COSMETIC PRODUCTS FROM TORCH GINGER

NATTAWUT WHANGSOMNUEK

DOCTOR OF PHILOSOPHY IN AGRICULTURAL INTERDISCIPLINARY

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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บทคัดย่อ

ดาหลา (*Etlingera elatior* (Jack) R.M. Sm.) เป็นพืชสมุนไพรในตระกูลขิงข่า ในประเทศไทยนิยมปลูกเพื่อใช้เป็นไม้ประดับหรือเป็นอาหารพื้นบ้าน การศึกษาครั้งนี้ทำการเลือกศึกษาส่วนดอกและส่วนใบของดาหลาที่ทำการรับซื้อจากเกษตรกร โดยนำตัวอย่างส่วนดอกและส่วนใบดาหลาแห้งมาทำการสกัดด้วยน้ำและทำให้แห้งด้วยวิธีแตกต่างกันคือวิธีแช่เยือกแข็งและวิธีทำแห้งแบบพ่นฝอยเพื่อเปรียบเทียบวิธีการทำแห้งที่เหมาะสมต่อสารสกัดทั้ง 2 ชนิดโดยทำการเปรียบเทียบปริมาณผลผลิตของการสกัดและสารประกอบกลุ่มฟีนอลิกทั้งหมด นำสารสกัดจากวิธีการทำแห้งที่เหมาะสมมาทำการตรวจวัดชนิดและปริมาณกรดอะมิโน และปริมาณสารประกอบกลุ่มฟีนอลิกด้วยด้วยวิธี GC-MS และ LC-MS ตามลำดับ จากนั้นศึกษาฤทธิ์ทางชีวภาพของสารสกัดส่วนดอกและส่วนใบดาหลาในการเป็นสารต้านอนุมูลอิสระด้วยการทดสอบฤทธิ์การขจัดอนุมูลอิสระในหลอดทดลองด้วยวิธีแตกต่างกัน 3 วิธีคือ อนุมูลเอบีทีเอส (2, 2’-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) อนุมูลดีพีพีเอช (2, 2-diphenyl-1-picrylhydrazyl) และอนุมูลซุปเปอร์ออกไซด์ และประเมินประสิทธิภาพในการเป็นสารช่วยบำรุงผิวด้วยการทดสอบฤทธิ์การยับยั้งเอนไซม์ไทโรซิเนสและคอลลาจิเนส จากนั้นทำการวิเคราะห์หาปริมาณและอัตราส่วนที่เหมาะสมของสารสกัดส่วนดอกและส่วนใบร่วมกันเพื่อใช้เป็นสารออกฤทธิ์ในเครื่องสำอางด้วยการทดสอบฤทธิ์การยับยั้งเอนไซม์ไทโรซิเนส และพัฒนาเป็นผลิตภัณฑ์เครื่องสำอางบำรุงผิวเรียกว่า TG จำนวน 3 ผลิตภัณฑ์คือ ครีมบำรุงผิวหน้า โฟมล้างหน้า และผงขัดผิวกาย ทำการศึกษาคุณสมบัติทางกายภาพของผลิตภัณฑ์ดังกล่าว รวมถึงศึกษาความคงตัวของผลิตภัณฑ์ภายใต้สภาวะเร่งที่สภาวะแตกต่างกัน จากนั้นศึกษาการระคายเคืองผิวหนังโดยใช้ Finn® Chamber และการปรับสภาพผิวให้กระจ่างใสของแต่ละผลิตภัณฑ์ในอาสาสมัครสุขภาพดีจำนวน 24 คนเป็นระยะเวลา 4 สัปดาห์ โดยการศึกษาการช่วยปรับสภาพผิวให้ขาวกระจ่างใสทำการเปรียบเทียบปริมาณเม็ดสีเมลานินก่อนและหลังการใช้ผลิตภัณฑ์ด้วยเครื่องทดสอบผิวหนังแบบหลายวิธี DermaLab® combo

ผลการวิจัยพบว่าสารสกัดส่วนดอกและส่วนใบที่ทำให้แห้งด้วยวิธีแช่เยือกแข็งมีปริมาณผลผลิตของสารสกัดและปริมาณสารประกอบกลุ่มฟีนอลิกทั้งหมดสูงกว่าวิธีทำแห้งแบบพ่นฝอย ดังนั้น

จึงคัดเลือกสารสกัดจากวิธีทำให้แห้งด้วยวิธีแช่เยือกแข็งเพื่อใช้ในการศึกษาขั้นต่อไป ส่วนปริมาณสารประกอบฟีนอลิกทั้งหมดพบว่าสารสกัดส่วนดอกและส่วนใบดาหลามีปริมาณเท่ากับ 38.68 ± 0.45 และ 246.52 ± 0.26 มิลลิกรัมสมมูลของกรดกรดแกลลิกต่อกรัมสารสกัด การวิเคราะห์ด้วย LC-MS พบว่าสารฟีนอลิกชนิดเด่นในสารสกัดทั้ง 2 ส่วนคือ ไอโซเควอซิทิน แคทีชิน และกรดแกลลิก นอกจากนี้กรดอะมิโนชนิดเด่นที่พบในสารสกัดดังกล่าวคือ ไลซีน และลิวซีน ซึ่งมีรายงานว่ามีประสิทธิภาพในกระบวนการสร้างคอลลาเจนและช่วยลดเลือนริ้วรอยที่ปรากฏบนผิวหนัง ส่วนฤทธิ์การต้านอนุมูลอิสระพบว่าสารสกัดจากดาหลามีประสิทธิภาพสูงในการขจัดอนุมูลเอบีทีเอส ดีพีพีเอช และซุปเปอร์ออกไซด์เทียบเท่ากับสารมาตรฐานโทรลอกซ์และกรดแกลลิก นอกจากนี้ยังมีประสิทธิภาพในการยับยั้งการทำงานของเอนไซม์ไทโรซิเนสและคอลลาจิเนส การศึกษาปริมาณสารสกัดที่เหมาะสมพบว่าสารสกัดผสมส่วนดอกและส่วนใบความเข้มข้น 1% ที่อัตราส่วน 1:1 มีประสิทธิภาพสูงที่สุดในการยับยั้งเอนไซม์ไทโรซิเนส ส่วนการศึกษาทางคลินิกวิทยาพบว่าผลิตภัณฑ์เครื่องสำอางจากดาหลาทั้ง 3 ชนิดช่วยลดปริมาณเม็ดสีเมลานินในบริเวณทดสอบอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$, bonferroni test) แต่ในช่วงสัปดาห์สุดท้ายของการทดสอบพบว่ามีค่าเฉลี่ยของเม็ดมีเมลานินมีค่าเพิ่มขึ้น ซึ่งอาจเป็นผลมาจากในช่วงการทดลองอยู่ในช่วงเวลาที่แดดร้อนจัด (เดือนมีนาคม – พฤษภาคม 2562) โดยสัมพันธ์กับค่าดัชนียูวีที่สูงขึ้นซึ่งเป็นสาเหตุหนึ่งของการสังเคราะห์เม็ดสีเมลานิน ส่วนการทดสอบความพึงพอใจหลังการใช้ผลิตภัณฑ์พบว่าทุกผลิตภัณฑ์อยู่ในช่วงความพอใจดีถึงดีมากในทุกหัวข้อ นอกจากนี้ยังไม่พบการระคายเคืองในอาสาสมัคร ดังนั้นสารสกัดจากส่วนดอกและส่วนใบดาหลาจึงสามารถนำมาใช้เป็นสารออกฤทธิ์ทางชีวภาพจากธรรมชาติที่มีศักยภาพในการช่วยปรับสภาพผิวให้กระจ่างใสและป้องกันผิวหนังเสื่อมสภาพเพื่อใช้พัฒนาเป็นผลิตภัณฑ์เครื่องสำอาง

คำสำคัญ : ดาหลา, สารต้านอนุมูลอิสระ, เอนไซม์ไทโรซิเนส, เอนไซม์คอลลาจิเนส, เครื่องสำอาง, การทดสอบทางคลินิก

Title COSMETIC PRODUCTS FROM TORCH GINGER
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ABSTRACT

Torch ginger (*Etlingera elatior* (Jack) R.M. Sm.) is a plant of the Zingiberaceae family that possesses medicinal properties and is widely known and cultivated in Thailand as an ornamental flower or locally consumed as food. In this study, torch ginger flowers and leaves were purchased from a cultivar. The dried flowers and leaf were extracted by water and dried via freeze drying and spray drying method. In comparing suitable drying processes each aqueous extract from both drying processes were measured for the yield of extract and total phenolic content. Their effective extracts from a proper dried method were examined for amino acid content and phenolic compounds using GC-MS and LC-MS, respectively. The extracts were evaluated for their antioxidant and enzyme activities. The former referred to assay via 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS), 2, 2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) and superoxide radical scavenging while the latter was an activity of the tyrosinase and collagenase enzyme inhibition to determine possible skin benefits. A suitable concentration of the flower and leaf extracts for formulation as cosmetic was assessed via inhibiting tyrosinase. Furthermore, the mixing of torch ginger flower and leaf extract were developed as cosmetic products including TG cream, TG foam and TG scrub. The cosmetic products were studies on their physical properties and also underwent an accelerated stability test under various conditions. The cosmetic products were also evaluated for skin irritation and its skin whitening effect among 24 healthy volunteers who used each product for four weeks. The whitening effects of each product were evaluated and compared with before the

treatment by measuring the amount of melanin using DermaLab® Combo and using Finn chamber® for skin irritation test.

The results showed that the flower and left extract from the freeze drying method produced the highest yield of extract and total phenolic content, higher than the spray drying method. Henceforth these extracts were used for further analysis. The total phenolic contents of the flower and leaf extracts were 38.68 ± 0.45 and 246.52 ± 0.26 mg GAE/g extract, respectively. The LC-MS analysis revealed that the major components of both extracts were isoquercetin, catechin, and gallic acid. In addition, the amino acid contents of these extracts, which include lysine and leucine, exhibited a compound that may be effective in improving the formation of collagen and reducing wrinkle appearance when used on skin. The antioxidant activity displayed that both extracts can be used as potential sources of natural antioxidants with ABTS, DPPH and superoxide radical scavenging, as well as trolox and gallic acid. The flower and leaf extracts were shown to inhibit tyrosinase and collagenase activities. Further, a suitable concentration of the flower and leaf extracts revealed that 1% of the flower and leaf extract in a ratio of 1:1 exhibited the highest tyrosinase inhibition activity. From the clinical study, each of the torch ginger products demonstrated that the melanin content in the skin significantly decreased after using the products on different tested areas ($p < 0.05$, bonferroni test). On the other hand, the melanin content was increased on the final week of testing, which may be due to the time of testing (March 2019 to May 2019) in Thailand being in the hot season. This period has a corresponding increase in UV exposure, which is one of the causes of melanin synthesis. The volunteer's satisfaction with each torch ginger products was between well to very well for all areas measured. Additionally, none of the volunteers suffered skin irritation of allergic reactions during the test period. In conclusion, torch ginger flower and leaf extract could potentially be a natural source to use as an active ingredient for whitening and anti-aging application in cosmetic products.

Keywords : Etlingera elatior, Antioxidant, Tyrosinase, Collagenase, Cosmetic, Clinical evaluation

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CHAPTER 1

INTRODUCTION

The skin is the largest living organ that has many functions, particularly important in protects the body from the harmful of physical, chemical and biological factors, which influenced by several factors such as solar radiation, pollutant exposures, chemical agent, hormonal changes, and metabolic processes. The prominence effects caused by environmental factors, particularly UV radiation exposure, damage skin through the generation of reactive oxygen species (ROSs), which are instigated of the dermatological changes, leading to skin aging (Działo *et al.*, 2016; Jadoon *et al.*, 2015). Simultaneously, ROSs are relative to extracellular matrix (ECM) protein degradation in the epidermal and dermal layers, which include collagen and elastin. Matrix metalloproteinases (MMPs) are enzyme involved in ECM degradation, and with it comprising collagenase (MMP-1) and gelatinase (MMP-9). The response of ECM degradation effects the integrity of the skin structure, resulting in wrinkles, dryness, and roughness (Campa and Baron, 2018; Limtrakul *et al*., 2016; Zakiah *et al*., 2018). One of the most important factors, inhibiting ECM degradation has been determined to combat the skin aging process through the generation of ROSs (Wittenauer *et al*., 2015).

In addition to the synthesis of melanin is the mechanism responsible for protecting the harmful of UV radiation, visible light and diminished the ROSs. On the other hand, the obtained of ROSs can induce the activities of tyrosinase and also melanogenesis. The overproduction and accumulation of melanin resulting in several skin pigmentation disorders including solar lentigos (age spot), melasma, freckles and post-inflammatory hyperpigmentation (Campa and Baron, 2018; D'Mello *et al*., 2016; Ortonne and Bissett, 2008; Ribeiro *et al*., 2015). The pigment of melanin are formed through oxidation and the amino acid tyrosine by cyclization. Tyrosinase is a

melanogenic enzyme that catalyzes the rate-limiting synthetic for melanin production. Melanogenesis is initial with hydroxylation L-tyrosine by tyrosinase converted to the L-3,4-dihydroxyphenylalanine (L-DOPA) and the oxidation L-DOPA to DOPA-quinone, eventually to melanin pigments (D'Orazio *et al*., 2013; Kim *et al*., 2018). Accordingly, tyrosinase inhibitors is possibly an important target for reducing the melanin synthesis (Sahin, 2018).

In current times, the desire to light complexion is becoming popular in Asia people for a beauty-looking skin. Skin whitening products purposed to obtaining a lightening skin appearance or hyper-pigmented treatment has become highly desirable in the cosmeceutical industries (Lorz *et al*., 2019). Meanwhile, there are many studies was looking for suppress the ROSs formation which delays the skin aging process in tandem with inhibiting the melanogenesis (Itoh *et al*., 2019; Kageyama and Waditee-Sirisattha, 2019; Limtrakul *et al*., 2016; Namjoyan *et al*., 2019; Tu and Tawata, 2015). The use of cosmetics containing anti-aging and skin whitening cosmetic is an important purpose for improving skin appearance in term of wrinkles and lightening as well as diminish dark spots on their skin (Mukherjee *et al*., 2011; Sahin, 2018; Wang *et al*., 2016).

Torch ginger (*Etlingera elatior* (Jack) R.M.Sm.) is a plant of the Zingiberaceae family that has medicinal properties and is widely cultivated in Southeast Asia (Chan *et al*., 2011[a]). The phytochemical composition of torch ginger flowers and leaves has been reported on a few times in the past decades. Extracts of both flower and leaf have revealed the presence of cosmeceutical activities that can improve skin problems (Chan *et al*., 2011[b]). The methanolic extracts of the flower reveal the presence of active compounds containing flavonoids, terpenoids, saponins, tannins and carbohydrates (Lachumy *et al*., 2010). Meanwhile, the leaf extract reveals a group of flavonoids, including isoquercetin, quercetin, catechin and caffeoylquinic acids (Chan *et al*., 2011[b]). Regarding pharmacological and biological properties, the flower extract exhibits the antioxidant activities of scavenging DPPH and chelating

ferrous-ions, as well as anti-pathogenic bacteria and anticancer cell lines (MCF-7 and MDA-MB-231). Leaf extracts exhibit the inhibition of melanogenesis and radical scavenging activities, as does the flower extract (Ghasemzadeh *et al*., 2015). The cultivation of torch ginger in Thailand widely cultivated in the Southern region, there are culture for cut flower to sale, consumed flower as local food, and herbal drink (Hemathulin *et al*., 2015). In addition, in the past several years, the exploitation of leaf has not been reported as it should be as well as flower.

This research aims to extraction and evaluate the bioactive compounds of the amino acid content and phenolic compounds and analysis the biological activities, including antioxidant, anti-tyrosinase and anti-collagenase activities of torch ginger flowers and leaves. Furthermore, to formulate the cosmetic products containing both of the flower and leaf extracts of torch ginger, and examine the skin irritation and their efficiency in human volunteers.

The objective of the study are as follows:

1. To extract the bioactive compounds from torch ginger flowers and leaves for uses as a cosmetic ingredient.

2. To examine the bioactive compounds and biological activities of torch ginger flower and leaf extracts.

3. To develop cosmetic products containing torch ginger flower and leaf extracts, include 3 products.

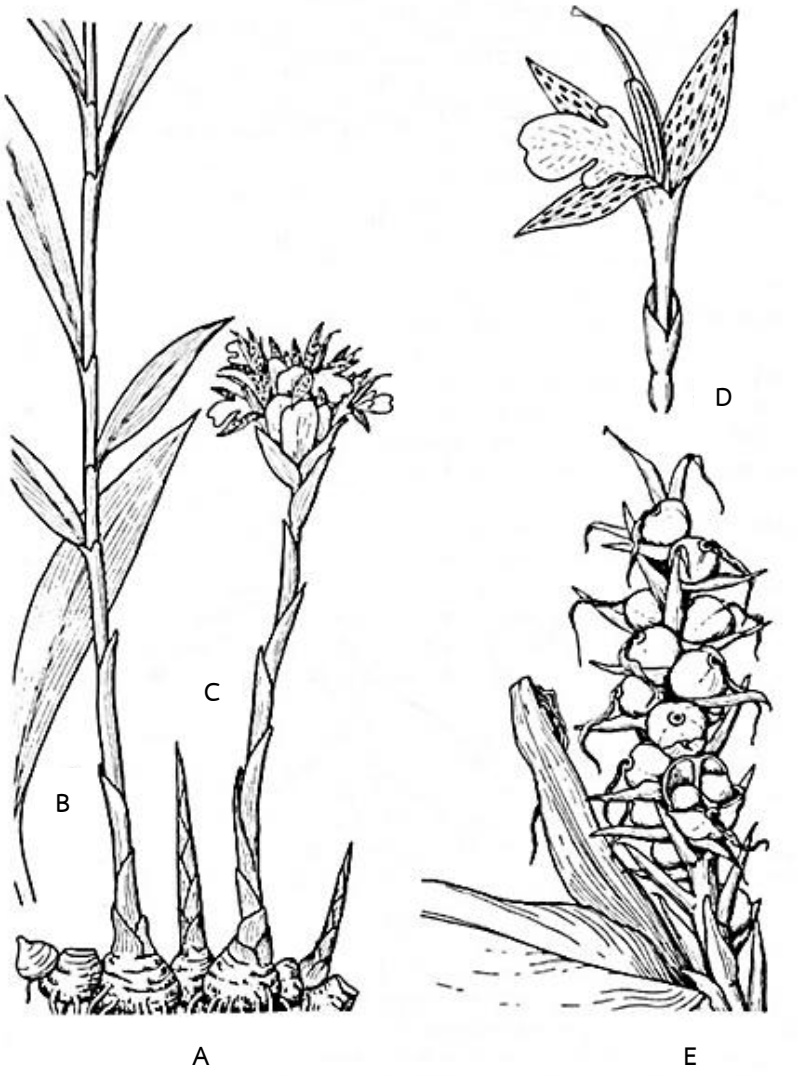
4. To investigate the efficiency of cosmetic products and their satisfaction in volunteers.

CHAPTER 2
LITERATURE REVIEWS

2.1 Zingiberaceae

The plant in Zingiberaceae are largest families known as ginger which consist of high biodiversity and distributed mainly in the tropical and subtropical region especially in the Southeast Asia, there are approximately 50 genera and 1,500 species belong in the family. Furthermore, there are about 30 genera and 300 species that found in Thailand (ke *et al*., 2000; Saensouk *et al*., 2016). The Zingiberaceae may reach above up to 10 m height from the ground and it belongs in the group of perennials with a rhizome in the underground, the rhizome is often ellipsoid or spherical tubers with the terminated root. The stem usually is short, replaced by pseudostem formed by leaf sheaths and shoot from the terminal portion of the rhizome. Inflorescence is shoot from the rhizome or is either on pseudostem, shapes of cylindric or fusiform or globose, sometimes a spike or raceme, nominal to many flowered and presence of bracts and bracteoles. The flower is bisexual that composed a stamen and pistil. There are inner whorl, the corolla consists of 3 petals. The inside of perianth have of 6 stamens, which the only one operative as a reproductive organ. And two of stamen transformed to lateral staminodes with the rest of two stamen transformed to labellum or lip. The initial ovary composed of 3-loculed, and 1 or 3-loculed when mature. Developed style is very thin that placed in a niche of the filament between of anther locules. The stigma is on above of anther. Finally, the fruit is a capsule and coexist like an infructescence, shape like pineapple or berrylike, as shown in Figure 1 (ke *et al*., 2000; Larsen and Larsen, 2006). In Thailand, has reported numerous genera of Zingiberaceae families' i.e. *Alpinia* (สกุลข่า), *Amomum* (สกุลกระวาน), *Boesenbergia* (สกุลกระชาย), *Curcuma* (สกุลขมิ้น-กระเจียว), *Globba* (สกุลข่าลิง), *Hedychium* (สกุลมหาหงส์), *Kaempferia* (สกุลเปราะ), *Zingiber* (สกุลขิง), *Caulokaempferia*, *Camptandra*,

Elettariopsis, *Geostachys*, *Plagiostachys*, *Scaphochlamyms*, and *Etlingera* (สกุลดาหลา) (Nontasit *et al*., 2015; Saensouk *et al*., 2016; Sirirugsa, 2008).



Figures 1 Diagram of the Zingiberaceae plant;
(A) rhizome, (B) leafy shoot, (C) flowering shoot, (D) flower, and (E) infructescence
Source: Larsen and Larsen (2006)

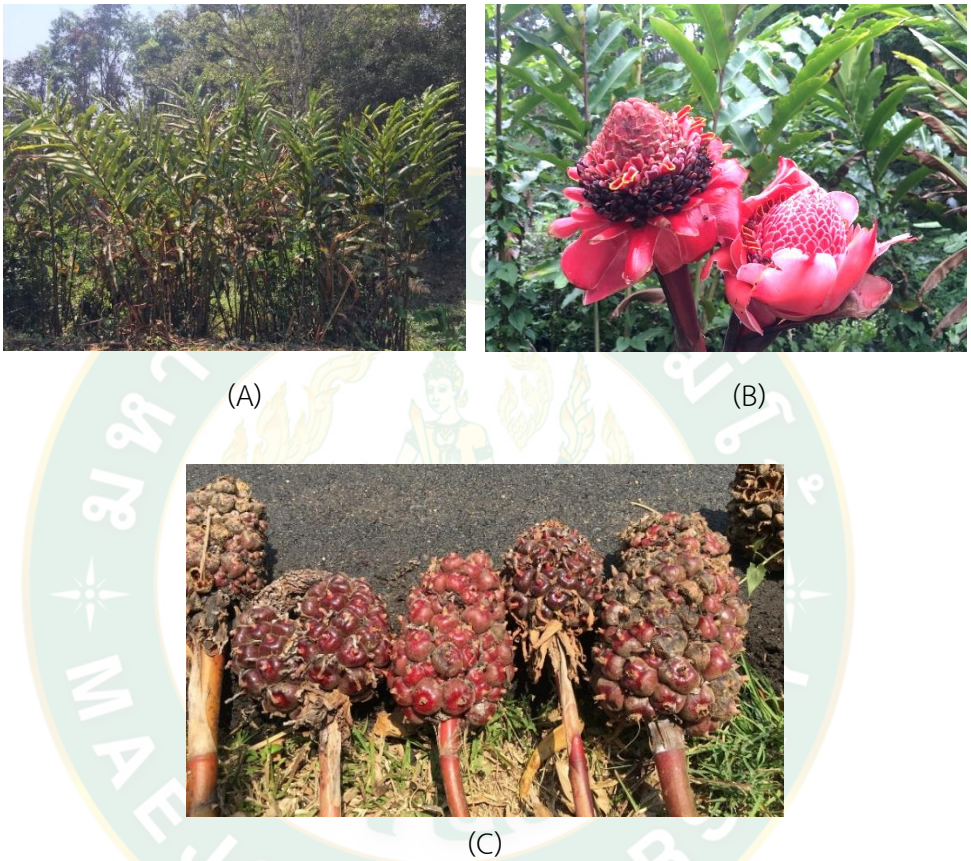
The utilization of Zingiberaceae has been reported for a long time as a food, spice, medicine, ornamental, herbal drink, as well as cosmetic purposes. The phytochemical studies reveal that most of bioactive compounds are the phenolic group, which is the beneficial to antioxidant, anti-inflammatory, anticancer and, pain relief etc. (Batubara *et al*., 2016; Jing *et al*., 2010; Ullah *et al*., 2014; Zahra, 2016). The Zingiberaceae has high biodiversity, including Thailand is the one area as well. Thus, plants belong in this family are of interesting for further studies.

2.2 Torch ginger

Torch ginger (*Etlingera elatior* (Jack) R.M.Sm.) are plant belong to the Zingiberaceae, the symnonyms of *E. elatior* are *Alpinia elatior*, *Elettaria speciosa*, *Nicolaia elatior*, *Nicolaia speciosa*, and *Phaeomeria speciosa*. This plant are widely cultivated in Southeast Asia area, and found slight in Australia farms, and Hawaii for as a spice and a commercially ornament flower (Chan *et al*., 2011[a]). The botanical characteristics of torch ginger include growth of up to 5-6 m tall from their underground groves, and the width of the stout rhizome is 3-4 cm in diameter. The leaves are entirely green, possess a lanceolate shape and are up to 81 cm in length, and the crushed leaf gave feel a charming sour fragrance. The tall stems, formed by the sheaths of leaves, are pseudostems that grow from the underground rhizomes. The waxy inflorescences are shaped like spearheads when young, and when in blossom, the bracts are larger and feature prominent red and pink coloration. The fruits are shaped like pineapples and have green to reddish coloration, featuring many black seeds inside, as shown in Figure 2 (Aswani *et al*., 2013; Chan *et al*., 2013; Larsen and Larsen, 2006).

In Thailand, the cultivation torch ginger is widely in the Southern region with slight in other regions, there are cultured for commercial flower especially pink and red flower. Furthermore, when comparative with another flower, torch ginger is a

large flower, colorful, not easy to bruised, and bear for several days, as well as the flower bloom all year round and a peak blooming January to April of the year (Soontornnon, 2008).



