# ULTRASONIC ASSISTED EXTRACTION OF *Etlingera elatior* LEAVES: OPTIMIZATION, CHARACTERIZATION AND KINETIC STUDIES

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**UNIVERSITI SAINS MALAYSIA**

**2020**

# ULTRASONIC ASSISTED EXTRACTION OF *Etlingera elatior* LEAVES: OPTIMIZATION, CHARACTERIZATION AND KINETIC STUDIES

**by**

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**Thesis submitted in fulfilment of the requirements**
**for the degree of**
**Master of Science**

**August 2020**

## ACKNOWLEDGEMENT

The writing of this dissertation has been one of the most significant academic challenges in life. It has been seen through to completion with the support and encouragement from numerous people whom I am thankful for. First and foremost, I would like to express my sincere gratitude to my supervisor Dr. Masrina Mohd Nadzir for the continuous support of my Master's study and research, for her patience, motivation and knowledge. Her guidance helped me in all the time of research and writing of this dissertation. The completion of this dissertation would not have been possible without her guidance and supervision. I would like to acknowledge Dr Salfarina Ramli for her kind effort for her assistance in my lab work.

Speical thanks to my parents, Gong Ngie Hea and Khor Mooi Eng , as well as my siblings for their support and financial assistance to allow me to complete my studies.I would like to express my gratitude to these friends of mine whom had provided me help and guidance throughout my studies: Farhana Nazira Idris, Lau Sin Mun and Chin Jing Yi. I would like to thank the School of Chemical Engineering, Universiti Sains Malaysia for providing me the opportunity to carry out the research. Finally, I would like to acknowledge the financial support from Fundemental Research Grant Scheme (FRGS) from the Ministry of Higher Education Malaysia (Grant number: 203/PJ KIMIA/ 6071379).

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## LIST OF SYMBOLS

| | |
|---|---|
| $b$ | washing coefficient in Unsteady diffusion model |
| $B$ | constant incorporating the characteristics of the carrier–active agent system in Power Law model |
| $\beta_o$ | intercept of the BBD model |
| $\beta_i$, $\beta_{ii}$, $\beta_{ij}$ | regression coefficients of BBD model |
| $C_0$ | initial yield of oil at t = 0 |
| $C_1$ | yield at equilibrium for the washing step |
| $C_2$ | yield at equilibrium for the diffusion step |
| $C_t$ | yield at any time, t |
| $i$ | values from 1 to the total number of variables |
| $j$ | values from 1 to the total number of variables |
| $K_1$ | Peleg's rate constant |
| $K_2$ | Peleg's capacity constant |
| $k_1$ | mass transfer coefficient for the washing step |
| $k_2$ | mass transfer coefficient for the diffusion step |
| $m_i$ | initial mass of seeds sample |
| $m_d$ | final mass of seeds sample |
| $n$ | diffusional exponent in Power Law model |
| $N$ | number of observations |
| $R^2$ | determination coefficient |
| $X_i$ | linear terms of BBD model |
| $X_i^2$ | quadratic terms for a single variable of BBD model |
| $X_i$, $X_j$ | interaction terms for variables of BBD model |
| $y_o$,$y_1$ | parameters in Parabolic Diffusion model |
| $t$ | time |

| | |
|---|---|
| $Y, y$ | response variable |

## LIST OF ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysis of variance |
| BBD | Box-Behnken design |
| CCD | Central composite design |
| CV | Coefficient of Variance |
| DMSO | Dimethyl sulfoxide |
| DOE | Design of experiment |
| DPPH | 2,2-diphenyl-1-picrylhydrazyl |
| EAE | Enzyme-assisted extraction |
| FC | Folin-Ciocalteu |
| FFD | Full factorial design |
| GAE | Gallic acid equivalents |
| GRG | Generalized Reduced Gradient |
| $IC_{50}$ | half maximal inhibitory concentration |
| L/S | Liquid to solid |
| MAE | Microwave-assisted extraction |
| MRPD | Mean relative percentage deviation |
| OFAT | One-factor-at-a-time |
| PSE | Pressure-assisted solvent extraction |
| ROS | Reactive oxygen species |
| Rpm | Revolution per minute |
| RSA | Radical scavenging activity |
| SEM | Scanning electron microscopy |
| UF | Ultrasound frequency |
| USD | United States Dollar |
| UV | Ultraviolet |

**PENGEKSTRAKAN BANTUAN ULTRASONIK DAUN *Etlingera elatior*: PENGOPTIMUMAN, PENCIRIAN DAN KAJIAN KINETIK**

## ABSTRAK

*Etlingera elatior* (*E. elatior*) merupakan sejenis pokok saka herba yang ditanam secara luas tetapi daunya digolongkan sebagai bahan buangan. Beberapa kajian menunjukkan bahawa daun *E. elatior* berpotensi untuk digunakan dalam aplikasi formulasi kosmetik. Dalam proses pengekstrakan, kajian pengoptimuman dan kinetik amat penting untuk membina proses yang dapat mencapai hasil pengekstrakan and kecekapan yang tinggi. Dalam kajian ini, ekstrak daun *Etlingera elatior* (*E.elatior*) telah diperolehi dengan menggunakan teknik pengekstrakan bantuan ultrasonik (UAE). Pelarut metanol didapati paling berkesan untuk pengekstrakan, diikuti oleh etanol, aseton, etil asetat dan n-heksana. Hasil tertinggi yang direkod daripada UAE adalah 21.10 ± 0.29% pada masa pengekstrakan 15 minit, suhu pengekstrakan 50 °C dan nisbah cecair kepada pepejal (L/S) 35 mL/g dengan kuasa ultrasonik tetap pada 150 W. Proces UAE juga dioptimumkan dengan menggunakan kaedah permukaan sambutan (RSM) melalui reka bentuk 'Box-Behnken' (BBD). Ramalan hasil ekstrak maksimum sebanyak 21.21 % telah diperolehi pada masa pengekstrakan 16 minit, suhu pengekstrakan 55 ° C, dan nisbah cecair kepada pepejal 38 mL/g (L/S). Berdasarkan imej Mikroskop Elektron Imbasan (SEM), proses ultrasonik menyebabkan rekahan pada permukaan daun *E. elatior*. Untuk kajian kinetik, model Patricelli didapati sangat berpadanan dengan data pengekstrakan dengan $R^2$> 0.99 dan nilai MRPD <10%. Di antara semua ekstrak pelarut daun *E. elatior*, ekstrak metanol menunjukkan jumlah kandungan fenolik tertinggi (228.35 ± 17.34 mg GAE/g) dan mempunyai aktiviti antioksida tertinggi (nilai $IC_{50}$ 66.25 ± 7.77 μg / mL). Terdapat

