galangal, as their bioactive compounds exhibit strong antimicrobial activity while also neutralizing oxidative damage, making them valuable candidates for acne treatment formulations.

Acne treatment typically depends on the severity of the condition. Mild to moderate acne is managed with topical agents, including retinoids, azelaic acid, and benzoyl peroxide, while inflammatory acne often requires a combination of topical agents and antibiotics, such as benzoyl peroxide and clindamycin (Nast et al., 2016; Fox et al., 2016). However, prolonged use of antibiotics can lead to bacterial resistance, contributing to treatment failures (Ashkenazi et al., 2003). Consequently, there is a growing interest in exploring natural plant-derived compounds as alternative therapies (Walsh et al., 2016). These compounds are rich in bioactive phytochemicals and offer a novel approach for acne management (Vaou et al., 2021).

Alpinia galanga (commonly known as galangal) is a medicinal plant belonging to the family Zingiberaceae. The rhizome of galangal is widely used as a spice and in traditional medicines in countries such as Indonesia, Sri Lanka, India, and Thailand. Its flowers and young shoots are also used as vegetables and flavoring agents. Galangal is reported to contain bioactive compounds like flavonoids and volatile oils, which exhibit antioxidant, anti-inflammatory, and antimicrobial properties (Proença et al., 2022; Yuandani et al., 2023).

Similarly, *Zingiber zerumbet* (L.) Roscoe ex Sm. (commonly known as bitter ginger) is another medicinal plant from the Zingiberaceae family. It is cultivated in several Asian countries for its aromatic rhizomes and flowers, which are used in traditional medicines and as a food additive. Bitter ginger contains diverse phytochemicals, such as polyphenols, terpenes, and alkaloids, which have demonstrated a range of pharmacological activities, including carminative, antipyretic, anti-diarrheal, anti-inflammatory, and antibacterial effects (Yang & Eilerman, 1999; Chouini & Paul, 2018; Koga et al., 2016; Devi et al., 2014).

The delivery of plant-based extracts for therapeutic applications can be further optimized using advanced formulations like hydrogels. Hydrogels are versatile polymeric materials known for their biocompatibility, high water content, and ability to sustain drug release. They have been extensively studied as carriers for active compounds, offering enhanced stability and controlled release. For example, hydrogels incorporating Aristoflex AVC have been shown to provide superior rheological properties, making them ideal for uniform application in pharmaceutical and cosmetic products (Ivaniuk et al., 2018). Furthermore, studies have demonstrated the adaptability

of hydrogels in encapsulating bioactives such as resveratrol, hyaluronic acid, and plant-derived extracts for skincare applications (Theansungnoen et al., 2022; Phosri et al., 2022). By leveraging these properties, hydrogels represent a promising platform for incorporating bioactive compounds like those found in bitter ginger and galangal. The combination of these extracts with hydrogel formulations could enhance their efficacy in combating acne-causing bacteria and improving skin health.

This study aimed to evaluate the effects of different solvents and plant parts on the biological activities of galangal and bitter ginger extracts. These evaluations included antioxidant activities, antimicrobial activities against *C. acnes, S. aureus, and S. epidermidis*. Additionally, the potential for utilizing these extracts in hydrogel formulations for skin health applications was explored. The phytochemical compositions of the extracts were analyzed using GC-MS and LC-MS/MS techniques.

1.2 Objectives of the Study

1.2.1 To study extraction techniques for galangal and bitter ginger.

1.2.2 To study phytochemical compounds of galangal and bitter ginger extracts.

1.2.3 To study antioxidant activities of galangal and bitter ginger extracts.

1.2.4 To study antimicrobial activities of galangal and bitter ginger extracts.

1.2.5 To perform develop hydrogels incorporating galangal and bitter ginger extracts as well as conduct stability testing.

This study aimed to evaluate the effects of different solvents and plant parts on the biological activities of galangal and bitter ginger extracts. These evaluations included antioxidant activities and antimicrobial activities against acne-causing bacteria (*C. acnes, S. aureus*, and *S. epidermidis*). Additionally, the potential for utilizing these extracts in hydrogel formulations for skin health applications was explored. The phytochemical compositions of the extracts were analyzed using LC-MS/MS techniques.

1.3 Scope of the Study

This study involves the extraction of bioactive compounds from the rhizomes, stems, and leaves of *Alpinia galanga* and *Zingiber zerumbet* using methanol and ethanol as solvents. The extracted phytochemicals are analyzed for their antioxidant capacity using DPPH assay and their antimicrobial efficacy against acne-causing bacteria, including *C. acnes*, *S. epidermidis*, and *S. aureus*. The study also focuses on formulating hydrogels with these extracts and evaluating their stability under different storage conditions. This research provides a comprehensive understanding of the potential of these plant extracts in cosmetic applications for managing acne.

1.4 Output of the Study

The primary output of this study is the development of stable and effective hydrogel formulations incorporating galangal and bitter ginger extracts. These hydrogels aim to address acne-associated conditions by evaluating the dual antioxidant and antimicrobial properties of the extracts. The findings from this study will contribute to the growing field of plant-based cosmetics and provide a sustainable alternative to conventional acne treatments, aligning with the increasing demand for eco-friendly and natural skincare solutions.

CHAPTER 2

LITERATURE REVIEW

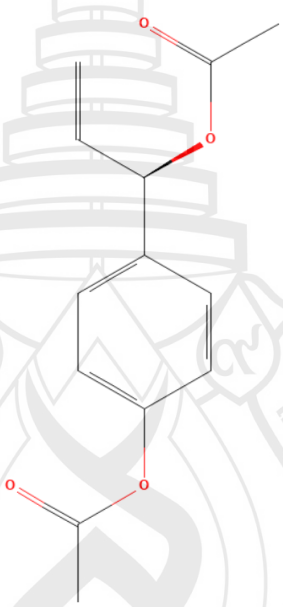
2.1 *Alpinia galanga*

The plant *Alpinia galanga* is a member of the Zingiberaceae family and has rhizomes with a strong aroma and visible nodes and internodes. Its seed is used for oral cleansing and to enhance digestive power and appetite, and it is also employed as a purgative. The rhizome is commonly used as a spice and a source of essential oil, while young shoots and flowers are utilized as a vegetable or spice. This plant is extensively used in various dietary and traditional medicinal practices such as Ayurveda, Unani, Chinese, and Thai folk medicine. It has a pungent and spicy taste with an aromatic ginger-like odor and is widely used as a condiment for food and as local medicine in China and Thailand (Chouni & Paul, 2017).

Source Austin (2002)

Figure 2.1 *Alpinia galanga* rhizome (1), stem (2), and leaf (3)

Crude extracts of the rhizome contain several compounds with antioxidant properties, such as kaempferol, pyrogallol, and apigenin (Köse et al., 2015; Chouni & Paul, 2017). In addition, ethanolic extract of *A. galanga* contains hydroxycinnamaldehyde, coumaryl alcohol, and 1′-acetoxychavicol acetate (AGA), which exhibits strong antibacterial properties against *Staphylococcus aureus*, a multidrug-resistant microbe. AGA is known to inhibit cell-membrane and cell-wall synthesis proteins, regulate osmotic balance, and block bacterial adhesion and invasion, making it a promising antibacterial compound (Zhang et al., 2021; Chouni & Paul, 2017).



Source National Center for Biotechnology Information (2024)

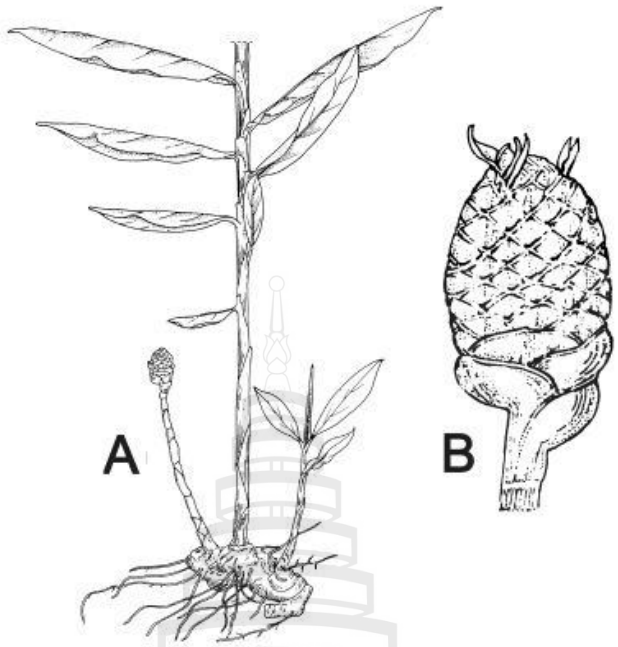
Figure 2.2 Structure of 1′-acetoxychavicol acetate (AGA)

Recent studies identified β-turmerone (58.71%) and α-turmerone (22.52%) as dominant constituents in the essential oil of *A. galanga* rhizomes, which contribute to its antimicrobial and anti-inflammatory properties (Aziz et al., 2024). The antioxidant capacity of A. galanga has been validated through its high total phenolic content (TPC, 233.79 mg GAE/g) and total flavonoid content (TFC, 143.13 mg QE/g), with notable radical scavenging activities measured through DPPH (IC_{50}: 79.34 µg/mL) and ABTS (IC_{50}: 88.94 µg/mL) assays, highlighting its potential for reducing oxidative stress (Aziz et al., 2024).

Furthermore, galangal has been explored for its incorporation into advanced delivery systems, such as hydrogels, to improve bioavailability and stability in topical applications. Such systems show promise in maintaining the activity of phenolic compounds and enhancing skin absorption, making *A. galanga* extracts a suitable candidate for anti-acne formulations (Aziz et al., 2024; Chouni & Paul, 2017).

2.2 *Zingiber zerumbet*

Zingiber zerumbet, commonly known as bitter ginger or shampoo ginger, is a perennial herbaceous plant from the Zingiberaceae family. Native to Asia and widely cultivated in Southeast Asia and the Pacific, this plant thrives in damp, shaded areas of lowland or hill slopes. It is recognized for its ornamental, culinary, and medicinal uses. The name "Zingiber" derives from a Sanskrit word referring to "bull's horn." Traditionally, the rhizome of Z. *zerumbet* has been used as a tonic, stimulant, and remedy for various ailments, including digestive issues, inflammation, and skin conditions (Yob et al., 2011). This plant is rich in bioactive compounds, such as zerumbone, a key constituent of its rhizome. Zerumbone is known for its anti-inflammatory, antimicrobial, and antioxidant properties, making it a valuable compound in therapeutic and cosmetic applications (Reddy et al., 2024). Preliminary phytochemical screening of ethanolic extracts has also revealed the presence of flavonoids, phenolics, tannins, and terpenoids, which contribute to the plant’s antioxidant and antibacterial activities (Manonmani & Mehalingam, 2018). Thin-layer chromatography (TLC) of rhizome extracts highlights zerumbone as the primary active compound, with an Rf value of 0.84 under specific solvent conditions. The abundance of flavonoids and phenolics further reinforces its potential as a source of natural antioxidants (Reddy et al., 2024).



Source Ibáñez et al. (2023)

Figure 2.3 Morphology of *Zingiber zerumbet* Rhizome (a) and Flower (b)

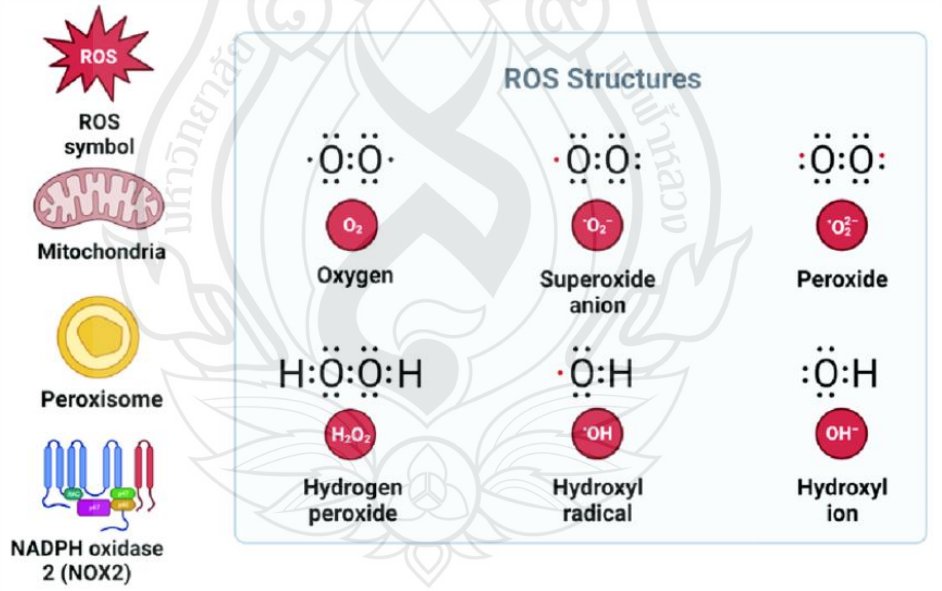
The antimicrobial properties of Z. *zerumbet* have been extensively studied. Ethanolic extracts of the rhizome exhibit significant activity against a range of pathogenic microorganisms, including *Staphylococcus aureus, Bacillus subtilis*, and *Escherichia coli*. In agar well diffusion assays, the ethanolic extract demonstrated a maximum zone of inhibition of 17.83 mm against *S. aureus*, outperforming standard antibiotics like gentamicin in some cases (Manonmani & Mehalingam, 2018). Recent studies found that combining bitter ginger extract and antibiotic solution can improve the antibacterial activity against multi-drug resistant bacteria (Assiry et al., 2023; Ramzan & Zeshan, 2023). This suggests that the extract could be an effective natural alternative for combating microbial infections.

The antioxidant and antimicrobial properties of *Z. zerumbet* make it a promising candidate for cosmetic formulations. Its ability to scavenge free radicals and inhibit bacterial growth aligns well with the requirements for skincare products, particularly those targeting acne-prone skin. Zerumbone, in particular, has shown potential for reducing inflammation and protecting the skin from oxidative stress, further supporting its use in anti-aging and anti-acne products (Reddy et al., 2024). Additionally,

the plant's traditional use in treating skin diseases highlights its relevance in modern formulations. Future research into delivery systems such as hydrogels could enhance the bioavailability and stability of Z. *zerumbet* extracts, optimizing their efficacy in cosmetic applications.

2.3 Oxidative Stress and Free Radicals

The term "oxidative stress" refers to an imbalance between the production of reactive oxygen species (ROS) and the ability of the antioxidant defense system to neutralize or remove them. This imbalance can lead to the accumulation of ROS in cells, causing damage to critical cellular components and, in severe cases, resulting in cell death (Poljšak & Dahmane, 2012). Such stress occurs when the natural antioxidant defenses are overwhelmed by excessive ROS production, disrupting the delicate balance required for cellular homeostasis.

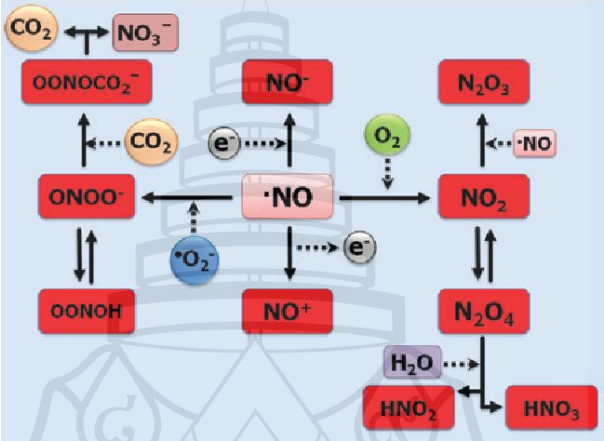


Source Masenga et al. (2023)

Figure 2.4 Structure of reactive oxygen species (ROS)

Free radicals are molecular entities or fragments characterized by one or more unpaired electrons in their outer atomic or molecular orbitals. These unpaired electrons make free radicals highly reactive and unstable, leading them to interact with

surrounding molecules in an attempt to achieve stability. Free radicals are produced continuously within cells through both enzymatic and non-enzymatic reactions. Enzymatic processes such as those in the mitochondrial respiratory chain, phagocytosis, prostaglandin synthesis, and the cytochrome P-450 system are significant contributors to free radical formation. Similarly, non-enzymatic reactions, such as the interaction of oxygen with organic compounds or ionizing reactions, can also generate free radicals (Martemucci et al., 2022; Lobo et al., 2010).