(A) (B)

(C)

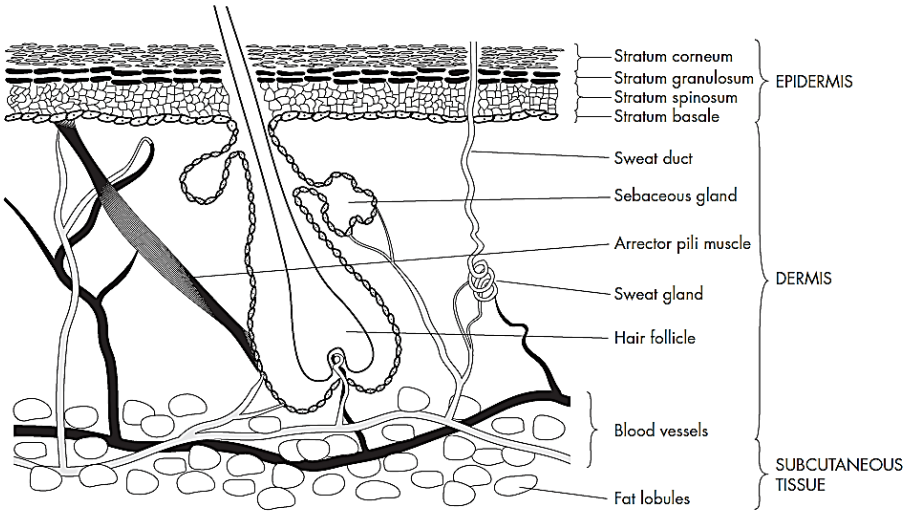
Figures 2. The physical appearances of torch ginger plant;
(A) plant, (B) flower and leaf, and (C) infructescences

The biological constituent of torch ginger has been reported a few times in the past decades. Torch ginger has been identified for its phytochemical content by various extraction solvent. The aqueous extract of torch ginger flower show the highest total phenolic content (618.9 mg GAE/100 g), follow by ethanol and methanol extraction of 520.4, and 361.0 mg GAE/100 g, respectively. Further, the aqueous extract showed the presence of phenolic acid (consisting of gallic acid, tannic acid, chlorogenic acid, and caffeic acid), flavonoids (i.e. quercetin, apegenin,

kaempferol, luteolin, myricetin, and anthrocyanin), and tannin. Flower extract had potential to be an antioxidant via DPPH and FRAP (ferric reducing antioxidant power) assay. It has been to have anticancer activity against with human breast carcinoma cell lines of MCF-7 and MDA-MB-231. In cosmeceutical properties, flower has been exhibited for the inhibition of tyrosinase and collagenase enzymes, and it has no toxicity on *Artemia salina* with IC_{50} value of 2.52 mg/mL (Ghasemzadeh *et al*., 2015; Jackie *et al*., 2011; Lachumy *et al*., 2010; Wijekoon *et al*., 2011; Wijekoon *et al*., 2013). The leaf of torch ginger contain flavonoids i.e. isoquercitrin, quercitrin and (+)-catechin, and caffeoylquinic acid (CQA) such as 3-CQA (chlorogenic acid), 5-CQA (neocholorogenic acid), and methyl 5-CQA. Moreover, the dominant phenolic compound in leaf is chlorogenic acid (Chan *et al*., 2011[b]; Chan *et al*., 2011[a]). There are exhibited the total phenolic content and antioxidant activity than those from flower and rhizomes approximately 10 to 20 times. Their biological activities of leaf contain radical scavenging activity, melanogenesis inhibition and antibacterial activity (Chan *et al*., 2007; Chan *et al*., 2008).

2.3 The skin

The skin is the largest organ of the body, is approximately 18,000 cm^2 covers the body's and more than 10% of the total body mass. The primary function of the skin is a protective barrier of the body from the external environment against UV radiation, chemicals, allergens, microorganism, moisture control, and body nutrients. Moreover, also function to regulate the body temperature control and blood pressure. The structure of skin be composed of three main layer i.e. epidermis, dermis and subcutaneous tissue (hypodermis), as shown in Figure 3 (Benson, 2012; มยุรี, 2559).



Figures 3. The structure and layer of skin
Source: Williams (2003)

2.3.1 Epidermis

The epidermis is the outermost layer of the skin which is composed of many layers of closely packed cells. The epidermis serves as a skin barrier provides to protect against exterior environment from pathogens, UV radiation and chemicals, and interior body as well. The epidermal thickness varies in a different type of skin which is further divided into 5 separate stratum layers (Benson, 2012; Hunter *et al*., 2003; Yousef *et al*., 2019; มยุรี, 2559).

1) Stratum corneum (or horny layer) is the outermost layer that composed of dead skin cell called “corneocytes” ranges from 15 to 20 layers, resulting in a total thickness layer approximately 10 – 20 μm. This layer contains hexagonal flat cells without a nucleus, together with lipids and desmosomes that renews within 6 to 30 days.

2) Stratum lucidum (if present) is a thin clear layer lower portion from stratum corneum that found on the palms and soles of feet. The cell of

this layer is flattened and compacted, which the cell nuclei and organelles disintegrate.

3) Stratum granulosum (or granular layer) is a viable layer which a part layer of keratin production and containing hydrolytic enzyme that degradation of the cell component of nuclei and organelles.

4) Stratum spinosum (or prickle cell layer) is found on the top basal layer which consist of 2 to 6 rows of polygonal keratinocytes. Each cell has a large cytoplasm that contains many filament and organelles.

5) Stratum basale (stratum germinativum or basal layer) is the most important layer that ability to division and replicate every 200 to 400 h. The mainly is keratinocytes that formed before moving up to the upper layer. Further, this layer contains the melanocytes that produce melanin which responsible for protecting the harmful of UV radiation that causes in skin pigmentation.

2.3.2 Dermis

The dermis lies lower of epidermis that acts as the epidermis structurally and nutritionally. The dermis is about 1 – 2 mm in thickness. It contains several specialized structures including sebaceous glands, eccrine and apocrine sweat glands, hair follicles, blood vessels, and nerves, which serves to regulate body temperature, provide oxygen and nutrients, support the immune response, tensile strength, elasticity. Further, this layer consists of extracellular matrix (ECM) which relative in the flexibility and elasticity of the skin such as the fibrous protein of collagen, elastin, and, glycosaminoglycans (GAGs). The GAGs is the matrix insert between collagen and elastin fibrils, it contains hyaluronic acid, dermatan sulfate, and chondroitin sulfate involved in maintaining the skin hydration, flexibility, and soften.

2.3.3 Subcutaneous tissue

The subcutaneous tissue (hypodermis) is an adipose tissue layer, arranges lobules, with interconnecting collagen and elastin fibers. This layer act to protective against physical shock including heat insulation. The connective tissue of this layer contains large blood vessels and nerves.

2.4 Skin aging

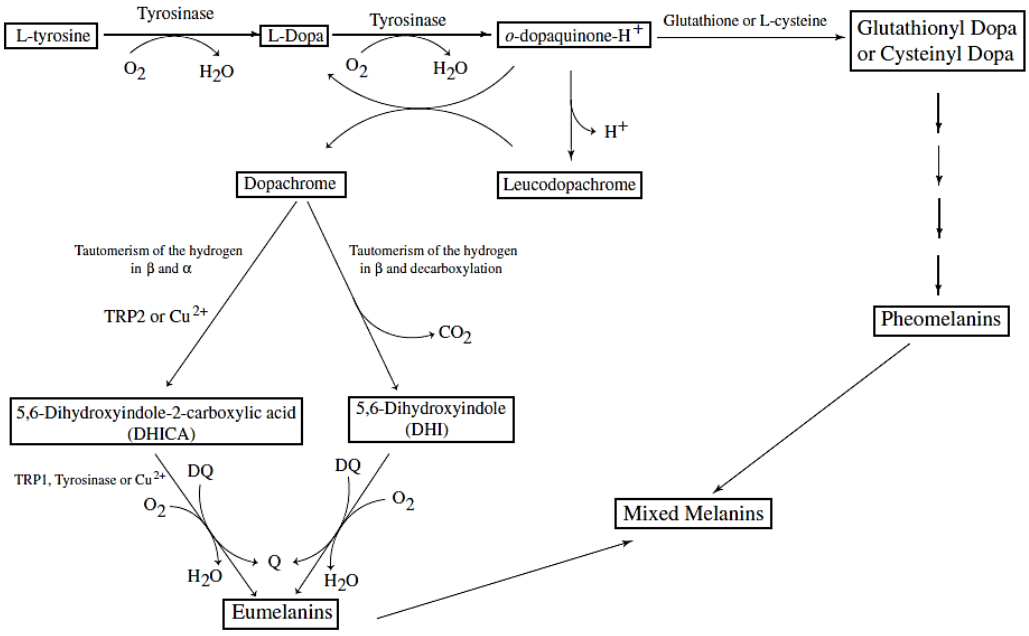
Skin aging is a dermatological change influenced by several factors, including age, hormonal changes, environmental exposures and metabolic processes (Jadoon *et al*., 2015). The oxidative stress processes is prominence major of skin aging and related dermatological diseases through the generation of reactive oxygen species (ROSs) (Ribeiro *et al*., 2015; Zakiah *et al*., 2018). The typical skin has a complex of enzymatic antioxidant (superoxide dismutase (SOD), catalase, and glutathione peroxidase) and non-enzymatic antioxidant (ascorbic acid, tocopherol, ubiquinone, and glutathione) that protect harmful free radicals and repair oxidative damages. Nonetheless, it is often insatiable, the natural antioxidant (such as phenolic compounds) is an alternative to serves as exogenous sources against the high level of free radical formation in the body (Soto *et al*., 2015; Taofiq *et al*., 2017). ROSs are related to ECM protein degradation in the epidermal and dermal layers, which include collagen and elastin fibrils. Matrix metalloproteinase (MMPs) are enzymes involved in ECM degradation and include MMP-1 (collagenase), MMP-3 (stromelysin) and MMP-9 (gelatinase). The response of ECM degradation effects the integrity of the skin structure, resulting in wrinkles, dryness roughness and flaccid skin (Limtrakul *et al*., 2016; Thring *et al*., 2009; Zakiah *et al*., 2018). Hence, inhibiting ECM degradation has been determined to combat the skin aging process through the generation of ROSs (Wittenauer *et al*., 2015).

Further, the skin aging process of each individual is differences, there are 2 main influenced include both intrinsic and extrinsic aging (Zakiah *et al*., 2018). The intrinsic aging (or chloronologic aging) caused by genetics, hormone changes, corporal changes and metabolic processes factor. This factor occurring in the normal aging process. Extrinsic aging (or premature, or photoaging) due to an environment which UV radiation is the main factor. Both intrinsic and extrinsic aging resulting in a thinning of epidermal and dermal layers further loses the function to act as a barrier against environmental conditions (Farage *et al*., 2008; Rinnerthaler *et al*., 2015).

2.5 Melanin

Melanin is a pigment responsible for the color of skin found in animal, human skin, hair, and eyes, is synthesized from oxidation and cyclization reaction of amino acid tyrosine, it is called melanogenesis (Solano, 2014). According to the chemical form, melanin is a divide into two categories include: eumalanin, a black or brown pigment, and pheomelanin, a red or yellow pigment (D'Orazio *et al*., 2013). The melanin production can be stimulating by several factors such as melanocyte stimulating hormone (α-MSH), cyclic adenosine monophosphate (cAMP)-elevating agents, UV radiation, and 8-methoxypsoralen (8-MOP), those factors leading to an increase in the activity of the melanin-producing enzyme (Li *et al*., 2014). Generally, the pigmentation generating have benefit for protecting human skin against harmful UV effects, along with protecting ROSs and oxidative stress processes. On the other hands, the excessive oxidative stress effect by UV radiation may be a cause of melanin accumulation as well (Pillaiyar *et al*., 2017). Besides, overproduction and accumulation of melanin pigments are the cause of several skin disorders such as solar melanosis, ephelides, melisma, senile lentigo, and post-inflammatory hyperpigmentation (Di Petrillo *et al*., 2016). Tyrosinase (EC 1.14.18.1) and tyrosinase-related proteins (TRP1 and TRP2) are melanogenic enzymes that catalyze the rate-limiting synthetic for melanin production. The tyrosinase structure contained two

copper atoms, and each atom is ligated to three histidines (Chang, 2009; Zolghadri *et al*., 2019). Melanogenesis initially occurs through hydroxylation of L-tyrosine by tyrosinase converted to L-3,4-dihydroxyphenylalanine (L-DOPA) and by the oxidation of L-DOPA to DOPA-quinone, and eventually to melanin pigments (D'Orazio *et al*., 2013; Kim *et al*., 2018). Moreover, in another mechanism, a process of protein glycosylation, Neu5Acα (2-6)Gal- and possibly sialyl(α2-3)gal-terminated glycans play an important role in melanogenesis and melanosome transfer to keratinocytes, as shown in Figure 5 (Diwakar *et al*., 2015). Biosynthesis of melanin pigment (Figure 4) (Pillaiyar *et al*., 2017) are following:



Figures 4. The melanin biosynthesis pathways

Source: Zolghadri *et al*. (2019)

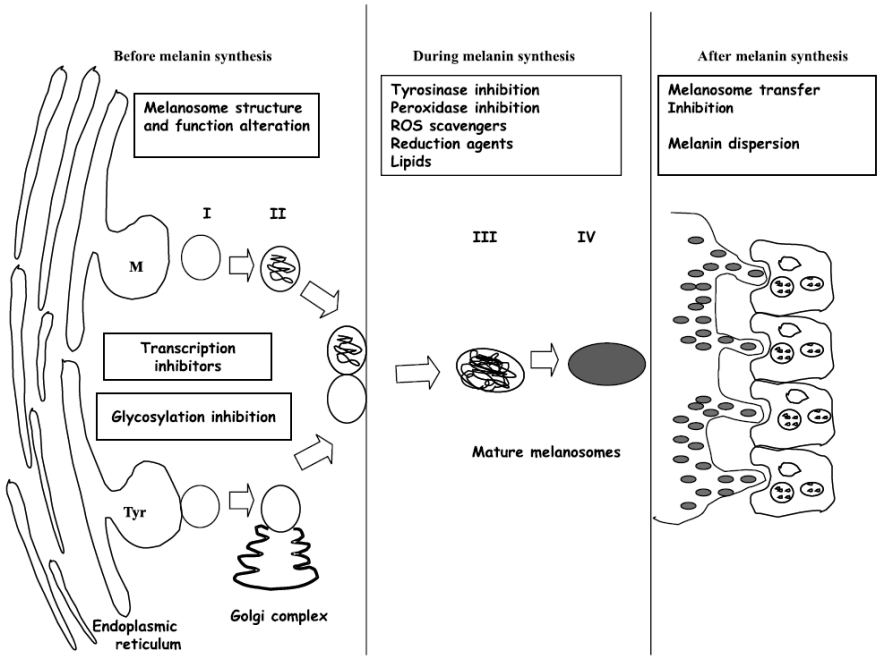
First step: the oxidation of L-tyrosine to L-dihydroxyphenylalanine (L-DOPA) by enzyme tyrosinase.

Second step: the oxidation of L-DOPA to dopaquinone by enzyme tyrosinase resulting quinone is substrate for formation of eumelanin or pheomelanin.

Third step: this step is non-enzyme-catalyzed process, transformed dopaquinone to leucodopachrome.

Forth step: this step is oxidized leucodopachrome to dopachrome, this step is very fast and non-enzyme-catalyzed process.

Fifth step: this step is catalyzed by TRP-2 which decomposes dopachrome and oxidized dihydroxyindole (DHI) and dihydroxyindole-2-carboxylic acid (DHICA) to melanin pigments.



Figures 5. The possible approaches to inhibit the melanogenesis pathway;
Tyr = tyrosinase; M = melanosomes
Source: Briganti *et al*. (2003)

Therefore, whitening ingredients (Table 1) that result in inhibited tyrosinase activity, including inhibitory effects on melanogenesis and the melanosome transfer

process, etc. are essentially significant for reducing melanin synthesis. Tyrosinase inhibitors are obtained from both natural and synthetic sources, such as hydroquinone, arbutin, kojic acid, L-ascorbic acid, tranexamic acid, ellagic acid, and thiamidol (Zolghadri *et al*., 2019). Lately, Baswan *et al*. (2019a); (Baswan *et al*., 2018; Baswan *et al*., 2019b) reported that cytidine, though not a tyrosinase inhibitor, inhibits melanin synthesis and the melanosome transfer process by interfering with glycosylation processes. In current times, the desire to lighten the complexion has become popular in Asian people seeking beautiful-looking skin. Skin whitening products that propose to lighten the skin's appearance or treat hyper-pigmentation have become highly desirable in the cosmeceutical industries (Lorz *et al*., 2019). The use of skin whitening cosmetics has an important role in achieving skin lightening, as well as in diminishing dark spots on the skin (Sahin, 2018; Wang *et al*., 2016).

Table 1. Skin whitening ingredients and their mechanism of depigmentation

Mechanism of action	Molecule	Reference
Melanosome transfer	niacinamide, protease inhibitor, cytidine, linoleic acid	(Couteau and Coiffard, 2016; Diwakar *et al*., 2015; Ortonne and Bissett, 2008)
Protein glycosylation inhibition	cytidine, glucosamine, N-acetyl glucosamine	(Baswan *et al*., 2019[a]; Baswan *et al*., 2018; Baswan *et al*., 2019[b]; Ortonne and Bissett, 2008)
Epidermal turnover accelerant	vitamin C, vitamin E, thioctic acid, retinoids, lactic acid, glycolic acid, salicylic acid	(Chang, 2009; Couteau and Coiffard, 2016; Ortonne and Bissett, 2008)
Anti-inflammatory	niacinamide, soy milk	(Couteau and Coiffard, 2016)
Free radical trapping agents	glycyrrhetinic acid, steroid, vitamin C, vitamin E	(Couteau and Coiffard, 2016)
Tyrosinase copper chelation	ellagic acid	(Ortonne and Bissett, 2008)
Tyrosinase inhibition	hydroquinone, mequinol, arbutin, azelaic acid, kojic acid, ellagic acid, resveratrol, oxyresvaretral	(Chang, 2009; Couteau and Coiffard, 2016; Ortonne and Bissett, 2008)

2.5.1 Tyrosinase inhibitor

Due to the desire to light complexion, several substances have been considered as a tyrosinase inhibitor. As described by Chang (2009), the different active compounds inhibiting tyrosinase activities are suppressed by dissimilar acting, as followings:

1) Reducing agents that cause of produce dopaquinone such as ascorbic acid, it react by reverse the reaction of DOPA to dopaquinone, then reduce the melanin formation process.

2) *o*-Dopaquinone scavenger e.g. a substance in group of thio-containing compounds, it can react with dopaquinone resulting to colorless form, which is delay the melanogenesis until all scavenger is eradicated.

3) Alternative enzyme substrates e.g. phenolic compounds, it reaction created a quinoid product that replaces from the regular reaction of dopachrome. Phenolic compounds are a good attraction for tyrosinase results to inhibited dopachrome formation.

4) Nonspecific enzyme inactivators i.e. acids or bases, it is not-specifically which result to denature the enzyme.

5) Specific tyrosinase inactivators e.g. mechanism-based inhibitors. Its react by catalyzed tyrosinase and binding with the enzyme by covalent bond. They reaction called “suicide reaction”.

6) Specific tyrosinase inhibitor, this group is widely distributed in the nature, it can be bind to enzyme and reduce its reaction.

2.6 Free radical

Free radical is the species capable of independent existence which comprises one or more unpaired electron in its outermost atomic or molecular orbital, an unpaired electron is a very unstable and highly reactive with other compounds. The free radicals can donate an electron or accept an electron from another molecule, then it is oxidant or reductants. These free radical can attack with both radical and non-radical molecule, and rapidly to react with other molecules and continuously, and can start a chain reaction. Then, the free radical are very short-lived, with the half-lived of milli- or micro- or nanoseconds (Devasagayam *et al*., 2004; Sarma *et al*., 2010). The oxygen-containing free radical is the most important in many biological system i.e. hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical (Lobo *et al*., 2010). These free radical lead to damaging biologically molecule in the body such as lipids, nucleic acid, proteins (Mohammed *et al*., 2015).

Generally, a free radical can be produced through the following (Kumar, 2011):

1. The homolytic cleavage of the covalent bond of the stable molecule, with each fragment remaining one paired electron. ($X : Y \longrightarrow X^{\bullet} + Y^{\bullet}$)

2. The loss of single electron from stable molecule. ($X : Y \longrightarrow X^{-} + Y^{+}$)

3. The addition of single electron to stable molecule. ($A + e^{-} \longrightarrow A^{-\bullet}$)

2.6.1 Sources of free radical

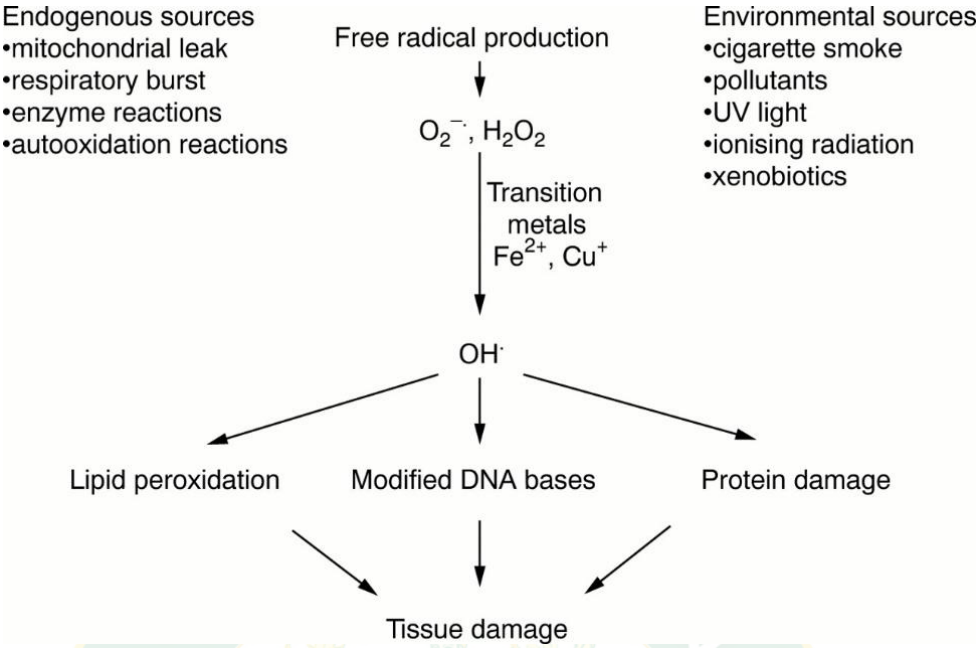
Free radicals can be generated by numerous of biochemical processes in the human body including endogenous sources, exogenous sources, and physiological factors, as shown in Figure 6.

2.6.1.1 Endogenous sources

Free radical are produced from the cellular metabolism, including enzymatic reactions in the respiratory chain, in phagocytosis, prostaglandin synthesis, and cytochrome P_{450} system as well. In the mitochondria are the major site of superoxide anion ($O_2^{-\bullet}$), is formed by addition the electron to the oxygen molecule. This process can be produced by nicotine adenine dinucleotide phosphate [NAD(P)H] oxidase, or xanthine oxidase, or mitochondrial electron transport system. In generally the transferred electron through mitochondria transport system for reduction oxygen to water, this reaction process may have electron leak that causes of superoxide production. Hydrogen peroxide (H_2O_2) is produced from the cellular reaction of oxidase enzyme i.e. amino acid oxidase and xanthine oxidase. In addition, the H_2O_2 with the presence of chloride ion can be transformed into the hypochlorous acid (HOCl) with play a vital role to kill the pathogen. The oxidative damage reaction of lipids, proteins, DNA and sugar can be produced the peroxyl radicals ($ROO^{\bullet}$). These radical including hydroperoxyl radical (HOO^{-}) that is a role in lipid peroxidation (Birben *et al*., 2012; Devasagayam *et al*., 2004; Halliwell, 2001).

2.6.1.2 Exogenous sources

The exogenous sources of free radical are environmental factors, including cigarette smoke, ozone exposure, hyperoxia, ionizing radiation, heavy metal ions, an environmental pollutant, ultraviolet light, ozone, drugs, and industrial solvents, etc. (Birben *et al*., 2012; Kumar, 2011)



Figures 6. Endogenous and environmental sources of free radicals
Source: Young and Woodside (2001)

2.6.1.3 Physiological factors

The free radical formation involving the physiological factors are through mental stress e.g. emotion and disease symptoms (Kumar, 2011).

2.6.2 Free radical generation step (Sarma *et al*., 2010)

The reaction of free radical generation in chemistry contained the radical addition and radical substitution, the chain reaction divided into 3 steps are initiation, propagation and termination as following:

Initiation: this step is increasing the number free radical by an attack with another stable molecule or may involve the reaction normal stable molecule reacting with other free radicals results in more form of free radicals.

Propagation: this step is a free radical continuously transformed to another form of free radical by a chain reaction with a transferred electron, atomic and molecule.

Termination: This step is the reaction of two free radicals resulting in a decrease of totally free radical with combined to more stable molecule (e.g. $2Cl^{\bullet} \longrightarrow Cl_2$).

2.6.3 Oxidative stress

In biological, oxygen metabolism occurring in the mitochondria may cause of formation the reactive oxygen species or free radicals. Oxidative stress is a status of unbalance between free radical production and antioxidant defenses. These oxidative stress may be a cause of some diseases, participatory with damage all macromolecule in biological membrane and molecule i.e. lipids, proteins, and nucleic acid, as shown in Figure 6. The oxidative stress can be found in the tissue injured by several conditions such as trauma, infection, heat injury, hyperoxia, toxins, and extremely exercise. These injuries may induce the radical producing enzymes along with disruption of the electron transport chain in the oxidative phosphorylation, finally produced the ROSs (Lobo *et al*., 2010; Yoshikawa and Naito, 2002). Furthermore, ROSs can be generated through exogenous sources e.g. UV radiation and chemical agents. The lipid oxidation by ROSs can destructive the cellular structure and leads to unexpired cell death. Moreover, the increase of oxidative stress induced by ROSs is a risk for skin including in the aging process. This is an incident of accelerating in the skin aging process and skin diseases such as dermatitis, sunburn, acne, eczema, vasculitis, psoriasis and cancer as well (Iriondo-DeHond *et al*., 2016; Naidoo and Birch-Machin, 2017).

2.6.4 Type of free radicals

In a biological system, free radical and relative substances can be divided into 3 main groups: reactive oxygen species (ROSs), reactive nitrogen species (RNSs), and reactive chloride species (RCSs), as shown in Table 2.

Table 2. Reactive species and its non-radicals

Radicals	Non-radicals
Reactive Oxygen Species (ROSs)	
Superoxide, Superoxide anion, $O_2^{\bullet-}$	H_2O_2 Ozone O_3
Hydroxyl, $^{\bullet}OH$	Hypobromous acid, HOBr
Hydroperoxyl, $HO_2^{\bullet}$	Hypochlorous acid, HOCl
Peroxyl, $RO_2^{\bullet}$	Singlet oxygen (O_2 $^1\Delta g$)
Alkoxyl, $RO^{\bullet}$	Organic peroxides, ROOH
Carbonate, $CO_3^{-\bullet}$	Peroxynitrite, $ONOO^-$
Carbon dioxide, $CO_2^{-\bullet}$	Peroxynitrous acid, ONOOH
Reactive nitrogen species (RNSs)	
Nitric oxide, $NO^{\bullet}$	Nitrous acid, HNO_2, Nitrosyl cation, NO^+
Nitrogen dioxide, $NO_2^{\bullet}$ $NO_2^{\bullet-}$	Nitroxyl anion, NO^-, Dinitrogen tetroxide, N_2O_4
	Dinitrogen trioxide, N_2O_3
	Peronitrite, $ONOO^-$
	Peroxynitrous acid, ONOOH
	Nitronium (nitryl) cation, NO_2^+
	Alkyl peroxynitrites, ROONO
	Nitryl (nitronium) chloride, NO_2Cl
Reactive chlorine species (RCSs)	
Atomic chlorine, Cl	Hypochlorous acid, HOCl
	Nitryl (nitronium) chloride, NO_2Cl
	Chloramines, Chlorine gas, Cl_2

Source: Halliwell and Whiteman (2004)

2.6.4.1 Reactive oxygen species (ROSs)

ROSs are natural by-products of cellular oxygen metabolic pathways and have a single unpaired electron in an outer orbit that is highly reactive molecule to damage other molecules. Normally, ROSs are generated in all aerobic organism in cellular metabolism. The ROSs are normally not contained only oxygen-centered radical e.g. superoxide radical ($O_2^{\bullet-}$) and hydroxyl radical ($OH^{\bullet}$), and also include some non-radical derivative of oxygen e.g. hydrogen peroxide (H_2O_2), singlet oxygen $^1\Delta_g$, and hypochlorous acid (HOCl) (Halliwell, 2001; Noori, 2012).