hubungan korelasi yang memuaskan di antara aktiviti antioksida dan jumlah kandungan fenolik ($R^2$= 0.75). Semua ekstrak pelarut mempunyai aktiviti perencatan tirosinase, tetapi ekstrak metanol menunjukkan aktiviti perencatan tirosinase tertinggi (nilai $IC_{50}$ 263.91 ± 13.61 μg/ml). Hanya ekstrak metanol dan etanol menunjukkan aktiviti antibakteria terhadap *B. subtilis* (bakteria gram-positif), tetapi tiada ekstrak pelarut mempunyai sebarang aktiviti antibakteria terhadap *E. coli* (bakteria gram-negatif). Kajian ini menunjukkan bahawa ekstrak daun *E. elatior* yang diperoleh dengan menggunakan kaedah UAE mempunyai potensi untuk aplikasi kosmeseutikal.

# ULTRASONIC ASSISTED EXTRACTION OF *Etlingera elatior* LEAVES: OPTIMIZATION, CHARACTERIZATION AND KINETIC STUDIES

## ABSTRACT

*Etlingera elatior* (*E. elatior*) is a herbaceous plant that is widely cultivated but the leaves are discarded as waste. Studies have shown that the leaves have potential in cosmetics formulation applications. In an extraction process, optimization and kinetic studies are important to develop a process that results in higher extraction yield and efficiency. In this study, the leaves extract of *E. elatior* were obtained by using ultrasonic-assisted extraction (UAE) technique. Methanol solvent was found to be most effective for extraction, followed by ethanol, acetone, ethyl acetate and *n*-hexane. The highest yield attained from UAE was 21.10 ± 0.29 % at an extraction time of 15 min, extraction temperature of 50 °C and liquid to solid (L/S) ratio of 35 mL/g at fixed ultrasonic power of 150 W. The UAE process was further optimized using response surface methodology by applying Box-Behnken design. The optimal conditions for the extraction were obtained at extraction time of 16 min, extraction temperature 55 °C and liquid to solid (L/S) ratio 38 mL/g and with methanol as solvent. Under these optimal conditions, the experimental yield of leaves extract obtained was 21.42 %, which matched well with the predicted model. Based on SEM images, ultrasonic treatment induced fissures on the surface of *E. elatior* leaves. For kinetics studies, Patricelli's model fitted well to the extraction data with $R^2 > 0.99$ and mean relative percentage deviation value of < 10 %. Among all solvent extracts of *E. elatior* leaves, methanolic extract exhibited the highest total phenolic content (228.35 ± 17.34 mg GAE/g) and has the highest antioxidant activity ($IC_{50}$ value 66.25 ± 7.77 μg/mL). A

reasonable correlation was found between antioxidant capacity and the TPC ($R^2$= 0.75). All solvent extracts possess tyrosinase inhibition activity, but methanolic extracts showed the strongest tyrosinase inhibition activity ($IC_{50}$ value 263.91 ± 13.61 µg/ml). Only methanolic and ethanolic extracts showed antibacterial activity towards *B. subtilis* (gram-positive bacteria), but none of the solvent extracts exhibited any antibacterial activity against *E. coli* (gram-negative bacteria). The study demonstrated that the properties of *E. elatior* leaves extract obtained by using UAE method may have potential for cosmeceutical applications.

# CHAPTER 1

# INTRODUCTION

## 1.1 Background

Cosmeceuticals are products that cleanse or improve the skin with integrated biologically active substances or functional ingredients that have therapeutic properties on the surface applied (Pieroni et al., 2004; Wanjari and Waghmare, 2015). Cosmeceuticals are usually available in the form of lotions or creams and are mostly aimed at dermatological issues (Choi and Berson, 2006). Some cosmeceuticals are derived from natural sources while some are synthetic, which are produced or formulated by chemical process (Beerling and Sahota, 2014). Among the favourable characteristics of cosmeceuticals are safety, formulation stability, effectiveness, and low cost (Dureja et al., 2005).

According to Dureja et al. (2005), the term 'natural' can be defined as a substance or ingredient that is made by nature or naturally derived from plants or animal products. The regulatory definition of "natural cosmetics" in Natural Cosmetics Act introduced in the United States congress in 2019 defines the term "natural" as cosmetics product sold, labeled, or represented must contain at least 70% natural substances, excluding water and salt (Hanssen and Jackson, 2020). A report by Grand View Research Inc. (2019) estimated that the global natural cosmetics (cosmetics with natural ingredients) market sales was USD 34.12 billion in 2018 and forecasted to increase with a compound annual growth rate of 5.01% from 2019 to 2025. In 2018, skin care represented the largest share of natural cosmetics market at USD 10.31 billion.

Natural ingredients used in cosmetics are becoming more popular in modern cosmetics formulations (Fowler et al., 2010). The inherent relationship between mankind and nature affects people's decision in favor of natural products for drugs, cosmetics and care products (Varol, 2018). Costa (2015) reported that globally, the number of products in skincare cosmetics consist mainly of natural ingredients has increased from 900 in 2005 to more than 6000 in 2012. Due to technological advancement, pharmaceutical and cosmetic companies have emphasized the importance of researching active or functional natural ingredients by spending large amounts of research and development budget to meet market demand (Chang, 2011; Mohd-Nasir and Mohd-Setapar, 2018). By incorporating natural ingredients, cosmetic products can be marketed as 'natural' or 'organic' to meet the demand of consumers.