Source Tharmalingam et al. (2017)

Figure 2.5 Structure of reactive nitrogen species (RNS)

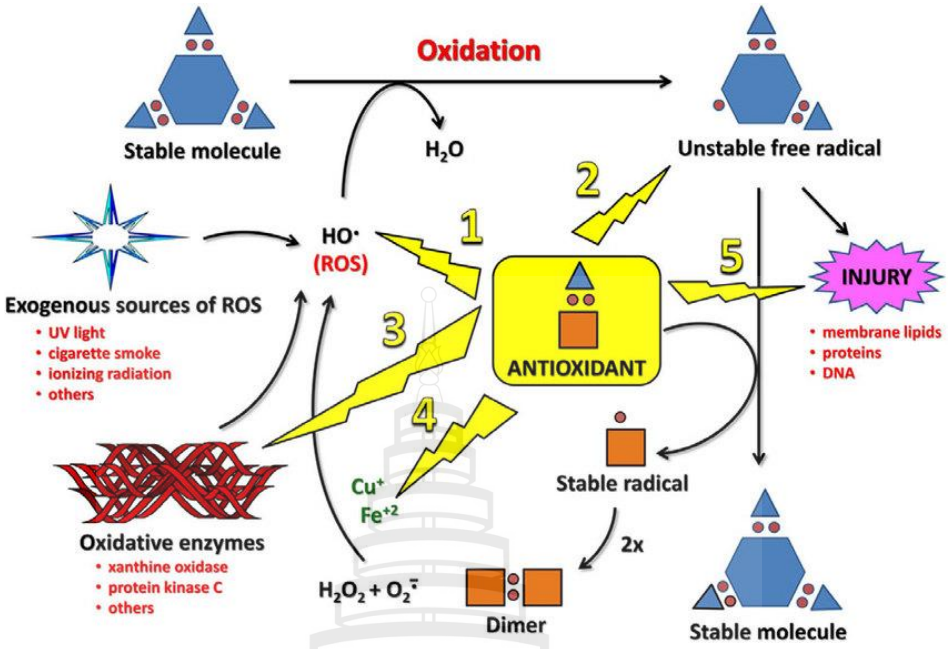
External factors further exacerbate the formation of free radicals. Exposure to X-rays, cigarette smoke, air pollutants, and industrial chemicals are significant sources of oxidative stress. These external contributors introduce additional reactive molecules into the system, intensifying the imbalance between ROS production and antioxidant defenses (Martemucci et al., 2022). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are two classes of reactive molecules derived from oxygen and nitrogen. Both exist in radical and non-radical forms and are constantly produced in the body as part of normal cellular metabolism. Examples of ROS include superoxide anions ($O_2 \cdot^-$) and hydroxyl radicals (·OH), while RNS includes nitric oxide (NO·) and peroxynitrite ($ONOO^-$). Collectively referred to as reactive oxygen and nitrogen species (RONS), these molecules influence various biological processes. However, when their levels become excessive, they contribute to oxidative damage in cellular structures, such as lipids, proteins, and DNA (Liguori et al., 2018).

The effects of oxidative stress extend to the aging process and the development of age-related diseases. Excessive ROS and RNS levels accelerate molecular and cellular damage, contributing to the progression of conditions such as cardiovascular diseases, neurodegenerative disorders, and chronic inflammation. This underscores the critical role of maintaining a balance between ROS production and the antioxidant defense system to prevent the detrimental effects of oxidative stress (Poljšak & Dahmane, 2012; Liguori et al., 2018).

2.4 Antioxidants

Antioxidants are vital substances that play a crucial role in neutralizing free radicals, which can otherwise cause significant damage to living organisms at the cellular level. Free radicals, due to their high reactivity, can initiate oxidative chain reactions, leading to the degradation of cellular components such as lipids, proteins, and DNA. Antioxidants prevent or mitigate such oxidative damage by interrupting these chain reactions. As described by Munteanu and Apetrei (2021), antioxidants serve as a protective mechanism, eliminating or neutralizing free radicals to maintain cellular health and function.

Antioxidants are defined as substances that, even at relatively low concentrations compared to the oxidizable substrate, can significantly delay or prevent its oxidation. This definition, originally proposed by Halliwell & Gutteridge (1995), emphasizes the efficiency of antioxidants in protecting biological systems. The presence of reactive oxygen species (ROS) is essential for maintaining cellular homeostasis, as ROS participate in cell signaling and immune responses. However, excessive ROS levels lead to oxidative stress, causing cellular imbalance and damage. Antioxidants act as a defense mechanism to balance oxidative stress and antioxidant protection, enabling living organisms to maintain homeostasis (Huyut et al., 2017; Öztaşkın et al., 2017).



Source de Oliveira Silva et al. (2017)

Figure 2.6 Mechanisms of antioxidants combating against oxidative stress

Antioxidants can be categorized based on their mechanisms of action into two primary types. The first type, known as primary antioxidants, directly scavenges free radicals to inhibit oxidative reactions. Phenolic compounds, such as α-tocopherol, serve as primary antioxidants due to their ability to neutralize free radicals. These compounds donate electrons or hydrogen atoms to unstable free radicals, stabilizing them and preventing further oxidative damage. This direct scavenging action is critical in breaking the chain reaction of oxidation (Altay et al., 2019; Gülçin et al., 2008). The second type of antioxidant works through indirect mechanisms and is referred to as secondary antioxidants. These compounds do not directly interact with free radicals but instead utilize alternative pathways to prevent oxidation. Secondary antioxidants can bind metal ions, which catalyze the production of free radicals, thereby reducing their availability for oxidative reactions. They also deactivate singlet oxygen, absorb harmful UV radiation, or convert hydroperoxides into non-radical species. By employing these diverse mechanisms, secondary antioxidants provide an additional layer of protection against oxidative damage (Altay et al., 2019; Gülçin et al., 2008).

Living organisms rely on a sophisticated antioxidant defense system to regulate ROS levels and maintain oxidative balance. This system includes both endogenous antioxidants, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, and exogenous antioxidants derived from dietary sources. Endogenous antioxidants are enzymes that neutralize ROS within cells, ensuring minimal oxidative damage to cellular structures. Exogenous antioxidants, on the other hand, are obtained from food and include compounds like vitamins C and E, carotenoids, and polyphenols. Together, these antioxidants form a comprehensive defense network, allowing organisms to effectively mitigate oxidative stress and sustain cellular integrity (Huyut et al., 2017; Öztaşkın et al., 2017).

The ability of antioxidants to inhibit oxidation is fundamental not only for cellular health but also for broader biological functions, such as aging, immunity, and disease prevention. The balance between ROS production and antioxidant defense is a critical determinant of overall health, emphasizing the importance of antioxidants in both endogenous systems and dietary supplementation.

2.5 Correlation Between Antioxidant and Antimicrobial Properties

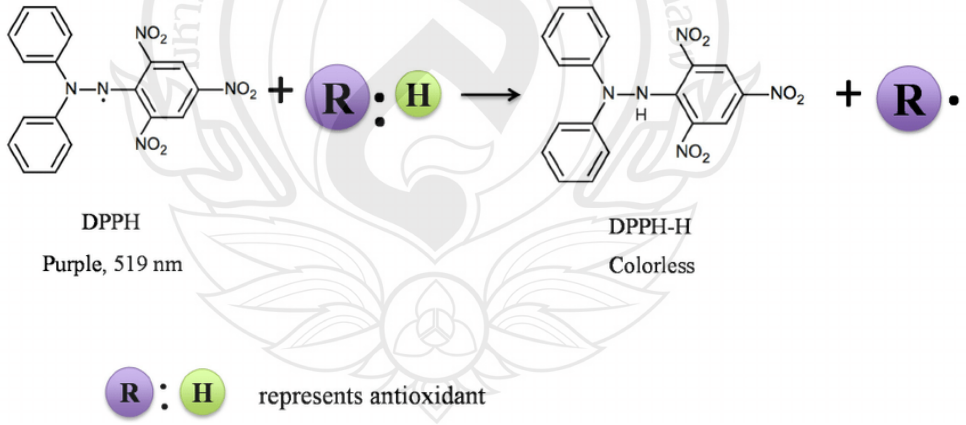
Phytochemical compounds are well-known for their antioxidant and antimicrobial properties, which are critical in protecting cells from oxidative damage and microbial infections. These properties depend significantly on the structural features of the compounds, such as the presence and arrangement of functional groups. A study by Liu et al. (2020) observed that the antimicrobial effectiveness of these compounds decreases as the number of hydroxyl and/or methoxy groups on the aromatic ring structure increases. This reduction in antimicrobial activity suggests that these groups, while beneficial for certain biological functions, may limit the compounds' ability to disrupt microbial membranes or interfere with microbial metabolic processes.

On the other hand, the study also noted that the presence of carboxylic acid groups in these compounds does not appear to influence their antioxidant or antimicrobial functionality. This neutrality indicates that not all functional groups

contribute equally to the biological activity of phytochemicals, emphasizing the complex relationship between structure and function. Understanding how these structural variations affect the dual properties of phytochemicals is essential for optimizing their application in therapeutic and cosmetic formulations. By striking a balance between the structural features that enhance antioxidant and antimicrobial properties, researchers can better tailor these compounds for specific uses.

2.6 Evaluation of Antioxidant Activity

Various techniques have been developed to assess and explore the antioxidant potential and efficacy of commercial antioxidants, food items, medicinal and pharmaceutical products, as well as biological samples. The notion of antioxidant capacity was initially established in the field of chemistry and later extended to other fields such as biology, medicine, epidemiology, and nutrition, as discussed by Floegel et al. (2011). Several techniques have been developed for measuring the antioxidant potential of a substance in vitro such as FRAP assay (ferric ion reducing antioxidant power assay), DPPH· radical scavenging assay, and ABTS·+ radical scavenging assay.



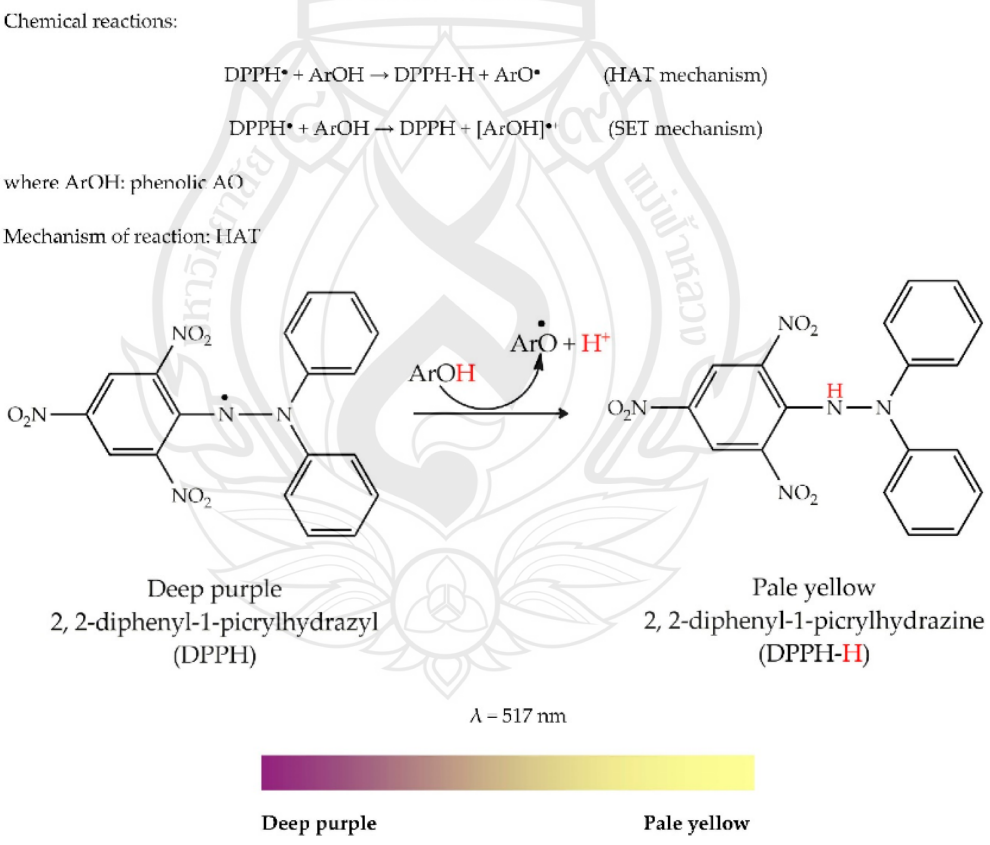
Source Liang and Kitts (2014)

Figure 2.7 Mechanisms of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with antioxidant

Various techniques have been developed to evaluate and explore the antioxidant potential of substances in a wide range of applications. These techniques are used to assess the efficacy of commercial antioxidants, food items, medicinal and

pharmaceutical products, as well as biological samples. The concept of antioxidant capacity originated in the field of chemistry, where it was used to describe the ability of substances to inhibit oxidative reactions. Over time, this concept has been extended to other disciplines, including biology, medicine, epidemiology, and nutrition, demonstrating its relevance across diverse fields of study (Floegel et al., 2011).

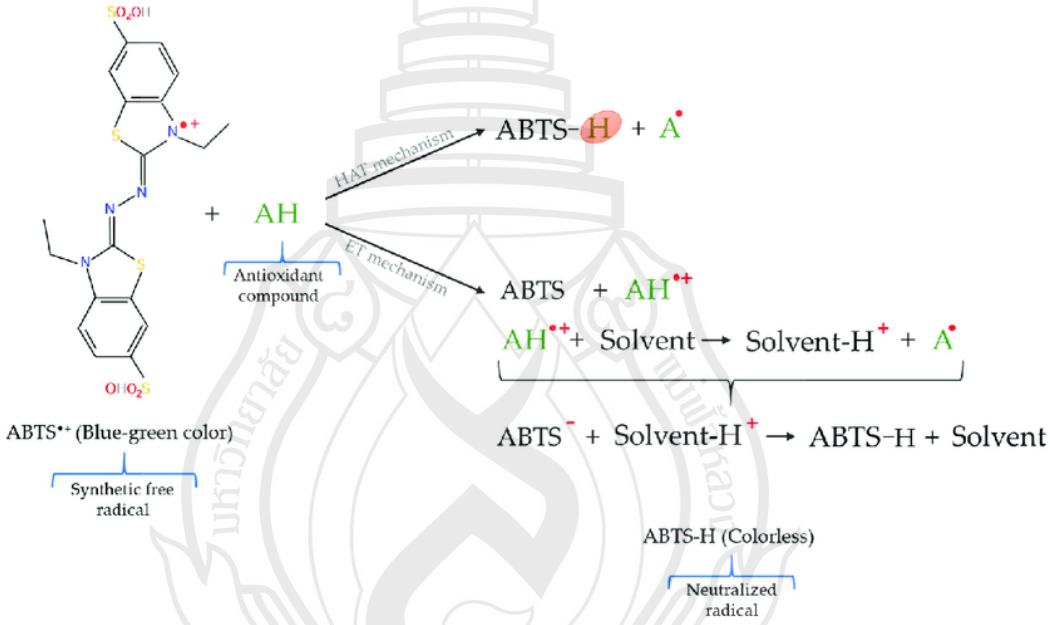
To measure the antioxidant potential of a substance, several in vitro methods have been developed. These methods are designed to simulate oxidative reactions in controlled environments, allowing researchers to quantify the ability of a compound to neutralize free radicals or reduce oxidative agents. Commonly used assays include the FRAP assay (ferric ion reducing antioxidant power assay), the DPPH· radical scavenging assay, and the ABTS·+ radical scavenging assay. Each of these assays evaluates antioxidant activity based on specific mechanisms.



Source Bibi Sadeer et al. (2020)

Figure 2.8 Mechanism of Ferric reducing antioxidant power (FRAP)

The FRAP assay measures the ability of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), providing a quantitative assessment of reducing power. This method is particularly useful for assessing hydrophilic antioxidants and is widely used in food science and nutritional studies. The DPPH· radical scavenging assay, on the other hand, assesses the ability of antioxidants to donate hydrogen atoms to stabilize the DPPH· radical, which is a stable free radical used as a standard in antioxidant evaluations. The ABTS·+ radical scavenging assay measures the capacity of antioxidants to neutralize the ABTS·+ radical cation, providing insights into the total antioxidant potential of both hydrophilic and lipophilic compounds.



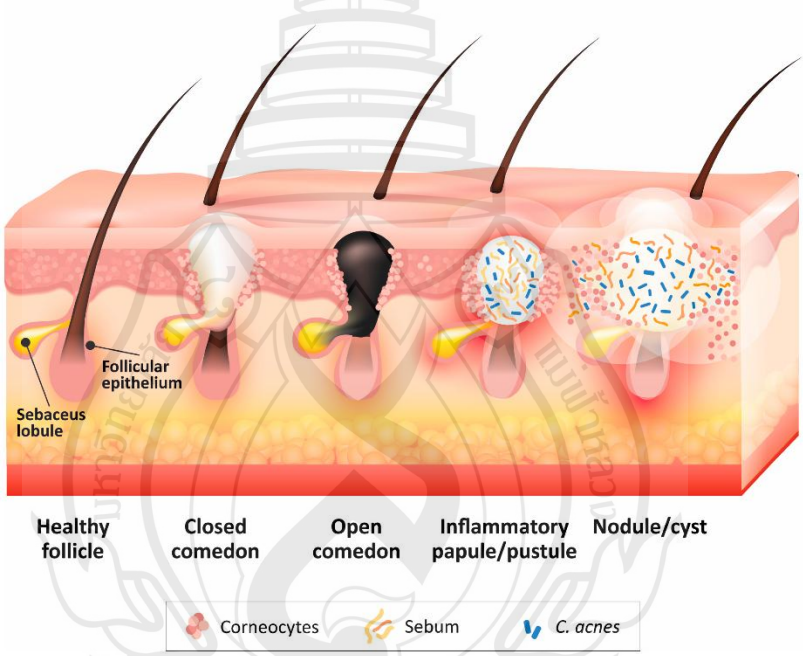
Source Echegaray et al. (2021)

Figure 2.9 Mechanism of ABTS •+ reaction

These techniques have become fundamental tools in antioxidant research, providing valuable insights into the antioxidant properties of various substances. By evaluating antioxidant activity through these methods, researchers can better understand the potential applications of antioxidants in health, nutrition, and pharmaceutical sciences.