1) Superoxide anion radical ($O_2^{\bullet-}$)

Superoxide anion radical is the most important one generated through direct auto-oxidation of the oxygen molecule in the electron transport chain in mitochondria. The mitochondria typically generate energy using 4 electrons for reducing oxygen to water. Some of an electron leak from the mitochondrial chain reaction directly react with the oxygen generated the $O_2^{\bullet-}$. Further, the superoxide anion radical produced by enzyme include xanthine oxidase, lipoxygenase, cyclooxygenase, and NADPH dependent oxidase. The superoxide anion radical occurring in two forms i.e. $O_2^{\bullet-}$ and hydroperoxyl radical (HO_2). Furthermore, the $O_2^{\bullet-}$ play an important role to generate other ROS such as hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), or singlet oxygen. Additionally, the $O_2^{\bullet-}$ can be reacted with nitric oxide ($NO^{\bullet}$) generate the peroxynitrite ($ONOO^-$) that cause of formation of toxic compounds e.g. hydroxyl radical ($OH^{\bullet}$) and nitric dioxide ($NO^{\bullet}$) (Lee *et al*., 2004; Phaniendra *et al*., 2015; Salaja *et al*., 2011).

2) Hydroxyl radical ($OH^{\bullet}$)

Hydroxyl radical is the most highly reactive free radical molecules and is the neutral form of hydroxide ion. It is one of the most react with all both organic and inorganic molecules in the living cells comprising DNA,

polypeptides, proteins, lipids, carbohydrates, especially thiamine and guanosine. The $OH^{\bullet}$ can be formed by 2 main mechanisms: 1) radiolysis of water by a reaction between H_2O_2 and metal ion (Fe^{2+} or Cu^{2+}), this reaction is the Fenton reaction (equation 1) and formed by hemolytic fission between superoxide radical and H_2O_2, it is the Haber-Weiss reaction (equation 2). It attacks and damages cell membrane and lipoproteins by the lipid peroxidation that may be a cause of atherosclerosis. In addition, the $OH^{\bullet}$ is the most potent reactive species generated in the biological system and react rapidly with everything in a living organism (Phaniendra *et al*., 2015; Salaja *et al*., 2011).

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\bullet} + OH^{-} \quad \text{(equation 1)}$$

$$O_2^{\bullet-} + H_2O_2 \longrightarrow O_2 + OH^{\bullet} + OH^{-} \quad \text{(equation 2)}$$

3) Hydrogen peroxide (H_2O_2)

Hydrogen peroxide is generated in dismutation reaction from superoxide catalyzed by superoxide dismutase (equation 3). This hydrogen peroxide is a non-radical form and mainly produced by enzymatic reaction e.g. amino acid oxidase, xanthine oxidase, etc. The H_2O_2 has no direct effect on DNA damaged but it is a cause of $OH^{\bullet}$ generated that has an effect on DNA damage in the presence of transition metal ion. This substance is poor oxidizing and reducing agent then it is as a poorly reactive (Phaniendra *et al*., 2015). Hydrogen peroxide can be generated the $OH^{\bullet}$ with the presence of $O_2^{\bullet-}$ and metal ion (equation 3). Moreover, this can degrade some heme proteins e.g. hemoglobin, to release iron ion, and cause of cell death and tissue injury (Lee *et al*., 2004).

$$O_2 + O_2^{\bullet-} + 2H_2O \longrightarrow H_2O_2 + O_2 \qquad \text{(equation 3)}$$

4) Singlet oxygen ($^1O_2$)

Singlet oxygen is not a free radical when compared with other reactive oxygen species, singlet oxygen is gentler and nontoxic for living tissue. Meanwhile, this singlet oxygen is involved in cholesterol oxidation. This singlet oxygen is generated in some radical reactions and activation of neutrophils and eosinophils (equation 4), as well as the enzymatic reaction of lipoxygenases, dioxygenases, and lactoperoxidase. It can be an oxidizing agent which cause damage to tissue and DNA (Kumar, 2011; Lee *et al*., 2004; Phaniendra *et al*., 2015).

$$HOCl + H_2O_2 \longrightarrow {}^1O_2 + H_2O + Cl^- \qquad \text{(equation 4)}$$

2.6.4.2 Reactive nitrogen species (RNSs)

RNS is a free radical contained a nitrogen component with generated by from the interaction of generated free radical in biological to form more insistent reactive molecules resulting in several biological damages (Patel *et al*., 1999).

1) Nitric oxide and nitric monoxide ($NO^{\bullet}$)

A free radical nitric oxide has a single unpaired electron and generated from L-arginine by the nitric oxide synthase (NOS) via electron transfer of NADPH. These species normally is not a strong reactive free radical, except in the situation the overproduction of $NO^{\bullet}$ related in ischemia-reperfusion, neurodegenerative, and chronic inflammatory disease e.g. rheumatoid arthritis or inflammatory bowel disease. The most important of $NO^{\bullet}$ reaction occurs at low

radical concentrations with the transition of metal such as iron and copper in the protein and reaction with other radical species. Besides when the presence of human blood plasma the $NO^{\bullet}$ can be diminish the ascorbic acid and uric acid which may lead to lipid peroxidation (Lee *et al*., 2004; Metodiewa and Koska, 2000; Patel *et al*., 1999; Zorov *et al*., 2005).

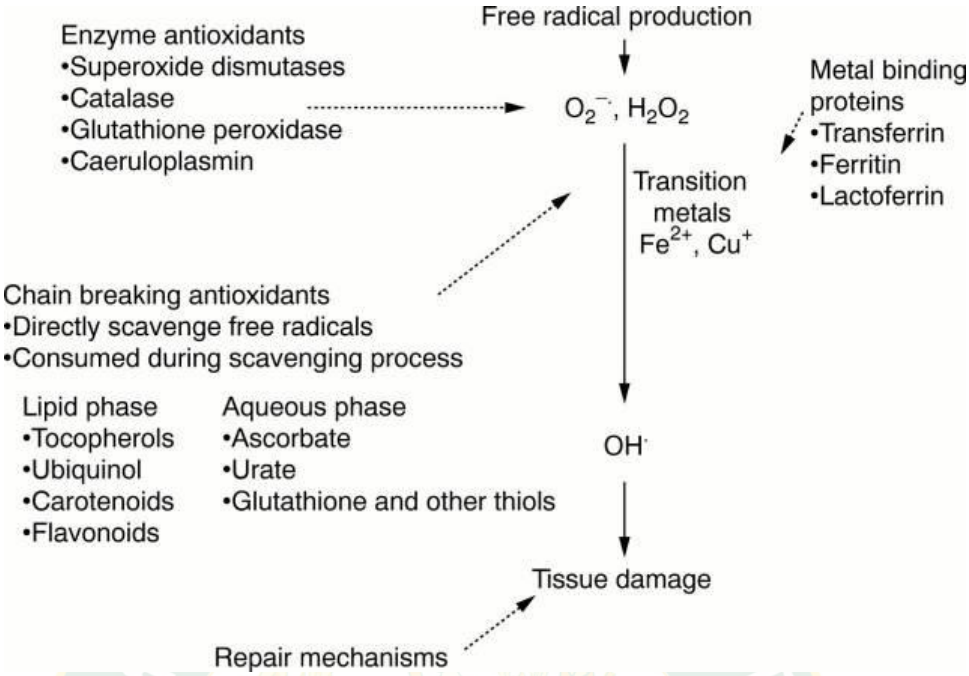
2) Peroxynitrite ($OONO^-$)

Peroxynitrite ($ONOO^-$) is a potent chemically unstable and formed by the non-enzymatic reaction of NO and superoxide anion radical (equation 5). The $ONOO^-$ is a short-life, it can be reactive with all biological molecules, and may cause of tissue injury. This unstable reactive molecule is damaging tissue formation at an inflammatory local site, it related to the various neurodegenerative disorder and kidney disease (Lee *et al*., 2004; Patel *et al*., 1999; Singh *et al*., 2009).

$$NO + O_2^{\bullet-} \longrightarrow ONOO^- \qquad \text{(equation 5)}$$

2.7 Antioxidant

Antioxidants are a stable molecule that neutralizes free radicals or donates an electron to free radical species resulting inhibit the damaging effect of oxidants (Lobo *et al*., 2010). These antioxidants inhibiting the oxidation processes that can prevent or delay the cellular damage affected by free radicals species (Sarma *et al*., 2010). The antioxidant involved in the defense free radicals by hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, an enzyme inhibitor, synergism, and metal-chelating agents, as shown in Figure 7 (Lobo *et al*., 2010; Lu *et al*., 2010).



Figures 7. Antioxidant defense against free radical species
Source: Young and Woodside (2001)

An Antioxidant can be classified into 3 main categories are 1) primary antioxidant: this group is related to preventive antioxidants, which suppressed the formation of free radicals. This preventing oxidant formation include superoxide dismutase, catalase, and glutathione peroxidase. Consequently, it may be substances dissolve in water or oil e.g. ascorbate, glutathione, uric acid, tocopherol, ubiquinol, and carotenoids, etc. 2) secondary antioxidant: it is involved to be ROS scavenger, which acting as suppress chain initiation and breaking chain propagation reaction such as glutathione reductase, glucose-6-phosphate dehydrogenase, glutathione-s-transferase, ubiquinone (coenzyme Q10). 3) tertiary antioxidant: it is acted by repairing the oxidized molecule through a source like dietary or consecutive antioxidants (Lobo *et al*., 2010; Mehta and Gowder, 2015; Noori, 2012).

Moreover, the antioxidant can be classified by their sources as following (Basu *et al*., 1999; Kumar, 2011; Sarma *et al*., 2010):

1) Natural antioxidants, the antioxidant in this group contain (1) intracellular antioxidant e.g. enzymes (such as catalase, superoxide dismutase (SOD), glutathione S-transferases (GSTs), glutathione peroxidases (GPx), glutathione reductase (GSR), thiols protein, histones, dehydrogenase (quinone), and protease and (2) extracellular antioxidants or low molecular weight antioxidants such as ascorbic acid, α-tocopherol, uric acid, bilirubin, polyphenol, and carotenoids, etc.

2) Cell membrane antioxidants i.e. beta-carotene, ubiquinone, tocopherol, ascorbate, polyphenol, flavonoids, glucosinolates, and procyanidines.

3) Protein antioxidants such as albumin, transferrin, ferritin, metallothionein, and ceruloplasmin.

4) Synthetic antioxidants, these groups are usually applied in industrial for preventing the lipid oxidation that may cause of physical spoilage i.e. propyl gallate, 2-butylated hydroxyanisole, 2-butylate hydroxyanisole, butylated hydroxytoluene, tertiary butylhydroquinone, trolox, and gallic acid.

2.7.1 Example of antioxidant agent

2.7.1.1 Ascorbic acid

Ascorbic acid or vitamin C (Figure 8) is very fertile water-soluble and found in both animal and plant. Their antioxidant mechanism is a natural antioxidant which can be together of intracellular and extracellular antioxidants (Birben *et al*., 2012). Ascorbic acid plays an important role in amino acid metabolism and acts as co-enzyme in biochemical reaction in the human body. It can donate a hydrogen atom to lipid radicals, quenching of singlet oxygen, and remove the oxygen molecule, further, it is an excellent electron donor and

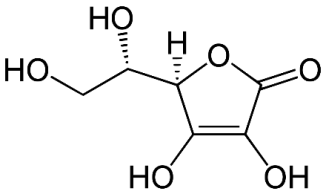
antioxidant properties as well (Lee *et al*., 2004). In addition, ascorbic acid has reported suppressing the free radical formation caused by pollutant and cigarette smoke, and promote the returning of tocopherol radical to the active form (Sarma *et al*., 2010).

2.7.1.2 Alpha-tocopherol (α-tocopherol)

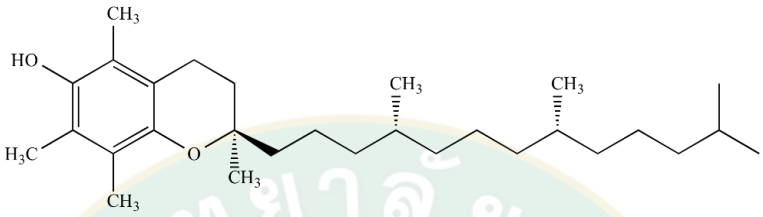
The α-tocopherol (Figure 8) is a most bioactive stereo-isomer of vitamin E, it is an effective radical scavenger at the cell membrane. The α-tocopherol is well known as a lipid-soluble substance then it acting mainly against lipid peroxidation and resulting protect the membrane from oxidative substance (Salaja et al., 2011). Moreover, the antioxidant reaction of α-tocopherol may lead produce α-tocopheroxyl radicals, but it can returning back to the active form by other antioxidants e.g. ascorbate, retinol, or ubiquinol (Lobo *et al*., 2010).

2.7.1.3 Carotenoids

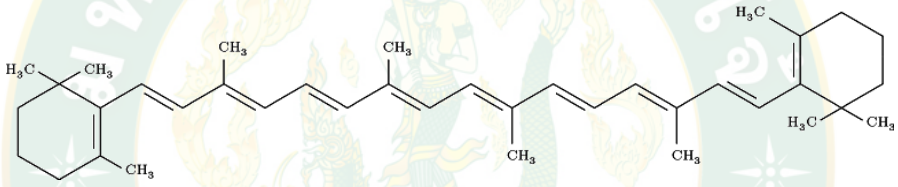
Carotenoids are a natural pigment, it is an important substance in the photosynthesis in higher plant, mosses, fern, and algae. The carotenoids, beta-carotene (Figure 8), lutein, and zeaxanthin exhibit potent antioxidant properties acting as singlet oxygen quenchers and scavenging the ROS such as $ROO^{\bullet}$, $OH^{\bullet}$, and, $O_2^{\bullet-}$ (Fiedor and Burda, 2014; Ramel *et al*., 2012; Young and Lowe, 2018).



Ascorbic acid (vitamin C)



α-tocopherol (vitamin E)



Beta-carotene

Figures 8. Structure of some radical scavenger

Source: Birben *et al*. (2012)

2.8 *In vitro* methods for testing antioxidant activity

Antioxidant activity is not accomplished using a single method. There are numerous analytical methods for evaluating antioxidants capacity of the focused sample. Contrariwise, the testing antioxidant model is many aspects and it is difficult to compare between different models. Depending upon the chemical reactions involved between free radical and antioxidant compounds can cursorily be categorized into 2 types: 1) hydrogen atom transfer reaction (HAT) and 2) electron transfer reaction (ET) (Dontha, 2016).

2.8.1 Trolox equivalent antioxidant capacity (TEAC) assay or 2, 2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$) cation decolorization assay

The ABTS radical cation decolorization assay was improved and described by Re *et al*. (1999). The model of ABTS assay desires 2, 2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) which oxidized with potassium persulphate or manganese dioxide to generated the ABTS cation radical ($ABTS^{\bullet+}$) (equation 6) giving a bluish-green color which absorbed at 734 nm (Pisoschi and Negulescu, 2012). The ABTS assay is performing by the reaction of 7 mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand for 12 h in the dark at room temperature and formed by nitrogen atom loss of an electron (Gupta, 2015). The ABTS inhibition activity is assessed the capacity of a sample (e.g. A-OH) to diminished $ABTS^{\bullet+}$ radical by obviate initial oxidation or directly react with the $ABTS^{\bullet+}$ radical cation (equation 7) (Apak *et al*., 2016).

$$ABTS + \text{oxidizing agent (e.g. } K_2S_2O_8) \longrightarrow ABTS^{\bullet+} \qquad \text{(equation 6)}$$

$$ABTS^{\bullet+} + A\text{-}OH \longrightarrow ABTS + AO^{\bullet} + H^{+} \qquad \text{(equation 7)}$$

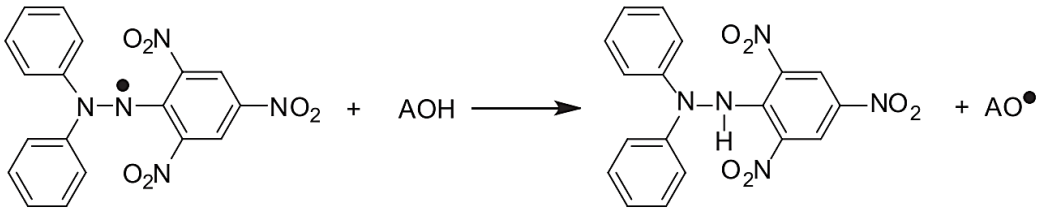
$ABTS^{\bullet+}$ is suitable for multiple substances medium to measure both the lipophilic and hydrophilic antioxidants due to it can be soluble in aqueous and organic media (Re *et al*., 1999; Wojdylo *et al*., 2007). The capacity of decolorization as a percentage of inhibition of the ABTS radical scavenging is considered with both the concentration and time and is comparative calculated with the scavenging activity of trolox (water-soluble vitamin E analogue) standard substance under the same condition (Dontha, 2016). A limitation of this model is that the $ABTS^{\bullet+}$ used in this assay is a stable radical but not found in the human body, then it represents a non-physiological radical (Alam *et al*., 2013). The advantages of this model were

reported to reduce labor time, material cost, and sample volume, as well as simplicity and reproducibility (Dontha, 2016).

2.8.2 DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay

DPPH is a one of stable radical at room temperature, which gives a deep violet color solution in methanol and has maximum absorption band at 517 nm (Alam *et al*., 2013). This method is widespread to measure antiradical or antioxidant of natural plant extract and purified phenolic compounds (Shalaby and Shanab, 2013). The reaction of DPPH was considered for determining radical quenching kinetic, which respect to the HAT mechanism. This assay based on the capacity of the stable DPPH radical reacts with a hydrogen-donating antioxidant molecule (equation 8, Figure 9), then this given to the reduced form (DPPH-H) result in decolorization with the loss of violet to pale yellow color (Apak *et al*., 2016; Dontha, 2016).

$$DPPH^{\bullet} + A\text{-}OH \longrightarrow DPPH_2 + AO^{\bullet} \qquad \text{(equation 8)}$$



Figures 9. The reaction of DPPH assay

Source: de Oliveira *et al*. (2014)

DPPH assay is a simple, easy and rapid for screening antioxidant activity with needs only a UV – vis spectrophotometer. Further, many antioxidant agents that rapidly react with peroxyl radical may react slowly or may be inert to the DPPH radical that due to steric inaccessibility (Prior *et al*., 2005). The DPPH assay is also has limited like ABTS, it represents a non-physiological radical, which not found to the biological system. Further, the assay color can be interfered by the sample that contained anthocyanins and carotenoids which also have an overlap maximum absorbance at 515 nm leading to an exaggeration of antioxidant capacity (Shalaby and Shanab, 2013).

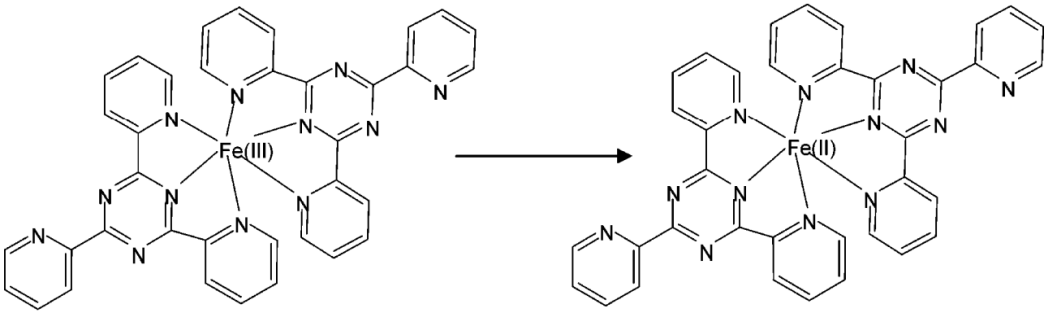
2.8.3 Ferric reducing antioxidant power (FRAP) assay

The FRAP method is a chemical reaction based on the reduction of Fe^{3+} to Fe^{2+} by the sample that contained tripyridyltriazine tridentate ligands generating a colored complex with Fe (II) (equation 9, Figure 10). The reduction results by antioxidant (A-OH) is monitored the absorbance at 593 nm (Apak *et al*., 2016). Previously, FRAP is used for measuring the antioxidant in plasma, then popularly useful to determine the antioxidant activity of numerous biological compounds from plant extract by measured the reduction of 2,4,6-tripyridyl-s-triazine (TPTZ) to colored solution (Figure 6) (Dontha, 2016).

FRAP assay is simple, rapid, inexpensive, do not require specialized equipment. FRAP reaction is performed by mixed the test sample together with FRAP reagent (10 mM of TPTZ in 40 mM of HCl, 20 mM $FeCl_3$ and 0.3 M acetate buffer at pH 3.6), and allowing the mixture to stand for 10 min at 37°C. The results are expressed as $FeSO_4$ equivalent capacity. FRAP is non-specific, in that any reaction has redox potential lower than that reaction conditions of 0.77V, that will drive the ferrous (Fe^{3+} to Fe^{2+}) ion formation. Therefore, the reduction activity is directly related to combining or total reducing power of electron donating antioxidant

substance that present in the reaction. This method unsuitable to detect slowly-reacting polyphenolic compounds and thiols such as glutathione and proteins, in fact not all antioxidant reduce Fe^{3+} to Fe^{2+} at a rate fast enough to measure within the observation time, typically several polyphenol compounds react too slowly and required longer reaction time (approximately 30 min) (Badarinath *et al*., 2010; Dontha, 2016; Gupta, 2015).

$Fe(TPTZ)_2^{3+}$ + A-OH ⟶ $Fe(TPTZ)_2^{2+}$ + $AO^{\bullet}$ + H^+ (equation 9)



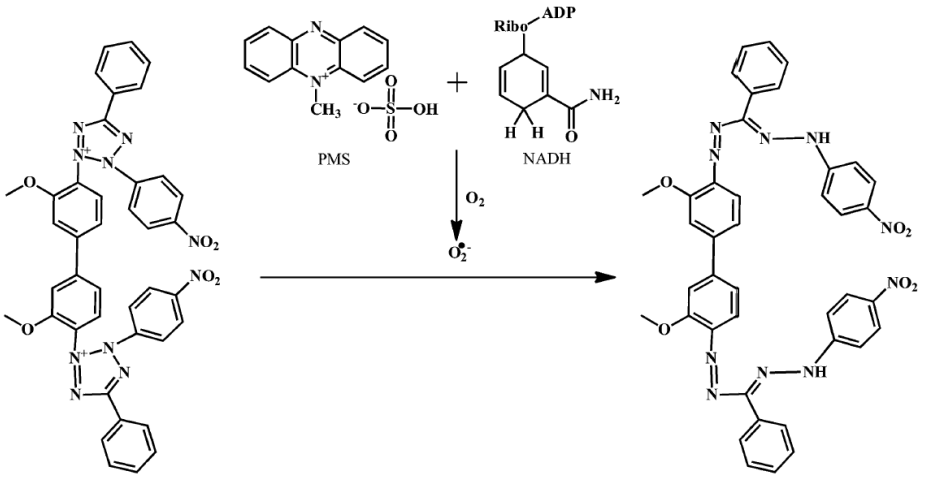
$Fe(TPTZ)_2^{3+}$ + reducing antioxidant ⟶ $Fe(TPTZ)_2^{2+}$ (intense blue color)

Figures 10. The reaction of FRAP assay
Source: Prior *et al*. (2005)

2.8.4 Superoxide radical scavenging assay

Superoxide anion is initiated from the reduction of one electron of a free oxygen molecule by the membrane-bound enzyme of nicotinamide adenine dinucleotide phosphate oxidase (Chun *et al*., 2003). Generally, superoxide anion is a weak oxidant, but the overproduction of superoxide anion stimulate to redox imbalance with harmful to many physiological functions. Eventually, it combines with other reactive species produce powerful and dangerous nitric oxide or hydroxyl

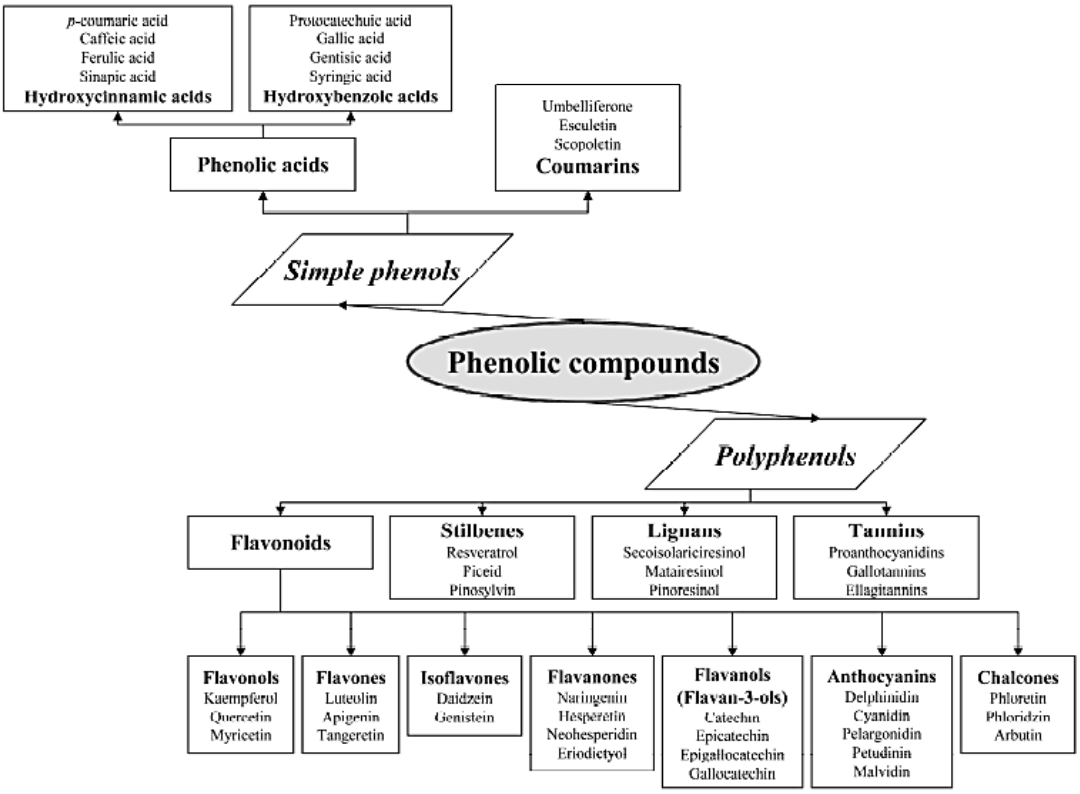
radicals with promoting to oxidative stress (Alam *et al*., 2013; Dontha, 2016; Zou *et al*., 2015).



Figures 11. The reaction of NBT reduction by superoxide anion radical
Source: Nimse and Pal (2015)

The superoxide radical scavenging capacity of the sample is determining using the method of Nishikimi *et al*. (1972). The superoxide radical is generated by the PMS (phenazine methosulphate) – NADH (nicotinamide adenine dinucleotide) system by oxidation of NADH and the reaction operate by the reduction of NBT (nitroblue tetrazolium), as shown in Figure 11. The decreasing of the absorbance at 560 nm indicating antioxidant substance in the mixture reaction diminished the superoxide radical anion. The capacity to consumption the superoxide anion is compared with the standard substances e.g. gallic acid, ascorbic acid, α-tocopherol, BHA, and curcumin under the same condition (Arulmozhi *et al*., 2007; Nimse and Pal, 2015).

2.9 Phenolic compound



Figures 12. The classification of phenolic compounds
Source: Soto *et al*. (2015)

Phenolic compounds are the one largest group of secondary metabolites in plants world, produced for the response under the distinct stress conditions e.g. infection, UV radiation, ozone, pollutant, etc. The phenolic compounds playing important role in widely biological properties especially antioxidant property which higher than vitamin C, vitamin E, and carotenoids (Dey *et al*., 2016; Tanase *et al*., 2019). The phenolic compounds synthesized through the pentose phosphate, shikimate, and phenylpropanoid pathways. The classification of phenolic groups are based on their carbon skeleton in the structure, which can be separated into the

subsequent groups: phenolic acids, flavonoids, stilbenes, lignans, tannins, and coumarins, as shown in Figure 12 (Mark *et al*., 2019).