The safety of cosmetics has been a top priority among consumers. Consumer attitude toward synthetic cosmetics has been generally negative and perceived as unsafe or causing side effects (Beerling and Sahota, 2014). In some developing countries such as Iran, the safety of cosmetic products is poorly regulated, for example heavy metal content in cosmetics products that exceeds the permitted limit under EU cosmetics regulation 1223/2009 (Zafarzadeh et al., 2018). Some of the harmful synthetic additive added to cosmetic products are preservatives and fragrances (Zulaikha et al., 2015). These chemical additives are the main causes of dermatological problems such as skin irritation, phototoxicity, contact allergy, and others (De Groot and Frosch, 1998; Lindberg et al., 2004). Various plant extracts after being thoroughly researched can be a safe and economically feasible alternative to synthetic product (Ribeiro et al., 2015).

Many plant extracts contain antioxidants and phenolic compounds. Plant phenolics are distributed throughout plants and consist of a large variety of

compounds, for instance flavonoids such as quercetin, kaempferol, flavonols, flavones and different classes of non-flavonoids such as phenolic acids, tannins, and stilbenes (Kornsteiner et al., 2006). Natural phenolic compounds are an abundant source of antioxidants (Anitha, 2012). Antioxidant is a beneficial compound that controls the formation of free radicals. An imbalance between antioxidant and free radicals known as oxidative stress which plays a major part in skin aging and various skin conditions (Kruk and Duchnik, 2014). Skin aging is caused by the accumulation of oxidative stress when the natural antioxidant function of the skin is adversely affected by reactive oxidative species (ROS) (Campa and Baron, 2018). Sunlight contains ultraviolet radiation that can be damaging to the skin and long term exposure will lead to changes in connective tissue which causes skin disorders (Sies and Stahl, 2004; Yamakoshi et al., 2003). In such case, topical administration of antioxidants will contribute in a beneficial way to protect the endogenous cutaneous system which can minimize the UV-radiation induced oxidative effects and also reduces oxidative stress-induced damages (Burke, 2004).

Fair skin is an integral part of female beauty standards in various Asian cultures, which leads to a rapid growth of sales of skin whitening and lightening cosmetic products (Li et al., 2008). Human skin color is determined by melanin pigment. Melanin acts as a barrier to the skin against damaging UV light by absorbing UV rays from sunlight and prevents the generation of ROS (Herrling et al., 2008). Over-active production of tyrosinase will cause over-production of melanin. Numerous plant compounds contain tyrosinase inhibiting properties, which leads to reduction of melanin production (Lall and Kishore, 2014).

Natural antimicrobial agents have drawn the attention of cosmetic and pharmaceutical companies searching for an alternative to replace synthetic

antimicrobials in topical cosmetics products (Ribeiro et al., 2015). Other than rising consumer preference for natural materials, microorganisms are increasingly more resistant to conventional antimicrobials (Aslam et al., 2018). Phenolic compounds present in plants provide defense against microbial attacks (Cowan, 1999) by the interaction between cell membrane or cell wall of microorganisms, which alters the membrane permeability of microorganisms, and eventually result in cell death (Bouarab-Chibane et al., 2019).

In general, plant-based products contains an abundant source of vitamins, antioxidants, essential oils, proteins, and other bioactive compounds (Alğin Yapar, 2017). The properties of the extract are based on the extract's composition and concentration. Bioactive compounds from various types of plants are applied in cosmetics to provide care for the body, to improve the physiological functions of the skin, and to supplement nutrition for healthy skin (Ribeiro et al., 2015). Bioactive compounds are widely used in the pharmaceutical, food and chemical industries. Extraction is used to selectively separate the bioactive compounds from active or inert components of plants (Ahmad et al., 2013). However, no single method has been established as the standard for the extraction of active compounds from plants (Azmir et al., 2013), as the choice of the extraction method depends on the nature of bioactive compounds present in the plant. Application of plant extracts requires careful consideration regarding to method of extraction, solid extract to liquid solvent ratios and the concentration of active compounds present in the plant (Aburjai and Natsheh, 2003).

## 1.2 Problem statement

According to a survey conducted by Chen (2009), 94% of users have an opinion that chemical-based cosmetics would cause side effects while only 6% of consumers view that natural cosmetics contain any side effects. More cosmetics companies are taking advantage of the growth of consumer preference for natural cosmetics by incorporating natural ingredient into their products. According to Beerling and Sahota (2014) the natural cosmetics market is projected to rise significantly over the coming years to meet increasing consumer demand.

Clinical research has shown scientific evidence for the use of certain plant-based ingredients, and their proposed biological mechanisms are widely studied (Baumann et al., 2009). Various species and parts of plants have been used as traditional or modern cosmetics formulation. For example, the root extract of *Cichorium intybus L.* contains organic acids such as hydroxycinnamic acid and chlorogenic acid that acts as skin moisturizer and skin protection function (Maia Campos et al., 2017). The distillate solution in of *Achillea millefolium* can be used to cure Oral Mucositis (Miranzadeh et al., 2015). Leaves of lavender plant, *Lavanda officinalis* contains essential oils that are used as fragrance or perfume (Njenga Waithaka et al., 2016).

*Etlingera elatior* is a fast-growing, long-lived perennial plant that thrives in tropical and subtropical regions and tolerates acidic soil and low sunlight conditions. It is cultivated mainly for its inflorescence which is commonly used as a spice in local cooking (Rojas-Sandoval, 2014). However, the leaves of *E. elatior* does not have any commercial uses and can be harvested without any additional cost. Furthermore, the pruning of leaves does not affect the growth of inflorescence.

According to research conducted by Chan et al. (2007), macerated methanolic extracts from fresh *E. elatior* leaves were found to contain high values of total phenolic content, antioxidant activity and antibacterial properties. Whangsomnuek et al. (2019) also reported that the aqueous extract of macerated *E. elatior* leaves contains tyrosinase inhibition properties that could reduce melanin content in the skin. The topical cream containing *E. elatior* extract tested on the skin of human subjects demonstrated skin lightening effects and showed no signs of skin irritation or allergic reaction. Hence, the leaves extract of *E. elatior* may have the potential to be developed as a source of natural whitening ingredient for cosmeceutical applications. There are several published studies on the properties of *E. elatior* leaves using maceration extraction method in the past few years (Chan et al., 2008; Chan et al., 2007; Whangsomnuek et al., 2019), but none has reported on the ultrasonic-assisted extraction (UAE) method on *E. elatior* leaves.