2.7 *Acne Vulgaris*

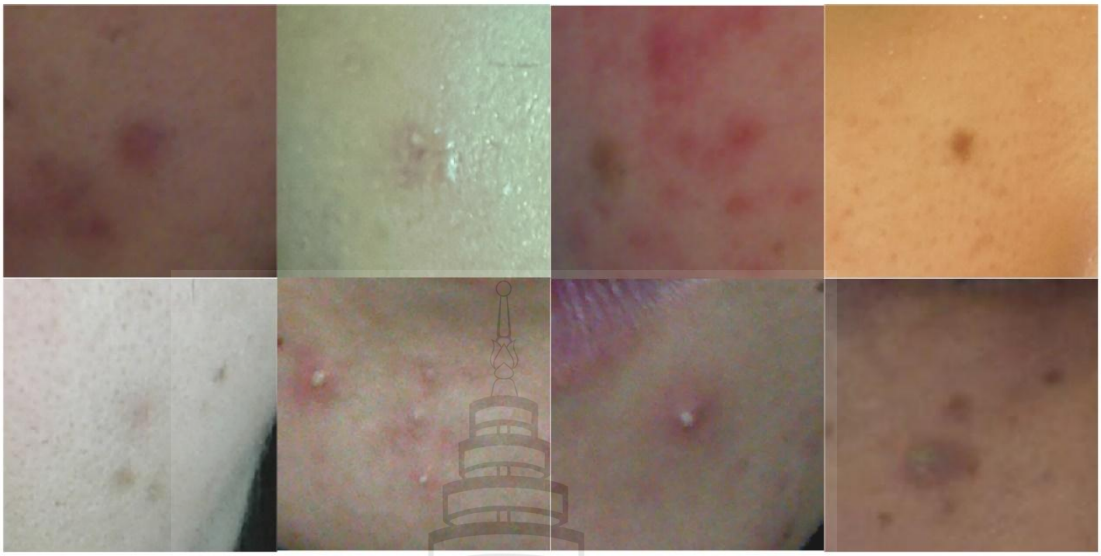
Acne vulgaris is recognized as one of the most prevalent skin disorders, particularly affecting adolescents worldwide. This condition arises due to inflammation within the hair follicles and sebaceous glands of the skin, leading to the development of various lesions, including pimples. While the exact etiology of acne vulgaris remains incompletely understood, it is widely accepted that the disease is multifactorial. Key contributing factors include increased production of sebum, obstruction of hair follicles, and bacterial proliferation, primarily involving *Cutibacterium acnes* (Kim et al., 2021).



Source Dragicevic and Maibach (2024)

Figure 2.10 Pathogenesis of *Acne Vulgaris*

Emerging studies have provided insights into the genetic diversity of *C. acnes*, revealing that this bacterium is composed of distinct cluster groups with unique pathogenic traits. These cluster groups differ in their ability to induce inflammation and exhibit varying secretome profiles, which play a pivotal role in acne pathogenesis. The differential inflammatory potential and secretome characteristics underscore the complex interactions between *C. acnes* and the host's skin environment (Hazarika, 2021).



Source Kim et al. (2024)

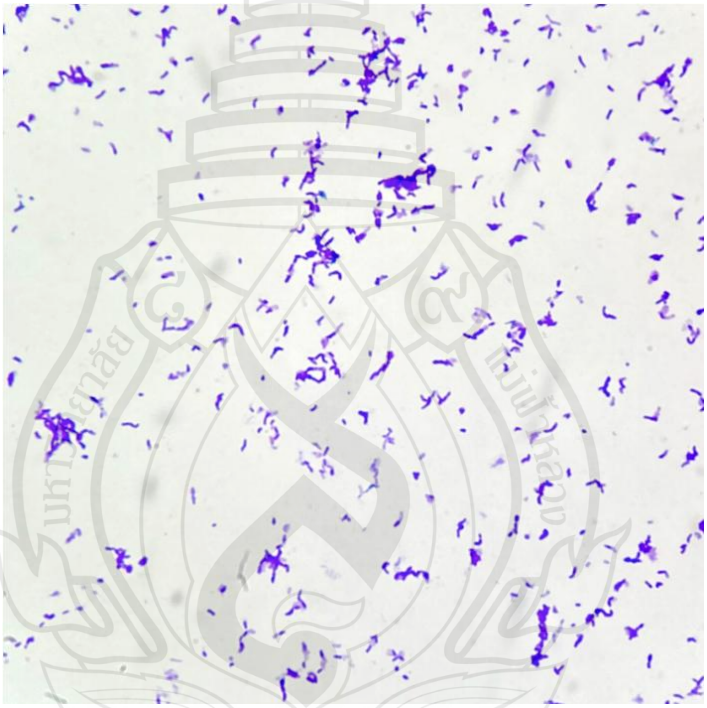
Figure 2.11 Samples of acne

Clinically, acne vulgaris manifests in multiple forms, including comedones, papules, and pustules. Comedones, the hallmark of this condition, are further categorized into two types. Open comedones, commonly known as blackheads, occur when the follicular opening is exposed to air, resulting in oxidation of the follicular contents. In contrast, closed comedones, or whiteheads, are characterized by clogged follicles that lack an opening, thereby trapping sebum and keratin beneath the skin surface. Papules are small, raised lesions measuring less than 1 cm in diameter, whereas pustules, which resemble papules, are inflamed and filled with pus, reflecting their more advanced inflammatory state (Heng & Chew, 2020).

These findings illustrate the multifaceted nature of acne vulgaris, emphasizing the interplay between genetic factors, bacterial involvement, and inflammation in the progression of this skin disorder. Continued research into the molecular and microbial underpinnings of acne is essential for advancing therapeutic strategies to address this common and often distressing condition.

2.8 Microbial Growth and Acne

Cutibacterium acnes (C. *acnes*) is a Gram-positive bacterium that is naturally commensal and lipophilic, meaning it thrives in lipid-rich environments. Morphologically, *C. acnes* is slightly curved and rod-shaped, with dimensions ranging from 0.4 to 0.7 µm in width and 3 to 5 µm in length, which has led to its classification as a diphtheroid or coryneform bacterium. It is an aerotolerant anaerobe, enabling its survival on the skin surface through specialized enzymatic systems that neutralize oxygen, a unique adaptation for an anaerobic organism.

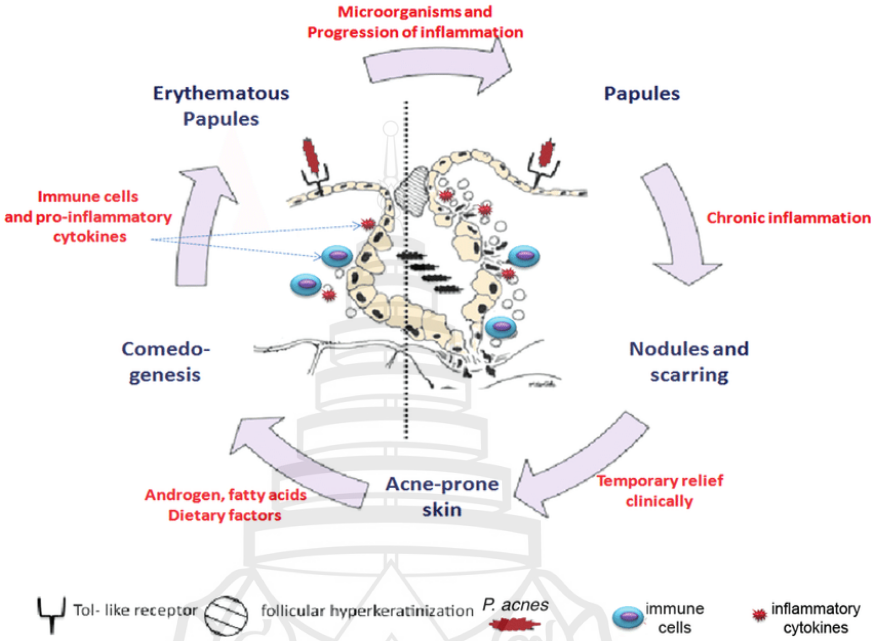


Source Pu and Garrett (2024)

Figure 2.12 Gram stain of *C. acnes*

Although *C. acnes* was historically regarded as a primary cause of acne, recent studies have redefined its role, revealing its dual nature. It can act as an opportunistic pathogen, contributing to various inflammatory conditions, including acne, while simultaneously playing an essential role in maintaining healthy skin. The microbial composition of the skin, as elucidated through advanced amplification and sequencing methods, underscores its vast diversity, encompassing microorganisms from bacteria,

fungi, and viruses across multiple kingdoms. Among skin bacteria, four major phyla dominate: Actinobacteria (notably Corynebacterineae and Propionibacterineae), Firmicutes (e.g., Staphylococcaceae, Proteobacteria, and Bacteroidetes).



Source Rocha et al. (2014)

Figure 2.13 Role of *Cutibacterium acnes* in the pathogenesis of inflamed acnes

In terms of distribution, C. *acnes* is predominantly located in sebaceous regions such as the face, back, and pre-thoracic areas, which provide the anaerobic and lipid-rich environments conducive to its growth. By contrast, *Staphylococcus* and *Corynebacterium* species are commonly associated with more humid areas of the skin, while a diverse bacterial community is observed in drier regions. Notably, *C. acnes* is the most abundant bacterium within the skin microbiota, and its population density does not significantly vary between healthy skin and skin affected by acne.

The development of inflammatory acne, however, is believed to derive from imbalances in the skin microbiota, particularly involving the selection of specific pathogenic phylotypes of *C. acnes*. Among these, phylotypes IA-1 are strongly linked to inflammatory acne and severe acne. These strains produce elevated levels of porphyrins, which can stimulate inflammatory processes, exacerbating skin conditions. The dynamic nature of the cutaneous microbiota further complicates its interactions, as bacteria like *S. epidermidis* and *C. acnes* may influence each other's behavior.

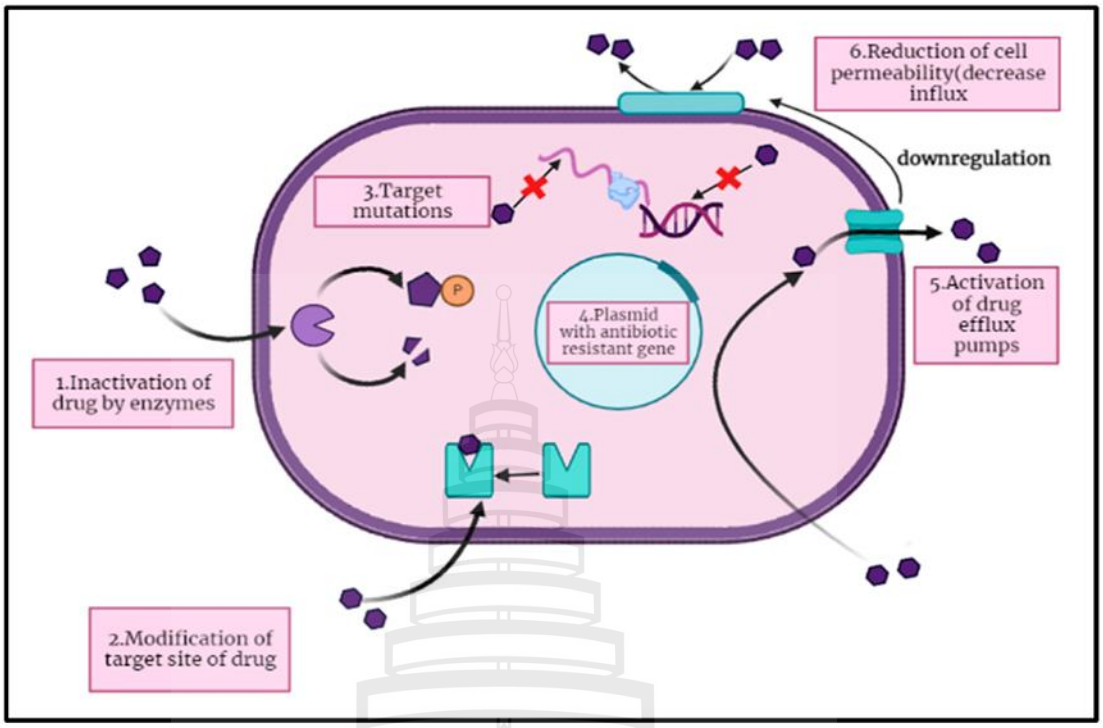
Additionally, external factors such as hormonal fluctuations, stress, and environmental changes can promote the selection of more virulent strains of *C. acnes*. These pathogenic strains are capable of producing virulence factors, including biofilms and surface proteins, which significantly enhance their inflammatory capacity.

Importantly, the commensal microbiota also exerts a profound influence on skin health. It modulates the immune response and contributes to epidermal development, highlighting its dual role in maintaining skin homeostasis and its potential involvement in disease processes (Mayslich et al., 2021).

2.9 Antimicrobial Agents and Antimicrobial Resistance

Antimicrobial agents refer to a class of substances capable of inhibiting or restricting the growth of microorganisms, even when used at low concentrations. These agents are broadly categorized into antibiotics and chemotherapeutics. Antibiotics are naturally occurring compounds synthesized by microorganisms such as yeasts and molds, which leverage these substances to gain a competitive edge in their natural habitats. On the other hand, chemotherapeutics are synthetic substances specifically designed and produced to target microbial activity. In addition to these, certain minerals, such as copper, exhibit antibacterial properties when applied at high concentrations, further diversifying the arsenal of antimicrobial agents available (Cromwell, 2002).

Antibiotics have historically been a mainstay in acne treatment, owing to their ability to combat microbial populations contributing to the condition. Despite their effectiveness, the widespread and prolonged use of antibiotics presents a significant drawback: the emergence of antibiotic resistance. This phenomenon occurs when bacteria adapt to the presence of antibiotics, rendering the drugs less effective or even obsolete. The development of resistance is influenced by various factors intrinsic to the interaction between bacterial populations and antimicrobial agents. Such adaptations not only compromise the efficacy of existing treatments but also pose a broader threat to public health by limiting the options available to combat bacterial infections (Sinha et al., 2014).



Source Muteeb et al. (2023)

Figure 2.14 Bacterial antibiotic resistance pathways

This double-edged nature of antibiotics underscores the necessity of exploring alternative antimicrobial strategies and highlights the importance of prudent antibiotic use to mitigate the growing challenge of microbial resistance.