2.9.1 Classification of phenolic compounds

In current times, approximately 8,000 different phenolic compounds structure are found in plants (Tanase *et al*., 2019). Diverse phenolic compounds can be classified by the basic skeleton structure (Kulbat, 2016; Mark *et al*., 2019; Soto *et al*., 2015) with followings:

1) Phenolic acid: it contains phenols ring with at least one carboxylic acid group, which found in the free and bound forms in the plants, and this group can be separated into 2 sub-group of hydroxybenzoic acid (C6-C1) and hydroxycinnamic acid (C6-C3), that obtained from benzoic acid and cinnamic acid, respectively. The hydroxybenzoic acid groups such as benzoic acid, salicylic acid, gallic acid, quinic acid, and shikimic acid. Hydroxycinnamic acid group contains cinnamic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid, caffeic acid, rosmaric acid, and sinapic acid.

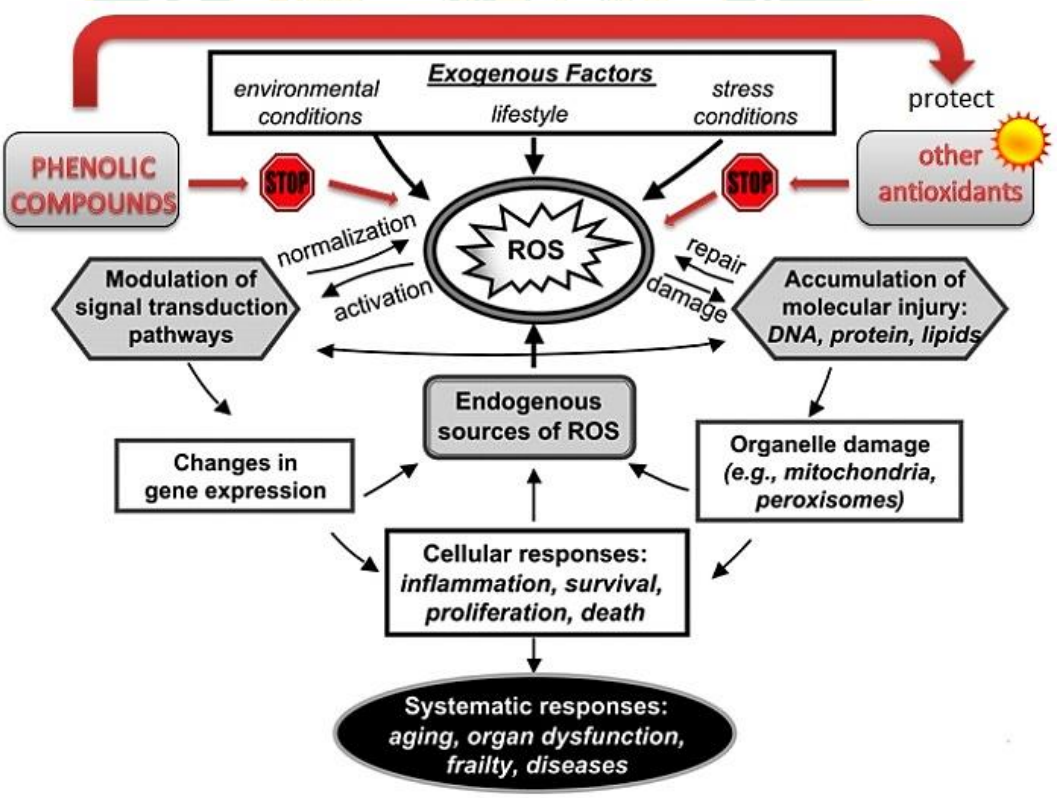
2) Coumarins: it produced from cinnamic acid and *p*-coumaric acid, the coumarins of umbelliferone and scopoletin are beneficial in the health effects.

3) Flavonoids: these are the largest group of phenols compounds in the plants' secondary metabolites. The chemical structure is based on 15 carbon atoms (C6-C3-C6) in the basic skeleton which consisted of two aromatic rings. The flavonoids synthesized through the phenylpropanoid pathway resulting in the generation of coumaroyl-CoA by phenylalanine in the shikimic acid pathway, and malonyl-CoA in the malonic acid pathway. The most important i.e. flavonols, flavones, isoflavones, flavanones, flavanols, and anthocyanins.

4) Stilbenes: it is a small group and synthesized from *p*-coumaroyl-CoA along with three molecules of malonyl-CoA. The stilbene are most in a unit of *trans*-resveratrol (3,5,4'-trihydroxy-*trans*-stilbene).

5) Lignans: this group is formed by phenylpropane of 2 units. The most well-known lignan such as secoisolariciresinol, matairesinol, and its derivative of pinoresinol, sesamin, and phyllathin.

6) Tannins: it has a molecular weight ranging from 500 to 4000 and may be separated into 2 categories are hydrolysable tannins and condensed tannins (proanthocyanin).



Figures 13. Effect of phenolic compounds on oxidative stress damage inhibition
Source: Działo *et al*. (2016)

Phenolic compounds have been reported for their several biological properties including antimicrobial activity, antivirus, anti-inflammatory, antitumor, antioxidant, and anti-aging. In antioxidant properties, phenolic compounds easily donate hydrogen atom from the hydroxyl group located at the aromatic ring in their structure against oxidation reaction of lipid or other biomolecules, as well as electron donation and metal chelating capacities (de Beer *et al*., 2002; Dey *et al*., 2016; Tanase *et al*., 2019). Then phenolic acid can be defenses the oxidation mechanism leading the neutralizing the oxidative stress reaction, as shown in Figure 13 (Kulbat, 2016). In addition, after donating a hydrogen atom to oxidation species, phenolic compounds turn into resonance-stabilized radical, then formed do not easily react in other radical reaction (Lee *et al*., 2004). These phenolic compounds have been reported for their benefit in prevention or treat several diseases such as cancers, diabetes, neurodegenerative, cardiovascular diseases, including skin aging (Mark *et al*., 2019).

Currently, the natural phenolic compounds have been widespread for the food industry or cosmetics purposes i.e. natural coloring, preservatives, antioxidant, and nutraceutical functional products, etc. such as followings:

1) Flavonoids are the largest and most several structures among phenolic compounds. In current times, it has been more than 6,000 identified, and glycosides are formed that presents in plants. These groups are most popularly studied for the pharmaceutical and cosmeceutical properties. Flavonoids exhibit the chelating the Fe^{2+} and Cu^{2+} and scavenging the $RO_2^{\bullet}$ or $HO^{\bullet}$ species. The several studies reveal the properties which can reduce the inflammation, antitumor, anti-angiogenic actions, antimicrobial, anti-allergic, protect the cardiovascular. Moreover, flavonoids have the potential to be formulated in cosmetic as anti-aging and whitening agents (Ozcan *et al*., 2014; Soto *et al*., 2015; Tanase *et al*., 2019).

2) Tannins are found in the complex with other molecule include alkaloids, polysaccharides, and proteins. Tannins are numerous in the grape (seed and skin), apple juice, strawberries, raspberries, pomegranate, walnuts, peach, olive, plum, cocoa, and chocolate (Ozcan *et al*., 2014). Tannins are water-soluble compound at 25-30^{o}C. Normally, tannins can be precipitating some molecule such as binding the proteins, pigments, basic molecules, large-molecule compounds, and metal ions. The biological properties of tannins comprising antioxidants effects, antimicrobial, anti-tumor activities, as well as inhibit HIV (Mark *et al*., 2019; Okuda and Ito, 2011).

3) Hydroxycinnamic acids are a group of phenolic acid almost found in human diets such as grains, fruits, and vegetables. In plants, phenolic acid obtained through shikimate pathway by several steps for transforming intermediate of the pentose phosphate pathway and glycolysis to important aromatic amino acid form (phenylalanine, tyrosine, and tryptophan) (Alam *et al*., 2016; Teixeira *et al*., 2013) such as following:

- *p*-Coumaric acid (4-hydroxycinnamic acid) is synthesized from tyrosine and phenylalanine. The *p*-coumaric acid is widespread in many fruits, vegetables or cereals (e.g. apple, grape, orange, tomato, and berries). A lot of studies revealed *p*-coumaric acid and its derivatives exposed the antioxidant, antimicrobial, antitumor, anti-inflammatory properties. Of note, it has low toxicity with LD_{50} value of 2850 mg/kg BW in mice (Pei *et al*., 2016; Taofiq *et al*., 2017). The cosmeceutical properties of p-coumaric acid can suppress the collagenase activity and matrix metalloproteinases (MMPs) associated with the decrease of dermal extracellular matrix leading to anti-aging properties (Seok and Boo, 2015).

- Caffeic acid (3,4-dihydroxycinnamic acid) is accumulated in fruits, vegetables, mushrooms, and herbs, which synthesized through hydroxylation of *p*-coumaric acid. Biological properties of caffeic acid and its derivatives expressed

antitumor, anti-inflammatory, antioxidant activities, and as an inventive for malathion-induced neuropeptides (Zheng *et al*., 2017). Moreover, caffeic acid demonstrated to suppress the melanin production in the murine B16 melanoma cell line (Maruyama *et al*., 2018).

- Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is distributed in several beverages (e.g. coffee, beer), fruits, vegetables, cereals, flowers, and nut. It has reported the effective for antioxidant, antitumor, anti-inflammatory, and UV absorbing activities. The ferulic acid can inhibit the tyrosinase activity by directly binding to the enzyme structure leading to anti-melanogenesis processing (Maruyama *et al*., 2018; Taofiq *et al*., 2017).

- Chlorogenic acid is the most important of hydroxycinnamic acid derivatives and synthesized by the esterification of caffeic acid and L-quinic acid. There are most abundant in some crop plants e.g. apples, pears, and coffee. The main of chlorogenic acid form in coffee contained 3-caffeoylquinic acid, 4-caffeoylquinic acid, 5-caffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid. They are effective for anti-inflammatory, inhibit α-glucosidase, anti-type II diabetes, and anti-AIDS drugs. Moreover, chlorogenic acid is effective for decreasing the ROSs and oxidative stress processes and suppressed the melanogenesis in murine B16 melanoma cell line (Li *et al*., 2014; Liang and Kitts, 2016; Taofiq *et al*., 2017).

2.10 Cosmetic dosage forms

A variety of skin care products in the current times exist in three main categories; 1) drugs: use to prevent or ameliorate diseases by modifying the structure and/or function of the body, 2) cosmetics: use to improve beautifying of sensory aspect of normal and/or non-diseased skin, and 3) cosmeceuticals: an intermediate of cosmetic products use to enhance the skin function (Epstein, 2009). In cosmetics

or pharmaceuticals, the topical dosage form is defined as a vehicle of cosmetics and active ingredients to address the problems presented for skin treatment and prevention of skin degeneration process (Draelos, 2001). The cosmetic dosage forms of the above three product groups can also be classified by their physical properties include solutions (e.g. serum), collodion, suspensions, emulsions (e.g. lotions), semisolids (e.g. foams, ointments, creams, gels), solids (e.g. loose powders, compressed powders, sticks), and spray (Government of Canada, 2013; Garg *et al*., 2015; Mishra *et al*., 2014; Surini *et al*., 2018; Ueda *et al*., 2009).

2.10.1 Creams

Creams are semisolid dosage form that contains one or more ingredients dissolved or dispersed in the suitable formulation for application to the skin. They are a combination of oil and water phases together which are divided into two types including water-in-oil (W/O) and oil-in-water (O/W) emulsions. W/O emulsion creams are good spreadability and more moisturizing as they provide an oily skin barrier which are reducing transepidermal water loss from the skin. Meanwhile, O/W emulsion creams are more comfortable and cosmetically accepted, because they rapidly readily rub into the skin, less greasy and more readily removed by water.

2.10.2 Gels

Gels (sometime called jellies) are transparent or translucent semisolid dosage form of one or more active ingredients in suitable hydrophilic or hydrophobic formulations. They are stabilized or set by a three-dimensional lattice system. Gels can separate owing as a phase system in two types include single-phase and two-phase system. Single-phase gels consist of organic macromolecules uniformly distributed throughout a liquid with no obvious boundary between the dispersed macromolecule and liquid. They are prepared by either a fusion process of the

gelling agents, humectants and preservatives. Hydrophilic gels or hydrogels are commonly consist of water, glycerol, or propylene glycol which gelled by suitable substances such as tragacanth, starch, cellulose derivatives, carboxyvinyl polymers, or magnesium aluminium silicates.

2.10.3 Lotions

Lotions are emulsion dosage form which low- to medium- viscosity topical preparation intended for application to normal skin. Typically, many characteristics of lotions are similarly with creams and applied with bare hands providing cosmetically properties to the skin.

2.10.4 Serums

Serums are solution dosage form containing the concentrated biologically active ingredients dissolve in mixtures formulations. Commonly, serums are formulated without heating that is an advantage to avoid degraded of bioactive compounds that may affect from heat. Due to gel-based dosage forms, serums are comfortable to use because they have a high content of water in a formulation which can hydrate the skin and good spreadability.

2.10.5 Powders

Powders are solids or compressed of solids dosage form in a dry, finely divided state for applied to the skin.

CHAPTER 3
MATERIALS AND METHODS

3.1 Chemicals

3.1.1 Folin-Ciocalteu's reagent (BDH Prolabo Chemicals, France)

3.1.2 Sodium carbonate (BDH Prolabo Chemicals, France)

3.1.3 ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), Sigma-Aldrich, USA)

3.1.4 Gallic acid (Sigma, Germany)

3.1.5 Kojic acid (Sigma, Germany)

3.1.6 L-DOPA (L-3,4-dihydroxyphenylalanine, Sigma-Aldrich, China)

3.1.7 Potassium persulfate (Sigma-Aldrich, USA)

3.1.8 Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich, USA)

3.1.9 Tyrosinase from mushroom (Sigma-Aldrich, USA)

3.1.10 Chlorogenic acid (Sigma-Aldrich, USA)

3.1.11 Methanol (Sigma-Aldrich, USA)

3.1.12 Acetic acid (Sigma-Aldrich, USA)

3.1.13 DPPH (2, 2-diphenyl-1-picrylhydrazyl, Sigma-Aldrich, USA)

3.1.14 Sodium phosphate monobasic (Sigma-Aldrich, USA)

3.1.15 Sodium phosphate dibasic (Sigma-Aldrich, USA)

3.1.16 Nitro blue tetrazolium (NBT, Sigma-Aldrich, UK)

3.1.17 Phenazine methosulfate (PMS, Sigma-Aldrich, Ukraine)

3.1.18 NADH (Sigma-Aldrich, USA)

3.1.19 Collagenase type I, *Clostridium histolyticum* (Calbiochem®, USA)

3.1.20 (-)-Epigallocatechin gallate (EGCG, Calbiochem®, USA)

3.1.21 N-[3-(2-Furyl)acryloyl]-Leu-Gly-Pro-Ala (FALGPA, Sigma-Aldrich, Switzerland)

3.1.22 Sodium hydroxide (NaOH, Sigma, USA)

3.1.23 Sodium lauryl sulfate (Namsiang Co., Ltd., Thailand)

3.1.24 Cosmetic ingredient; Glycerin (and) Hydrogenated lecithin (and) Hydroxypropyl methylcellulose stearoxy Ether (and) Squalane (and) Sodium methyl stearoyl taurate (Nikkomulese LH), Dimethicone (DC 350), Squalane, Cetyl alcohol, Stearyl alcohol, Disodium EDTA, Glycerin, Butylene glycol, Acrylates/C10-30 Alkyl Acrylate Crosspolymer (Carbopol ultrez 21 polymer), Allantoin, Sodium cocoyl alaninate (and) Water (Amilite® ACS-12), Cocamidopropyl betaine (Zohartaine ABC 45), Sodium PCA (and) Aqua (Ajidew® NL-50), Sodium methyl cocoyl taurate (Tuaranol WS conc), Cetyl Ethylhexanoate (CIO), Triethylhexanoin (Trifat), Butyrospermum parkii butter (Shea butter), Ceteareth-20 (Eumulgin® B2), Sorbitan stearate (Span 60), Petrolatum (Petroleum jelly), α-tocopheral, PEG 40 hydrogenated castor oil, Caprylhydroxamic Acid (and) 1,2-Hexanediol (and) Butylene Glycol (Spectrastat BHL), Methylpropanediol (and) Ethylhexyl-glycerin (and) Caprylhydro-

xamic acid (Spectrastat E) were purchased from Namsiang Co., Ltd., Thailand. All chemicals used were of cosmetic grade.

3.2 Instruments

3.2.1 Hot-air oven (Memmert Model 30-1060, Germany)

3.2.2 High-speed disintegrator machine (Siripanya Trading Co., Ltd. Thailand)

3.2.3 Centrifuge (Hettich Mikro 200R, Germany)

3.2.4 Rotary evaporator (KNF RC 900, USA)

3.2.5 Freeze dryer (Labconco Model 7750020, USA)

3.2.6 Mini spray dryer (Büchi Model B-290, Switzerland)

3.2.7 Spectrophotometer (Thermo ScientificTM Evolution 260 Bio, USA)

3.2.8 Vortex mixer (LMS Model VTX-3000L, Japan)

3.2.9 Liquid chromatography (LC) system (Agilent technologies 1100 series, Germany) - Mass detector (MS) system (Agilent technologies LC/MSD SL, USA)

3.2.10 Gas chromatography (GC) system (Agilent technologies Model 6890N, Germany) - Mass detector (MS) system (Agilent technologies Model 5973 inert, USA)

3.2.11 Microplate reader (Biochrom EZ read 400, UK)

3.2.12 96-well plate (NUNC^{TM}, Denmark)

3.2.13 Analytical balance 2 position (Ohaus NV2101/2, USA)

3.2.14 Analytical balance 4 position (Sartorius BSA224S-CW, Germany)

3.2.15 Auto pipette P20, P200, P1000 (Biohit, USA and Drawell, China)

3.2.16 Viscometer (DV-1 Qingtian, China)

3.2.16 pH meter (SI Analytics®, Germany)

3.2.17 Hot plate stirrer (Xylem D-82362, Germany)

3.2.18 Colorimeter (Colar CF-18, China)

3.2.19 High speed homogenizer (Siripanya Trading Co., Ltd. Thailand)

3.2.20 Finn chambers® (SmartPractice, USA)

3.2.21 DermaLab® Combo (Cortex Technology, Denmark)

3.3 Torch ginger materials

Torch ginger flowers and leaves were purchased from a cultivator in the Reso District of Narathiwat Province, Thailand.

3.4 Torch ginger preparation

Torch ginger flowers and leaves were rinsed several times with distilled water and cut into small pieces and subsequently air-dried (while being protected from sunlight) with a hot-air oven at 50°C until dry. Dried samples were ground into fine powder using a high-speed disintegrator machine and then stored in amber zip lock bags at room temperature for further study.

3.5 Torch ginger extraction

The powdered samples (100 g each) were extracted with 1,000 mL of distilled water at 50°C for 8 h to obtain the aqueous extracts. The samples were centrifuged at 4,000 rpm for 5 min at an ambient temperature and filtered through Whatman No. 1 filter paper to separate the pellets. Then, the aqueous samples were concentrated via evaporation under vacuum pressure. The samples were dried via freeze drying and spray drying to obtain the dried flower and leaf extracts then the extracts obtained from both drying process were compared the extraction yield, total phenolic content, and ABTS activity for selected the suitable drying method for torch ginger extracts. The yields of the extracts obtained using the following equation:

$$\text{Extraction yield (\%)} = [SE / PS] \times 100.$$

In this equation, SE is the weight of the sample extract obtained (g) and PS is the weight of the powdered sample used (g).

3.6 Determination of total phenolic content

The total phenolic contents of the extracts were measured using the Folin-Ciocalteu method with modified from Hammerschmidt and Pratt (1978). For this method, 200 µL of different concentrations of the extracts were added to a test tube, followed by 1,000 µL of 10% (v/v) Folin-Ciocalteu's reagent and 800 µL of sodium carbonate (7.5% w/v). The mixture was incubated for 60 min at an ambient temperature. Then, the absorbance was measured at 765 nm using a spectrophotometer. The total phenolic content was expressed as the gallic acid equivalent (GAE) in milligrams per gram of dried extract (mg GAE/g extract).

3.7 Phenolic compounds analysis by LC-MS

The phenolic compounds of extracts were determined using a liquid chromatography–mass spectrometry (LC-MS) system (Agilent 1100 series LC/MSD SL). The following column was used: LiChroCART (150 x 4.6 mm) Purospher STAR 120 RP-18e (5µm). Phenolic compounds were determined as described by Peñarrieta *et al*. (2007), with some modifications. The mobile phase consisted of acetonitrile (solvent A), and a 10 mM ammonium formate buffer (pH 4) with formic acid (solvent B) was used. The elution flow rate was 1.0 mL/min. The elution solvent was performed using the following difference conditions: a constant of 100% of B solvent from 0 min to 5 min; 0% to 20% of solvent A from 5 min to 10 min; a constant of 20% of solvent A from 10 min to 20 min; and 20% to 40% of solvent A from 20 min to 60 min. Results were acquired from the mass spectrometer and diode array at wavelengths of 270, 330, 350 and 370 nm. Results from the LC-MS system were compared with apigenin, catechin, eriodictyol, gallic acid, hydroquinin, isoquercetin, kaempferol, quercetin, rutin and tannic acid standard compounds to quantify the amounts of phenolic compounds.

3.8 Chlorogenic acid analysis by HPLC

Chlorogenic acid content was quantified using high-performance liquid chromatography (HPLC; Agilent Technologies 1200 series) with C18 column, 5 mm; 250 mm x 4.6 mm; detection was carried out by UV absorbance at 280 nm. The chlorogenic acid were determined as described by Erdem *et al*. (2016), with slight modification. The mobile phase consisted of absolute methanol (A) and 2% aqueous solution of acetic acid (B) was used for determine chlorogenic acid content with elution flow rate of 0.5 mL/min. The gradient of elution was performed using different conditions as follows: a constant of 12% of A in B solution for the first 9 min; a linear gradient of 12% to 15% of A in B solution from 0 min to 5 min; a linear gradient of 15% to 30% of A in B solution from 5 min to 15 min; a isocratic elution of

30% of A in B solution from 15 min to 19 min; and a linear gradient of 30% to 50% of A in B solution from 19 min to 27 min. The result from the HPLC system was compared with chlorogenic acid standard compound to quantify the amount of chlorogenic acid in torch ginger extracts.

3.9 Amino acid analysis by GC-MS

The amino acid analysis was evaluated as described by AOAC (2000), with slight modification. The gas chromatography (GC; Agilent Model 6890N, Germany) to a mass spectrometer (MS; Agilent Model 5973 inert, USA) was performed the following method of 994.12 (performic acid oxidation with acid hydrolysis-sodium metabisulfite) method and 998.15 (ion exchange chromatographic method) method with the Zebron ZB-AAA (10 m x 0.25 mm, 5 µm film thickness) capillary column (Phenomenex, USA). Results from GC-MS system were compared with amino acid standard substances to quantify the amount of amino acid compounds.

3.10 Determination of antioxidant activities

Antioxidant activities of torch ginger flower and leaves extracts were evaluated by the scavenging effects on three different radical of 2, 2'-azino-bis (3-ethylbenztohiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$), 2, 2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), and superoxide anion radical (O_2^-) assays. The antioxidant capacities of torch ginger extracts were compared with standard substances i.e. trolox and gallic acid.

3.10.1 Determination of 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity

The ABTS radical scavenging activity was determined as described by Re *et al*. (1999), with some modifications. The $ABTS^{\bullet+}$ was generated by mixing an

equal volume of the 7 mM solution of ABTS with 2.45 mM of potassium persulfate and allowing the mixture to stand for 12 h in the dark at room temperature. Before performing the assay, the $ABTS^{\bullet+}$ solution was diluted to achieve a final absorbance of 0.7 ± 0.5 at 734 nm. A total of 200 µL of different concentration of each extract was mixed with 1,800 µL of the $ABTS^{\bullet+}$ solution and allowed to stand for 6 min at an ambient temperature (with distilled water used for a control reaction). Then, the absorbance at 734 nm was measured using a spectrophotometer. Trolox was used as a reference substance. The results were expressed as trolox equivalent antioxidant capacity (TEAC) in milligrams per gram of dried extract (mg TEAC/g extract).

3.10.2 Determination of 2, 2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical scavenging activity

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging was evaluated as described by Mensor *et al*. (2001), with some modifications. In this process, 400 µL of different concentration of each extract was mixed with 2,000 µL of DPPH (0.3 mM) in methanol. After 20 min of incubation at an ambient temperature in darkness (with methanol used as the control reaction), the absorbance at 517 nm was measured using a spectrophotometer. Gallic acid was used as a reference substance. The results were expressed as gallic acid equivalent antioxidant capacity (GEAC) in milligrams per gram of dried extract (mg GEAC/g extract).

3.10.3 Determination of superoxide anion radical scavenging activity

Superoxide anion radical scavenging was measured using the nitro blue tetrazolium (NBT) reduction method described by Nishikimi *et al*. (1972), with slight modification. All solutions were prepared with 100 mM of phosphate buffer (pH 8). The reaction mixture contained 100 µL of the different concentrations of the

extracts, 1,000 µL of 156 µM NBT, 1,000 µL of 468 µM NADH and 100 µL of 60 µM phenazine methosulphate (PMS), and it was incubated at an ambient temperature for 5 min (with phosphate buffer used as a control reaction). Subsequently, the absorbance at 560 nm was measured using a spectrophotometer. Gallic acid was used as a reference substance. The results were expressed as mg GEAC/g extract.

The abilities of the extracts to scavenging radical activity were obtained using the following equation:

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

In this equation, $Abs_{control}$ is the absorbance of the control without sample extracts and Abs_{sample} is the absorbance reading with the presence of sample extracts.

3.11 Determination of enzymatic activities

3.11.1 Determination of tyrosinase inhibition activity

Tyrosinase inhibition was evaluated using the dopachrome method described by Masuda *et al*. (2005), with some modifications. Solutions of 200 U/mL of tyrosinase from mushrooms and 2.5 mM of L-DOPA (3, 4-dihydroxy-L-phenylalanine) were prepared with 20 mM of phosphate buffer (pH 6.8). Tyrosinase reactions were performed in a 96-well plate, with each well containing 20 µL of different concentrations of the extracts, 40 µL of tyrosinase solution and 140 µL of phosphate buffer. The 96-well plate was allowed to stand for 10 min, and then the reaction was started by adding 40 µL of L-DOPA solution (with phosphate buffer used as a control reaction). The 96-well plate was then incubated at an ambient temperature for 20 min, and the absorbance of tyrosinase activity was measured at 492 nm using a microplate reader, with kojic acid used as a reference substance.

Each sample was completed with a blank plate of the sample test, except for the tyrosinase solution. The percentage of tyrosinase inhibition activity was obtained using the following equation:

$$\text{Tyrosinase inhibition (\%)} = [\{(A - B) - (C - D)\} / (A - B)] \times 100.$$

In this equation, A is the absorbance of the control reaction without sample, B is the absorbance of the sample blank reaction without sample, C is the absorbance of the sample test with the presence of sample extracts, and D is the absorbance of the sample blank with the presence of sample extracts.

3.11.2 Determination of collagenase inhibition activity

The collagenase inhibition activities of the extracts were measured using the method described by (Zakiah *et al*., 2018), with slight modification. In this process, 50 µL of difference concentration of each extract, 50 µL of 50 mM of tricine buffer (pH 7.5) and 50 µL of 125 U/mL of *Clostridium histolyticum* collagenase (type IA) solution were added to a 96-well plate, which was allowed to stand for 15 min. A tricine buffer was used as the control reaction. The reaction was started using 50 µL of 0.5 mM of FALGPA (N-[3-(2-Furyl)acryloyl]-Leu-Gly-Pro-Ala), and collagenase inhibition activity was measured immediately using a microplate reader, with the absorbance determined to be 340 nm for 20 min. Epigallocatechin gallate (EGCG) was used as a reference substance. The percentage of collagenase inhibition activity was obtained using the following equation:

$$\text{Collagenase inhibition (\%)} = [1 - (O_s / O_c)] \times 100.$$

In this equation, O_s is the corrected absorbance of enzyme with the presence of sample extracts and O_c is the corrected absorbance of enzyme without sample.