The UAE method stands out among unconventional extraction methods as an alternative. This method requires less energy to operate than microwave extraction and it is also easier to handle and safer than supercritical fluid extraction as the extraction is conducted under conditions of atmospheric pressure (Medina-Torres et al., 2017). Furthermore, the ultrasonic effects on plant cells disruption allows for more effective extraction of low molecular weight compounds as compared to hydrodistillation extraction. This extraction process offers the advantage of higher extraction yield and shorter extraction time as compared to conventional extraction techniques such as reflux extraction (Dent et al., 2015). The efficiency of an extraction process from plant materials depends largely on extraction parameters such as type of solvent extraction time, extraction temperature and liquid to solid ratio. Table 1.1 shows the difference

of extraction yield between ultrasonicated and conventional (non-sonicated) extraction reported in literature.

Table 1.1 Comparison of ultrasonic-assisted extraction with non-sonicated control group

| Extract | parameter | Yield | | Reference |
|---|---|---|---|---|
| | | Sonicated | (Control) Non-sonicated | |
| Water soluble extracts from roots of *Valeriana Officinalis* | Ethanol solvent, 120 min, 25 °C, L/S ratio 1:6.7 mL/g, 20 kHz, 600 W. | 10. 3% | 8.3% | (Hromádková et al., 2002) |
| Polysaccharides from *Lycium Barbarum* | Ethanol solvent, 30 min, 60 °C, L/S ratio 30 mL/g, 300 W, 28 kHz | 26.38 % | 22.45 % | (Muatasim et al., 2018) |
| Sarch from *P. erosus* root | Acetone solvent, 10 min, 25 °C, 40 kHz | 24.76 % | 16.86 % | (González-Lemus et al., 2018) |
| Phenolic content from *Salvia officinalis* | Ethanol solvent, 11 min, 60 °C, 1:100 mL/g, 400 W, 30 kHz | 6775.5 mg GAE/100 g dry sample | 6399.8 mg GAE/100 g dry sample | (Dent et al., 2015) |

The kinetics study of an extraction process is useful to understand the factors that affect the extraction rate and to describe the mechanism of UAE extraction, which allows prediction for the extraction behavior that is helpful for scaling-up the process. The optimization of process parameters is useful for scaling up in commercial production. Response surface methodology (RSM) is an experimental design that analyzes the effects of several factors and each of their interactive effects on the response variables. The RSM is advantageous over One-Factor-At-A-Time (OFAT)

technique whereby one variable is studied at a time and does not involve any interactions between the variables. This method also provides a statistical model that elucidates the effects of independent factors on the response. It requires less time, cost and materials to conduct the experiment as less experimental trials are required than conventional optimization methods. In an extraction process, optimization helps to increase the extraction yield while minimizing cost and maximizing efficiency.

## 1.3 Research objectives

i. To screen the extraction parameters of UAE of *E. elatior* leaves extract using one-factor-at-a-time (OFAT) method followed by optimization by using response surface approach (RSM) via Box-Behnken Design (BBD).

ii. To evaluate the kinetics of UAE of *E. elatior* leaves extract using mathematical models.

iii. To characterize the total phenolic content, antioxidant, antibacterial, and tyrosinase inhibition activities of *E. elatior* leaves extract extracted with different solvents.

## 1.4 Scope of Study

This study focused on the extraction of *E. elatior* leaves extract by using UAE techniques. Initially, the effects of different process parameters such as type of solvent (methanol, ethanol, acetone, ethyl acetate and n-hexane), extraction time (5 - 35 min), extraction temperature (35 - 65 °C) and L/S ratio (15 - 45 mL/g) on the extraction yield were investigated using OFAT technique. The UAE parameter of ultrasound frequency and ultrasound power were kept constant. The best parameter values were selected and were further optimized using RSM along with BBD. The morphology of *E. elatior*

leaves subjected to UAE was analyzed using SEM. Several mathematical models (power law, Peleg, parabolic diffusion, and Patricelli) was used to evaluate the model that best fits the extraction process involved in UAE of *E. elatior* leaves extract. The leaves extract extracted at optimized conditions along with extractions using different solvents were then characterized in terms of total phenolic content, antioxidant, tyrosinase inhibition and antibacterial activities.

## 1.5 Thesis Organization

This thesis consist of five chapters and each chapter highlights the details of this study.

**Chapter 1** provides an overview of this study including the background and definition of cosmeceuticals, their applications, properties and consumer preference. The problem statement, scope of study, and objectives of this project are included in this chapter.

**Chapter 2** reviews the literature related to this research. It introduces and describes the taxonomy, geographical distribution and applications of *E. elatior*. The principal application and influencing factors of UAE method were included. Statistical tools used to perform optimization study was discussed. Finally, the antioxidant, antibacterial and anti-tyrosinase activities of plant extracts and their application are provided in this chapter.

**Chapter 3** specifies the materials and equipment used and the methodology to conduct this research. This chapter provides a clear description on the methodology of this research, including the UAE of *E. elatior* leaves extract, optimization of process parameters, morphological analysis of *E. elatior* leaves, kinetics study of UAE

process; the characterization of total phenolic content, antioxidant, tyrosinase inhibition, and antibacterial activities of *E. elatior* leaves extract.

**Chapter 4** presents the results and discussions of this research. The first part of this chapter contains the results and discussions of the screening of extraction processes of UAE techniques using OFAT method followed by statistical optimization by applying RSM, along with the results and validation of the statistical optimization of process parameters. The morphological analysis of *E. elatior* leaves subjected to UAE and the kinetic study of extraction using UAE technique are reported. The second part reports the results and discussion of the characterization of antioxidant, total phenolic content, anti-tyrosinase, and antibacterial activities of *E. elatior* leaves extract.

**Chapter 5** concludes the research and suggests some potential application for *E. elatior* leaves extract. Recommendations for future improvements to this research project are proposed.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Introduction

This chapter provides a brief overview of the existing literature and research related to the study. The chapter begins with the introduction of classification, habitat, characteristics, and applications of *E. elatior* plant. The second part of the chapter discusses the beneficial properties of plant extracts and their application in cosmetic formulations. The third part introduces and compares the different types of extraction method and the influencing parameters of UAE. The fourth part focuses on screening method of OFAT and optimization methods of RSM in extraction, followed by extraction kinetics used for extraction of plant sample.