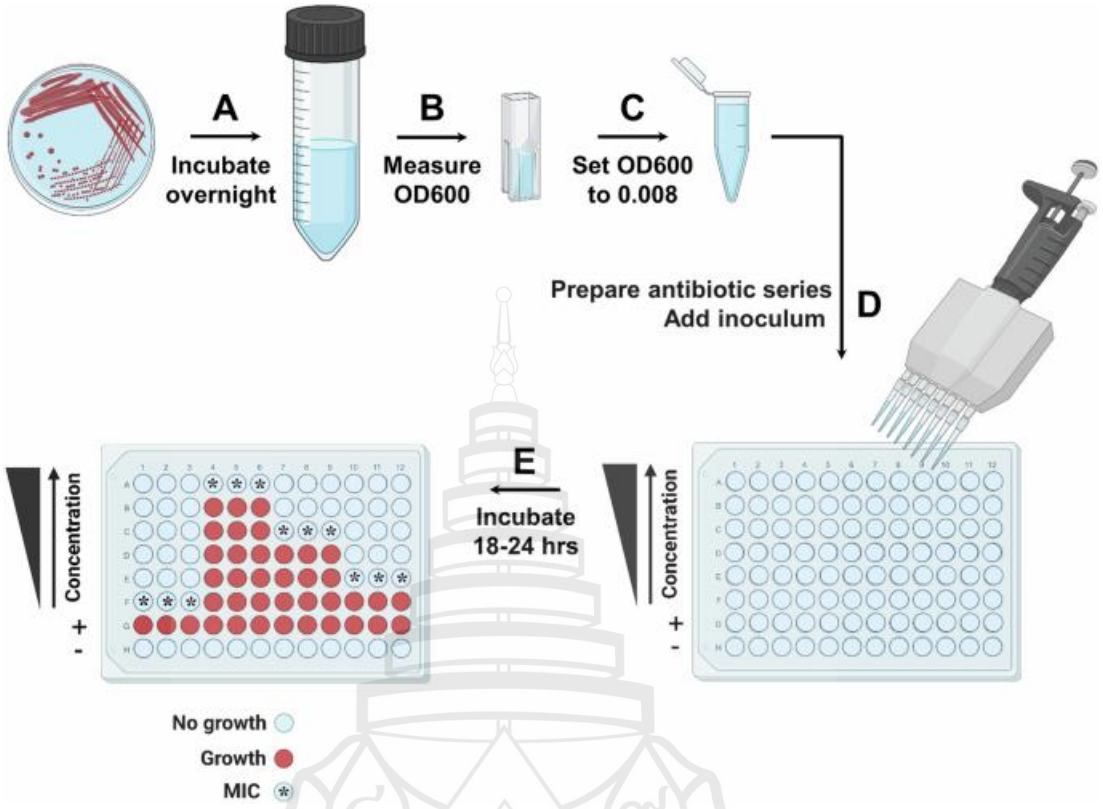
2.10 Evaluation of Antimicrobial Activity

The evaluation of antimicrobial susceptibility is a critical process with numerous practical applications, including its pivotal role in drug discovery, epidemiology, and the prediction of therapeutic efficacy. This assessment has been instrumental since the "golden era" of the 1960s, a transformative period during which nearly all major groups of antibiotics—such as tetracyclines, cephalosporins, aminoglycosides, and macrolides—were discovered. These advancements not only addressed significant challenges in chemotherapy but also laid the groundwork for modern antimicrobial treatments. However, the continued efficacy of these revolutionary compounds is now under threat due to the alarming rise of microbial

resistance. This growing resistance has far-reaching consequences, with multidrug-resistant bacteria contributing to an increasing number of treatment failures, elevating antimicrobial resistance to a global public health crisis.

To combat this challenge, the discovery of novel antibiotics remains an urgent priority. Natural products have historically served as a rich source of new antimicrobial agents and continue to be a cornerstone in the search for innovative drug molecules. These products originate from diverse sources, including prokaryotic bacteria, eukaryotic microorganisms, plants, and animals. Among the antimicrobial compounds identified to date, the majority have been derived from microbial and plant sources, emphasizing the importance of these natural reservoirs.

Several methodologies exist to evaluate the in vitro antimicrobial activity of an extract or a purified compound. Among the most widely used techniques are the disk-diffusion method and broth or agar dilution techniques, which are straightforward and effective for assessing antimicrobial potency. For antifungal testing specifically, the poisoned food technique is commonly employed. To gain a more detailed understanding of the antimicrobial efficacy of an agent, advanced methods such as the time-kill test and flow cytofluorometric analysis are recommended. These sophisticated techniques provide valuable insights into the nature of the inhibitory effect—whether it is bactericidal or bacteriostatic—along with determining whether the effect is time-dependent or concentration-dependent. Additionally, they measure the extent of damage inflicted on the tested microorganisms, enabling a comprehensive evaluation of the agent's antimicrobial impact (Balouiri et al., 2016).



Source Kadeřábková et al. (2024)

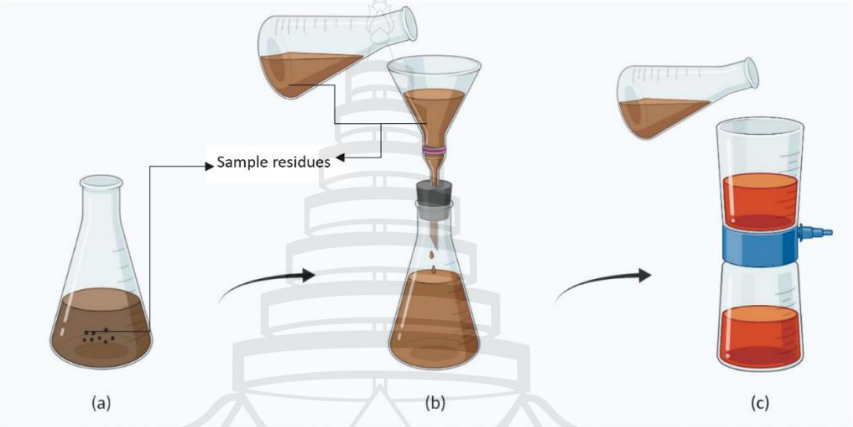
Figure 2.15 Broth microdilution technique

2.11 Maceration

Maceration is widely regarded as an efficient and cost-effective technique for extracting bioactive compounds from plant materials. The process involves immersing coarsely ground or powdered plant material into suitable solvents, such as methanol, ethanol, acetone, ethyl acetate, or hexane. To optimize the extraction efficiency, the plant material is first reduced to smaller particles through grinding, thereby increasing its surface area and enhancing its interaction with the solvent. The mixture is then left to soak for an extended period, during which it is periodically agitated to ensure uniform contact between the plant material and the solvent. Afterward, the mixture is filtered using a filtration medium to separate the extract from the plant residue.

The choice of solvent plays a pivotal role in determining the success of the maceration process, as its polarity directly influences the extraction of specific plant

components. Solvent polarity must align with the chemical nature of the targeted bioactive compounds to achieve maximum efficiency. During maceration, the cell structure of the plant material is disrupted, allowing the chemical constituents to be exposed and dissolved in the solvent. This process facilitates the removal of a broad spectrum of plant-derived compounds, ranging from simple phenolics to complex bioactive substances.



Source Kumar et al. (2023)

Figure 2.16 Process of maceration extraction (a) Sample and solvent mixed and kept for 7 days. (b) Straining of liquid and pressing the remaining marc. (c) Filtering out more clear liquid.

Maceration is a versatile method that has been extensively utilized for the extraction of bioactive compounds in laboratory research. Its scalability also makes it a valuable approach for industrial applications, supporting both small-scale and large-scale production needs. Its straightforward implementation and adaptability to various plant materials further underscore its widespread adoption in the field of natural product extraction (Farooq et al., 2022).

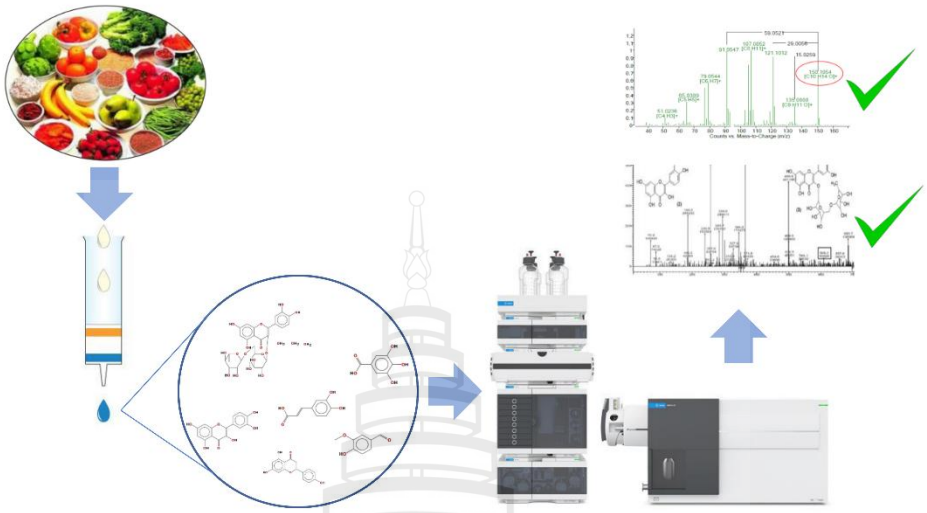
2.12 LC-MS/MS Analysis

The development of a new medication, from its initial discovery to its release in the market, is a complex and time-intensive process. This journey typically spans more than a decade and incurs costs exceeding 1 billion euros. It begins with an

extensive search for a drug-like compound or biological therapeutic, followed by rigorous testing and validation through a variety of advanced tools and methodologies. These include genetic, cellular, and animal model studies, all of which are essential steps before the compound can progress to clinical development. To streamline this process, the pharmaceutical industry, in collaboration with academic institutions, employs early-phase strategies to identify candidates with characteristics suitable for drug formulation.

Ensuring the safety of a drug is a multifaceted process that involves evaluating factors such as the active substance's properties, potential impurities, and degradation products. To guarantee both the quality and quantity of drug products, a wide range of analytical methods is utilized. These methods include titrimetric, chromatographic, spectroscopic, and electrochemical techniques. Among these, liquid chromatography (LC) is particularly prominent in the pharmaceutical industry due to its high resolving power. LC is highly effective for analyzing and quantifying impurities and degradation products in both bulk drug materials and pharmaceutical formulations.

The choice of detection systems in LC is critical to the successful identification of all components. Ultraviolet (UV) detectors are commonly employed because they allow the monitoring of multiple wavelengths simultaneously. However, UV detectors may not always provide precise identification of all substances, necessitating additional capabilities to enhance detection. To address this, combining LC with mass spectrometry (MS) has become a cornerstone in pharmaceutical research and development. This integration, referred to as LC-MS, offers exceptional throughput, selectivity, and sensitivity, making it a powerful tool for structural characterization of non-volatile compounds. LC-MS effectively marries the separation power of LC with the mass analysis capabilities of MS, resulting in an analytical technique that excels in resolving complex structural problems.



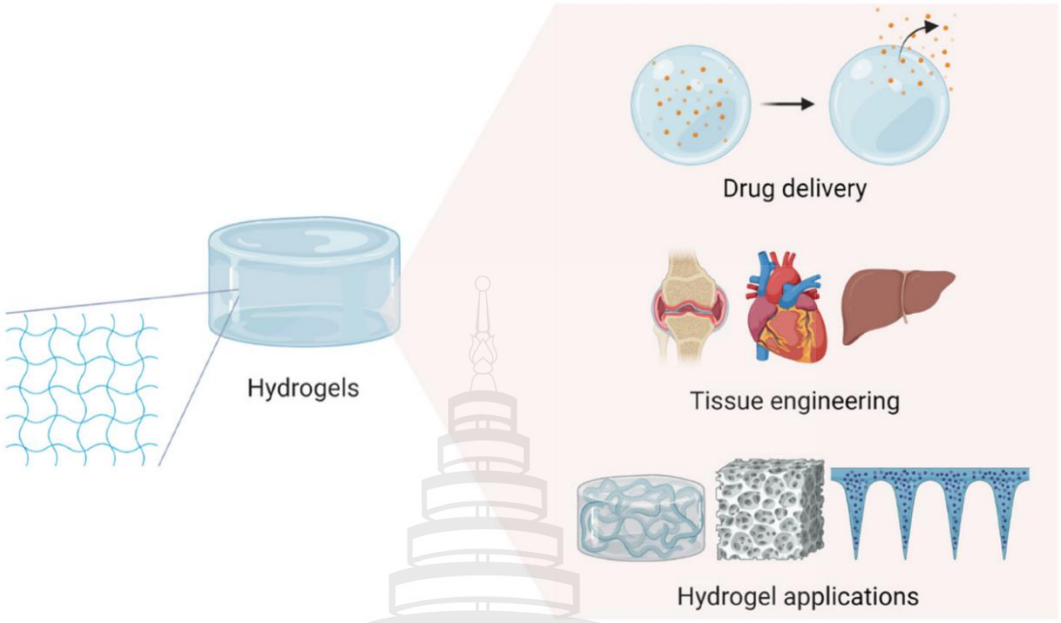
Source López-Fernández et al. (2020)

Figure 2.17 Determination of plant components using LC MS/MS technique

LC-MS has found applications across all phases of drug development, including the discovery, preclinical, and clinical stages. It is also instrumental in conducting metabolism studies in both in vitro and in vivo environments, as well as identifying impurities and degradation products in pharmaceuticals. Advances in LC-MS interface technologies and the continuous refinement of structural analysis tools have further solidified its role as an indispensable asset in pharmaceutical science (Beccaria & Cabooter, 2020).

2.13 Development of Hydrogels

Hydrogels are three-dimensional polymeric networks capable of retaining large quantities of water while maintaining their structural integrity. Their high water content, softness, and biocompatibility make them ideal candidates for cosmetic and biomedical applications, particularly as drug delivery systems. Hydrogels can encapsulate bioactive compounds and release them in a controlled manner, enhancing the stability and bioavailability of these compounds.



Source Choi et al. (2024)

Figure 2.18 Application of hydrogels on different fields

The study by Ivaniuk et al. (2018) on vaginal gel formulation highlights the potential of hydrogels for delivering active ingredients effectively. The research emphasized the use of Aristoflex AVC as a gelling agent and propylene glycol as a solvent to enhance the release of bioactive compounds like resveratrol. This study demonstrated that a concentration of 15% propylene glycol optimized the release of resveratrol over six hours, suggesting that a similar approach could ensure the effective release of phytochemicals from galangal and bitter ginger. Furthermore, a gelling agent concentration of 1.5% provided a balance between stability and spreadability, ensuring consumer-friendly properties. The rheological analysis indicated pseudoplastic flow and thixotropic behavior, critical for ease of application and maintaining efficacy during use.

Given these properties, hydrogels represent a promising choice for formulating cosmetic products that leverage the antioxidant and antimicrobial properties of galangal and bitter ginger extracts. By encapsulating these phytochemicals within a hydrogel matrix, their stability and penetration into the skin can be improved, offering enhanced therapeutic benefits.

2.14 Stability Testing

Stability testing is a crucial process in the development of cosmetic formulations to ensure the efficacy, safety, and performance of active ingredients under various storage conditions. According to Pongsua (2016), the stability of water-soluble polysaccharides extracted from edible mushrooms was evaluated through a series of rigorous tests. These tests involved exposing the extracts to repeated heating-cooling cycles and varied storage conditions, including refrigeration (4°C), elevated temperatures (45°C), and ambient room temperature over a three-month period.

The results demonstrated that the polysaccharides retained their antioxidant properties, as evidenced by stable ABTS radical scavenging capacities. Furthermore, the gel cream formulations incorporating these polysaccharides exhibited no significant changes in viscosity, pH, or visual appearance throughout the stability testing phases. Such findings underscore the robustness of these bioactive compounds, validating their use as stable and effective ingredients in cosmetic products.

The methodology employed by Pongsua (2016) aligns closely with industry standards, highlighting the importance of replicating real-world conditions to predict product behavior over its shelf life. These stability assessments are essential for ensuring that formulations maintain their desired functional properties, such as moisturizing and antioxidant efficacy, when subjected to varying environmental stresses.

This study provides a foundation for incorporating similar stability testing protocols in hydrogel formulations containing plant extracts, such as galangal and bitter ginger, to ensure their viability and effectiveness in cosmetic applications.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Chemicals and Instruments

Table 3.1 Chemicals

Chemicals	**Chemical formula**	**Company**
Ammonium Acryloyldimethyltaurate/VP Copolymer	$C_7H_{16}N_2O_4S$	CLARIANT
Ethanol	C_2H_6O	KRUNGTHEPCHEMI
Methanol	CH_3OH	KRUNGTHEPCHEMI
Phenoxyethanol	$C_8H_{10}O_2$	Chemipan
Tetra sodium EDTA	$C_{10}H_{12}N_2Na_4O_8$.	Chemipan

Table 3.2 Instruments

Instruments	**Model**	**Company**
Climate chamber	KBF720	BINDER
Colorimeter	UltraScan VIS	HunterLab
Field Emission Scanning Electron Microscope	MIRA4	TESCAN
Incubator shaker	SI4	Shel Lab
Overhead stirrer	RW20 Digital	IKA
PH meter	PH700	EUTECH
Rotary evaporator	Hei-VAP	Heidolph
Tray dryer	Model SGO5	Shel Lab
Viscometer	DV-II+ Pro	Brookfield

3.2 Methodology

3.2.1 Plant Materials and Extraction

Fresh rhizomes, stems, and leaves of bitter ginger (*Zingiber zerumbet*) were purchased from Nakhon Si Thammarat, Thailand and galangal (*Alpinia galanga*) were obtained from local farmers in Chiang Rai, Thailand, in October 2024. To ensure quality and consistency, plant materials were carefully selected, washed thoroughly with tap water, and cut into small pieces before drying. Drying was conducted in a tray dryer at 60°C, a temperature chosen to preserve bioactive compounds while ensuring complete dehydration. The dried materials were then ground into a fine powder using a hammer mill.

For extraction, the method was adapted from Mahae and Chaiseri (2009). A total of 30 g of powdered sample was extracted using absolute ethanol or methanol at a ratio of 1:6 (w/v). The mixtures were continuously agitated in an incubator shaker at 150 rpm at room temperature for 24 hours to facilitate the extraction of polar phytochemicals. After incubation, the solutions were filtered through Whatman® No.1 filter paper using a Buchner funnel to separate the residues. The filtrates were then concentrated at 60°C under reduced pressure using a rotary evaporator to remove the solvents and obtain semi-solid crude extracts.