3.12 Determination of the suitable ratio of torch ginger extracts for cosmetic formulations

The effective suitable ratio of torch ginger flower and leaves extracts for formulated as cosmetic products were determined on tyrosinase inhibition assay. The concentration of flower and leaves extracts of 0.5% and 1.0%, with the ratio of 1:1, 2:1, and 3:1 (leaves: flowers) were used to evaluate the tyrosinase inhibition activity.

3.13 Formulation of cosmetic products containing torch ginner extracts

The cosmetic products contained torch ginger leaf and flower extract of this study purposed to formulate torch ginger facial (TG) cream, torch ginger facial (TG) foam and body (TG) scrub containing residues fibers of torch ginger flower after the extraction process. All products in this study formulated followed the requirement of the entrepreneur that awarding a scholarship under the program of Research and Researcher for industry (RRi).

3.13.1 Torch ginger facial (TG) cream

The TG cream was formulated with the effective amount of flower and leaf extract from the topic of 3.12. The formulation ingredients of TG cream are shown in Table 3. The process of this formulation was prepared by the addition of the oil phase to the water phase, both of phase was heated up to 80°C before mixed. Stirring was continued by mixer homogenizer until the temperature was

approximate 50°C, added sodium hydroxide, and preservative respectively. Then, the stirring was also continued until the emulsion cooled at ambient temperature.

3.13.2 Torch ginger facial (TG) foam

The TG foam was also formulated with the effective amount of flower and leaf extracts as torch ginger cream (topic 3.13.1). The ingredients: disodium EDTA, Amilite® ACS-12, Zohartaine ABC 45, Ajidew® NL-50, Tauranol WS conc, Allantoin, Spectrastat BHL, torch ginger extracts, and distilled water (Table 4) were incorporated addition and the continuous agitation until all ingredients are well soluble.

Table 3. The formulations of TG cream

Phase	Trade Name	INCI Name	%
A (Oil Phase)	Nikkomulese LH	Glycerin (and) Hydrogenated lecithin (and) Hydroxypropyl methylcellulose stearoxy Ether (and) Squalane (and) Sodium methyl stearoyl taurate	4.00
	DC 350	Dimethicone	3.00
	Squalane	Squalane	5.00
	Cetyl alcohol	Cetyl alcohol	0.30
	Stearyl alcohol	Stearyl alcohol	0.30
B (Water Phase)	Na_2EDTA	Disodium EDTA	0.10
	Glycerin	Glycerin	3.00
	Butylene glycol	Butylene glycol	4.00
	Carbopol ultrez 21 polymer	Acrylates/C10-30 alkyl acrylate crosspolymer	0.25
	1Flower: 1Leaf extracts	*Etlingera elatior*	1.00
	DI water	Aqua	q.s. 100
C	NaOH (18% w/v)	Sodium hydroxide (for adjusting pH to 5.5)	0.30
D	Spectrastat BHL	Caprylhydroxamic acid (and) 1,2-hexanediol (and) Butylene glycol	2.00

Table 4. The formulations of TG foam

No.	Trade Name	INCI Name	%
1	Na_2EDTA	Disodium EDTA	0.10
2	Amilite® ACS-12	Sodium cocoyl alaninate (and) Water	8.00
3	Zohartaine ABC 45	Cocamidopropyl betaine	10.00
4	Ajidew® NL-50	Sodium PCA (and) Aqua	3.30
5	Tauranol WS conc	Sodium methyl cocoyl taurate	3.30
6	1Flower: 1Leaf extracts	*Etlingera elatior*	1.00
7	Allantoin	Allantoin	0.15
8	Spectrastat BHL	Caprylhydroxamic acid (and) 1,2-hexanediol (and) Butylene glycol	1.80
9	DI water	Aqua	q.s. 100

Table 5. The formulations of TG scrub

No.	Trade Name	INCI Name	%
1	CIO	Cetyl Ethylhexanoate	6.00
2	Squalane	Squalane	6.00
3	Trifat	Triethylhexanoin	5.00
4	Shea butter	Butyrospermum parkii butter	9.00
5	Cetyl alcohol	Cetyl alcohol	9.00
6	Stearyl alcohol	Stearyl alcohol	9.00
7	Eumulgin® B2	Ceteareth-20	2.40
8	Span® 60	Sorbitan stearate	1.20
9	Petroleum jelly	Petrolatum	24.00
10	DC 556	Dimethicone	10.00
11	α-tocopherol	α-tocopherol	0.50
12	PEG 40 hydrogenated castor oil	PEG-40 hydrogenated castor oil	2.00
13	Spectrastat E	Methylpropanediol (and) Ethylhexyl-glycerin (and) Caprylhydroxamic acid	1.00
14	Torch ginger flower fiber	*Etlingera elatior*	15.00

3.13.3 Torch ginger body (TG) scrub

Each ingredients: Cetyl ethylhexanoate, Squalane, Triethylhexanoin, Shea butter, Cetyl alcohol, Stearyl alcohol, Eumulgin® B2, Span® 60, Petroleum jelly, Dimethicone, α-tocopherol, PEG 40 hydrogenated castor oil, Spectrastat E, and dried residues fiber of torch ginger flower (Table 5) were incorporated to heated up until its melting approximately 75-80°C and continuous agitation. The mixture was then cooled down and continuous agitation before added into the product container.

3.13.4 The physical properties test of torch ginger products

The prepared products of TG cream, foam, and body scrub were evaluated immediately for their physical properties such as pH, color, viscosity, spreadability, and feel on skin.

3.13.5 Stability testing of torch ginger products

The stability of torch ginger facial cream was evaluated by centrifugation method at 4,000 rpm, 20°C for 30 min for their mechanical stress (Censi *et al*., 2018). Then, the accelerated stability of all torch ginger products was kept in a closed container under various conditions of room temperature, 4°C, and 45°C for 3 months. The further stability test of TG cream and foam were testing for the heating/cooling method (45°C, 48 h, and alteration with 4°C, 48 h for 1 cycle), which repeated for 6 cycles (Leelapornpisid *et al*., 2014). During the stability tested, the physiochemical appearance of all formulations, which include change of color or odor, pH, viscosity, phase separation and precipitation was investigated.

3.14 Clinical evaluation in volunteers

The clinical study in healthy volunteers was evaluated for skin irritation test and assessed the efficiency of torch ginger cosmetic products i.e. TG cream, TG foam, and TG scrub as well as satisfaction analysis.

3.14.1 Ethical approval

The protocol of the clinical study was obtained approving by the University of Phayao Human Ethics Committee, University of Phayao. The ethics committee gave a certificate, along with recommendation and informed consent form for the volunteers in this study, whose identification code is 3/018/61, as shown in Appendix A.

3.14.2 Location and duration time of this study

The study was started on March 2019 - May 2019 at Center of Excellence in Agricultural Innovation for Graduate Entrepreneur, Building 60 years of Faculty of Science, Maejo University.

3.14.3 Subjects of study

Twenty-four Thai volunteers, both male and female (aged 25 – 55 years) were selected using the following inclusion and exclusion criteria.

Inclusion criteria

1. Healthy skin, no skin diseases such as dermatitis.
2. No irregular skin color at a test site.

3. No past or present history of any skin irritation from plant extract, torch ginger extract or cosmetic ingredients, etc.

4. No any wound, blemish, and any skin diseases.

5. Subjects agree to sign an informed consent form.

6. Comfortable involved in this study.

Exclusion criteria

1. Subjects who were participating in any other clinical study.

2. Subject who had bruised skin or abnormal skin.

3. Subject who using, receiving or taking any preparation such as antihistamine drug or any other drugs.

Discontinuation criteria

1. Have skin irritation.

2. Subjects who want to quit from the experiment for any reason.

3. Subjects who could not practice following instruction criteria of the study.

Termination of study criteria

1. Subject had intensely side effect for test products.

2. Subject had an intensely irritating effect in ratio of 1: 3.

3.14.4 Skin irritation testing

Before participating in the study, each volunteer was received the information protocol that contained term and conditions of the clinical testing (Appendix B), and signed an informed consent form (Appendix C). The skin irritation

test was done using modified Draize model described by Bashir and Maibach (2009) using Finn chambers®. Skin irritation was performed on the upper outer arm of volunteers, with each chamber was saturated by TG cream, TG foam, and TG scrub, along with 1% w/v of sodium lauryl sulfate (used as a positive reaction) and deionized water (as a negative reaction), and it was covered for 48 hours. Subsequently, observed and scored the erythema and edema at 1 h, 24 h and 7 day after removed the patch using Draize scoring system, as shown in Table 6. Finally, the primary dermal irritation index (PDII) of each test substances were calculated by the sum of the average of erythema and edema formation score at the time of 1, 24 and 48 h using Draize scoring system (Table 7).

Table 6. Draize-FHSA Scoring System

Topics	Score
Erythema formation	
No erythema	0
Very slightly erythema	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema to eschar formation	4
Edema formation	
No edema	0
Very slightly edema	1
Slightly edema – edges of area well defined	2
Moderate edema (raised > 1 mm)	3
Severe edema (raised > 1 mm and extending beyond the area of exposure	4

Source: Bashir and Maibach (2009)

Table 7. Classification system of skin irritation

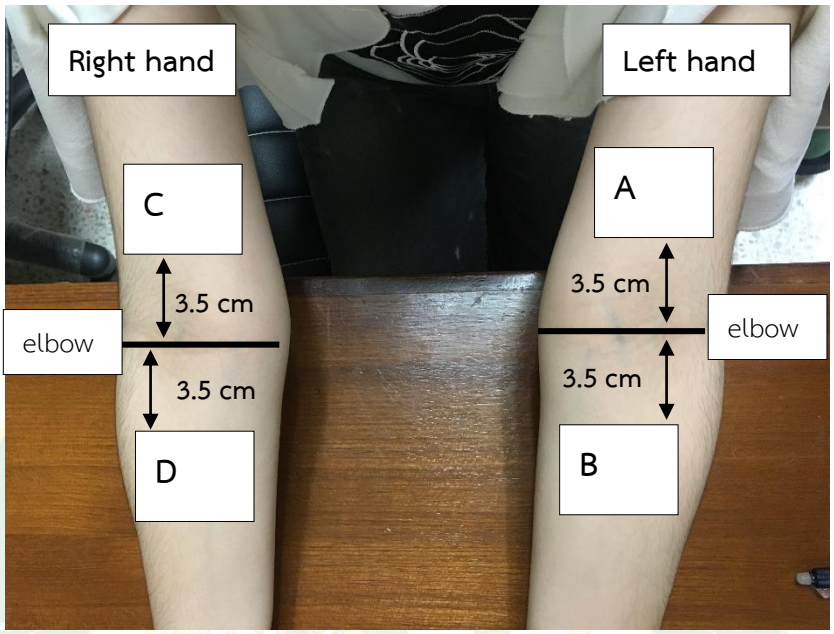
Primary dermal irritation index (PDII)	Classification of skin irritation
< 0.5	non-irritating
0.5 – 2.0	slightly irritating
2.1 - 5.0	moderately irritating
> 5.0	severely irritating

3.14.5 Skin whitening testing in human volunteers

Skin improving test of torch ginger cosmetic products were performed with twenty-four healthy volunteers. They were prohibited to apply any cosmetic products before 3 days to start of the study. Each volunteer was instructed to apply each torch ginger cosmetic products on each site of their forearms. For the first month, TG cream (A) and TG scrub (B) were tested on their upper inner arm and forearm, respectively. Next, the second month was tested for torch TG foam (C) at the right-hand on their upper inner arm. An untreated area at the right-hand was used as a control (Figure 14). Each volunteer was tested for reducing melanin content by applied twice a day, morning and evening on each skin site of each product for 4 weeks, except TG scrub was used approximately 4 times per week. Before the study, the volunteers were rested in suitable room at 20^{o}C and 50% relative humidity (RH) for 15 min (Jaros *et al*., 2019). The study had been conducted on day 0 for the initial value, 1st, 2nd, 3rd and 4th weeks. The melanin content and CIE-L* value were measured with using the skin color probe of DermaLab® Combo. The volunteers were asked to fill out the questionnaire for their satisfaction after using each products by each individual on day 30 (Appendix D). The ability to reduce melanin pigment was obtained using the following equation:

$$\text{Reduction activity (\%)} = [(M_{d0}-M_{dm}) / (M_{d0})] \times 100.$$

In this equation, M_{d0} is the melanin content of the initial day and M_{dm} is the melanin content of the measuring day.

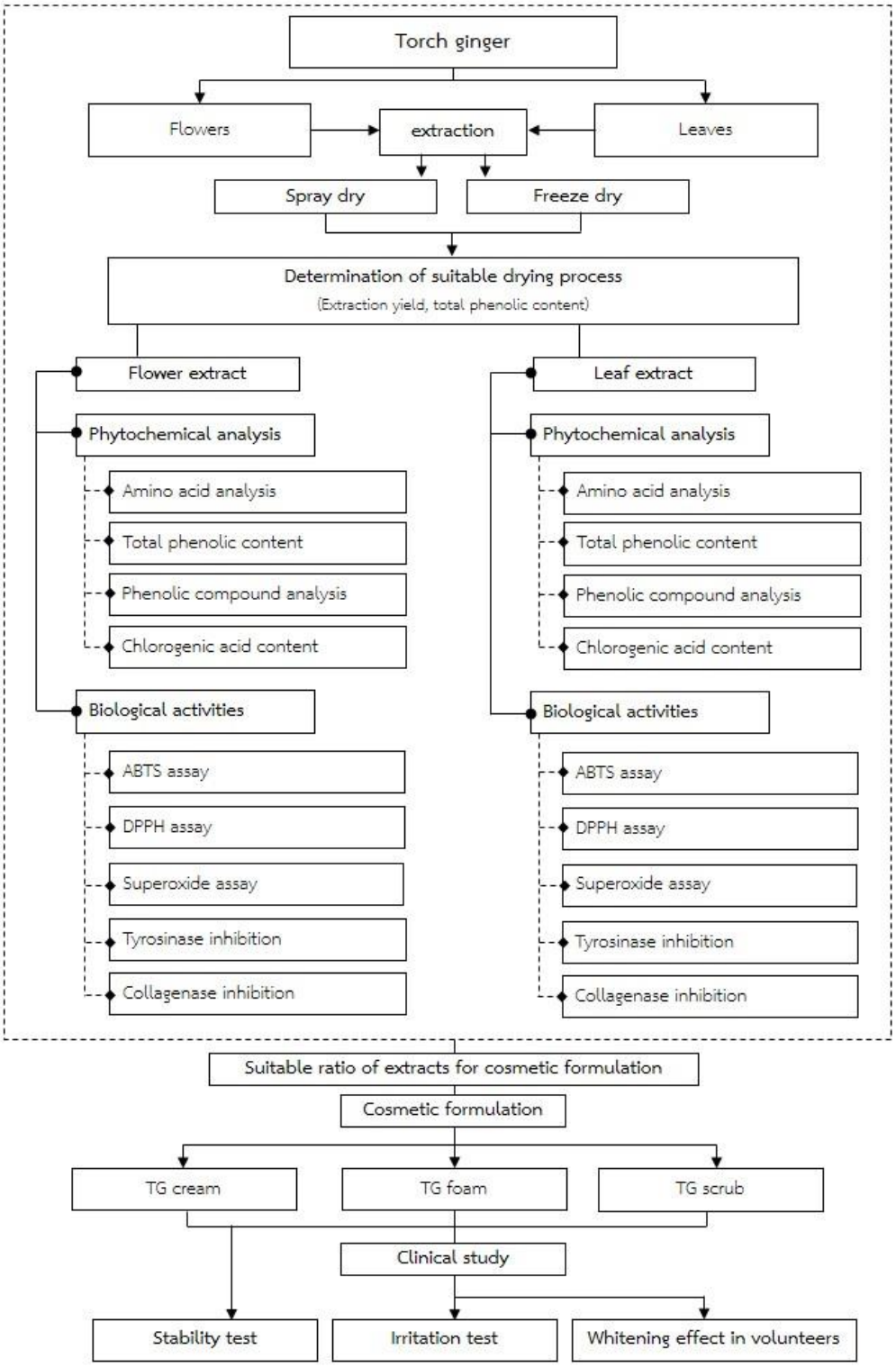


Figures 14. Profile of test site of TG products;
(A) TG cream, (B) TG scrub, (C) TG foam, and (D) untreated area 1, 2

3.15 Statistical Analysis

Data are expressed as mean ± S.D. (mean ± standard deviation). The data were analyzed by the use of one-way analysis of variance (ANOVA) with Tukey's HSD test. Exceptions of the clinical study, data are expressed as mean ± S.E. (mean ± standard error) and the repeated measures ANOVA with pairwise comparisons by the Bonferroni method was used to analyze the skin whitening effect in volunteers. Statistical analysis was processed using the Statistical Package for the Social Sciences (SPSS), version 17.0 for windows. All statistical significance was determined to be $p < 0.05$.

3.16 Research plans



CHAPTER 4
RESULTS AND DISCUSSION

4.1 Extraction of torch ginger

The yields of aqueous extracts from the different drying method, the freeze dried both flower and leaf extracts exhibited the yield significantly higher than that of its spray dried. The highest yields from the freeze dried of both flower and leaf extracts were approximately 20%, as shown in Table 8. Ghasemzadeh *et al*. (2015) reported that the aqueous extract of torch ginger presented the total phenolic, total flavonoid and total tannin content significantly higher than that ethanolic extract. Furthermore, aqueous extraction has been recommended for the extraction of antioxidant compounds from natural plant (Tiwari *et al*., 2011). Comparative the superoxide radical scavenging activity, the aqueous extract also notice the highest activity compared to methanolic extract (Altemimi *et al*., 2017). From the previous reports, where it is found that the aqueous extraction suitable for produce the bioactive compounds such as phenolic and antioxidant compounds from torch ginger. Furthermore, the yields of fresh torch ginger extracted by methanol, ethyl acetate and n-hexane to be 2.36%, 0.54% and 0.21%, respectively (Maimulyanti and Prihadi, 2015).

Table 8. The yield of torch ginger extracts

Part	Spray dry (% w/w)	Freeze dry (% w/w)
Flowers	15.92 ± 1.63 [a]	23.90 ± 4.09 [b]
Leaves	12.02 ± 2.48 [a]	24.30 ± 1.98 [b]

Values are expressed as mean ± standard deviation (n = 3). For groups in the same extract, not sharing the same letter indicates significantly different at $p < 0.05$.

Additionally, from the results the drying method by freeze dryer presented the highest values include yields of extraction and total phenolic content compared to the extracts from spray dryer (Table 8 and 9). Hence, torch ginger flower and leaf extracts obtained from freeze drying method were selected for further analysis.

4.2 Total phenolic content

Previous research tends to focus on the phenolic compounds of herbal extracts for their biological activity. Therefore, this study determined the total phenolic content of torch ginger, which is shown in Table 9. The results indicated both total phenolic content of flower and leaf extracts from freeze dried were appropriately for produced total phenolic content than spray drying method. Further, the total phenolic content of leaf extracts in comparison to the flower extracts as approximately 6 times greater, while rhizomes of torch ginger were 326 ± 76 mg GAE/100 g in fresh weight (Chan *et al*., 2008).

Table 9. Total phenolic content of torch ginger extracts

Part	Spray dry (mg GAE/g extract)	Freeze dry (mg GAE/g extract)
Flowers	35.97 ± 2.02 [a]	38.68 ± 0.45 [a]
Leaves	205.68 ± 1.95 [a]	246.52 ± 0.26 [b]

Values are expressed as mean ± standard deviation (n = 3). For groups in the same extract, not sharing the same letter indicates significantly different at $p < 0.05$.

Generally, the phenolic compounds of the plant exhibit significant potential for scavenging free radicals compared to vitamin C, E and carotenoids, and the compound scavenges free radicals by trapping them, along with chelate metals, and donating hydrogen to the free radicals formed through the oxidation reaction

(Brewer, 2011; Soto *et al*., 2015). Consequently, previous reports indicate that phenolic compounds are beneficial in antimicrobial, anti-inflammatory and anti-aging effects, and they can be absorbed through the skin barrier (Soto *et al*., 2015).

4.3 Phenolic compound analysis by LC-MS

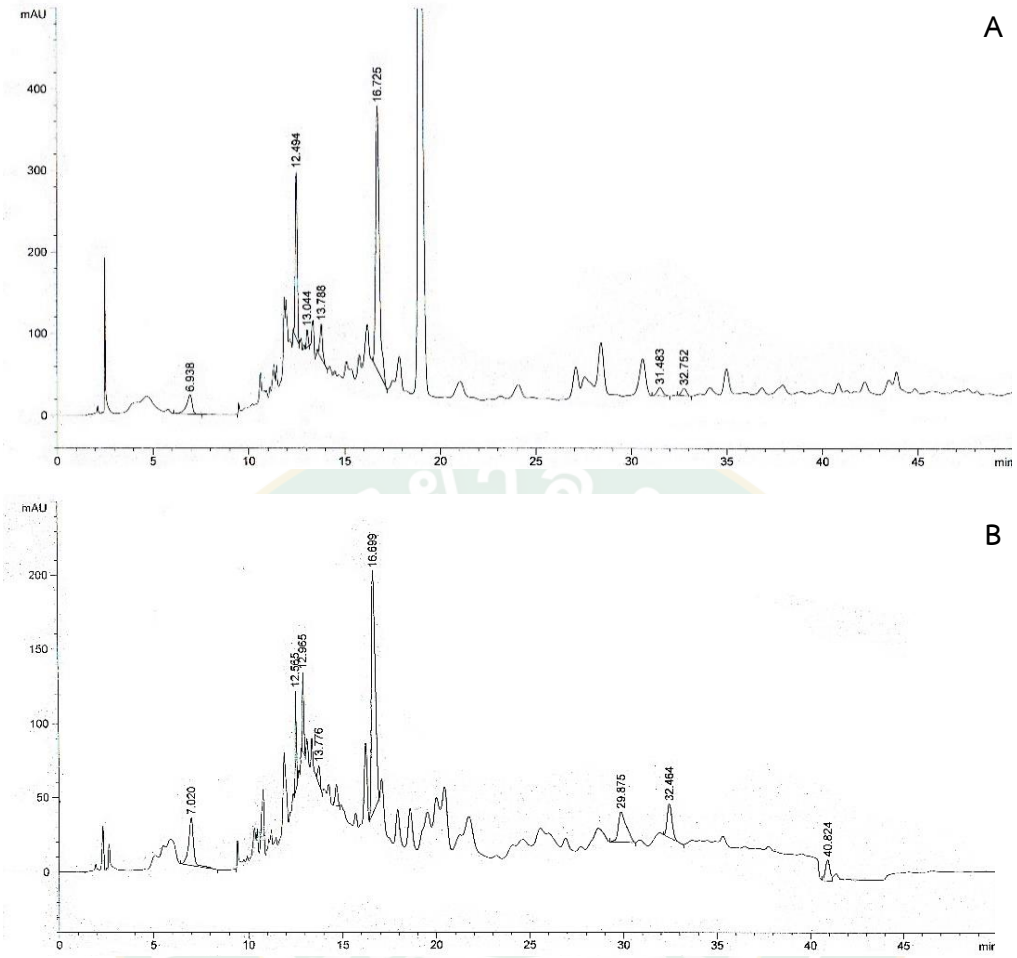
Phenolic compounds are bioactive plant substances that are widely studied as sources of natural antioxidant compounds useful as natural additives in the cosmetic and food industries (Soto *et al*., 2015). Because of this importance, this study analyses 10 phenolic compounds from flower and leaf extracts of torch ginger with a focus on apigenin, catechin, eriodictyol, gallic acid, hydroquinin, isoquercetin, kaempferol, quercetin, rutin and tannic acid. The phenolic acid content of both extracts is shown in Table 10, with that of the leaf extract exhibiting higher content than the flower extract. The leaf extract showed high contents of isoquercetin, catechin, gallic acid and tannic acid, while the flower extract exhibited high contents of isoquercetin, catechin and gallic acid. Chromatograms of the extracts are shown in Figure 15 A-B.

Table 10. Phenolic content of torch ginger extracts

Phenolic	Flowers	Leaves	Phenolic	Flowers	Leaves
Apigenin	ND	4.72	Isoquercetin	55.36	69.37
Catechin	40.69	65.75	Kaempferol	ND	ND
Eriodictyol	1.78	3.39	Quercetin	12.64	31.24
Gallic acid	35.47	50.01	Rutin	11.66	24.16
Hydroquinin	ND	ND	Tannic acid	15.17	49.10

Each phenolic compound expressed in mg/100 g dried extract. ND: non-detectable

Previous studies have reported gallic acid exhibiting significant antioxidant activity, along with oxidative stress damage via ROSs and suppressed cellular melanin synthesis in B16F10 cells through the inhibition of tyrosinase activity (Kashif *et al*., 2017; Su *et al*., 2013). Catechin could be considered a metal chelator and may bind with the Zn^{2+} ion inside the collagenase structure and replace the binding of the substrate, which is also involved in inhibiting the activities of tyrosinase and elastase enzyme (Hong *et al*., 2014; Kim *et al*., 2004). Eriodictyol is also well-known for its antioxidant, anti-inflammatory activities (Rossato *et al*., 2011), as well as its anti-tyrosinase activity, with an IC_{50} value of approximately 150 μM (Zhang *et al*., 2016). Choi and Shin (2016), report that quercetin is a potent tyrosinase inhibitor, in addition to inhibiting melanogenesis, which is shown by many studies through a cellular test of B16F10/B16F1 melanoma cells, human melanoma of the vagina (HMVII) cells and normal human epidermal melanocytes (NHEM). It has also been reported as effective in a 20-50 μM concentration of quercetin. Furthermore, quercetin has anti-inflammatory benefits, as well as antioxidant and anti-allergen properties (Działo *et al*., 2016; Li *et al*., 2016). Meanwhile, apigenin has been reported to be a collagenase and hyaluronidase inhibitor, in addition to possessing anti-aging and anti-acne bacteria (Brand-Garnys *et al*., 2007; Działo *et al*., 2016). Diverse phenolic compounds, such as tannic acid, rutin and isoquercetin, are considered to possess antioxidant properties (Kim and Lim, 2017; Krishnamoorthy *et al*., 2012). The present study showed that the phenolic compound contained in torch ginger flowers and leaves could be included as an ingredient in skin care products that provide a variety of properties used to promote healthy skin, especially for improving skin appearance in terms of wrinkles and pigmentation.