## 2.2 *Etlingera elatior*

*Etlingera elatior* (*E. elatior*) is a species of herbaceous perennial plant that belongs to the Zingiberaceae family (Choon and Ding, 2016). It is known as torch ginger or wax flower in English and 'kantan' in Bahasa Malaysia (Yunus et al., 2012). Zingiberaceae consists of 50 genera and 1500 species identified around the world. *Etlingera elatior* is among the most well-known species of Zingiberaceae that is present in the tropical and subtropical areas (Jaafar et al., 2007). It is a clumping plant that grows in large colonies and it can be propagated sexually (seeds) and asexually (rhizomes) (Choon and Ding, 2016). The rhizomes grow on shallow ground, with size of 3-4 cm diameter. A single colony can bear up to 200 or more flowers, with 11-13 flowers open simultaneously (Khaw, 2001).

Figure 2.1 Plant of *E. elatior* (Chan et al., 2011)

*Etlingera elatior* is indigenous to Peninsular Malaysia, Southern Thailand and Indonesia (Chan et al., 2011). This species is commonly grown and was found to be naturalized in tropical and subtropical regions worldwide. This plant thrives at lower altitudes in wet and humid areas in tropical and subtropical regions (Nontasit et al., 2015). It is categorized as an invasive species in China, Costa Rica and Hawaii where it is naturalized and has the potential to form dense thicket and competes with the native flora species (Weber et al., 2008). It is cultivated throughout South-east Asia, Australia (Queensland), Polynesia and is introduced throughout tropical Africa to Central and South America (Kunnath et al., 2013). Detailed descriptions of *E. elatior* are given in Table 2.1.

Table 2.1 Botanical description of *E. elatior* (Acevedo-Rodríguez, 2005; Khaw, 2001)

| Traits | Characteristics/ Description |
|---|---|
| Plant height | Up to 6 m tall |
| Stem | Pseudostems (leaf shoots) |
| Length of stem | 3-6 m tall |
| Leaves | Multiple, dark green |
| Length of leaves | 38-85 cm long |
| Width of leaves | 8-18 cm wide |
| Shape of leaves | Lanceolate, hairless |
| Texture of leaves | Glabrous |
| Infloresence | Flower with oval shaped head containing spirally overlapping flowers. Flower has bright red or pink bracts.<br>The base of the flower is covered by crimson-pink bracts, |
| Size of flower | Receptacle of inflorescence 4-9 cm long, reach up to 17 cm |
| Peduncle | Horizontal, 60-150 cm long and 0.8-1.5 cm wide |
| Fruit | Greenish or redish with many and black seeds, short-pubescent |
| Size of fruit | 2-2.5 cm in diameter |

### 2.2.1 Application of Etlingera elatior

Local people in Malaysia consume the hearts of young shoots, inflorescences, and fruits of *E. elatior* as a condiment, cooked as vegetable or consumed raw (Chan et al., 2009). The inflorescence of *E. elatior* has a distinct flavor and aroma and is most commonly used in the preparation of traditional cooking such as ulam, asam laksa and other condiments (Wijekoon et al., 2011). This plant is also being known for its medicinal uses. In Malaysia, the inflorescence is used to relieve earache and the decoction of leaves are used to clean wounds (Chan et al., 2013).

Local traditions believe that the consumption of inflorescence can help in lowering diabetes and hypertension (Wijekoon et al., 2011; Mai et al., 2009). When combined with other aromatic herbs, the leaves of *E. elatior* are used by post-partum women for cleansing and removal of body odour (Chan et al., 2009). The local people in Porehu District, Indonesia use various parts of *E. elatior* as a traditional medicinal herb to treat illness with symptoms related to typhoidal fever (Sabilu et al., 2017).

*Etlingera elatior* is also popularly planted as ornamental and landscape plant in gardens because of their bright-coloured and attractive inflorescence. The inflorescence from the tight bud to the blooming stage is also sold as a cut flower or used as floral decorations in countries such as Australia, Brazil, Hong Kong, Thailand and the United States (Choon and Ding, 2016). Due to its fragrant smell and medicinal properties, it is also used as an ingredient for products such as soap, shampoo, and perfume (Jo et al., 2010).

## 2.3 Phenolic compounds in plants

Phenolic compounds are secondary compounds that are usually found in varying concentrations in various species of plants. They are widely studied for their biological effects, especially for antioxidant properties (El Gharras, 2009). Phenolic compounds contain one or more benzene rings, with one or more hydroxyl substituents (Dai and Mumper, 2010). Phenolic compounds encompass a large variety of structures, from simple monomers to complex polymers with diverse molecular weight (Cheynier, 2005). Phenolic compounds are synthesized for plant development especially for pigment production and structural support for plants, and for protection against pathogens (Bhattacharya et al., 2010). Phenolic compounds are not evenly distributed throughout the plants. Insoluble phenols are mostly located in the cell walls

to provide mechanical strength whereas soluble phenols are highly distributed in the vacuoles of plant cells (Shahidi and Yeo, 2016). The production of many phenolic compounds by plants is significantly influenced by the amount of light received, as plants contain higher levels of phenolic compounds when exposed to more sunlight (Sun et al., 2017). Phenolic compounds are divided into several classes as shown in Figure 2.2 (Vardhan and Shukla, 2017).