The extracts were transferred into amber glass vials and stored at 16°C, away from light, to maintain their stability and prevent degradation. Extraction yields were calculated in triplicate to ensure accuracy and reproducibility. This procedure was carefully optimized to enhance yield while preserving the bioactive components for further analysis.

3.2.2 Acne-Causing Bacteria

The acne-causing bacteria utilized in this study included *Cutibacterium acnes* DMST 14916, *Staphylococcus epidermis* TISTR 518, and *Staphylococcus aureus* TISTR 746. *C. acnes* was cultured in brain heart infusion broth under anaerobic conditions at 37 °C for a duration of 3 to 5 days. In contrast, *S. aureus* and *S. epidermidis* were grown in nutrient broth at 37 °C for 24 to 48 hours. All bacterial strains were

sourced from the Biology and Biotechnology Laboratory, part of the Scientific and Technological Instruments Center at Mae Fah Luang University, Thailand.

3.2.3 Antioxidant Activity Assay

The antioxidant potential of the bitter ginger and galangal extracts was assessed using a DPPH assay, following a slightly modified method from Mahae and Chaiseri (2009). In brief, 50 µL of various concentrations of the extracts were added to 200 µL of a 0.1 mM DPPH solution. The reaction mixtures were kept under dark conditions at room temperature for 30 minutes. The absorbance of the solutions was measured at 517 nm using a microplate reader. Ascorbic acid served as the positive control. The percentage of antioxidant activity (I%) was determined using the formula

$$I\% = \left[\frac{(A517\ control - A517\ sample)}{A517\ control}\right] \times 100$$

where A_{517}control is the absorbance of the control solution without the extracts, and A_{517}sample is the absorbance of the solution containing the extracts (or ascorbic acid). The value, which represents the concentration of the extracts (or ascorbic acid) required to inhibit DPPH activity by 50%, was subsequently calculated. The IC_{50} value, which represents the concentration of the extracts (or ascorbic acid) required to inhibit DPPH activity by 50%, was subsequently calculated.

3.2.4 Antimicrobial Activity Assay

The antimicrobial properties of bitter ginger and galangal extracts against *C. acnes* DMST 14916, *S. epidermidis* TISTR 518, and *S. aureus* TISTR 746 were assessed using a broth microdilution assay, adapted slightly from earlier studies (Wu et al., 2020; Pata et al., 2011). Serial dilutions of the extracts were prepared in 10% DMSO. Bacterial cells were cultured to the logarithmic growth phase (OD_{600nm} = 0.5–0.8) and diluted to a density of approximately 10^6 cells/mL (OD_{600nm} = 0.001). The bacterial cultures were treated with varying concentrations of the extracts (0.50–31.68 mg/mL) and incubated at 37 °C for 24 hours for *S. epidermidis* and *S. aureus*, and for 72 hours under anaerobic conditions for *C. acnes*. DMSO (10% v/v) was employed as a negative control, while tetracycline served as a positive control.

The minimum inhibitory concentration (MIC) of the extracts was determined using the resazurin dye solution technique (Elshikh et al., 2016). Following incubation for 24 or 72 hours, 0.06% resazurin dye solution (10 µL) was added to the bacterial suspensions and further incubated for 4–6 hours. The MIC value was defined as the lowest concentration of the extracts capable of inhibiting bacterial growth without altering the color of the resazurin dye. The maximum bactericidal concentration (MBC) was evaluated by plating the bacterial suspensions on agar plates and determining colony counts.

3.2.5 Scanning Electron Microscopic Analysis

The antimicrobial properties of bitter ginger and galangal extracts against *C. acnes* DMST 14916 and *S. epidermidis* TISTR 518 were examined using scanning electron microscopy (SEM), based on the method described by Lau et al. (2004). The bacterial cells were cultured to the logarithmic growth phase (OD_{600nm} = 0.5–0.8), centrifuged at 3500 × g for three minutes, and washed twice with PBS (pH 7.4). The harvested cells were then diluted to a concentration of approximately 10^8 cells/mL (OD_{600nm} = 0.1). *C. acnes* and *S. epidermidis* were exposed to bitter ginger and galangal extracts at 10 × MICs for 60 minutes under anaerobic and aerobic conditions, respectively, with untreated cells serving as controls. A 10 µL aliquot of each sample was spread onto a coverslip and heat-fixed using a flame. The bacterial cells were dehydrated in a graded ethanol series (30%, 50%, 70%, 90%, and 100%) for 30–60 minutes in each solution. The dried cells were then coated with gold-palladium and visualized using a Field Emission Scanning Electron Microscope.

3.2.6 LC-MS/MS Analysis

Phytochemical analysis of bitter ginger and galangal extracts was conducted using the LC-MS/MS method, based on the procedure described by Phosri et al. (2022). Extract samples (500 ppm) were prepared in absolute methanol, filtered through 0.2 µm NYL filters, and transferred into 1.5 mL glass vials. For LC analysis, the samples were injected into an Agilent Poroshell EC-C18 column (2.1 mm × 150 mm, 2.7 µm) with a guard column of the same material. The separation was performed using a linear gradient of mobile phase A (0.1% formic acid in water) and mobile phase B (acetonitrile) at a flow rate of 0.2 mL/min.

Four extracts were selected for LC-MS/MS analysis: bitter ginger rhizome ethanol extract (BRE), bitter ginger rhizome methanol extract (BRM), galangal rhizome ethanol extract (GRE), and galangal rhizome methanol extract (GRM). These extracts were chosen based on their strongest antimicrobial activity, as demonstrated in Table 4.2, where the rhizome extracts of both plants exhibited the highest inhibition against *Cutibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. Rhizomes generally contain higher concentrations of bioactive secondary metabolites, such as flavonoids, phenolics, and terpenoids, which are known for their antimicrobial properties. Additionally, both ethanol and methanol extracts were included to compare the effect of solvent polarity on phytochemical composition, as methanol efficiently extracts polar compounds while ethanol is effective for a broader range of polar and non-polar compounds.

Data acquisition for MS analysis was performed on an Agilent G6454B Q-TOF Mass Spectrometry unit equipped with a Dual AJS ESI ion source. The capillary voltage was set to 4000 V, nozzle voltage to 500 V, and voltages for skimmer1, fragmentor, and OctopoleRFPeak were set to 65 V, 150 V, and 750 V, respectively. The scan range was 100–1100 m/z with a scan rate of 1.00 spectra/s. Internal reference compounds of m/z 121.05087300 and m/z 922.00979800 were used for the positive mode, while m/z 112.98558700 and m/z 1033.98810900 were used for the negative mode. MS/MS data were collected at collision energies of 10, 20, and 40 eV.

3.2.7 Statistical Analysis

Statistical analysis was conducted using Statistix version 9.0 software. Differences between the control and sample groups were evaluated using analysis of variance (ANOVA). Significant differences between groups were determined using a student's t-test, and results were considered statistically significant when the p-value was less than 0.05 ($p < 0.05$).

3.2.8 Development of Hydrogel Formulations

Hydrogel formulations were developed following a modified approach based on the method described by Ivaniuk et al. (2018). A total of six formulations were created to incorporate extracts derived from the rhizomes of Bitter Ginger and Galangal. These included a hydrogel base without additives (F1), a base containing a preservative (F2), and four experimental formulations (F3–F6). The experimental formulations (F3–

F6) each contained 0.3% of one of the rhizome extracts: GRE (Galangal rhizome ethanol extract), GRM (Galangal rhizome methanol extract), BRE (Bitter Ginger rhizome ethanol extract), or BRM (Bitter Ginger rhizome methanol extract). The detailed composition of all formulations is outlined in **Table 3.3**.

The concentration of 0.3% plant extract was selected to ensure optimal solubility and formulation stability. Preliminary trials indicated that increasing the extract concentration beyond this level resulted in precipitate formation, which could compromise the homogeneity, viscosity, and long-term stability of the hydrogel system. Since plant extracts contain a diverse range of bioactive compounds with varying solubility, exceeding their solubility limit within the gel matrix could lead to phase separation or sedimentation. By maintaining the extract concentration at 0.3%, the formulation remained physically stable, ensuring even distribution of bioactives while preserving the hydrogel's structural integrity and usability for cosmetic applications.

To prepare the hydrogels, Aristoflex AVC was dispersed in deionized water while stirring continuously with an overhead stirrer at room temperature until a uniform gel base was formed. Tetra EDTA and phenoxyethanol were added to the base as a chelating agent and preservative, respectively. For the experimental formulations (F3–F6), the plant extracts were dissolved in 95% ethanol before being incorporated into the hydrogel base. Gentle mixing was applied to ensure even distribution of the extracts throughout the gel. The final hydrogel formulations were left to equilibrate at room temperature for 24 hours before undergoing stability testing and characterization.

Table 3.3 Formula of hydrogel preparations

Ingredients (%)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Tetra EDTA	0.1	0.1	0.1	0.1	0.1	0.1
Aristoflex AVC	1.5	1.5	1.5	1.5	1.5	1.5
Ethanol 95%	-	-	5	5	5	5
GRE Extract	-	-	0.3	-	-	-
GRM Extract	-	-	-	0.3	-	-
BRE Extract	-	-	-	-	0.3	-
BRM Extract	-	-	-	-	-	0.3
Phenoxyethanol	-	1	1	1	1	1
Deionized water	*qs.*100	*qs.*100	*qs.*100	*qs.*100	*qs.*100	*qs.*100

Note F1: Hydrogel base, F2: Hydrogel base with preservative, F3: Hydrogel with 0.3% GRE extract, F4: Hydrogel with 0.3% GRM extract, F5: Hydrogel with 0.3% BRE extract, F6: Hydrogel with 0.3% BRM extract

3.2.9 Stability Testing

The stability of the hydrogel formulations was assessed using a cyclic temperature stress test to replicate extreme storage conditions. The samples were exposed to four heating and cooling cycles in a controlled climate chamber. Each cycle comprised two phases: heating at 45°C for 24 hours, followed by cooling at 4°C for an additional 24 hours.

The color, pH, and viscosity of each formulation were evaluated both before the cyclic test began and immediately after its completion. Color analysis was conducted using a calibrated colorimeter to measure L* (lightness), a* (red-green), and b* (yellow-blue) values. pH measurements were taken with a calibrated pH meter, and viscosity was determined using a viscometer operating at a controlled shear rate. To ensure precision and reliability, all measurements were conducted in triplicate.

CHAPTER 4

RESEARCH RESULTS

4.1 Yields and Antioxidant Activity of Crude Extracts of Bitter Ginger and Galangal

Bitter ginger and galangal (Figure 4.1) were divided into three parts: rhizomes, stems, and leaves, which were then subjected to extraction. Crude extracts of bitter ginger and galangal were obtained using both ethanol and methanol as solvents. Extracts from the rhizomes (BRE, BRM, GRE, and GRM), stems (BSE, BSM, GSE, and GSM), and leaves (BLE, BLM, GLE, and GLM) were produced, as shown in Table 4.1 The yields for rhizome extractions were as follows: BRE (5.17 ± 0.63%), BRM (7.30 ± 0.09%), GRE (5.47 ± 0.40%), and GRM (6.94 ± 0.50%). For stem extractions, the yields were BSE (5.03 ± 0.76%), BSM (1.06 ± 0.13%), GSE (1.72 ± 0.06%), and GSM (2.72 ± 0.62%). The leaf extractions yielded BLE (2.14 ± 0.34%), BLM (2.14 ± 0.37%), GLE (5.37 ± 0.94%), and GLM (5.67 ± 0.36%). Statistical analysis revealed no significant difference in the yields of galangal rhizomes extracted with ethanol or methanol (5.47% vs. 6.94%) or in the yields of ethanolic and methanolic extracts of bitter ginger leaves (2.14% vs. 2.14%) and galangal leaves (5.37% vs. 5.67%) ($p > 0.05$). However, the yields of ethanolic and methanolic extracts from bitter ginger rhizomes (5.71% vs. 7.30%) showed significant differences ($p < 0.05$), as did the yields from bitter ginger stems (5.03% vs. 1.06%) and galangal stems (1.72% vs. 2.72%) ($p < 0.05$).

The antioxidant activity of the extracts was assessed using a DPPH radical scavenging assay. Ascorbic acid was used as the positive control, with an IC_{50} value of 1.4 ± 0.2 µg/mL. The IC_{50} values for the rhizome extracts were BRE (1.19 ± 0.06 mg/mL), BRM (0.99 ± 0.04 mg/mL), GRE (0.08 ± 0.01 mg/mL), and GRM (0.06 ± 0.01 mg/mL). For the stem extracts, the IC_{50} values were BSE (1.42 ± 0.04 mg/mL), BSM(0.46 ± 0.03 mg/mL), GSE (0.15 ± 0.01 mg/mL), and GSM (0.28 ± 0.02 mg/mL). The IC_{50} values of the leaf extracts were BLE (0.27 ± 0.03 mg/mL), BLM (0.17 ± 0.02

mg/mL), GLE (0.30 ± 0.01 mg/mL), and GLM (0.17 ± 0.02 mg/mL). Statistical analysis revealed that the methanolic extract of bitter ginger stems (BSM) had a significantly lower IC_{50} value compared to its ethanolic counterpart (BSE) ($p < 0.05$). Additionally, the ethanolic extraction of galangal stems (GSE) was more effective at extracting antioxidants compared to the methanolic extraction (GSM) ($p < 0.05$).

Methanolic extracts of bitter ginger and galangal leaves (BLM and GLM) demonstrated higher antioxidant activity than their ethanolic counterparts (BLE and GLE) ($p < 0.05$). Similarly, the methanolic extract of bitter ginger rhizomes (BRM) exhibited greater antioxidant activity than its ethanolic extract (BRE) ($p < 0.05$). However, no significant difference was observed between the antioxidant activities of methanolic and ethanolic extracts of galangal rhizomes (GRM and GRE) ($p > 0.05$). Among all extracts, GRM and GRE exhibited the highest antioxidant activity.

(a) (b)

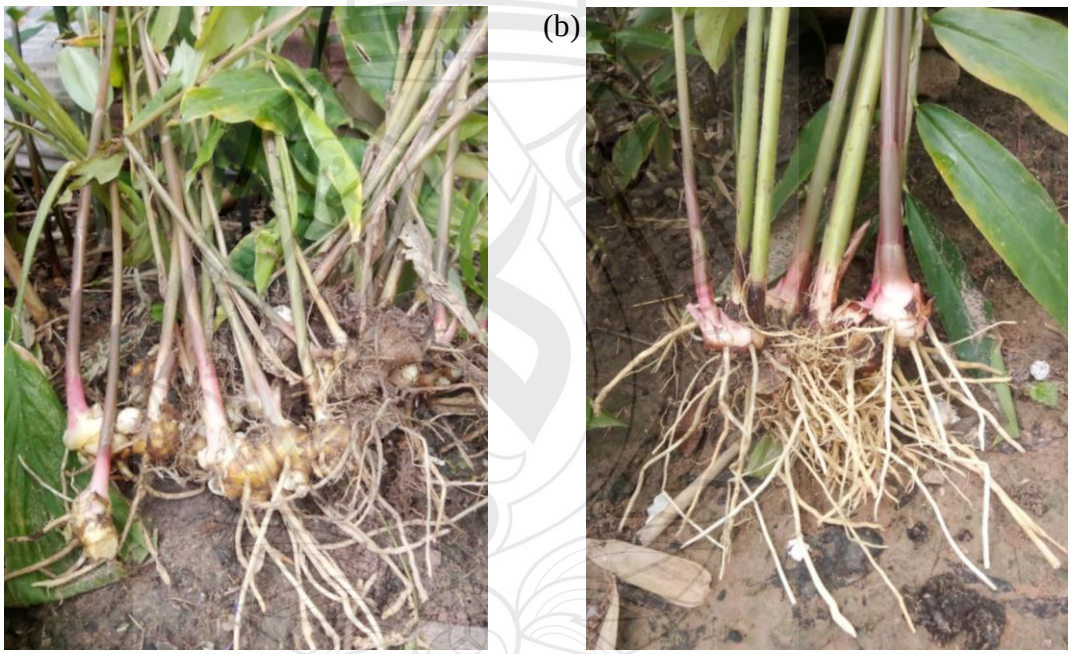


Figure 4.1 Whole plants of bitter ginger (*Zingiber zerumbet* (L.) Roscoe) (a) and galangal (*Alpinia galanga* (L.) Wild) (b)

Table 4.1 Yields and DPPH radical scavenging activity of crude extracts of galangal and bitter ginger

Chemicals	**Yields ± SD (%)**	**IC_{50}**
Ethanolic extract		
Bitter ginger rhizome (BRE)	5.71 ± 0.63^{b}	1.19 ± 0.06^{b}
Bitter ginger stem (BSE)	5.03 ± 0.76^{c}	1.42 ± 0.04^{a}
Bitter ginger leaf (BLE)	2.14 ± 0.34^{de}	0.40 ± 0.02^{e}
Galangal rhizome (GRE)	5.47 ± 0.06^{b}	0.08 ± 0.01^{h}
Galangal stem (GSE)	1.72 ± 0.06^{e}	0.15 ± 0.01^{g}
Galangal leaf (GLE)	5.37 ± 0.94^{b}	0.27 ± 0.03^{f}
Methanolic extract		
Bitter ginger rhizome (BRM)	7.30 ± 0.09^{a}	0.99 ± 0.04^{c}
Bitter ginger stem (BSM)	1.06 ± 0.13^{f}	0.46 ± 0.03^{d}
Bitter ginger leaf (BLM)	2.14 ± 0.37^{de}	0.30 ± 0.01^{f}
Galangal rhizome (GRM)	6.94 ± 0.50^{b}	0.06 ± 0.01^{h}
Galangal stem (GSM)	2.72 ± 0.62^{d}	0.28 ± 0.02^{f}
Galangal leaf (GLM)	5.67 ± 0.36^{b}	0.17 ± 0.02^{g}
Ascorbic acid	-	1.4 ± 0.2*

Note *The value is expressed in µg/mL. Values (mean ± SD) are the averages of three samples of each plant extract, analyzed individually in triplicate. Superscript letters within the same column indicate significant ($p < 0.05$) differences of means within the plant extracts.