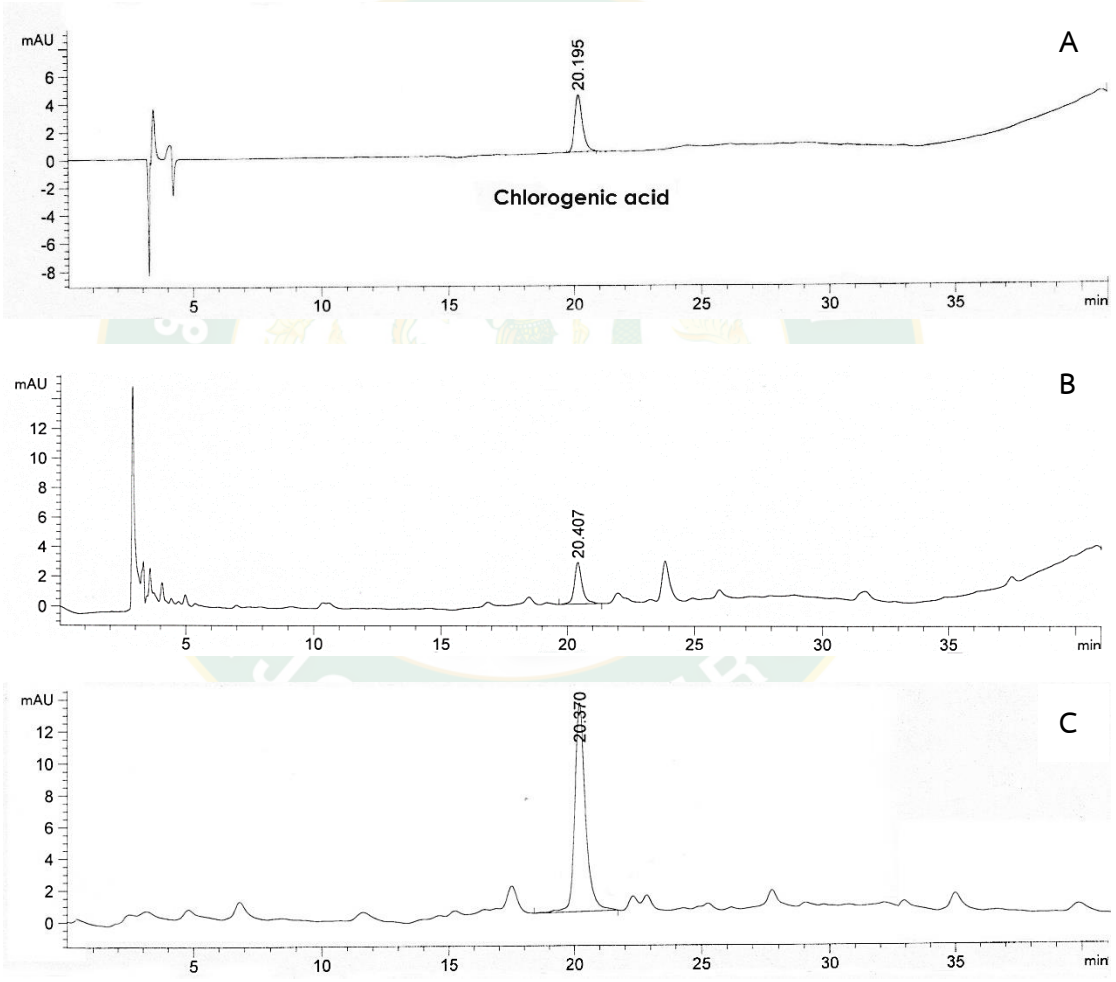


Figures 15. LC-MS chromatogram of torch ginger extracts;
(A) flower and (B) leaf extracts showing the peaks of gallic acid (RT: 6.94 and 7.02 min), catechin (RT: 12.50 and 12.57 min), tannic acid (RT: 13.04 and 12.97 min), rutin (RT: 13.79 and 13.78 min), isoquercetin (RT: 16.73 and 16.70 min), eriodictyol (RT: 31.48 and 29.88 min), quercetin (RT: 32.75 and 32.46 min) and apigenin (RT: 40.82 min)

4.4 Chlorogenic acid analysis by HPLC

Chlorogenic acid (or 3-caffeoylquinic acid: 3CQA) is hydroxycinnamic acid derivative obtained through esterification of caffeic acid and L-quinic acid, the other

isomers of them include 4- CQA or cryptochlorogenic acid and 5CQA or neochlorogenic acid (Naveed *et al*., 2018; Taofiq *et al*., 2017). Chlorogenic acid play an important substance involving in anti-inflammatory, antioxidant and antityrosinase (Li *et al*., 2014; Yun *et al*., 2012), and it found in natural dietary fruits and vegetable such as peach, prune, potato, green coffee bean and also in leaves of torch ginger. (Chan *et al*., 2009; Maalik *et al*., 2016).



Figures 16. HPLC chromatogram of chlorogenic acid;
(A) chlorogenic acid standard , (B) torch ginger flower, and (C) leaf extracts

The results exhibited the chlorogenic acid of flower and leaf extracts from torch ginger were found to be 5.65 and 27.58 mg/kg extract, respectively. The HPLC chromatogram presented the retention time of chlorogenic acid at 20.20 min. The HPLC chromatograms of the extracts and chlorogenic acid standard are shown in Figure 16 A-C. Further, Li *et al*. (2014) reported that after 48 h chlorogenic acid suppress the melanogenesis in B16 melanoma cell with a decrease of melanin pigment level. Therefore, the chlorogenic acid that contains in torch ginger extracts has the potential to be antioxidant and antityrosinase substances for suppressing the melanogenesis process.

4.5 Amino acid analysis by GC-MS

Amino acids have been shown to play an important role as antioxidants and as cosmetic ingredients performing skin improvement functions (Adhikari *et al*., 2018; Hwang and Winkler-Moser, 2017). Therefore, both the flower and leaf extracts were analyzed for amino acid contents, as shown in Table 11. The flower extract exhibited overall higher amino acid contents than the leaf extract, with the flower extract exhibiting the highest values for lysine, glutamic acid and aspartic acid, in that order. Of the 19 amino acids present in torch ginger extracts, cysteine, glycine, histidine, leucine, lysine, proline and tyrosine have been reported to have strong benefits as cosmetic properties, with cysteine, glycine and histidine exhibiting anti-inflammatory properties (Hasegawa *et al*., 2012). Proline is involved in correcting wrinkles by enhancing the elasticity of the stratum corneum. Further, concerning collagen formation, the combination of proline and branched-chain amino acids has benefits in recovering the biosynthesis of collagen after UV exposure, while the lysine and leucine amino acids are significantly involved in the formation of collagen (Collier *et al*., 2016; Kalamajski and Oldberg, 2010; Liu *et al*., 2017; Murakami *et al*., 2012). The results of this study suggest that several amino acids present in torch ginger exhibit cosmetic properties used for restoring and resolving skin aging problems.

Table 11. Amino acid contents of torch ginger extracts

Amino acid	Torch ginger extracts (mg/100 g extract)	
	Flowers	Leaves
Alanine	261	74.35
Aspartic acid	1177	94.67
Cysteine	35	24.65
Glutamic acid	1250	202.82
Glycine	176	41.16
Histidine	437	19.36
Hydroxylysine	<5.00	<5.00
Hydroxyproline	29	7.79
Isoleucine	581	83.64
Leucine	838	112.74
Lysine	2891	138.50
Methionine	<5.00	<5.00
Phenylalanine	685	141.67
Proline	216	28.02
Serine	394	122.65
Threonine	146	12.05
Tryptophan	60	9.99
Tyrosine	372	26.08
Valine	523	57.41

4.6 Determination of antioxidant activity

4.6.1 Determination of 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical scavenging activity

The antioxidant activity of extracts was examined using an ABTS assay based on direct reduction of ABTS radical via electron transfers, which measured the decay of absorbance at 734 nm (Cerretani and Bendini, 2010). The results were

expressed as trolox-equivalent antioxidant capacity and are shown in Table 9. In this study, both extracts exhibited increases in antioxidant activity as extract concentration increased. The leaf extract exhibited the highest ABTS radical scavenging capacity activity with approximately 24 times greater activity than that of the flower extract. Of note, the ABTS scavenging capacity activity of the leaf extract closely resembled that of trolox, the reference substance (Figure 17A, Table 12). Further, flower and leaf extracts showed IC_{50} values of 0.69 ± 0.01 and 0.04 ± 0.00 mg/mL, respectively. This finding indicates that both flower and leaf extracts of torch ginger are potent for radical scavenger activities, especially the leaf extract, when compared with trolox.

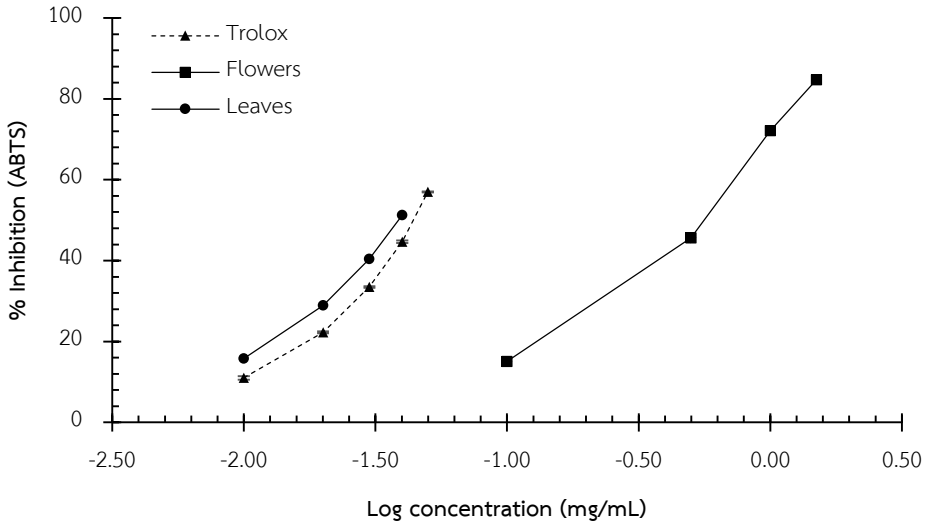
4.6.2 Determination of 2, 2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical scavenging activity

The DPPH radical scavenging assay is based on the extract acting as a hydrogen atom donor in the reduction of DPPH radical scavenging (Szabo *et al*., 2007). The results indicated that the radical scavenging capacity is dependent on the distributed dose of torch ginger flower and leaf extracts, with the amounts of activity shown in Figure 17B. In this assay, the leaf extract demonstrated higher antioxidant capacity than the flower extract, as well as exhibiting higher ABTS radical scavenging assay results (Table 12). Further, flower and leaf extracts exhibited IC_{50} values of 0.44 ± 0.00 and 0.09 ± 0.00 mg/mL, respectively. In a previous study of torch ginger flower, Lachumy *et al*. (2010) determined, using this assay, the IC_{50} value of methanolic extract to be 9.14 mg/mL, with an ethanolic extract exhibiting the potential range of 0.04-0.14 mg/mL (Ghasemzadeh *et al*., 2015). The aqueous extract of torch ginger flower in the present study could be considered to possess moderate capacity when compared with methanolic and ethanolic extracts, whereas the aqueous leaf extract exhibited higher capacity than the solvent extracts.

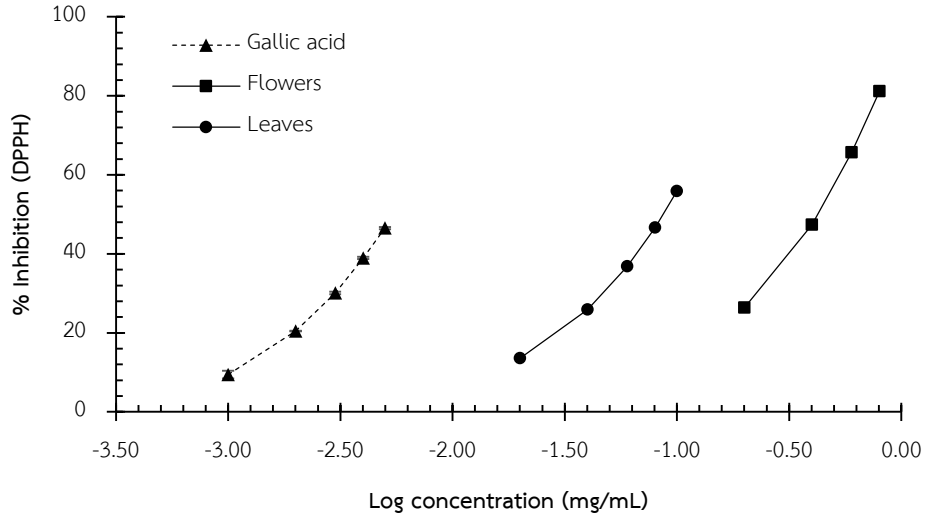
4.6.3 Determination of superoxide anion radical scavenging activity

The superoxide anion radical is well-known as an initial radical that plays an important role in acutely damaging cells and tissues by forming other ROSs, such as singlet oxygen and hydroxyl radicals (Sasikumar and Kalaisezhiyen, 2014). As shown in Figure 17C, the leaf extract exhibited higher superoxide anion scavenging capacity than the flower extract, in addition to exhibiting higher capacity in the ABTS and DPPH methods. Further, the leaf extract exhibited a very different IC_{50} value than that of the flower extract at a rate of approximately 7 times greater, as shown in Table 12. Skin aging processes and dermatological conditions are most commonly the result of oxidative stress from many endogenous and exogenous factors, such as metabolism, pollution or exposure to UV radiation (Gianeti and Maia Campos, 2014; Stojiljković *et al*., 2014). The antioxidant has been reported to be involved in preventing cellular damage from harmful free radicals by inhibiting free radical formation, interrupting the autoxidation chain reaction and protecting the cellular antioxidant defense mechanisms that take place in the oxidation process, which results in slowing the skin aging process and reducing dermatological conditions (Costa and Santos, 2017; Sarma *et al*., 2010; Soto *et al*., 2015).

Moreover, cosmetic products containing antioxidants from natural sources have been increasing in popularity over the past few decades and their popularity is expected to grow because these products may exhibit less side effects than synthetic compounds while reducing oxidative stress (Costa and Santos, 2017). Therefore, torch ginger flower and leaf extracts, as potent antioxidants, are demonstrated to be effective as cosmetic ingredients to prevent the harms of cellular damage and skin aging and to protect the skin from free radicals.



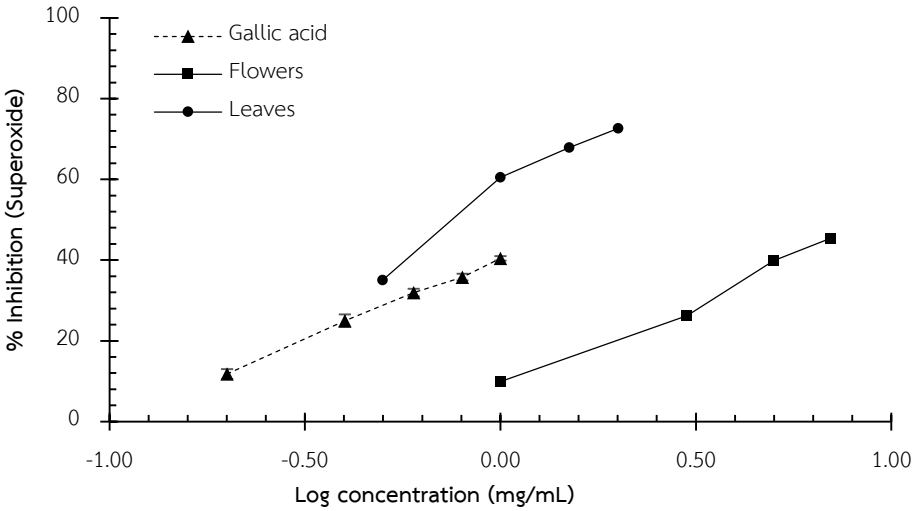
(A)



(B)

Figures 17. Antioxidant activity of torch ginger extracts;

(A) ABTS, (B) DPPH, and (C) superoxide radical scavenging activities of torch ginger extracts



(C)

Figure 17. Antioxidant activity of torch ginger extracts (Cont.);
(A) ABTS, (B) DPPH, and (C) superoxide radical scavenging activities of torch ginger extracts

4.7 Determination of enzymatic activities

4.7.1 Determination of tyrosinase inhibition activity

Tyrosinase is a crucial rate-limiting enzyme in direct melanin synthesis. However, overproduction of melanin pigment may cause hyperpigmentation and the production of a skin melanoma. Tyrosinase is involved in catalysing the hydroxylation of L-tyrosine to L-DOPA (3, 4-Dihydroxy-L-Phenylalanine) and the oxidation of L-DOPA to DOPA quinone in the biosynthesis pathway of melanin (Nesterov *et al*., 2008). While melanin pigmentation is normally responsible for preventing skin damage caused by ROSs or UV radiation exposure, overproduction and over distribution of melanin pigment is a primary cause of melasma, age spots, skin darkening and other issues (Pintus *et al*., 2015). Therefore, cosmetic products containing tyrosinase inhibitors are becoming more common for their skin-whitening properties.

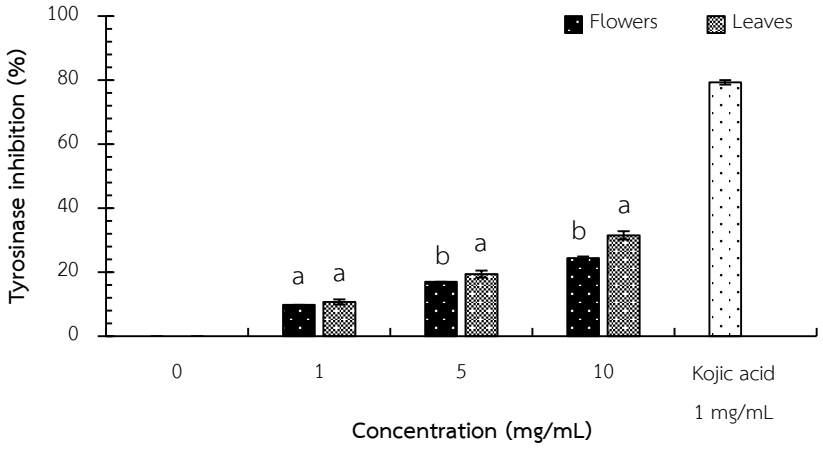
Table 12. The antioxidant capacities of torch ginger extract

Part	ABTS assay		DPPH assay		Superoxide assay	
	mg TEAC /g extract	IC_{50} (mg/mL)	mg GEAC /g extract	IC_{50} (mg/mL)	mg GEAC /g extract	IC_{50} (mg/mL)
Flowers	43.41 ± 0.56	0.69 ± 0.01 [a]	9.85 ± 0.08	0.44 ± 0.00 [a]	219.65 ± 4.38	6.39 ± 0.13 [a]
Leaves	1030.74 ± 10.82	0.04 ± 0.00 [b]	56.86 ± 0.65	0.09 ± 0.00 [b]	314.12 ± 12.79	0.88 ± 0.03 [c]
Trolox	-	0.04 ± 0.00 [b]	-	-	-	-
Gallic acid	-	-	-	0.01 ± 0.00 [c]	-	1.37 ± 0.05 [b]

Values are expressed as means ± standard deviation (n = 3). For IC_{50} values in the same column, not sharing the same letter indicates significantly different at $p < 0.05$.

4.7.1 Determination of tyrosinase inhibition activity (Cont.)

In this study, mushroom tyrosinase was used to determine the role of torch ginger in the process of melanogenesis. Different concentrations of flower and leaf extracts (consisting of 1, 5 and 10 mg/mL) and kojic acid (1 mg/mL) were incubated with the tyrosinase solution, and the reactions were stopped by adding L-DOPA. The results revealed that all concentrations of flower and leaf extracts inhibited tyrosinase activity. Furthermore, leaf and flower extracts at 10 mg/mL concentrations exhibited tyrosinase inhibition activity at 31.48 ± 1.28% and 24.37 ± 0.52%, respectively. Kojic acid demonstrated higher inhibition activity than both flower and leaf extracts. The flower and leaf extracts presented IC_{50} values of 25.77 ± 0.88 and 18.08 ± 0.74 mg/mL, respectively. These results indicate that both extracts of torch ginger significantly inhibit tyrosinase activity in a dose-dependent manner (Figure 18). According to these findings, the properties of torch ginger are capable of suppressing tyrosinase activity, therefore acting as whitening ingredients in cosmetic uses.



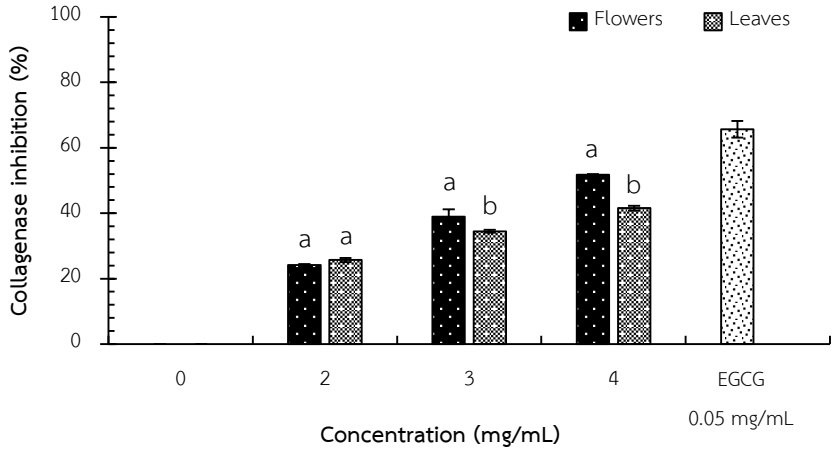
Figures 18. Effect of torch ginger extracts on tyrosinase inhibition activity

Values are expressed as means ± standard deviation (n = 3). For groups in the same concentration, not sharing the same letter indicates significantly different at $p < 0.05$.

4.7.2 Determination of collagenase inhibition activity

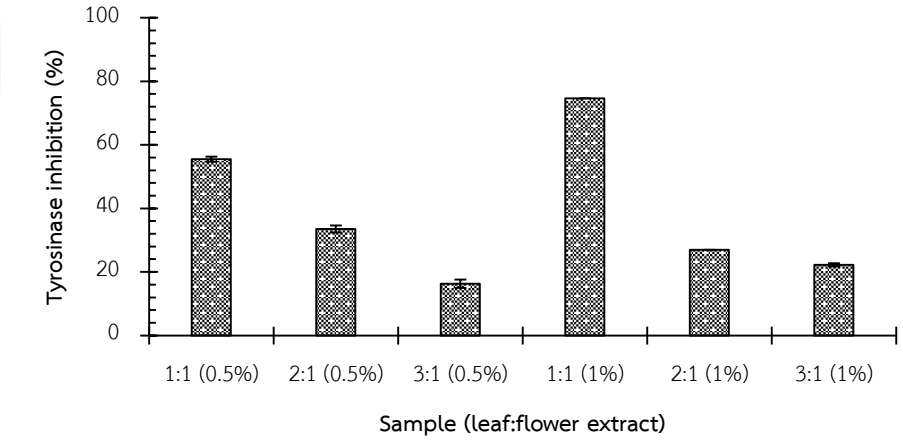
Collagenase is a proteolytic enzyme belonging to the MMPs group and is responsible for breaking down collagen (Apraj and Pandita, 2016). Thus, inhibiting collagenase activity delays the skin aging process that produces wrinkles, leading to the appearance of youthful, healthy and glowing skin (Mukherjee *et al*., 2011; Ndlova *et al*., 2013). Different concentrations of torch ginger flower and leaf extracts (consisting of 2, 3 and 4 mg/mL) were used, along with the reference substance, EGCG (0.05 mg/mL). Collagenase from *C. histolyticum* was used to measure the anti-aging properties of torch ginger. The results demonstrate that both flower and leaf extracts significantly inhibited collagenase activity in a dose-dependent manner, as shown in Figure 19. The collagenase inhibition activities of the flower and leaf extracts at 4 mg/mL were 51.73 ± 0.20% and 41.54 ± 0.75%, respectively. At 0.05 mg/mL, the reference substance, EGCG, exhibited inhibition of 65.67 ± 2.52%. Finally, the IC_{50} values of flower and leaf extracts were 3.89 ± 0.17 and 5.02 ± 0.47 mg/mL, respectively.

The results show the leaf extract inhibiting tyrosinase activity more than the flower extract, while the flower extract demonstrates higher anti-collagenase activity than the leaf extract. Previous studies reveal that plant extracts possess anti-aging, wrinkle-prevention properties through different mechanisms, depending on their phenolic compounds, antioxidant properties, anti-collagenase activities and anti-elastase activities (Pientaweeratch *et al*., 2016; Zakiah *et al*., 2018). These results indicate that torch ginger could potentially be a natural source to use as an active ingredient for anti-aging and anti-wrinkle benefits in cosmetic products.



Figures 19. Effect of torch ginger extracts on collagenase inhibition activity

Values are expressed as means ± standard deviation (n = 3). For groups in the same concentration, not sharing the same letter indicates significantly different at $p < 0.05$.



Figures 20. Effect of torch ginger mixed extracts on tyrosinase inhibition activity

Different ratio (0.5% and 1%) of torch ginger mixed extract are expressed as leaf: flower. Values are expressed as means ± standard deviation (n = 3).

4.8 Determination of the suitable ratio of torch ginger extracts for cosmetic formulations

The research proposed developing cosmetic products for purpose of skin whitening along with anti-aging properties. According to the results of bioactive

compounds include amino acid content and phenolic compounds along with biological activities of torch ginger extracts. The leaf extract presented the strongest antioxidant inhibitory property, higher than that of trolox and gallic acid, and showed the highest of phenolic contents, as well as moderate anti-collagenase and amino acid contents. On the other hand, the flower extract showed more potential to inhibit collagenase activity, and more amino acids, than the leaf extract, while also exhibiting moderate phenolic compound levels and antioxidant activity. Meanwhile, both the flower and leaf were shown to be capable of suppressing melanogenesis through inhibiting tyrosinase activity. Therefore, both flower and leaf extract suitable for mixing together for their synergistic benefits to be formulation in cosmetic products as a whitening ingredient, along with anti-aging properties. A suitable concentration and ratio of mixed flower and leaf extracts were investigated on their potential to inhibit tyrosinase activity. As shown in Figure 20, the results demonstrated that all concentrations of mixed flower and leaf extracts inhibited tyrosinase activity. In addition, flower and leaf extracts at a concentration of 1% and in a ratio of 1:1 exhibited the highest tyrosinase inhibition activity at 74.61 ± 0.00%. These results indicate that 1% of mixed flower and leaf extracts of torch ginger in a ratio of 1:1 is a suitable concentration for use in formulating the cosmetic products.

4.9 Formulation of cosmetic products containing torch ginner extracts

4.9.1 Torch ginger facial (TG) cream

The TG cream (Figure 21) was evaluated for its physical properties, including pH, texture, color, viscosity (Pa.s), consistency, spreadability, feel on skin, and the centrifugation test. As shown in Table 13, after the cosmetic formulation, the physical appearance of torch ginger facial cream was assessed. The texture and consistency were found to be tender, with a very good spreadability and soft felling

on the skin. The TG cream was stable and showed no phase separation when centrifuged at 4000 rpm for 30 min.

Table 13. The physical properties of the TG cream formulation

Parameter	pH	color			Viscosity	Centrifugation
		L^*	a^*	b^*	(Pa.s)	test
TG cream	5.5	60.69 ± 0.63	2.47 ± 0.26	20.43 ± 1.08	6.20 ± 0.02	stable

Values are expressed as means ± standard deviation (n = 3).

Figures 21. The appearance of torch ginger facial (TG) cream

4.9.1.1 Stability testing of TG cream

The stability test under accelerated conditions found the pH of all conditions did not change after testing, nor were separation and precipitation of the TG cream observed. The viscosity of all conditions compared to the start condition was maintained at room temperature and 4 ºC, and under heating/cooling cycles was not significantly different ($p > 0.05$). At 45 ºC, there was an obvious decrease in viscosity, which may be the effect of heat (Table 14). Moreover, the physical properties of color and odor were stable after the stability tests. Therefore, the TG cream could be stored long term, without any change in the pH and viscosity.

Table 14. The stability test of TG cream after three months and after H/C cycles

Conditions	pH	Viscosity (Pa.s)	Separation and precipitation
Initial	5.5	6.20 ± 0.02 [a]	x
RT	5.5	6.15 ± 0.02 [a]	x
4°C	5.5	6.17 ± 0.06 [a]	x
45°C	5.5	6.01 ± 0.03 [b]	x
H/C	5.5	6.15 ± 0.05 [a]	x

Value are expressed as means ± standard deviation. For viscosity values, not sharing the same letter indicates significantly different at $p < 0.05$. RT: Room temperature, H/C: Heating/cooling conditions.