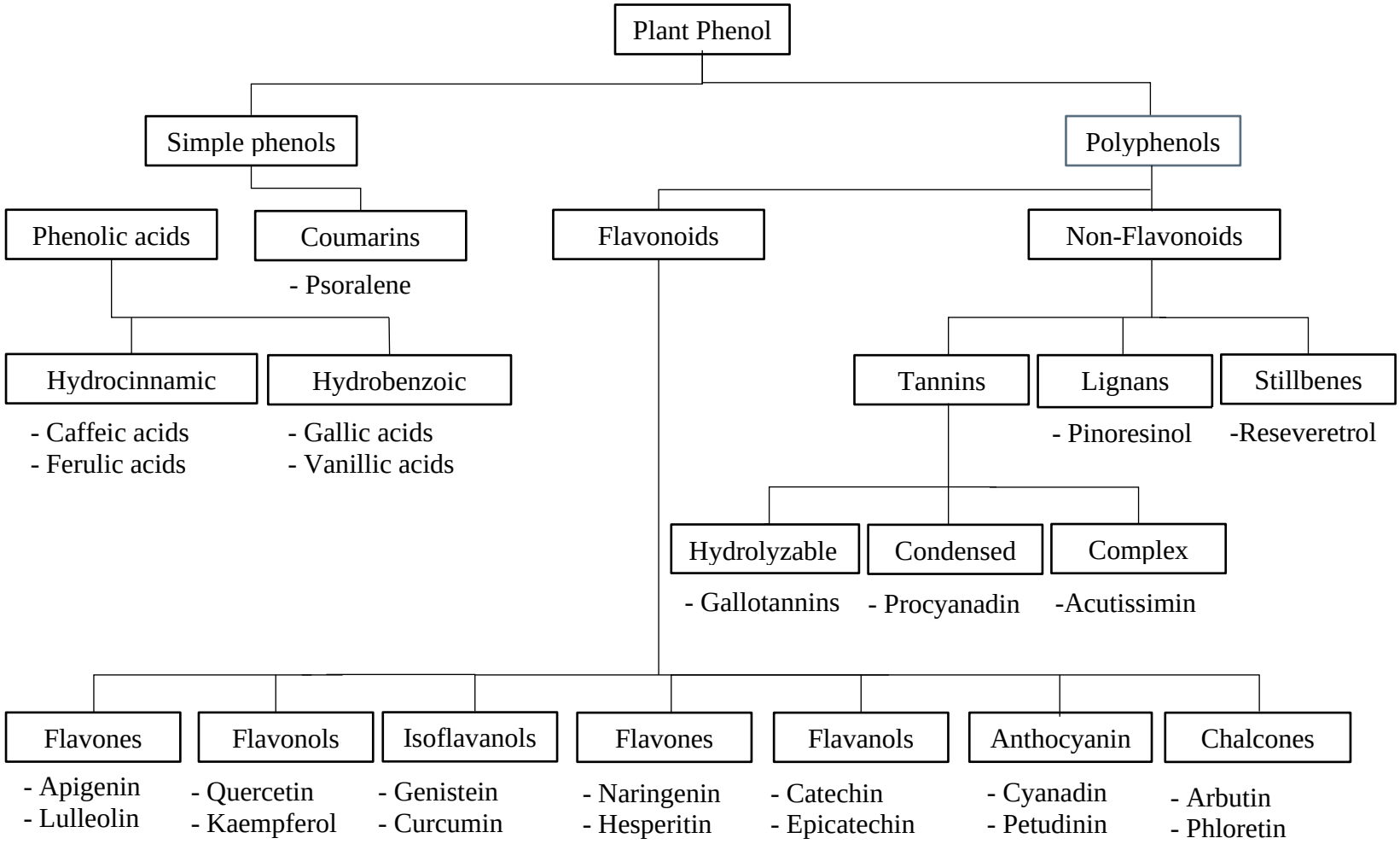


Figure 2.2 The classification and examples of phenolic compounds (Vardhan and Shukla, 2017)

### 2.3.1 Properties of phenolic compounds

Phenolic compounds are secondary compounds that are usually found in varying concentration in many species of plants. Phenolic compounds can decrease the level of reactive oxidative species (ROS) generated in the human body that can cause damages in cell structures, which is linked to many illnesses including cancer, inflammation, hypertension, diabetes and cardiovascular diseases (Valko et al., 2007). Certain compounds in plants also contain anti-tyrosinase properties that can inhibit melanin production which is responsible for skin colour. Plants with tyrosinase inhibition properties can be potentially used as skin whitening agents (Gillbro and Olsson, 2011). Phenolic compounds especially flavonoids possess antifungal, antiviral and antibacterial activity. Because of the presence of different hydroxyl group in phenolic compounds, plant extracts have antibacterial properties against the membrane of bacterial cells (Gyawali and Ibrahim, 2014). Plant extracts that contain aromatic compounds that are used as flavouring to improve the taste of foods and essential oils in plants have an aromatic smell and is widely used as fragrance (Schwab et al., 2008).

### 2.3.2 Antioxidant activity

Antioxidants are compounds that slow down or inhibit the oxidation of an oxidizable matter. Examples of plant antioxidants include ascorbic acid, tocopherols, phenolic compounds, and terpenoids (Grassmann, 2005). Among all secondary metabolites, phenolic antioxidants contribute most to the plant's antioxidant activity (Kasote et al., 2015).

Oxidative stress is linked to an imbalance between the reactive oxygen species (ROS) and the capacity to counterbalance their action by antioxidative systems. In

humans, oxidative stress is linked to several chronic health problems like cardiovascular, cancer and aging (López-Alarcón and Denicola, 2013). Free radicals are produced in aerobic processes, reaction to pathogenic infections, during intensive physical activity and the exposure to pollutants and toxins. Excessive free radicals are detrimental to cell biomolecules, damaging almost all substrates in the cell (Pisoschi and Pop, 2015).

There are several ways that phenolics act as antioxidants. The hydroxyl groups in phenolic compounds can donate hydrogen ions which can react with reactive oxygen species (Valentão et al., 2003) that break the cycle of formation of new radicals in a termination reaction, which provides chemical stability to the radicals. Phenolic structure contains a benzenoid ring and hydrogen-bonding potential of the hydroxyl group. These properties enable phenolics to interact with the enzyme, thus acting as an antioxidant by inhibiting some enzymes that generate free radicals (Parr and Bolwell, 2000).

### 2.3.3 Anti-tyrosinase activity

Eumelanin and pheomelanin are types of melanin that are produced by melanocytes through the melanogenesis process. It is a pigment that is mainly found in the skin, hair, and eyes and is produced by melanocytes through melanogenesis (Zolghadri et al., 2019). Melanin is responsible for photoprotection against ultraviolet radiation damage and skin photo-carcinogenesis.