4.2 Antimicrobial Activity of Bitter Ginger and Galangal Extracts

The antimicrobial properties of bitter ginger and galangal extracts were evaluated against acne-causing bacteria, including *C. acnes* DMST 14916, *S. aureus* TISTR 746, and *S. epidermidis* TISTR 518, using a broth microdilution assay. As shown in Table 4.2, most ethanol and methanol extracts from bitter ginger and galangal demonstrated bactericidal activity against *C. acnes*. Extracts derived from plant rhizomes exhibited the widest spectrum of antimicrobial activity, effectively targeting

C. acnes, *S. aureus*, and *S. epidermidis*. The minimum inhibitory concentrations (MICs) of BRE, BRM, and GRM for C. *acnes* were 3.96 mg/mL, while the MIC of GRE was 7.92 mg/mL.

The minimum bactericidal concentrations (MBCs) of BRE, BRM, GRE, and GRM against C. *acnes* were 3.96 mg/mL, 7.92 mg/mL, 15.84 mg/mL, and 7.92 mg/mL, respectively. For S. *aureus*, the MICs of BRE, BRM, GRE, and GRM were 7.92 mg/mL, 15.84 mg/mL, >31.68 mg/mL, and 31.68 mg/mL, while the MBCs were 7.92 mg/mL, >31.68 mg/mL, >31.68 mg/mL, and >31.68 mg/mL, respectively. Against S. *epidermidis*, the MICs of BRE, BRM, and GRE were 15.84 mg/mL, while that of GRM was 7.92 mg/mL. The MBCs of BRE, BRM, GRE, and GRM against S. *epidermidis* were >31.68 mg/mL. While extracts from the stems and leaves of the plants exhibited antimicrobial activity against *C. acnes*, they showed no effect on *S. aureus* and *S. epidermidis*. The findings indicated that rhizome extracts from bitter ginger (BRE and BRM) and galangal (GRE and GRM) demonstrated broad-spectrum antimicrobial activity against these acne-associated bacteria. Consequently, these extracts were selected for further experiments in this study.

Table 4.2 The antimicrobial activity of crude extracts of galangal and bitter ginger.

Extract	*Cutibacterium acnes* DMST 14916		*Staphylococcus aureus* TISTR 746		*Staphylococcus epidermidis* TISTR 518	
	MIC (mg/mL)	**MBC (mg/mL)**	**MIC (mg/mL)**	**MBC (mg/mL)**	**MIC (mg/mL)**	**MBC (mg/mL)**
Ethanol extract						
Bitter ginger rhizome (BRE)	3.96	3.96	7.92	7.92	15.84	>31.68
Bitter ginger stem (BSE)	31.68	>31.68	Nd	Nd	Nd	Nd
Bitter ginger leaf (BLE)	31.68	31.68	Nd	Nd	Nd	Nd
Galangal (GRE)	7.92	15.84	>31.68	>31.68		
Galangal stem (GSE)	>31.68	>31.68	Nd	Nd	Nd	Nd
Galangal leaf (GLE)	7.92	31.68	Nd	Nd	Nd	Nd
Methanol extract						
Bitter ginger rhizome (BRE)	3.96	7.92	15.84	>31.68	15.84	>31.68
Bitter ginger stem (BSE)	>31.68	>31.68	Nd	Nd	Nd	Nd
Bitter ginger leaf (BLE)	3.96	>3.96	Nd	Nd	Nd	Nd
Galangal (GRE)	3.96	7.92	31.68	>31.68	7.92	>31.68
Galangal stem (GSE)	15.84	15.84	Nd	Nd	Nd	Nd
Galangal leaf (GLE)	15.84	15.84	Nd	Nd	Nd	Nd
Tetracycline	1*	4*	2*	4*	64*	256*

Note Nd, not detected in concentration range of 0.50-31.68 mg/mL. *The value is expressed in µg/mL

4.3 Effects of Bitter Ginger and Galangal Extracts Observed via SEM

The antimicrobial effects of ethanol and methanol extracts from bitter ginger and galangal rhizomes were evaluated using *C. acnes* DMST 14916 and *S. epidermidis* TISTR 518 via scanning electron microscopy (SEM). The results are displayed in Figures 4.2 and 4.3, respectively. As shown in Figure 4.2, untreated *C. acnes* cells, used as control cells, were approximately 1 micron in size and exhibited a smooth surface with no ruptures (Figure 4.2a). However, C. *acnes* cells treated with rhizome extracts, including BRE, BRM, GRE, and GRM, displayed significant surface damage and shrinkage (indicated by arrows) (Figure 4.2b–e).

Similarly, as illustrated in Figure 4.3, untreated *S. epidermidis* cells, which served as controls, were round-shaped, approximately 1 micron in size, and had smooth surfaces without abnormalities (Figure 4.3a). In contrast, *S. epidermidis* cells treated with BRE exhibited notable shrinkage of the cell surface (arrows) (Figure 4.3b), while those treated with BRM showed minor shrinkage and surface ruptures (arrows) (Figure 4.3d). Cells treated with GRE and GRM demonstrated slight shrinkage but retained a significant amount of cell debris on their surfaces (arrows) (Figure 4.3c,e).

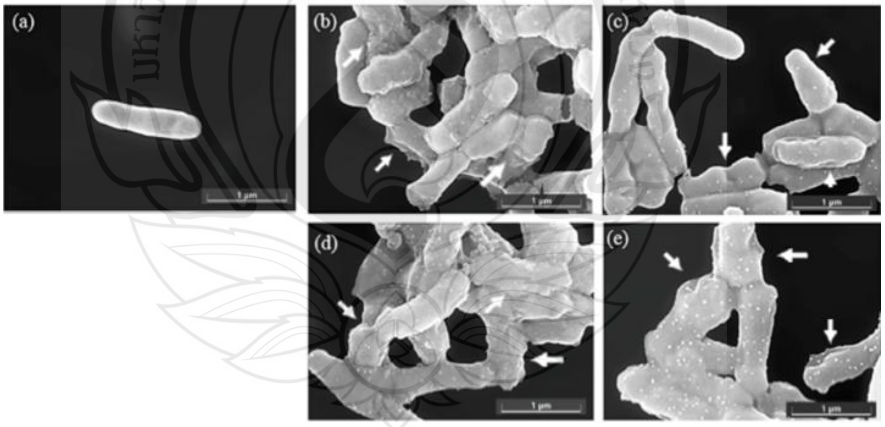


Figure 4.2 SEM images of *Cutibacterium acnes* DMST 14916 after incubation with bitter ginger and galangal extracts at 10× MICs for 60 min. The density of bacterial cells was used at approximately 1 x 10^8 cells/mL. Cells of *C. acnes* were treated without any sample as controls (a). The cells were treated with the ethanol extracts of the bitter ginger rhizome (BRE) (b) and galangal rhizome (GRE) (c) and the methanol extracts of the bitter ginger rhizome (BRM) (d) and galangal rhizome (GRM) (e). Scale bar: 1 µm.

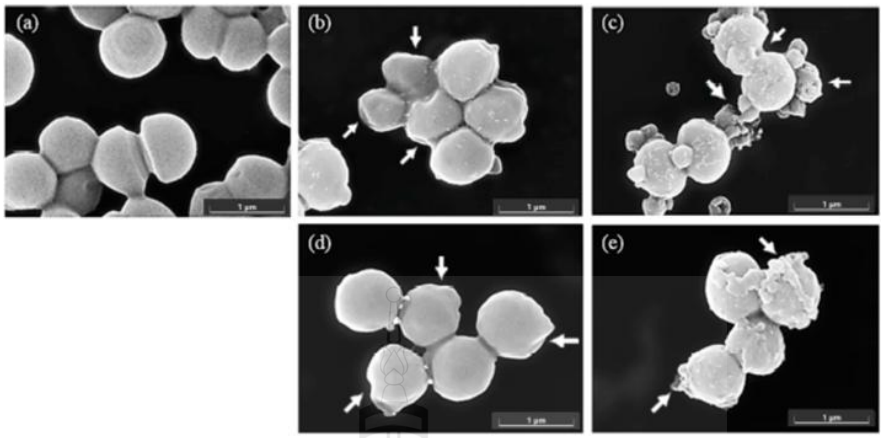


Figure 4.3 SEM images of Staphylococcus epidermidis TISTR 518 after incubation with bitter ginger and galangal extracts at 10 × MICs for 60 min. The density of bacterial cells was used at approximately 1×10^8 cells/mL. Cells of S. epidermidis were treated without any sample as controls (a). The cells were treated with the ethanol extracts of the bitter ginger rhizome (BRE) (b) and galangal rhizome (GRE) (c) and the methanol extracts of the bitter ginger rhizome (BRM) (d) and galangal rhizome (GRM) (e). Scale bar: 1 µm.

4.4 Determination of Phytochemicals in Bitter Ginger and Galangal Extracts via LC-MS/MS

LC-MS chromatograms were evaluated (Figure 4.4), phytochemical substances that were acquired from LC-QTOF-MS/MS were analyzed in the extracts of bitter ginger rhizome (Tables 4.3 and 4.4). Results shown in Table 4.3 demonstrate that twenty phytochemicals in BRE were identified, including sugar (allose and D-(+)-turanose), fatty acid derivatives (3-hydroxyphenyl-valeric acid), phenolic derivatives (1,3-dicaffeoylquinic acid and piceatannol 4'-galloylglucoside), flavonoid derivatives (apigenin 7-galactoside, 8-C-beta-D-glucofuranosylapigenin 2''-O-acetate, myricetin 3-(2''-p-hydroxybenzoylrhamnoside), tectorigenin and cirsimaritin), alkaloid (piperic acid), ubiquinones (myrsinone), catecholamine (n-acetyldopamine), 6a-hydroxymaackiain, canescacarpin, lauryl hydrogen sulfate, trifluoroacetic acid, N-undecylbenzene sulfonic acid, sodium tetradecyl sulfate, and 2-dodecylbenzene sulfonic acid. Similarly, Table 4.4 indicates twenty phytochemicals that were identified in BRM, including sugar

(sucrose), fatty acid derivatives (3-hydroxyphenyl-valeric acid), phenolic derivatives (1,3-dicaffeoylquinic acid, piceatannol 4'-galloylglucoside, 2,4,2'-trihydroxy-6'',6''-dimethyl-3'-prenylpyrano[2'',3':4',5']chalcone), flavonoid derivatives (apigenin 7-galactoside, 8-C-beta-D-glucofuranosylapigenin 2''-O-acetate, myricetin 3-(2''-p-hydroxybenzoylrhamnoside), tectorigenin and cirsimaritin), ubiquinones (myrsinone), catecholamine (n-acetyldopamine), diterpenoids (triptophenolide), trifluoroacetic acid, N-undecylbenzene sulfonic acid, 2-dodecylbenzene sulfonic acid, cis-ß-D-glucosyl-2-hydroxycinnamate, demethoxycurcumin, thyrotropin releasing hormone, and 7E,9E, 11-dodecatrienyl acetate.

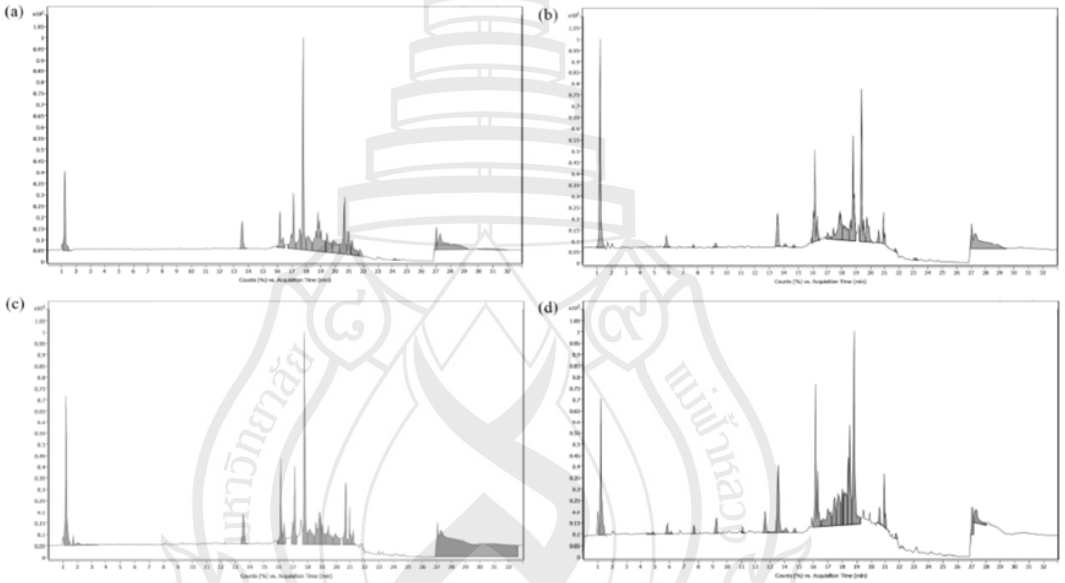


Figure 4.4 LC-MS chromatograms of ethanolic and methanolic extractof bitter ginger and galangal rhizomes: The LC-MS chromatograms of the ethanol extracts of the bitter ginger rhizome (BRE) (a) and galangal rhizome (GRE) (b) and the methanol extracts of the bitter ginger rhizome (BRM) (c) and galangal rhizome (GRM) (d).

In Tables 4.5 and 4.6, the phytochemicals that are contained in the extracts of galangal rhizome are presented. As the results show in Table 4.5, twenty phytochemicals in GRE were identified and included sugar (sucrose), fatty acid derivatives (3-hydroxyphenyl-valeric acid), phenolic compounds and derivatives (sweroside and methylsyringin), flavonoids and flavonoid derivatives (amoritin and (+)-myristinin A), ubiquinones (myrsinone), catecholamine (n-acetyldopamine),

diterpene and diterpenoids (sagequinone methide A and gamma-crocetin), coumarins (dihydrosamidin and phenprocoumon), sesquiterpenes (10-hydroxymelleolide), nivalenol, 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)p-(3,4-dihydro-6-methoxy-2-naphthyl)phenol, -1-propanol, and (2-butylbenzofuran-3-yl)(4-hydroxyphenyl) are all examples of compound names.ketone, cortisone acetate, dinoterb, and thyrotropin releasing hormone are the active ingredients. As can be seen in Table 4.6, twenty different phytochemicals were found in BRM. These phytochemicals included sugar (sucrose), phenolic compounds and derivatives (myzodendrone), flavonoids and flavonoid derivatives (neobavaisoflavone), ubiquinones (myrsinone), coumarins (phenprocoumon), diterpene and diterpenoids (sagequinone methide A), sesquiterpenes and derivatives (molephantinin), lignan (Gmelinol), and ubiquinones (myrsinone). Triptophenolide, 2-dodecylbenzenesulfonic acid, lauryl hydrogen sulfate, and 2-(4-Allyl-2,6-dimethoxyphenoxy) are the components of this compound.The chemical formula for -1-(4-hydroxy-3-methoxyphenyl)-1-propanol, (2-Butylbenzofuran-3-yl)(4-hydroxyphenyl)ketone, gibberellin A120, nivalenol, thyrotropin releasing hormone, sodium tetradecyl sulfate, and N-undecylbenzenesulfonic acid**.**

Table 4.3 Analysis of phytochemical constituents in the ethanol extract of bitter ginger rhizome (BRE) performed via LC-QTOF-MS-MS.