4.9.2 Torch ginger facial (TG) foam

The TG foam was investigated for its physical properties, include pH, color, foam forming, and feel on skin. The TG foam presented the physical appearance was found to be soft and smooth feeling after applied on the skin. After filling the TG foam into the pump-bottles with foam maker, it was good to generated foam forming and easy to wash (Table 15, Figure 22).

Table 15. The physical properties of the TG foam formulation

Parameter	pH	color	Feel on skin	Foam forming
TG foam	6.0	amber orange	soft & smooth	good

Figures 22. The appearance of torch ginger facial (TG) foam

Table 16. The stability test of TG foam after three months and after H/C cycles

Conditions	pH	Color	Feel on skin	Foam forming
Initial	6.0	amber orange	soft & smooth	good
RT	6.0	amber orange	soft & smooth	good
4ºC	6.0	amber orange	soft & smooth	good
45ºC	5.9	rust	soft & smooth	good
H/C	6.0	amber orange	soft & smooth	good

RT: Room temperature, H/C: Heating/cooling conditions.

4.9.2.1 Stability testing of TG foam

The accelerated stability conditions of TG foam was found the pH of all condition sable at 6.0, except at 45 ºC the pH value was slightly decrease. The color of all conditions compared to the start condition was maintained at room temperature, 4°C, and 6 cycles of heating/cooling conditions. For 45°C, there was an observable increase the color change, which may be the heat affected (Table 16). Furthermore, the feeling on the skin and foam forming were stable after the

accelerated stability test. Hence, the TG foam could be stored long term, with an escape from the heat.

4.9.3 Torch ginger body (TG) scrub

In this study, The TG scrub (Figure 23) was evaluated for its physical properties, including pH, color, texture, consistency, spreadability and feel on skin. After TG scrub formulation, the physical appearance was investigated (Table 17). The texture was found to be soft, with good spreadability and a soft feeling on the skin. The TG scrub was stable and showed no melted when standing at room temperature (25 – 30°C). Further, it easy to scrub and rinse off after applying.

Table 17. The physical properties of the TG scrub formulation

Parameter	color			Texture	Spreadability
	L^*	a^*	b^*		
TG scrub	4.17 ± 0.06	5.73 ± 0.06	3.23 ± 0.29	Soft	good

Values are expressed as means ± standard deviation (n = 3).

4.9.3.1 Stability testing of TG scrub

The stability test under accelerated conditions comprising room temperature, 4°C, 45°C for 3 month. The color of all conditions did not change after testing. The TG scrub presented no separated under room temperature and 4°C for three month. After 45°C condition, it was shown the ingredients obvious separated after cooled at room temperature. Moreover, the physical properties of color, texture, spreadability, and odor were stable after the accelerate stability tests. Therefore, the TG scrub can be stored at room temperature and below 45°C.

Figures 23. The appearance of torch ginger body (TG) scrub

4.10 Clinical evaluation in volunteers

4.10.1 Skin irritation testing

The dermatological test for irritation and allergy effects on human volunteers was performed to ensure the safety of the cosmetic products from torch ginger. The 24 volunteers were tested with TG cream, TG foam, TG scrub 1% w/v of sodium lauryl sulfate, and deionized water. The results are shown in Table 18. All cosmetic products from torch ginger were found to be non-irritating, with a low Primary Dermal Irritation Index value (PDII < 0.5), whereas sodium lauryl sulfate (SLS), which was used as a positive control, was revealed to be slightly irritating (PDII range from 0.5 to 2.0).

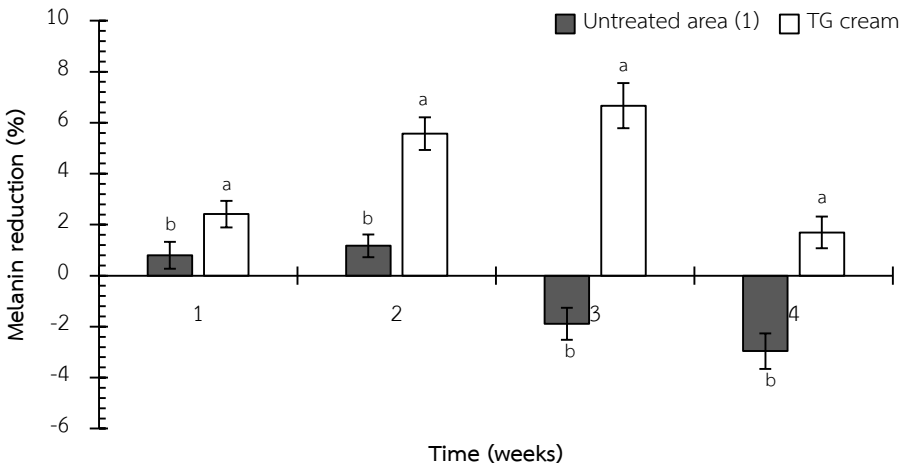
Table 18. The Primary Dermal Irritation Index (PDII) value and skin irritation reaction in 24 volunteers

Test substances	PDII	Classification of skin irritation
TG cream	0.00	Non-irritating
TG foam	0.26	Non-irritating
TG scrub	0.00	Non-irritating
1% w/v SLS (positive)	0.80	Slightly irritating
DI water (negative)	0.00	Non-irritating

4.10.2 Skin whitening testing in human volunteers

4.10.2.1 Skin whitening testing of TG cream

The whitening effect of all TG cosmetic products containing torch ginger extracts were evaluated and compared with before the treatment. This was done by measuring the amount of melanin using DermaLab® Combo (Cortex Technology, Denmark), to confirm the efficiency after using TG products. As shown in Table 19, the results demonstrated that the melanin content in the skin when using TG cream was significantly and continuous decrease after one week, until week three of testing ($p < 0.05$). In week four, the melanin content increased, which may be due to the time of testing (March 2019 to April 2019) in Thailand being in the summer season. This period has a corresponding increase in UV exposure, which is the one of the causes of melanin synthesis (Brenner and Hearing, 2008; Pintus *et al*., 2015); the UV index is shown in Figure 27. In addition, there are several different influencing factors of skin color which depends on volunteer's gender, age, working environment and lifestyles (Wang *et al*., 2019). The L^* value was used for indicating skin lightening after applying the TG cream, and it was found that the L^* value continuously increased from the 1^{st} to 3^{rd} week ($p < 0.05$), as did the melanin content. For the untreated areas (1), the data still showed constant melanin content till week two of testing with no significant difference ($p > 0.05$), but the content increased from the 3^{rd} to the 4^{th} week. However, the L^* value of the untreated area was slightly increased until week three and slightly decreased subsequently till week of testing. In addition, the melanin reduction activity of the TG cream reached its highest level of 6.67% after three week of testing (Figure 24). There were significantly differences in melanin reduction observed between the TG cream-treated and untreated areas of the volunteers during the testing period.



Figures 24. The effect of TG cream on melanin reduction activity

Values are expressed as means ± standard error. For groups in the same week, not sharing the same letter indicates significant difference at $p < 0.05$.

4.10.2.2 Skin whitening testing of TG foam

The whitening effects of TG foam containing flower and leaf extracts of torch ginger was investigated for four weeks and evaluated by comparison with before treatment as same as TG cream. The results demonstrated that the melanin content in the skin was significantly decrease until week two of the testing ($p < 0.05$). In week three the melanin content was increased approximately 0.2%, which may be due to the treated areas at the forearm that often exposed the UV radiation, along with the highest UV index in the period of testing (April 2019 to May 2019); the UV index is shown in Figure 27. However, it was found that the L^* value continuous slightly increased from the 1st to 3rd week, which is shown in Table 19. For the untreated areas (2), the results showed melanin content was constant throughout the period of testing with no significant difference ($p > 0.05$). Further, the L^* value also showed still constant from the 1st to the 4th week, as did the melanin content. Additionally, the melanin reduction activity of the TG foam showed reached its highest level to be 4.55% after two weeks of investigated (Figure 25). These results

were also significantly differences in melanin reduction observed between TG foam-treated and untreated areas (2) during the testing period.

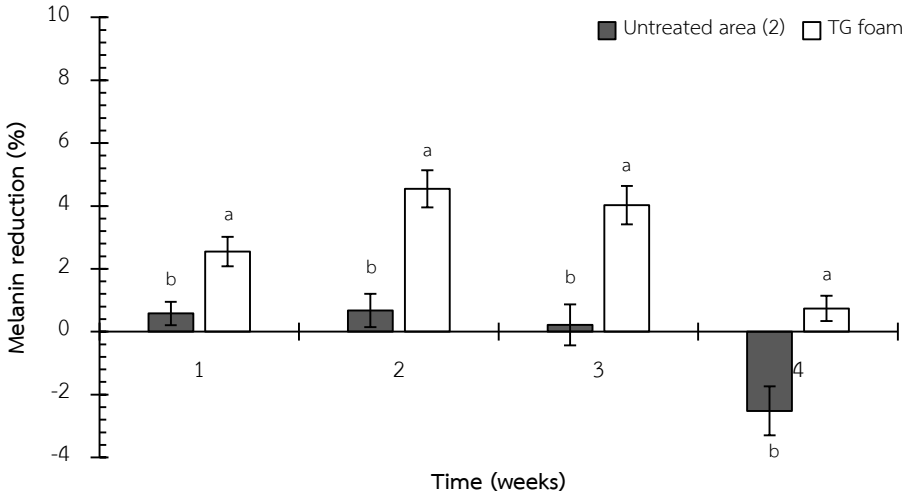
4.10.2.3 Skin whitening testing of TG scrub

The skin whitening effect after applying TG scrub containing dried residues fiber of torch ginger flower, which is residue from extraction processed. The whitening effect was evaluated the melanin for four weeks, which compared with before treatment as same as TG cream and foam. As shown in Table 19, the data showed that the significantly decreased of melanin content after one week, until week two of the testing ($p < 0.05$). In week three the results showed melanin increasing and stable till week four of testing. Furthermore, the L^* value was found an increase from the 1^{st} to 2^{nd} week and slightly decrease at week three, as did the melanin content. These results may be due to a corresponding increase in UV index between the times of testing (March 2019 to April 2019) and other influenced factors, as did the TG cream. In addition, the melanin reduction activity of the TG scrub reached 6.35% at the highest after two weeks of testing (Figure 26). These results were also found significantly differences in melanin reduction investigated between the TG scrub-treated and untreated areas (1) during the testing period, as did the TG cream and foam. According to these finding, the residue flower fiber which is agricultural residues which are capable uses for further utilized as a scrub fiber to obtain a lighten skin; hence, it is one approach to reducing agricultural waste, which is more environmentally friendly as well.

Table 19. The melanin content and L^* value after application of TG products for four weeks

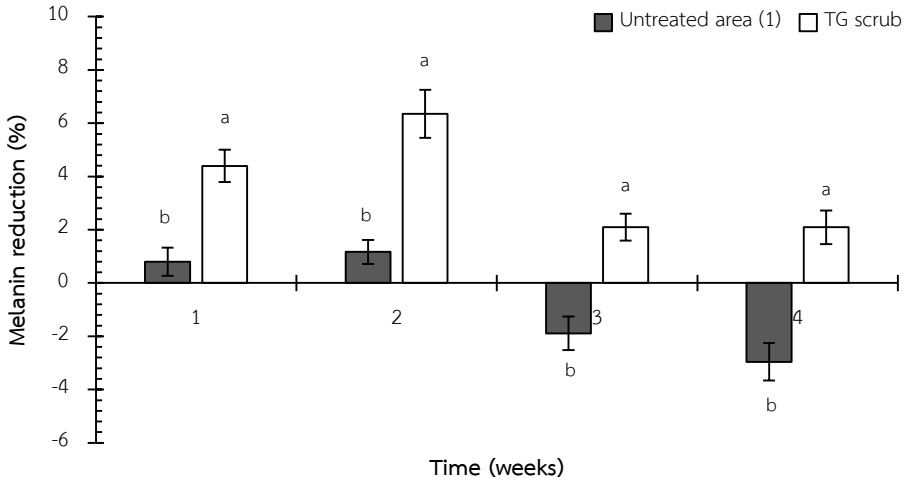
Product	Parameter	Baseline	Week 1	Week 2	Week 3	Week 4
TG cream	Melanin content (%)	36.00 ± 0.33 ^{a}	35.13 ± 0.38 ^{b}	33.93 ± 0.53 ^{c}	33.60 ± 0.44 ^{c}	35.39 ± 0.55 ^{ab}
	L^* value	37.39 ± 0.40 ^{a}	38.54 ± 0.47 ^{b}	39.41 ± 0.47 ^{c}	41.00 ± 0.65 ^{d}	39.13 ± 0.70 ^{bc}
TG foam	Melanin content (%)	36.49 ± 0.41 ^{a}	35.56 ± 0.43 ^{b}	34.83 ± 0.60 ^{b}	35.02 ± 0.56 ^{b}	36.22 ± 0.62 ^{ab}
	L^* value	36.80 ± 0.53 ^{a}	38.11 ± 0.57 ^{b}	38.60 ± 0.65 ^{b}	38.83 ± 0.77 ^{b}	37.52 ± 0.84 ^{ab}
TG scrub	Melanin content (%)	36.79 ± 0.57 ^{a}	35.17 ± 0.64 ^{b}	34.46 ± 0.81 ^{bc}	36.02 ± 0.62 ^{ab}	36.02 ± 0.78 ^{ab}
	L^* value	35.95 ± 0.64 ^{a}	37.35 ± 0.72 ^{b}	37.70 ± 0.79 ^{b}	37.20 ± 0.65 ^{b}	36.72 ± 0.82 ^{ab}
Untreated area (1)	Melanin content (%)	36.44 ± 0.37 ^{a}	36.15 ± 0.34 ^{a}	36.01 ± 0.34 ^{a}	37.13 ± 0.39 ^{ab}	37.52 ± 0.47 ^{b}
	L^* value	37.57 ± 0.42 ^{a}	38.07 ± 0.47 ^{ab}	38.33 ± 0.46 ^{b}	37.95 ± 0.42 ^{a}	36.54 ± 0.80 ^{a}
Untreated area (2)	Melanin content (%)	36.89 ± 0.46 ^{a}	36.67 ± 0.49 ^{a}	36.64 ± 0.39 ^{a}	36.81 ± 0.41 ^{a}	37.82 ± 0.43 ^{a}
	L^* value	36.36 ± 0.56 ^{a}	36.68 ± 0.65 ^{a}	36.96 ± 0.54 ^{a}	36.87 ± 0.50 ^{a}	35.56 ± 0.63 ^{a}

Values are expressed as means ± standard error. For each parameter of each product, not sharing the same letter indicates significant difference at $p < 0.05$.



Figures 25. The effect of TG foam on melanin reduction activity

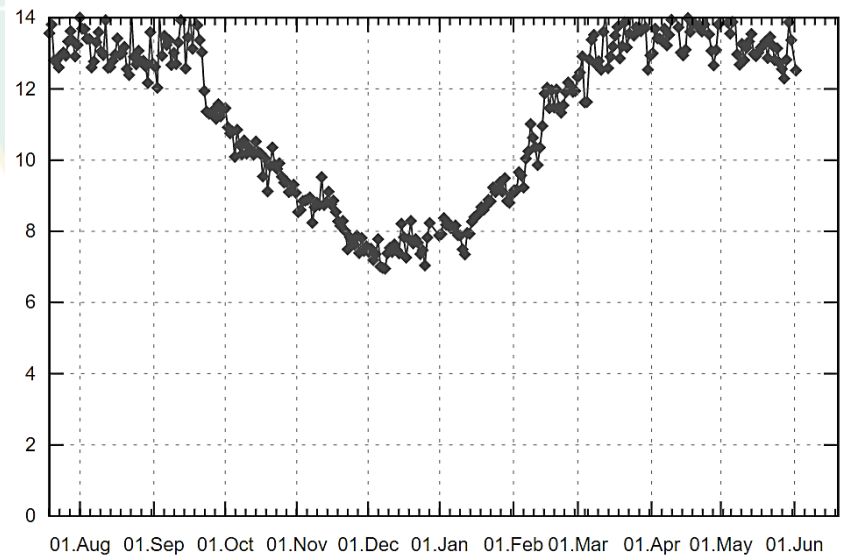
Values are expressed as means ± standard error. For groups in the same week, not sharing the same letter indicates significant difference at $p < 0.05$.



Figures 26. The effect of TG scrub on melanin reduction activity

Values are expressed as means ± standard error. For groups in the same week, not sharing the same letter indicates significant difference at $p < 0.05$.

Traditionally, commercial skin whitening agents for suppressing tyrosinase activity have included hydroquinone, arbutin, kojic acid, azelaic acid, ascorbic acid, ellagic acid, and tranexamic acid. These have been widely used in formulations of cosmetic products, with some drawbacks and side effects (Pillaiyar *et al*., 2017; Smit *et al*., 2009). In the past few decades, research has demonstrated that natural bioactive compounds are increasingly being used in whitening cosmetic formulations. In addition, there are several plant extracts that have been shown to be effective agents to suppress the overproduction of melanin or to regulate melanin synthesis, such as *Cassia fistula* flowers (Limtrakul *et al*., 2016), *Asphodelus microcarpus* (Di Petrillo *et al*., 2016), *Magnolia officinalis* (Wu *et al*., 2018), *Dendrobium tosaense* (Chan *et al*., 2018), and *Kummerowia striata* (Lee *et al*., 2019).



Figures 27. The UV index in Chiang Mai city from August 2018 to June 2019
Source: WeatherOnline (2019), © weatheronline.co.uk

Meanwhile, natural extracts are also uses as anti-aging of skin appearance. It is well known that bioactive compound from plants such as phenolic compounds and amino acid are potent for skin wrinkle improvement, as well as anti-collagenase and anti-elastase activities. Their antioxidant has been reported in preventing cellular damage from the oxidation process, which results in delay the skin aging process and maintains the skin homogeneity for beautiful-looking skin (Adhikari *et al*., 2018; Costa and Santos, 2017; Działo *et al*., 2016; Zakiah *et al*., 2018). In addition, natural skin care products are becoming popular for their uses in contemporary formulations by consumer demand, who are increasingly concerned with purchasing environmentally friendly products (Ribeiro *et al*., 2015). The results of the present study indicated that extracts of torch ginger flowers and leaves achieved a decent skin whitening effect on human volunteers; therefore, these extracts act as the active whitening ingredient in TG cream and foam.

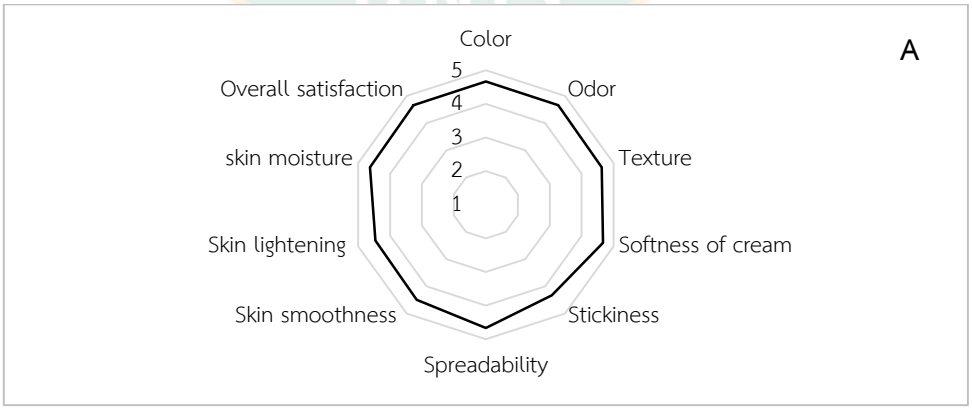
The dynamic of cosmetic substances' permeation and penetration through the skin depends on several factors, including formulation or vehicle which is presented to the skin; physicochemical properties i.e. lipophilicity, polarity, molecular weight, and charge; and skin condition such as the partition coefficient between the stratum corneum or exposure duration. Generally, applied substances reach into the systemic circulation through intracellular or intercellular route or absorbed via hair follicles and sweat glands (Petry *et al*., 2017; Tampucci *et al*., 2018). The main route of transferring is via discrimination through the lipid bilayers, which is dependent on the nature of the skin site and hydrophilic and lipophilic balance for the substance to absorb through a membrane barrier (WHO, 2006). Transcellular penetration occurring between corneocytes which possibly only small molecules soluble in water and fats. Additionally, due to the skin acting as a barrier, several ingredients transferred into the skin at low rate of permeability. Then, the addition of a chemical compound that increases interaction with compounds of the skin resulting in an increase the permeation capacity are important for cosmetics formulations. The

permeation of phenolic compounds depends on their subclass of phenolic compounds belongs, molecular size, structure, and etc. Meanwhile the penetration of epidermis, phenolic compounds may earn non-enzymatic or enzymatic reaction (Działo *et al*., 2016). The experiment of skin permeation of polyphenol compounds for used as anti-aging cosmetic ingredients exhibited catechin, epigallocatechin gallate, resveratrol, quercetin, rutin, protocatechuic acid and EGCG formulated into oil in water (O/W) emulsions could be pass into the stratum corneum barrier and were found in the epidermis and dermis layers (Soto *et al*., 2015). The hydrophilic protocatechuic acid exhibited the highest permeation rate followed by catechin, rutin and quercetin respectively (dal Belo *et al*., 2009). Further, propylene glycol presented the vehicle properties for delivery the different polarity of antioxidants and promote rutin release. The combination of chlorogenic acid, resveratrol, curcumin and quercetin in oil in water (O/W) microemulsions, which contained sucrose laurate or di-2-ethylhexyl sodium sulfosuccinate can be increasing delivery efficiency of phenolic compounds into the dermis layer (Zillich *et al*., 2015). Of note, an approximately 90% of polyphenolic compounds were retained in the stratum corneum layer meanwhile 10% was quantified in the underlying skin (Soto *et al*., 2015). A novel aspect has been developed for promising enhancement the permeation and penetration in the delivery system, such as encapsulation; nanocosmeceutical such as nanoemulsion, gold nanoparticles, dendrimers. But these still concern in toxicity for use in cosmeceuticals and in Thailand they are classified as “controlled cosmetics” which requires controlled ingredients under notification of Food and Drug Administration (FDA) for formulated in cosmetics before launching in the market (Ganesan and Choi, 2016; Kaul *et al*., 2018; Montenegro, 2014; Morganti and Coltelli, 2019; Soto *et al*., 2015).

4.11 Satisfaction test

The volunteers were asked to fill in a questionnaire (Appendix D) after using the cosmetic products comprising TG cream, TG foam and TG scrub for 30 days. The satisfaction level was determined using a 5-point scale, in which the point value represented the volunteer's feelings about how well the products worked, from very well (5) to very poor (1).

For the TG cream, the results revealed that the volunteer's satisfaction was between well to very well for all areas measured, as shown in Figure 28A. Further, the most satisfying areas of the TG cream were color, odor, softness of cream, spreadability, and overall satisfaction, which have a mean of 4.67, while the lowest was the cream's stickiness, with a mean of 4.33. As shown in Figure 28B, the results indicated that the volunteer's feeling reflected after used the TG foam was ranged in well to very well for all areas measured. The highest satisfying area of the TG foam was overall satisfaction, which has a mean of 4.67, while the lowest was the foam's odor, with a mean of 4.33. Ultimately, the results of TG scrub showed that the most satisfying area was overall satisfaction, which is has a mean of 4.63, whereas the lowest was the product's texture, with a mean of 4.25 (Figure 28C). Additionally, none of the volunteers suffered skin irritation or any reactions during the test period.



Figures 28. The satisfaction of the volunteers with the TG products;
(A) TG cream, (B) TG foam, and (C) TG scrub

Figure 28. The satisfaction of the volunteers with the TG products (Cont.); (A) TG cream, (B) TG foam, and (C) TG scrub

4.12 The prototype of cosmetic products from torch ginger

Cosmetic products from torch ginger established using a brand of “GINGiNA”, which “GINGi” refer to torch ginger and “NA” refer to nature; therefore, the definition of GINGiNA is torch ginger being from nature. The products of GINGiNA include 1) Torch Ginger Extract Facial Cream (or TG cream), 2) Torch Ginger Extract Facial Foam (or TG foam), and 3) Torch Ginger Extract Body Scrub (or TG scrub), which are shown in Figure 29 – 31.

4.12.1 Torch Ginger Extract Facial Cream

Figures 29. Prototype of packaging of torch ginger extract facial cream

4.12.2 Torch Ginger Extract Facial Foam

Figures 30. Prototype of packaging of torch ginger extract facial foam

4.12.3 Torch Ginger Extract Body Scrub

Figures 31. Prototype of packaging of torch ginger extract body scrub

CHAPTER 5
CONCLUSION

In this study, both the flower and leaf extracts of torch ginger exhibited potential as bioactive compounds with effective amino acids and phenolic compounds, as well as beneficial biological properties for cosmetic and cosmeceutical applications. It is worth noting that the aqueous extract of torch ginger leaves demonstrated higher potential as an antioxidant than that of the reference substances, including trolox and gallic acid. The flower extract, on the other hand, exhibited more significant collagenase activity than that of the leaf extract. These findings reveal that both the flower and leaf extracts of torch ginger possess anti-aging properties, especially the flower extract, which exhibits significant anti-collagenase activity and amino acid content while also possessing a moderate phenolic compound and antioxidant activity. The leaf extract was found to have the highest amount of antioxidant activity and phenolic compounds, as well as moderate anti-collagenase and amino acid contents. Further, torch ginger exhibits strong potential in improving and restoring skin damage, especially in addressing skin aging issues and preventing wrinkles, through the inhibition of free radicals and collagenase. Additionally, these extracts can suppress the melanogenesis process to provide a skin lightening effect useful in cosmetic and whitening applications.

For the clinical study, the results demonstrate that both the flower and leaf extracts of torch ginger could potentially be a natural whitening ingredient for commercial cosmetics. The results revealed that with the use of the TG cream, TG foam, and TG scrub the melanin content decreased and the L* value increased compared with initial values. The data showed an increased melanin reduction after application of TG products to the skin when compared to an untreated are. Further, all products were also considerably stable under accelerated stability, which is the

desirable properties for uses it commercially. Additionally, they were found to be safe, with no reported irritation of the skin of the volunteers, and was also found to be satisfactory by the volunteers. Therefore, cosmetic products containing torch ginger flower and leaf extracts are interesting to be a potent whitening cosmetic for improving skin appearance.

This is the new finding about a combination of both flower and leaf extracts of torch ginger for utilisation as active ingredients in formulation of cosmetic products which provide skin benefits. Besides that, this study may be an alternative way to value-adding agricultural products as facial cosmetic products, especially a leaf of torch ginger which considered as agricultural waste with no report on utilized in economic cost-benefit. Moreover, it is worthy to support torch ginger that widely cultivated the southern region of Thailand to be potent functional ingredients for cosmeceutical and other benefits. Above all, it can be an increasing household income of cultivator for having a better livelihood and communities further.