Tyrosinase is the primary contributor to melanogenesis or pigmentation in human skin (Kim and Uyama, 2005). Tyrosinase is a copper-containing metalloenzyme with dinuclear copper ions that is vital for the synthesis of melanin. Plants contain various phenolic compounds, many of these compounds were known to

be weak or strong tyrosinase inhibitor. Some of the phenolic compounds reported having tyrosinase inhibition properties are flavones, flavanones, flavanols, coumarins, stilbenes, phenolic acids and lignans (Chang, 2009). The mechanism of tyrosinase inhibition involves several different ways, which are: 1) by reducing the intermediate o-dopaquinone to L-dopa by reducing agents, 2) by introducing o-dopaquinone scavengers, 3) by some phenols with enzymatic reaction products that do not proceed further to the next cycle step in the catalytic cycle of tyrosinase, 4) by enzymatic denaturation with non-specific enzyme inactivators, or by specific tyrosinase inhibitors. Tyrosinase catalyzes specific tyrosinase inactivators to form a covalent bond with the enzyme, resulting in irreversible inactivation of the enzyme (Lee et al., 2016).

Based on Figure 2.3, tyrosinase functions in two activities in the melanin synthesis pathway. The first activity is the monophenolase activity where it hydroxylates l-tyrosine to l-dopa and second diphenolase activity where tyrosinase oxidises l-dopa to *o*-dopaquinone (Zolghadri et al., 2019). The production of eumelanin and pheomelanin can be controlled by inhibiting the activity of tyrosinase (Chang, 2009).

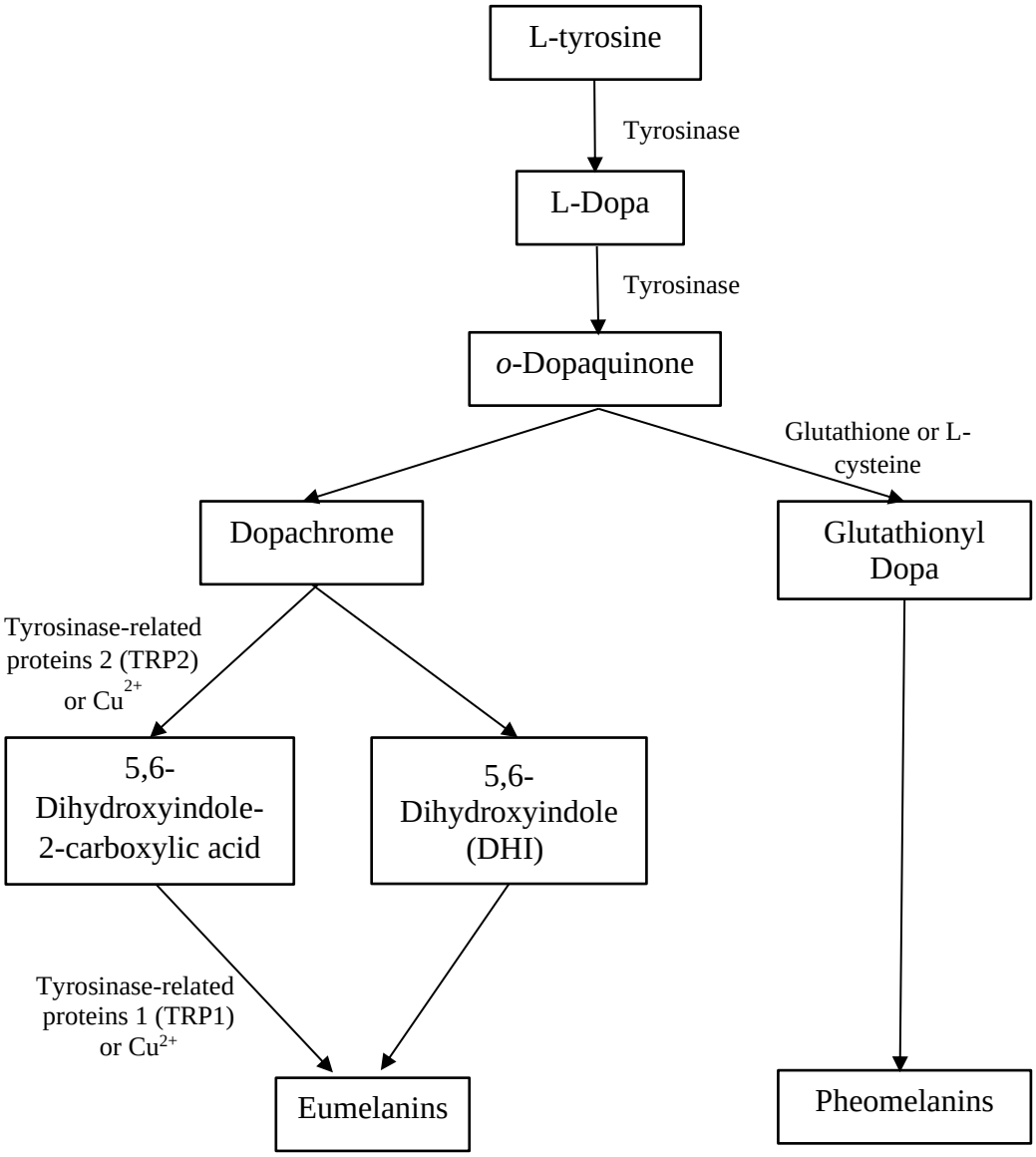


Figure 2.3 Schematic diagram of synthesis of eumelanins and pheomelanins (Zolghadri et al., 2019)

### 2.3.4 Antibacterial activity

Phenolic compounds especially flavonoids derived from plants contain antifungal, antiviral, and antibacterial activity (Cushnie and Lamb, 2005). Resistance to antimicrobial drugs has been an alarming global problem. Numerous types of bacteria or diseases are treated with a range of antibiotics, many bacterial strains have developed resistance to synthetic antibiotic (Pinho et al., 2014). In this case, phenolic

compounds have the ability to inhibit bacteria strains resistant to antibiotics, such as methicillin-resistant *Staphylococcus aureus* and glycopeptide antibiotics resistant *Enterococci* (Zuk et al., 2014). Because of the presence of different hydroxyl groups in phenolic compounds, they have varying antibacterial properties against the membrane of bacterial cells. Hydrophobic phenolic groups in contact with the lipid bilayer of membrane cause higher membrane permeability of cells, disrupting membrane structure which leads to leakage of cytoplasmic constituents. The destruction of the cell membrane allows further entry of more antibacterial agents (Gyawali and Ibrahim, 2014).