RT (min)	*m/z*	MS/MS Fragments	Formula	Tentative Identification	Mass	Ion Species
1.201	179.0566	59.0144, 71.0144	C6 H12 O6	Allose	180.064	(M-H)-
1.264	341.1094	89.0249, 179.0552, 341.1089	C12 H22 O11	D-(+)-Turanose	342.117	(M-H)-
16.393	431.0987	285.0402, 431.0984	C21 H20 O10	Apigenin 7-galactoside	432.106	(M-H)-
17.104	473.109	284.0323, 413.087, 473.107	C23 H22 O11	8-C-beta-D-Glucofuranosylapigenin 2”-O-acetate	474.116	(M-H)-
17.835	515.1211	284.0326,455.0974, 515.1196	C25 H24 O12	1,3-Dicaffeoylquinic acid	516.128	(M-H)-
17.839	583.1079	284.0316,515.1195, 583.1062	C28 H24 O14	Myricetin 3-(2″-p-hydroxybenzoylrhamnoside)	584.115	(M-H)-
17.911	299.0563	112.9856,284.0333, 300.0592	C16 H12 O6	Tectorigenin	300.064	(M-H)-
18.512	193.0871	124.0155, 193.0870	C11 H14 O3	3-Hydroxyphenyl-valeric acid	194.094	(M-H)-
18.589	557.1303	284.0324, 557.1307	C27 H26 O13	Piceatannol 4′-galloylglucoside	558.138	(M-H)-
18.847	217.0508	68.9983, 158.0374, 173.0603	C12 H10 O4	Piperic acid	218.058	(M-H)-
18.856	299.0559	63.0237, 151.0025, 255.0304	C16 H12 O6	6a-Hydroxymaackiain	300.063	(M-H)-
18.919	293.1761	71.0141, 177.0915, 236.1057	C17 H26 O4	Myrsinone	294.183	(M-H)-
18.929	337.1085	119.0503, 217.0506	C20 H18 O5	Canescacarpin	338.116	(M-H)-
19.104	313.072	112.9856, 283.0243	C17 H14 O6	Cirsimaritin	314.079	(M-H)-
19.512	194.0823	180.0603, 194.0822	C10 H13 N O3	n-acetyldopamine	195.09	(M-H)-
19.949	265.1482	96.9603, 265.1479	C12 H26 O4 S	Lauryl hydrogen sulfate	266.156	(M-H)-
20.322	112.9856	68.9961	C2 H F3 O2	trifluoroacetic acid	113.993	(M-H)-
20.908	311.1691	183.0123, 311.1691	C17 H28 O3 S	N-Undecylbenzene sulfonic acid	312.176	(M-H)-
21.79	293.1797	96.9605, 293.1794	C14 H30 O4 S	Sodium tetradecyl sulfate	294.187	(M-H)-
21.827	325.1844	119.0504, 183.0124	C18 H30 O3 S	2-Dodecylbenzene sulfonic acid	326.192	(M-H)-

Table 4.4 Analysis of phytochemical constituents of the methanol extract of bitter ginger rhizome (BRM) performed via LC-QTOF-MS-M

RT (min)	*m/z*	MS/MS Fragments	Formula	Tentative Identification	Mass	Ion Species
1.254	341.1092	89.0243, 179.0555, 341.1091	C12 H22 O11	Sucrose	342.1165	(M-H)-
8.134	325.093	145.0294, 265.0748	C15 H18 O8	cis-β-D-Glucosyl-2-hydroxycinnamate	326.1003	(M-H)-
16.394	431.0992	255.0254, 285.0401, 431.0965	C21 H20 O10	Apigenin 7-galactoside	432.1063	(M-H)-
17.103	473.1095	284.0325, 413.0876, 473.1086	C23 H22 O11	8-C-beta-D-Glucofuranosylapigenin 2"-O-acetate	474.1166	(M-H)-
17.782	515.1215	284.0328, 455.0978, 515.1204	C25 H24 O12	1,3-Dicaffeoylquinic acid	516.1285	(M-H)-
17.828	583.1081	515.1198, 583.1049	C28 H24 O14	Myricetin 3-(2"-p-hydroxybenzoylrhamnoside)	584.1151	(M-H)-
17.834	299.0563	112.9853, 284.0324	C16 H12 O6	Tectorigenin	300.0636	(M-H)-
18.542	193.0869	53.0034, 177.0556	C11 H14 O3	3-Hydroxyphenyl-valeric acid	194.0942	(M-H)-
18.635	557.1306	284.0327, 497.1038, 557.1301	C27 H26 O13	Piceatannol 4'-galloylglucoside	558.1377	(M-H)-
18.828	337.1084	119.0505, 217.0507, 337.1076	C20 H18 O5	Demethoxycurcumin	338.116	(M-H)-
18.912	361.1635	71.0143, 236.1053	C16 H22 N6 O4	Thyrotropin releasing hormone	362.1708	(M-H)-
18.932	293.1762	71.0144, 236.1054, 293.1754	C17 H26 O4	Myrsinone	294.1835	(M-H)-

Table 4.4 (continued)

RT (min)	*m/z*	MS/MS Fragments	Formula	Tentative Identification	Mass	Ion Species
19.037	313.072	255.0296, 283.0249, 313.0704	C17 H14 O6	Cirsimaritin	314.0793	(M-H)-
19.516	194.0825	61.9868, 135.0073, 194.0825	C10 H13 N O3	n-Acetyldopamine	195.0898	(M-H)-
20.11	405.1709	119.0503, 285.1133, 405.1706	C25 H26 O5	2,4,2′-Trihydroxy-6″,6″-dimethyl-3′-prenylpyrano[2″,3″:4′,5′]chalcone	406.1781	(M-H)-
20.442	221.1547	205.1226, 221.1543	C14 H22 O2	7E,9E,11-Dodecatrienyl acetate	222.162	(M-H)-
20.655	311.169	183.0122, 311.1685	C20 H24 O3	Triptophenolide	312.1759	(M-H)-
20.911	311.1689	183.0122, 311.1687	C17 H28 O3 S	N-Undecylbenzenesulfonic acid	312.1761	(M-H)-
21.14	112.9856	68.9962	C2 H F3 O2	Trifluoroacetic acid	113.9929	(M-H)-
21.889	325.1845	119.0508, 183.0128	C18 H30 O3 S	2-Dodecylbenzenesulfonic acid	326.1918	(M-H)-

Table 4.5 Analysis of phytochemical constituents of the ethanol extract of galangal rhizome (GRE) performed via LC-QTOF-MS-MS

RT (min)	*m/z*	MS/MS Fragments	Formula	Tentative Identification	Mass	Ion Species
1.261	341.1093	59.0141, 89.0245, 179.0559	C12 H22 O11	Sucrose	342.1166	(M-H)-
5.826	357.1194	149.0605, 357.1176	C16 H22 O9	Sweroside	358.1266	(M-H)-
16.007	311.1138	149.0606, 311.1186	C15 H20 O7	Nivalenol	312.121	(M-H)-
16.1	385.1505	101.0243, 177.0918, 385.1467	C18 H26 O9	Methylsyringin	386.1578	(M-H)-
17.468	387.1453	149.0607, 263.1074, 341.1392	C21 H24 O7	Dihydrosamidin	388.1525	(M-H)-
17.863	373.1661	251.1079, 327.1600	C21 H26 O6	2-(4-Allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-1-propanol	374.1733	(M-H)-
17.868	251.1081	93.0342, 251.1070	C17 H16 O2	p-(3,4-Dihydro-6-methoxy-2-naphthyl)phenol	252.1152	(M-H)-
17.874	327.1605	251.1076, 327.1587	C20 H24 O4	Sagequinone methide A	328.1677	(M-H)-
18.068	279.1029	173.0607, 119.0499, 279.1021	C18 H16 O3	Phenprocoumon	280.1101	(M-H)-
18.178	293.1183	83.0498, 119.0503, 187.0762, 293.1170	C19 H18 O3	(2-Butylbenzofuran-3-yl) (4-hydroxyphenyl)ketone	294.1255	(M-H)-
18.428	505.2594	251.1072, 343.1382, 459.2162	C31 H38 O6	Amoritin	506.2666	(M-H)-
18.505	193.0868	178.0625, 193.0867	C11 H14 O3	3-Hydroxyphenyl-valeric acid	194.0941	(M-H)-
18.804	415.1765	177.0919, 263.1070, 369.1708	C23 H28 O7	10-Hydroxymelleolide	416.184	(M-H)-
18.916	361.1633	71.0138, 293.1768, 361.1623	C16 H22 N6 O4	Thyrotropin releasing hormone	362.1707	(M-H)-

Table 4.5 (continued)

RT (min)	*m/z*	MS/MS Fragments	Formula	Tentative Identification	Mass	Ion Species
18.936	293.176	71.014, 236.1059, 293.1762	C17 H26 O4	Myrsinone	294.1833	(M-H)-
19.173	547.2699	59.0141, 147.0448, 263.1077, 395.1645, 455.1865, 547.2698	C33 H40 O7	(+)-Myristinin A	548.2771	(M-H)-
19.384	401.1977	177.0917, 263.1079, 355.1918	C23 H30 O6	Cortisone acetate	402.2049	(M-H)-
19.389	355.192	177.0921, 263.1076, 309.1497	C22 H28 O4	gamma-Crocetin	356.1993	(M-H)-
19.473	194.0824	108.0214, 178.0503	C10 H13 N O3	n-Acetyldopamine	195.0897	(M-H)-
20.290	239.0674	123.0328, 239.0671	C10 H12 N2 O5	Dinoterb	240.0747	(M-H)-

Table 4.6 Analysis of phytochemical constituents of the methanol extract of galangal rhizome (GRM) performed via LC-QTOF-MS-MS

RT (min)	*m/z*	MS/MS Fragments	Formula	Tentative Identification	Mass	Ion Species
1.279	341.109	89.0242, 101.024, 341.1086	C12 H22 O11	Sucrose	342.1164	(M-H)-
15.95	311.114	149.0608, 311.1120	C15 H20 O7	Nivalenol	312.1211	(M-H)-
16.306	341.124	71.0140, 133.0661, 341.1231	C16 H22 O8	Myzodendrone	342.1317	(M-H)-
17.509	313.145	112.9855, 175.0399, 251.1082	C19 H22 O4	Gibberellin A120	314.1522	(M-H)-
17.516	359.15	251.1075, 313.1433	C20 H24 O6	Molephantinin	360.1575	(M-H)-
18.064	279.103	173.0608, 279.1026	C18 H16 O3	Phenprocoumon	280.1103	(M-H)-
18.173	293.119	119.0505, 187.0763, 293.1183	C19 H18 O3	(2-Butylbenzofuran-3-yl) (4-hydroxyphenyl)ketone	294.1259	(M-H)-
18.475	355.155	59.0139, 131.0498, 251.1058, 355.1525	C21 H24 O5	Tephrowatsin C	356.1626	(M-H)-
18.483	401.161	131.0518, 263.1071, 355.1555	C22 H26 O7	Gmelinol	402.1684	(M-H)-
18.546	327.16	263.1079, 295.1341	C20 H24 O4	Sagequinone methide A	328.1677	(M-H)-
18.569	373.166	163.0779, 263.1069, 327.1605	C21 H26 O6	2-(4-Allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-1-propanol	374.1733	(M-H)-
18.841	321.1137	173.0596, 279.1028	C20 H18 O4	Neobavaisoflavone	322.121	(M-H)-
18.913	361.163	71.0146, 236.1053, 361.1630	C16 H22 N6 O4	Thyrotropin releasing hormone	362.1707	(M-H)-
18.934	293.1762	71.0141, 236.1056, 293.1751	C17 H26 O4	Myrsinone	294.1835	(M-H)-
19.977	265.148	96.9603, 265.1485	C12 H26 O4 S	Lauryl hydrogen sulfate	266.1557	(M-H)-

Table 4.6 (continued)

RT (min)	*m/z*	MS/MS Fragments	Formula	Tentative Identification	Mass	Ion Species
20.305	239.067	151.0757, 207.0409, 239.0666	C10 H12 N2 O5	Dinoterb	240.0747	(M-H)-
20.744	311.169	119.0503, 183.0123, 311.1682	C20 H24 O3	Triptophenolide	312.1759	(M-H)-
20.917	311.169	119.0497, 183.0124, 311.1684	C17 H28 O3 S	N-Undecylbenzenesulfonic acid	312.1761	(M-H)-
21.776	293.18	96.9607, 293.1796	C14 H30 O4 S	Sodium tetradecyl sulfate	294.1871	(M-H)-
21.887	325.185	79.9579, 183.0126, 325.1828	C18 H30 O3 S	2-Dodecylbenzenesulfonic acid	326.192	(M-H)-

4.5 Development of Hydrogels Containing Rhizome Extracts of Bitter Ginger and Galangal

Six unique hydrogel formulations (Figure 4.5) were developed, each incorporating specific compositions of bitter ginger and galangal rhizome extracts. The base formulation (F1) comprised key ingredients such as Tetra EDTA, Aristoflex AVC as a gelling agent, and water. Formulations F3-F6 incorporated 0.3% extracts of either Galangal rhizome ethanol extract (GRE), Galangal rhizome methanol extract (GRM), Bitter ginger rhizome ethanol extract (BRE), or Bitter ginger rhizome methanol extract (BRM), as detailed in Table 3.6.

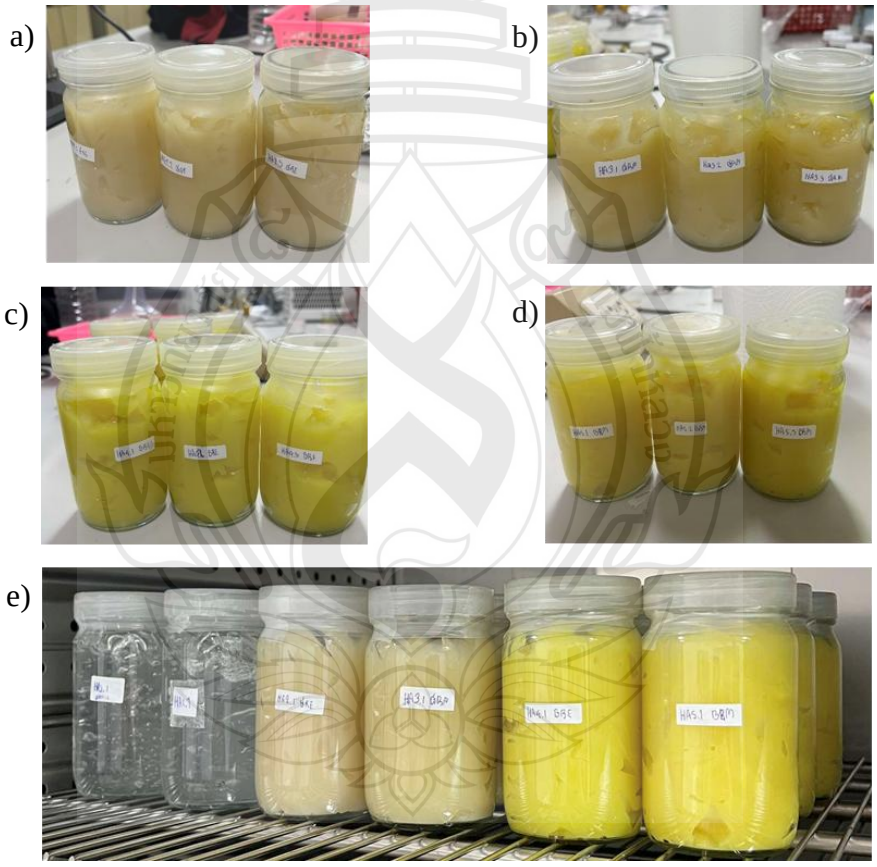


Figure 4.5 Formulations of hydrogels with GRE extract (a), hydrogels with GRM extract (b), hydrogels with BRE (c), hydrogels with BRM extract (d), and all hydrogels (e).

4.6 Stability Testing

Six hydrogel formulations were developed including; Hydrogel base (F1), Hydrogel base with preservative (F2), Hydrogel with 0.3% GRE extract (F3), Hydrogel with 0.3% GRM extract (F4), Hydrogel with 0.3% BRE extract (F5), and Hydrogel with 0.3% BRM extract (F6). The viscosity and torque (%T) of the hydrogel formulations were evaluated using a viscometer under controlled conditions. Measurements were conducted at a temperature of 25°C using a S06 spindle at a rotational speed of 100 rpm for 30 seconds. These parameters were kept constant to ensure consistency and accuracy in assessing the viscosity of the formulations before and after four cycles of stability testing.

Table 4.7 presents the viscosity (cPs.), torque (%T), temperature, speed (rpm), and spindle number used for the six hydrogel formulations. The initial viscosity of the hydrogel base was recorded at 8041.1 ± 70.29 cPs., which decreased slightly to 7740.0 ± 29.30 cPs. post-testing. Similarly, the hydrogel base with preservative exhibited a minor decrease in viscosity, from 8006.7 ± 88.05 cPs. to 7582.2 ± 59.48 cPs. Notably, formulations incorporating GRE and GRM extracts demonstrated superior viscosity stability. The GRE extract hydrogel experienced a minimal change, from 9160.0 ± 97.51 cPs. to 9155.6 ± 170.56 cPs., while the GRM extract hydrogel reduced from 9118.9 ± 74.39 cPs. to 8753.3 ± 184.78 cPs. Likewise, BRE and BRM extract hydrogels exhibited modest decreases in viscosity, from 9003.3 ± 61.10 cPs. to 8943.3 ± 72.59 cPs. and from 8923.3 ± 121.08 cPs. to 8826.7 ± 83.98 cPs., respectively. These findings suggest that the incorporation of plant extracts enhances the viscosity stability of the hydrogel formulations.