Some specific phenolic compounds were known to contain antimicrobial activity. Resveratrol is known as a natural phenolic compound that has antibacterial activity against *Arcobacter butzleri* and *A. cryaerophilus* (Ferreira et al., 2014). Studies on curcumin derived from turmeric showed antibacterial activity by damaging the cell membranes of *S. aureus* and *Escherichia coli* (Tyagi et al., 2015). Coumarins are phenolic substances from the roots of *Ferulago campestris* exhibit strong antibacterial activity against a variety of Gram-positive and Gram-negative bacteria strains (Basile et al., 2009).

## 2.4 Application of plant extracts in the cosmetics industry

Natural ingredients derived from plants are becoming more common in modern cosmetics formulations. Plant extracts contain properties such as antioxidant activity, anti-tyrosinase activity and antimicrobial activity that contains therapeutic effects on the skin and can also prevent and alleviate different skin conditions. Many studies have shown that most plant extracts are a safe and efficient alternative to synthetic ingredients (Ribeiro et al., 2015).

### 2.4.1 Skin whitening agent

Skin whitening agents are used in cosmetics to achieve skin lightening or whitening purposes, or in pharmaceutical application for the treatment of hyperpigmentation disorders. All of the skin whitening agents inhibit melanin production by inhibiting the tyrosinase enzyme (Gillbro and Olsson, 2011). Since tyrosinase catalyzes the cycle of melanin production, the colour of the skin can be altered by inhibiting the tyrosinase enzyme.

There are a variety of tyrosinase inhibitors used today that are naturally derived. Hydroquinone (1,4-dihydroxybenzene) is widely used as a whitening agent that can be extracted in small quantities from tea, wheat, berries, and coffee. Synthetic hydroquinone commonly used in cosmetics can cause side effects from long term application such as permanent depigmentation and ochronosis (discolouration of tissues), the usage of hydroquinone in cosmetics has been banned in several countries (Lee et al., 2016). Arbutin is a derivative of hydroquinone which is effective in treating hyperpigmentation disorders and also less cytotoxic than hydroquinone (Gillbro and Olsson, 2011). Kojic acid is a natural product of fungal metabolite derived from fungi species of *Acetobacter*, *Aspergillus*, and *Penicillium*. Although kojic acid is an effective treatment for melasma, the side effects reported are dermatitis and erythema (Saeedi et al., 2019). Flavonoids are a class of polyphenol derivatives that can be found in the leaves, seeds, bark, and flowers of plants. This compound can act as both substrates and inhibitors of tyrosinase. For example, flavonoids such as kaempferol and quercetin directly inhibit tyrosinase, while catechin inhibits tyrosinase by being a cofactor and some act as free radical scavenger such as rhamnetin (Lee et al., 2016).

### 2.4.2 Anti-inflammatory activity

In the human body, reactive oxygen species (ROS) may be mutagenic and have detrimental effects on the cells (Sakai et al., 2006). Excess ROS generated can cause oxidative stress that leads to damage in cell structure. Research shows evidence that oxidative stress can lead to many illnesses including cancer, inflammation, hypertension, diabetes and cardiovascular diseases (Valko et al., 2007). Inflammation is a defense response of cells against cell injury, allergies, bacterial or viral invasion, and functions to remove damaged or dead cells (Soto et al., 2015). Free radicals are produced during the process of inflammation. The formation of free radicals triggers the body's responses to activate cytokines and interleukins that causes skin inflammation, which triggers redness and swelling on the skin. Although the skin has its endogenous defense mechanism against oxidative stress, it is generally not sufficient to counteract with the ROS produced. Natural antioxidants can be supplemented through food consumption or external topical application (Działo et al., 2016).

Fu et al. (2014) found that the roots of *Glycyrrhiza uralensis* contain glycyrol that can suppress collagen-induced arthritis inflammation in *in vivo* subjects, while Zeng et al. (2014) reported that the leaves of *Artemisia argyi* have the ability to inhibit microglia-mediated inflammatory injuries *in vitro.*

### 2.4.3 Anti-aging activity

The signs of aging are loss of skin elasticity which appears to be wrinkled and dry. The skin-aging process can be caused by many factors, for example genetics, environmental contact, diet or smoking. Skin-aging is mainly contributed by oxidative stress reactions (Tan et al., 2018). Collagen and elastin synthesis in the skin also has

anti-aging benefits. Excess exposure to UV radiation and high levels of ROS will stimulate the production of collagenases and elastases, which leads to faster degradation of collagen and elastin in the skin (Abdul Karim et al., 2014). Since collagen and elastin are the main components of dermal tissue, the skin appears to be irregular and wrinkled when collagen content is lost in the skin due to aging (Ganceviciene et al., 2012). Long exposure to UV radiation is associated with damaging effects on human skin cells and causes oxidative stress. A large amount of oxidative stress can result in permanent DNA mutation and carcinogen toxicity. The skin is naturally equipped with endogenous protection which includes antioxidant protection and melanin production. However, the efficiency of the skin's antioxidant protection mechanism decreases across the aging process (Godic et al., 2014). Therefore, it is necessary to provide additional antioxidant protection to the skin from an external source (Dudonné et al., 2011).

Several researches have shown that plant extracts contain anti-aging properties, such as *Gastrodia elata* blume extract than contain anti-elastase properties on human dermal cells (Abarca-Vargas et al., 2016; Song et al., 2016). A study by Seok et al. (2016) reported that *Scutellaria radix* extract could provide UV protection and reduces skin irritation on tested *in vivo* in human trials.

### 2.4.4 Antimicrobial properties

Cosmetic products with antimicrobial activity can provide users protection against the harm of bacteria (Halla et al., 2018). Preservatives are used in cosmetics to prevent microbial growth during the lifespan of cosmetic products (Herman et al., 2013). However, synthetic preservative is the main cause of allergies to users. Methylparaben is the most commonly used preservative chemical on the market for