In addition to viscosity, torque (%T) values were measured before and after stability testing. The hydrogel base exhibited an initial torque of 80.4 ± 2.1, which decreased to 77.4 ± 0.9, while the hydrogel base with preservative dropped from 80.1 ± 2.6 to 75.8 ± 1.8. Hydrogels incorporating 0.3% GRE extract exhibited the highest initial torque at 91.2 ± 2.2, which remained stable at 91.6 ± 2.9 after testing. F4 hydrogel with 0.3% GRM extract showed a slight reduction from 91.6 ± 5.1 to 87.5 ± 5.5, while F5 hydrogel with 0.3% BRE extract decreased from 91.2 ± 2.2 to 89.4 ± 2.2, and F6

hydrogel with 0.3% BRM extract declined from 90.0 ± 1.8 to 88.3 ± 2.5. These results indicate that GRE extract contributed to greater torque stability, while GRM, BRE, and BRM extracts showed minor reductions in %T over time.

The pH values of all formulations exhibited a gradual decrease after the cycling test. The hydrogel base demonstrated an initial pH of 7.98 ± 0.04, which decreased slightly to 7.68 ± 0.03, while the GRE extract formulation experienced a more significant pH shift, from 7.55 ± 0.03 to 6.87 ± 0.02. Although the GRM and BRM extract formulations exhibited slightly larger pH adjustments, no significant changes were observed (Table 4.8).

Table 4.7 Cycling test results of hydrogels

Formula	**Needle**	**Temperature (°C)**	**speed**	**Viscosity (c. Ps)**			
				Before	**%T**	**After**	**%T**
F1	S06	25	100	8041.1 ±70.29	80.4±2.1	7740.0 ±29.30	77.4±0.9
F2				8006.7 ±88.05	80.1±2.6	7582.2 ±59.48	75.8±1.8
F3				9160.0 ±97.51	91.2±2.2	9155.6 ±170.56	91.6±2.9
F4				9118.9 ±74.39	91.6±5.1	8753.3 ±184.78	87.5±5.5
F5				9003.3 ±61.10	91.2±2.2	8943.3 ±72.59	89.4±2.2
F6				8923.3 ±121.08	90.0±1.8	8826.7 ±83.98	88.3±2.5

Note Values are given as mean ± S.D. from triplicate.

Table 4.8 pH measurement results of hydrogels

Formula	pH	
	Before	**After**
F1	7.98±0.04	7.68±0.03
F2	7.99±0.01	7.80±0.05
F3	7.55±0.03	6.87±0.02
F4	6.06±0.04	5.39±0.07
F5	7.88±0.04	7.44±0.07
F6	8.05±0.01	7.77±0.00

Note Values are given as mean ± S.D. from triplicate.

4.7 Colorimetric Analysis

Colorimetric analysis (Table 4.9), using L* (lightness), a* (red-green), b* (yellow-blue), and ΔE (Change in visual perception) values, provided further insights into the stability of the hydrogel formulations following the cycling test. The L* values for the hydrogel base and base with preservative demonstrated minimal shifts, with the hydrogel base increasing from 36.70 ± 0.17 to 38.50 ± 0.31. The ΔE vales of all samples ranges between 0.3-4.4, indicating color consistency. Formulations incorporating plant extracts exhibited similar, minor changes, suggesting that the extracts maintained stable visual properties.

Table 4.9 Colorimetric analysis of hydrogels before and after cycling test

Formula	Average						
	ΔL*		**Δa***		**Δb***		**ΔE**
	Before	**After**	**Before**	**After**	**Before**	**After**	
F1	36.70±0.17	38.50±0.31	-0.32±0.04	-0.05±0.08	-0.98±0.07	-0.80±0.12	1.83
F2	39.60±0.04	39.31±0.06	-0.23±0.06	-0.12±0.01	-0.37±0.05	-0.59±0.03	0.38
F3	46.60±0.06	46.77±0.18	-2.14±0.01	-1.57±0.03	1.10±0.08	1.87±0.18	0.97
F4	42.45±0.18	45.02±0.37	-1.71±0.05	-1.86±0.05	0.26±0.20	0.20±0.27	2.58
F5	51.20±0.83	51.60±0.67	-8.51±0.20	-6.79±0.10	18.32±1.15	14.29±0.83	4.40
F6	50.72±0.97	51.72±0.74	-7.41±0.20	-6.33±0.11	16.97±1.19	15.03±0.95	2.44

Note Values are given as mean ± S.D. from triplicate.

CHAPTER 5

CONCLUSION AND DISCUSSION

5.1 Conclusion

This study aimed to investigate the therapeutic potential of galangal (*Alpinia galanga*) and bitter ginger (*Zingiber zerumbet*) extracts for cosmetic and skincare applications, focusing on their antioxidant and antimicrobial properties, as well as their incorporation into hydrogel formulations. Using ethanol and methanol as solvents for extraction, bioactive compounds were obtained from the rhizomes, stems, and leaves of both plants. The phytochemical compositions were analyzed via LC-MS/MS, while biological activities were assessed through DPPH radical scavenging assays, and broth microdilution antimicrobial assays.

The results demonstrated that rhizome extracts of both plants exhibited superior antioxidant and antimicrobial activities against acne-associated bacteria, including *Cutibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. Furthermore, the development of hydrogel formulations incorporating these plant extracts showed promising stability and uniform distribution, enhancing their potential as effective carriers for topical delivery.

In summary, the findings of this study highlight the potential of galangal and bitter ginger extracts as natural, sustainable alternatives for managing acne and improving skin health. The successful integration of these extracts into hydrogel formulations reinforces their applicability in cosmetic and skincare product development.

5.2 Discussion

This study aimed to evaluate the potential of bitter ginger and galangal extracts as natural remedies for acne vulgaris, focusing on their antioxidant property,

antimicrobial property, and stability in hydrogel base. The findings highlight the significant impact of extraction solvents and plant parts on the biological activities of the extracts, reinforcing the importance of optimizing these factors for cosmetic and pharmaceutical applications.

Galangal (*Alpinia galanga*) and bitter ginger (*Zingiber zerumbet*) were chosen for this study due to their demonstrated antimicrobial efficacy against acne-associated bacteria. The broth microdilution assay results demonstrated that rhizome extracts of both plants (BRE, BRM, GRE, and GRM) exhibited significant antibacterial effects against *C. acnes*, *S. aureus*, and *S. epidermidis*, suggesting their potential as natural antimicrobial agents for acne treatment. These findings align with previous research indicating that bitter ginger extracts possess strong antibacterial properties against drug-resistant bacterial strains, including *Lactobacillus acidophilus, Streptococcus mutans, Enterococcus faecalis,* and *S. aureus* (Ramzan & Zeshan, 2023; Assiry et al., 2023). The notable antimicrobial efficacy of these extracts, particularly from the rhizomes, supports their selection over other potential plant candidates for this study. Since bacterial colonization is a key factor in acne pathogenesis, the ability of bitter ginger and galangal to inhibit multiple acne-associated bacteria makes them highly suitable for further exploration in topical antimicrobial formulations.

The selection of an optimal extraction solvent plays a critical role in determining the yield and bioactivity of plant-derived compounds (Altıok et al., 2008). Our results demonstrated that methanolic extraction produced a higher yield of bioactive compounds from bitter ginger and galangal rhizomes compared to ethanolic extraction. Specifically, bitter ginger rhizomes extracted with methanol (BRM) yielded 7.30%, whereas ethanolic extraction (BRE) yielded 5.71% ($p < 0.05$). This suggests that key bioactive compounds in bitter ginger, such as polyphenols and flavonoids, exhibit greater solubility in methanol, which aligns with previous studies highlighting the high polarity of methanol as beneficial for extracting phenolic-rich plant materials.

However, extraction yield alone does not fully determine effectiveness, as biological activity is also a key factor. Our results showed that BRM exhibited significantly stronger antioxidant activity than BRE, as indicated by its lower IC50 value in the DPPH assay (0.99 mg/mL vs. 1.19 mg/mL, $p < 0.05$). This suggests that

methanol extraction not only enhances compound yield but also extracts more potent antioxidant constituents.

In contrast, antimicrobial activity results varied, indicating that the choice of solvent influences not only compound yield but also bioactivity against different bacterial strains. While BRM exhibited strong antibacterial effects against C. acnes, its activity against *S. epidermidis* and *S. aureus* was comparable to BRE. This suggests that certain antimicrobial compounds may be better extracted by ethanol or that solvent-specific differences affect compound stability or interactions with bacterial cells.

The antimicrobial activity of bitter ginger and galangal extracts was evaluated against acne-associated bacteria (*C. acnes, S. aureus*, and *S. epidermidis*) using broth microdilution assays. The findings revealed that rhizome extracts exhibited the most potent antibacterial effects, with BRE, BRM, GRE, and GRM extracts demonstrating significant bacterial inhibition. These results are consistent with previous reports that bitter ginger rhizome extracts possess strong antimicrobial properties against drug-resistant bacterial strains, including *Lactobacillus acidophilus*, *Streptococcus mutans*, *Enterococcus faecalis*, and *Staphylococcus aureus* (Ramzan & Zeshan, 2023; Assiry et al., 2023).

A previous study found that bitter ginger rhizomes, extracted using 70% ethanol through maceration, exhibited strong antibacterial activity against *C. acnes* at various concentrations (Aji et al., 2022). Similarly, galangal rhizomes, extracted with ethyl acetate and methanol under reflux conditions, demonstrated antimicrobial activity against *C. acnes, S. aureus*, and *S. epidermidis* (Niyomkam et al., 2010). Our study builds upon these findings by demonstrating that not only rhizomes but also stems and leaves possess antibacterial activity, though with reduced potency.

The LC-MS/MS analysis of bitter ginger (*Zingiber zerumbet*) and galangal (*Alpinia galanga*) extracts in this study revealed a diverse range of bioactive phytochemicals, many of which are known for their antimicrobial properties. Galangal rhizome extract (GRE) contained phenolic compounds (sweroside, methylsyringin), flavonoids (amoritin, (+)-myristinin A), diterpenoids (sagequinone methide A, gamma-crocetin), and coumarins (dihydrosamidin, phenprocoumon), all of which have been previously associated with antibacterial and antifungal activity. Additionally, sesquiterpenes (10-hydroxymelleolide) and ubiquinones (myrsinone), also detected in

GRE, have been reported to exhibit membrane-disrupting effects on bacterial cells, contributing to antimicrobial potency. In bitter ginger rhizome extract (BRM), key compounds included flavonoids (neobavaisoflavone), phenolic derivatives (myzodendrone), diterpenoids (sagequinone methide A), and sesquiterpenes (molephantinin), suggesting a similar antimicrobial profile to galangal, but with distinct phytochemical differences that may contribute to its unique bacterial inhibition patterns.

Comparing these findings with previous studies, Wahab et al. (2022) reported that bitter ginger extracts contain high levels of zerumbone, a sesquiterpene known for its antimicrobial efficacy, further supporting the antibacterial potential of BRM observed in this study. Likewise, Nampoothiri et al. (2017) identified gallic acid and ellagic acid as major polyphenols in galangal, which are recognized for their bacterial growth inhibition through oxidative stress modulation and membrane destabilization. While these compounds were not detected in this study, the presence of other phenolic derivatives and flavonoids in GRE suggests a similar antimicrobial mechanism. The variations in phytochemical composition between this study and previous research highlight the influence of extraction methods, geographical origin, and environmental conditions on metabolite profiles. Nonetheless, the broad spectrum of antimicrobial compounds identified in both BRM and GRE reinforces their potential application in acne treatment formulations by effectively targeting bacterial proliferation and inflammation.

Interestingly, hydrogel formulations containing these extracts retained their antimicrobial efficacy after stability testing, suggesting that incorporating these bioactives into topical products does not compromise their antibacterial activity. This finding supports their potential use in acne treatment formulations.

To further investigate the mechanism of bacterial inhibition, scanning electron microscopy (SEM) was used to examine bacterial cell morphology following treatment with bitter ginger and galangal extracts. SEM images revealed significant cellular damage, including membrane shrinkage, surface rupture, and cytoplasmic leakage in *C. acnes* and *S. epidermidis* cells. These structural alterations align with previous TEM-based studies, which reported galangal extracts causing severe membrane and cell wall

damage in *S. aureus*, likely through lipid peroxidation and increased membrane permeability (Oonmetta-aree et al., 2006).

The ability of these extracts to disrupt bacterial membranes suggests that their antibacterial mechanism involves altering membrane integrity, leading to cell death via osmotic imbalance and intracellular leakage. Additionally, the extracts demonstrated antibiofilm activity, which is crucial in preventing bacterial colonization and resistance development. Given that biofilm formation plays a significant role in acne persistence, this property reinforces their therapeutic potential as anti-acne agents.

Oxidative stress plays a key role in acne pathogenesis, as free radical production contributes to inflammation, tissue damage, and delayed wound healing. Our findings revealed that methanolic extracts exhibited the highest antioxidant activity, particularly GRM and GRE, as determined by DPPH radical scavenging assays. These results align with previous reports indicating that the high polyphenol and flavonoid content in galangal and bitter ginger contributes to their strong antioxidant properties.

The superior antioxidant capacity of GRM compared to BRM suggests that galangal extracts may be more effective in neutralizing oxidative stress, making them promising candidates for formulations targeting acne-related inflammation and skin repair. However, the variability in antioxidant activity across plant parts and solvents highlights the importance of standardized extraction methods to ensure consistent bioactivity.

The rheological properties of hydrogel formulations were evaluated to assess stability, viscosity, and torque behavior. Viscometer analysis revealed that GRE and GRM hydrogel formulations exhibited the highest viscosity stability, with minimal reductions after stability testing. The % torque (%T) values indicated that hydrogels containing GRE extract maintained superior structural integrity, while GRM, BRE, and BRM formulations showed minor decreases in %T over time.

The hydrogel base and preservative-containing formulations exhibited greater viscosity loss, reinforcing the stabilizing effect of plant extracts. These results suggest that polyphenol-rich extracts enhance hydrogel stability by strengthening polymer network interactions, which is beneficial for topical applications requiring prolonged retention on the skin. Future studies should explore long-term storage stability,

bioactive release kinetics, and in vivo application to optimize these formulations for commercial use.

This study provides compelling evidence for the potential of bitter ginger and galangal extracts as effective topical agents for acne management. Their combined antimicrobial, antioxidant, and rheological benefits make them promising candidates for cosmetic and dermatological applications. However, challenges remain in ensuring consistent bioactivity, optimizing formulations, and evaluating long-term stability.

While this study highlights the potential of bitter ginger and galangal extracts as natural remedies for acne vulgaris, it is important to consider the sustainability of the extraction methods used. Methanol and ethanol were selected for their efficiency in isolating bioactive compounds, particularly polyphenols and flavonoids; however, methanol is not considered safe for cosmetic applications due to its toxicity, and ethanol, though safer, is still an organic solvent that may not fully align with the principles of green chemistry. To enhance the eco-friendliness of plant-based cosmetic formulations, future studies should explore alternative extraction techniques that minimize environmental impact and improve safety for human use. Green extraction methods, such as water-based extraction, glycerin-based maceration, supercritical CO_2 extraction, or natural deep eutectic solvents (NADES), offer promising solutions for replacing traditional organic solvents. These methods are not only safer but also maintain the integrity of bioactive compounds while reducing solvent waste and potential toxicity risks. Transitioning towards these sustainable extraction technologies will be essential for integrating bitter ginger and galangal extracts into commercially viable, environmentally friendly acne treatments, fully aligning with the growing consumer demand for natural and sustainable skincare solutions. Future research should focus on clinical validation, advanced formulation strategies (e.g., nanoemulsions or liposomes), and detailed mechanism studies to enhance their efficacy and commercial viability. Nonetheless, these findings establish a strong foundation for the future use of bitter ginger and galangal in acne treatment and natural skincare solutions.

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CURRICULUM VITAE

NAME Tanat Na Nongkhai

EDUCATIONAL BACKGROUND

2016-2020 Bachelor of Name
Beauty Technology
Mae Fah Luang University

SCHOLARSHIP

2003 Graduate Research Grant, Mae Fah Luang University (Grant no. 10/66)

2003 Neo Factory Scholarship

2024 Experiential Learning Program Grant

PUBLICATION

Na Nongkhai, T., Maddocks, S. E., Phosri, S., Sangthong, S., Pintathong, P., Chaiwut, P., Chandarajoti, K., Nahar, L., Sarker, S. D., & Theansungnoen, T. (2024). In Vitro Cytotoxicity and Antimicrobial Activity against Acne-Causing Bacteria and Phytochemical Analysis of Galangal (*Alpinia galanga*) and Bitter Ginger (*Zingiber zerumbet*) Extracts. *International Journal of Molecular Sciences*, *25*(20), 10869. https://doi.org/10.3390/ijms252010869

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