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APPENDICES

Appendix A

The Certificate of ethical clearance

คณะกรรมการจริยธรรมการวิจัยในมนุษย์ มหาวิทยาลัยพะเยา

UNIVERSITY OF PHAYAO HUMAN ETHICS COMMITTEE

19 หมู่ 2 ตำบลแม่กา อำเภอเมือง จังหวัดพะเยา 56000 เบอร์โทรศัพท์ 05446 6666

เอกสารรับรองโครงการวิจัย

คณะกรรมการจริยธรรมการวิจัยในมนุษย์ มหาวิทยาลัยพะเยา ดำเนินการให้การรับรองโครงการวิจัยตามแนวทางหลักจริยธรรมการวิจัยในคนที่เป็นมาตรฐานสากลได้แก่ Declaration of Helsinki, The Belmont Report, CIOMS Guideline และ International Conference on Harmonization in Good Clinical Practice หรือ ICH-GCP

ชื่อโครงการ : การทดสอบประสิทธิภาพและความปลอดภัยของผลิตภัณฑ์ความงามจากดาหลา
: Efficiency and safety testing of cosmetic products from torch ginger

เลขที่โครงการวิจัย : 3/018/61

ผู้วิจัยหลัก : ดร.ลภัสรดา มุ่งหมาย
สังกัดหน่วยงาน : คณะเภสัชศาสตร์ มหาวิทยาลัยพะเยา

ผู้วิจัยร่วม : ผู้ช่วยศาสตราจารย์ ดร.ดวงพร อมรเลิศพิศาล, นายณัฐวุฒิ หวังสมนึก
สังกัดหน่วยงาน : บัณฑิตวิทยาลัย มหาวิทยาลัยแม่โจ้

วิธีทบทวน : คณะกรรมการเต็มชุด (Full board)

รายงานความก้าวหน้า : ส่งรายงานความก้าวหน้าอย่างน้อยทุก 12 เดือน หรือส่งรายงานฉบับสมบูรณ์ หากดำเนินโครงการเสร็จสิ้นก่อน 1 ปี

ลงนาม

(ดร.แสงเดือน พรมแก้วงาม)

รองประธานคณะกรรมการจริยธรรมการวิจัยในมนุษย์

วันที่รับรอง : 25 กุมภาพันธ์ 2562

วันหมดอายุ : 25 กุมภาพันธ์ 2563

ทั้งนี้ การรับรองนี้มีเงื่อนไขดังที่ระบุไว้ด้านหลังทุกข้อ (ดูด้านหลังของเอกสารรับรองโครงการวิจัย)

Appendix B

The information sheet for research participant

แบบฟอร์ม UP-HEC 04.1 / 1

University of Phayao Human Ethics Committee	**ข้อมูลคำอธิบายสำหรับผู้เข้าร่วมในโครงการวิจัย** (Information Sheet for Research Participant)

ชื่อโครงการวิจัย

การทดสอบประสิทธิภาพและความปลอดภัยของผลิตภัณฑ์ความงามจากดาหลา

ผู้สนับสนุนการวิจัย

โครงการพัฒนานักวิจัยและงานวิจัยเพื่ออุตสาหกรรม สำนักงานกองทุนสนับสนุนการวิจัย

ผู้ทำวิจัย

ชื่อ อาจารย์ ดร.ลภัสรดา มุ่งหมาย

ที่อยู่ สาขาวิชาวิทยาศาสตร์เครื่องสำอาง คณะเภสัชศาสตร์ มหาวิทยาลัยพะเยา
19 หมู่ 2 ต.แม่กา อ.เมือง จ.พะเยา 56000

เบอร์โทรศัพท์ 086-6546-966

ผู้ร่วมในโครงการวิจัย

ชื่อ ผู้ช่วยศาสตราจารย์ ดร.ดวงพร อมรเลิศพิศาล

ที่อยู่ บัณฑิตวิทยาลัย มหาวิทยาลัยแม่โจ้
อาคารเทพศาสตร์สถิต 63 หมู่ 4 ต.หนองหาร อ.สันทราย จ.เชียงใหม่ 50290

เบอร์โทรศัพท์ 086-6546-966

ผู้ร่วมในโครงการวิจัย

ชื่อ นายณัฐวุฒิ หวังสมนึก

ที่อยู่ สาขาวิชาสหวิทยาการเกษตร บัณฑิตวิทยาลัย มหาวิทยาลัยแม่โจ้
63 หมู่ 4 ต.หนองหาร อ.สันทราย จ.เชียงใหม่ 50290

เบอร์โทรศัพท์ 087-765-0162

เรียน ผู้เข้าร่วมโครงการวิจัยทุกท่าน

ท่านได้รับเชิญให้เข้าร่วมในโครงการวิจัย เรื่อง การทดสอบประสิทธิภาพและความปลอดภัยของผลิตภัณฑ์ความงามจากดาหลา ซึ่งเป็นการยืนยันประสิทธิภาพของเครื่องสำอางบำรุงผิวที่มีส่วนผสมของสารสกัดจากดาหลา โดยทางผู้วิจัยได้แก่ อาจารย์ ดร.ลภัสรดา มุ่งหมาย ผศ.ดร.ดวงพร

อมรเลิศพิศาล และนายณัฐวุฒิ หวังสมนึก ก่อนที่ท่านจะตัดสินใจเข้าร่วมในการศึกษาวิจัยนี้ ขอให้ท่านอ่านเอกสารฉบับนี้อย่างถี่ถ้วน เพื่อให้ท่านได้ทราบถึงเหตุผลและรายละเอียดของการศึกษาวิจัยในครั้งนี้ หากท่านมีข้อสงสัยใดๆ เพิ่มเติม กรุณาซักถามจากทีมงานของผู้ทำวิจัยซึ่งจะเป็นผู้สามารถตอบคำถามและให้ความกระจ่างแก่ท่านได้

ท่านสามารถขอคำแนะนำในการเข้าร่วมโครงการวิจัยนี้จากครอบครัว เพื่อน หรือแพทย์ประจำตัวของท่านได้ ท่านมีเวลาอย่างเพียงพอในการตัดสินใจโดยอิสระ ถ้าท่านตัดสินใจแล้วว่าจะเข้าร่วมในโครงการวิจัยนี้ ขอให้ท่านลงนามในเอกสารแสดงความยินยอมของโครงการวิจัยนี้

ผู้วิจัยขอขอบพระคุณทุกท่านที่เสียสละเวลาอันมีค่าเพื่อศึกษาข้อมูลรายละเอียดเกี่ยวกับโครงการวิจัยนี้ ทางผู้วิจัยรู้สึกเป็นเกียรติอย่างยิ่งที่ได้ร่วมงานกับท่าน

<u>เหตุผลความเป็นมา</u>

สารออกฤทธิ์ทางชีวภาพจากพืชพันธุ์ธรรมชาติกำลังได้รับความนิยมในการนำมาเป็นส่วนผสมในผลิตภัณฑ์เครื่องสำอางในปัจจุบัน เนื่องจากกระแสความนิยมผลิตภัณฑ์ต่างๆที่มีส่วนผสมของสารจากธรรมชาติ ด้วยสาเหตุที่เชื่อว่าสารสังเคราะห์อาจก่อให้เกิดอันตรายต่อร่างกาย ขณะเดียวกันสารจากธรรมชาติน่าจะมีความปลอดภัยสูงกว่า และเป็นมิตรต่อสิ่งแวดล้อมมากกว่าเมื่อเปรียบเทียบกับสารสังเคราะห์ในเครื่องสำอางทั่วไป ผู้วิจัยจึงเลือกทำการศึกษาสารสกัดจากดาหลาซึ่งเป็นพืชสมุนไพรอยู่ในวงศ์ขิงข่า นิยมปลูกเป็นจำนวนมากทางภาคใต้ของประเทศไทยและมีรายงานว่ามีฤทธิ์ทางชีวภาพที่เหมาะสมในการพัฒนาผลิตภัณฑ์เป็นเครื่องสำอางบำรุงผิวต่อไปได้ ด้วยเหตุนี้ผู้วิจัยจึงทำการพัฒนาผลิตภัณฑ์เครื่องสำอางบำรุงผิวจำนวน 3 ชนิดได้แก่ ครีมบำรุงผิวผสมสารสกัดดาหลา โฟมล้างหน้าผสมสารสกัดดาหลา และผงขัดผิวจากดอกดาหลา ด้วยเหตุนี้งานวิจัยนี้จึงมีความจำเป็นต้องมีการประเมิน 2 ส่วนคือ การทดสอบประสิทธิภาพของเครื่องสำอางบำรุงผิวจากดาหลาในการช่วยปรับสภาพผิวให้กระจ่างใสจากการใช้จริง (โดยทำการวัดปริมาณเม็ดสีเมลานินที่ผิวหนัง) และการทดสอบการระคายเคืองของผลิตภัณฑ์ รวม 3 ผลิตภัณฑ์โดยใช้องค์ความรู้ทางวิทยาศาสตร์ เพื่อใช้ประเมินประสิทธิภาพและยืนยันความปลอดภัยของผลิตภัณฑ์จึงจำเป็นต้องผ่านการทดสอบกับผิวหนังของอาสาสมัครโดยตรง เพื่อให้ได้ผลการทดลองที่น่าเชื่อถือและมีการพิสูจน์ได้ทางวิทยาศาสตร์

<u>วัตถุประสงค์ของการศึกษา</u>

1. เพื่อทดสอบการระคายเคืองของผลิตภัณฑ์ 3 ชนิดคือ ครีมบำรุงผิวผสมสารสกัดดาหลา โฟมล้างหน้าผสมสารสกัดดาหลา และผงขัดผิวจากดอกดาหลาต่อผิวหนังของอาสาสมัคร

2. เพื่อทดสอบประสิทธิภาพในการปรับสภาพผิวให้กระจ่างในของผลิตภัณฑ์ 3 ชนิดคือ ครีมบำรุงผิวผสมสารสกัดดาหลา โฟมล้างหน้าผสมสารสกัดดาหลา และผงขัดผิวจากดอกดาหลา

3. เพื่อทดสอบความพึงพอใจของอาสาสมัครต่อผลิตภัณฑ์ครีมบำรุงผิวผสมสารสกัดดาหลา โฟมล้างหน้าผสมสารสกัดดาหลา และผงขัดผิวจากดอกดาหลา

วิธีการที่เกี่ยวข้องกับการวิจัย

งานวิจัยนี้แบ่งออกเป็น 2 ส่วน หลังจากท่านให้ความยินยอมที่จะเข้าร่วมในโครงการวิจัยนี้ ท่านจะได้รับการอธิบายรายละเอียดเกี่ยวกับโครงการวิจัยนี้ ขั้นตอนและวิธีการดำเนินการวิจัย และลงชื่อในหนังสือแสดงความยินยอมเข้าร่วมโครงการวิจัย โดยระยะเวลาในการเข้าร่วมตลอดโครงการครั้งนี้เป็นระยะเวลาประมาณ 67 วัน โดยแต่ละครั้งที่ทำการนัดหมายอาสาสมัครเพื่อรับการทดสอบด้วยเครื่องวิเคราะห์สภาพผิวจะใช้เวลาประมาณ 20-40 นาที ในช่วงเวลาที่อาสาสมัครสะดวกตั้งแต่ 09.00-16.00 น. โดยผู้วิจัยจะทำการนัดแนะเวลาเบื้องต้นขณะที่ท่านลงนามในหนังสือแสดงความยินยอมเข้าร่วมโครงการวิจัยเพื่อให้ท่านได้รับความสะดวกมากที่สุด ในการดำเนินการวิจัยท่านจะต้องปฏิบัติตัวดังต่อไปนี้

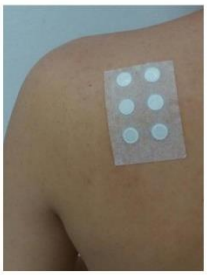
1. การทดสอบความระคายเคืองในอาสาสมัคร (อาสาสมัครจำนวน 24 คน)

1) ในวันที่นัดหมาย ผู้วิจัยจะทำการปิดแผ่นแปะทดสอบความระคายเคืองที่บรรจุตัวอย่างทดสอบผลิตภัณฑ์ ครีมบำรุงผิวผสมสารสกัดดาหลา โฟมล้างหน้าผสมสารสกัดดาหลา และผงขัดผิวจากดอกดาหลา และสารทดสอบการระคายเคือง sodium lauryl sulfate (SLS) ความเข้มข้น 1% w/v ในช่องทดสอบ โดยผู้วิจัยจะทำความสะอาดบริเวณตำแหน่งที่จะติดแผ่นแปะทดสอบก่อน และทำการติดแผ่นแปะทดสอบบริเวณแผ่นหลังของท่านด้านใดด้านใดด้านหนึ่ง (ภาพ 1) ไว้เป็นเวลานาน 48 ชั่วโมง โดยอาสาสมัครห้ามลอกออกเด็ดขาด

2) เมื่อครบระยะเวลาที่กำหนด 48 ชั่วโมง ผู้วิจัยจะทำการลอกแผ่นแปะทดสอบออก และทำการประเมินอาการระคายเคืองทันทีที่เวลา 1 ชั่วโมง 24 และ 7 วัน โดยผู้วิจัยจะทำการประเมินอาการระคายเคือง เช่น อาการแดง (erythema) และอาการบวม (edema) บนแต่ละตำแหน่งของผิวหนังที่ทำการแปะแผ่นทดสอบ

หมายเหตุ

- ท่านสามารถอาบน้ำได้ตามปกติระหว่างการทดสอบนี้
- ขอความร่วมมือท่านไม่แกะลอกแผ่นแปะออกด้วยตนเอง หากพบปัญหาระหว่างการทดสอบกรุณาติดต่อผู้วิจัยตามหมายเลขโทรศัพท์ที่ให้ไว้ข้างต้น



ภาพ 1 การติดแผ่นแปะทดสอบความระคายเคืองเบื้องต้นในอาสาสมัคร

2. การทดสอบประสิทธิภาพของผลิตภัณฑ์เครื่องสำอางผสมสารสกัดดาหลา
(อาสาสมัครจำนวน 24 คน)

I. การทดสอบประสิทธิภาพการปรับสภาพผิวให้กระจ่างใสของผลิตภัณฑ์ครีมบำรุงผิวผสมสารสกัดดาหลา

1) ท่านจะได้รับการอธิบายรายละเอียดต่างๆ เกี่ยวกับโครงการวิจัยนี้ ขั้นตอนการวิจัยผลิตภัณฑ์ครีมบำรุงผิวผสมสารสกัดดาหลา

2) อาสาสมัครจะได้รับการวิเคราะห์สภาพผิวก่อนเริ่มการทดสอบ (วิเคราะห์สภาพผิวครั้งที่ 1) ตามที่ผู้วิจัยนัดหมายด้วยเครื่องวิเคราะห์สภาพผิวหนังหลายพารามิเตอร์ รุ่น DermaLab® Combo ยี่ห้อ Cortex ประเทศเดนมาร์ก ซึ่งผู้วิจัยจะทำการวัดพารามิเตอร์ค่าสีเมลานิน (melanin index) และความชุ่มชื้น (moisture) และอ่านผลด้วยโปรแกรมสำหรับวิเคราะห์ผลของเครื่องมือดังกล่าว

3) ท่านจะได้รับผลิตภัณฑ์ทดสอบที่ผู้วิจัยจัดเตรียมไว้ให้ แล้วทาผลิตภัณฑ์ทดสอบปริมาณ ½ ช้อนชา บริเวณใบหน้าให้ทั่ว วันละ 2 ครั้ง เช้า-เย็น เป็นเวลานาน 30 วันติดต่อกัน

4) เข้ารับการวิเคราะห์สภาพผิวหนังหลังการทดสอบ ที่เวลา 1, 2, 3 และ 4 สัปดาห์ (วิเคราะห์สภาพผิวครั้งที่ 2, 3, 4 และ 5) ตามที่ผู้วิจัยนัดหมาย

II. การทดสอบประสิทธิภาพการปรับสภาพผิวให้กระจ่างใสของผลิตภัณฑ์โฟมล้างหน้าผสมสารสกัดดาหลา

1) ท่านจะได้รับการอธิบายรายละเอียดต่างๆ เกี่ยวกับโครงการวิจัยนี้ ขั้นตอนการวิจัยผลิตภัณฑ์โฟมล้างหน้าผสมสารสกัดดาหลา

2) อาสาสมัครจะได้รับการวิเคราะห์สภาพผิวก่อนเริ่มการทดสอบ (วิเคราะห์สภาพผิวครั้งที่ 1) ตามที่ผู้วิจัยนัดหมายด้วยเครื่องวิเคราะห์สภาพผิวหนังหลายพารามิเตอร์ รุ่น DermaLab®

Combo ยี่ห้อ Cortex ประเทศเดนมาร์ก ซึ่งผู้วิจัยจะทำการวัดพารามิเตอร์ค่าสีเมลานิน (melanin index) และความชุ่มชื้น (moisture) และอ่านผลด้วยโปรแกรมสำหรับวิเคราะห์ผลของเครื่องมือดังกล่าว

3) ท่านจะได้รับผลิตภัณฑ์ทดสอบที่ผู้วิจัยจัดเตรียมไว้ให้ แล้วใช้ผลิตภัณฑ์ทดสอบโดยใช้ทำความสะอาดผิวหน้า วันละ 2 ครั้ง เช้า-เย็น เป็นเวลานาน 30 วันติดต่อกัน

4) เข้ารับการวิเคราะห์สภาพผิวหนังหลังการทดสอบ ที่เวลา 1, 2, 3 และ 4 สัปดาห์ (วิเคราะห์สภาพผิวครั้งที่ 2, 3, 4 และ 5) ตามที่ผู้วิจัยนัดหมาย

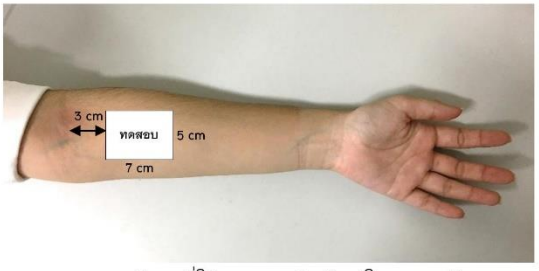
III. การทดสอบประสิทธิภาพการปรับสภาพผิวให้กระจ่างใสของผลิตภัณฑ์ผงขัดผิวจากดอกดาหลา

1) ท่านจะได้รับการอธิบายรายละเอียดต่างๆ เกี่ยวกับโครงการวิจัยนี้ ขั้นตอนการวิจัยผลิตภัณฑ์ผงขัดผิวจากดอกดาหลา

2) อาสาสมัครจะได้รับการวิเคราะห์สภาพผิวก่อนเริ่มการทดสอบ (วิเคราะห์สภาพผิวครั้งที่ 1) ตามที่ผู้วิจัยนัดหมายด้วยเครื่องวิเคราะห์สภาพผิวหนังหลายพารามิเตอร์ รุ่น DermaLab® Combo ยี่ห้อ Cortex ประเทศเดนมาร์ก ซึ่งผู้วิจัยจะทำการวัดพารามิเตอร์ค่าสีเมลานิน (melanin index) และความชุ่มชื้น (moisture) และอ่านผลด้วยโปรแกรมสำหรับวิเคราะห์ผลของเครื่องมือดังกล่าว

3) ท่านจะได้รับผลิตภัณฑ์ทดสอบที่ผู้วิจัยจัดเตรียมไว้ให้ แล้วใช้ผลิตภัณฑ์ทดสอบสครับผิวภายหลังจากท่านทำความสะอาดบริเวณตามปกติโดยใช้ถูบริเวณท้องแขนข้างซ้ายด้านในให้ห่างจากข้อพับ 3 เซนติเมตร (ภาพ 2) และใช้ผลิตภัณฑ์บริเวณดังกล่าวขนาดประมาณ 5x7 เซนติเมตร จำนวน 4 ครั้งต่อสัปดาห์ เป็นระยะเวลานาน 1 เดือน

4) เข้ารับการวิเคราะห์สภาพผิวหนังหลังการทดสอบ ที่เวลา 1, 2, 3 และ 4 สัปดาห์ (วิเคราะห์สภาพผิวครั้งที่ 2, 3, 4 และ 5) ตามที่ผู้วิจัยนัดหมาย



ภาพ 2 บริเวณที่ใช้ทดสอบผลิตภัณฑ์ในอาสาสมัคร

หมายเหตุ

- ก่อนที่ท่านจะได้รับการวิเคราะห์สภาพผิวหนังด้วยเครื่อง วิเคราะห์สภาพผิวหนังหลายพารามิเตอร์ ท่านจะต้องพักในห้องทดสอบที่มีอุณหภูมิ 20-25 องศาเซลเซียส เป็นเวลา 30 นาที
- หากผลิตภัณฑ์ทดสอบของท่านหมดก่อนสิ้นสุดการทดลองนาน 4 สัปดาห์ สามารถติดต่อผู้วิจัยเพื่อขอรับผลิตภัณฑ์ทดสอบเพิ่มเติม
- ระหว่างการเปลี่ยนการทดสอบประสิทธิภาพของผลิตภัณฑ์ครีมบำรุงผิวผสมสารสกัดดาหลาเป็นผลิตภัณฑ์โฟมล้างหน้าผสมสารสกัดดาหลา ขอความร่วมมืออาสาสมัครทำการพักผิวหน้าก่อนเริ่มทำการทดสอบขั้นต่อไปเป็นระยะเวลานาน 7 วัน

ความรับผิดชอบของอาสาสมัครผู้เข้าร่วมในโครงการวิจัย

เพื่อให้งานวิจัยนี้ประสบผลสำเร็จ ผู้วิจัยใคร่ขอความร่วมมือจากท่านโดยขอให้ท่านปฏิบัติตามคำแนะนำของผู้วิจัยโดยไม่ใช้ผลิตภัณฑ์ทาผิวอื่นบริเวณทดสอบที่กำหนด รวมทั้งแจ้งอาการผิดปกติต่างๆที่เกิดขึ้นกับทางระหว่างที่ท่านเข้าร่วมในโครงการวิจัยให้ผู้วิจัยได้ทราบ

ความเสี่ยงที่อาจได้รับ

ความเสี่ยงที่อาจเกิดขึ้นจากการวิจัยในครั้งนี้คือ อาการข้างเคียงระหว่างทดสอบประสิทธิภาพจากการใช้ผลิตภัณฑ์ทดสอบ ได้แก่อาการระคายเคืองและอาการแพ้ โดยในกรณีที่เกิดอาการแพ้หรืออาการระคายเคืองเล็กน้อยให้ใช้ยาแก้แพ้ 0.1% T.A. cream (triamcinolone cream) ทาบริเวณที่เป็นและ/หรือรับประทานยาแก้แพ้ และหยุดการทดสอบในอาสาสมัครรายดังกล่าว กรณีที่เกิดการระคายเคืองแบบรุนแรงให้หยุดใช้ผลิตภัณฑ์ทดสอบและรีบส่งแพทย์โดยทันที

ความเสี่ยงทางด้านจิตใจ เช่นอาสาสมัครอาจรู้สึกอึดอัดใจ ไม่สบายใจในการเข้าร่วมโครงการ รวมถึงเปิดเผยแผ่นหลังในการแปะทดสอบเพื่อประเมินอาการระคายเคืองเบื้องต้น หรือไม่สบายใจในการตอบแบบสอบถาม

ความเสี่ยงทางด้านเศรษฐกิจและสังคม เช่นการสูญเสียเวลาในการประกอบอาชีพ หรือละจากงานประจำที่ทำ เพื่อมาร่วมโครงการวิจัย เป็นต้น

หากท่านมีข้อสงสัยใดๆ เกี่ยวกับความเสี่ยงที่อาจได้รับจากการเข้าร่วมในโครงการวิจัย ท่านสามารถสอบถามจากผู้ทำวิจัยได้ตลอดเวลา

หากมีการค้นพบข้อมูลใหม่ ๆ ที่อาจมีผลต่อความปลอดภัยของท่านในระหว่างที่ท่านเข้าร่วมในโครงการวิจัย ผู้ทำวิจัยจะแจ้งให้ท่านทราบทันที เพื่อให้ท่านตัดสินใจว่าจะอยู่ในโครงการวิจัยต่อไปหรือจะขอถอนตัวออกจากการวิจัย

อันตรายที่อาจเกิดขึ้นจากการเข้าร่วมในโครงการวิจัยและความรับผิดชอบของผู้ทำวิจัย/ผู้สนับสนุนการวิจัย

หากพบอันตรายที่เกิดขึ้นจากการวิจัย ขอให้ท่านหยุดใช้ผลิตภัณฑ์ทดสอบและทางผู้วิจัยจะดำเนินการส่งท่านพบแพทย์ท่านจะได้รับการรักษาอย่างเหมาะสมทันที ผู้ทำวิจัย/ผู้สนับสนุนการวิจัยยินดีจะรับผิดชอบค่าใช้จ่ายในการรักษาพยาบาลของท่าน และการลงนามในเอกสารให้ความยินยอมไม่ได้หมายความว่าท่านได้สละสิทธิ์ทางกฎหมายตามปกติที่ท่านพึงมี

ในกรณีที่ท่านได้รับอันตรายใด ๆ หรือต้องการข้อมูลเพิ่มเติมที่เกี่ยวข้องกับโครงการวิจัย ท่านสามารถติดต่อกับผู้ทำวิจัยคือ อาจารย์ ดร.ลภัสรดา มุ่งหมาย หมายเลขโทรศัพท์ 086-6546-966 หรือ นายณัฐวุฒิ หวังสมนึก หมายเลขโทรศัพท์ 087-7650162 ได้ตลอด 24 ชั่วโมง

ประโยชน์ที่อาจได้รับ

ท่านจะไม่ได้รับประโยชน์ใดๆจากการเข้าร่วมในการวิจัยครั้งนี้ แต่ผลการศึกษาที่ได้ผู้วิจัยสามารถนำไปเผยแพร่เพื่อให้ผลการทดลองเป็นที่น่าเชื่อถือและสามารถพิสูจน์ได้ทางวิทยาศาสตร์ และสามารถใช้เป็นแนวทางในการพัฒนาผลิตภัณฑ์ที่มีส่วนผสมของผลผลิตทางการเกษตรของประเทศไทย ซึ่งถือได้ว่าเป็นการเพิ่มมูลค่าให้กับวัตถุดิบทางการเกษตรให้มีค่าสูงขึ้น และใช้เป็นทางเลือกให้แก่ผู้บริโภคในการเลือกใช้เครื่องสำอางที่มีส่วนผสมจากวัตถุดิบธรรมชาติ

ค่าตอบแทนสำหรับผู้เข้าร่วมวิจัย

ท่านจะได้รับเงินค่าตอบแทนการเดินทางและ/หรือค่าชดเชยสูญเสียรายได้/เสียเวลา หรือความไม่สะดวก ไม่สบาย จากการเข้าร่วมในการวิจัยหลังสิ้นสุดโครงการวิจัยเป็นจำนวนเงิน 300 บาท ตลอดการนัดหมายเพื่อทำการวัดประสิทธิภาพจำนวน 10 ครั้ง

ในกรณีที่ท่านเกิดอาการผิดปกติหรืออาการข้างเคียงจากการทดสอบประสิทธิภาพของผลิตภัณฑ์เครื่องสำอางบำรุงผิวจากดาหลา ท่านสามารถติดต่อผู้วิจัยได้ตลอดเวลาในการพาท่านไปรับการรักษาพยาบาล ณ สถานพยาบาลที่ใกล้ที่สุดเพื่อรับการรักษาอย่างทันท่วงที ทางผู้วิจัยยินดีรับผิดชอบค่าใช้จ่ายในส่วนของค่ารักษาพยาบาล รวมทั้งชดเชยรายได้ที่สูญเสียไประหว่างรับการรักษาพยาบาลดังกล่าว ภายในวงเงินไม่เกิน 2,000 บาท/ราย และหากอาสาสมัครเกิดอาการแพ้ขั้นรุนแรงในกลุ่มกว้างผู้วิจัยยินดีรับผิดชอบค่าใช้จ่ายค่ารักษาพยาบาลและค่าชดเชยรายได้ที่สูญเสียระหว่างการรักษาพยาบาลเพิ่มเติมภายในวงเงินไม่เกิน 3,000 บาท/ราย

การเข้าร่วมและการสิ้นสุดการเข้าร่วมโครงการวิจัย

การเข้าร่วมในโครงการวิจัยครั้งนี้เป็นไปโดยความสมัครใจ หากท่านไม่สมัครใจจะเข้าร่วมโครงการศึกษาวิจัยหรือมีความประสงค์ที่จะถอนตัวภายหลังตัดสินใจเข้าร่วมโครงการวิจัยแล้ว ท่าน

สามารถถอนตัวได้ตลอดเวลา เช่นในกรณีที่ท่านเกิดอาการข้างเคียงจากผลิตภัณฑ์ทดสอบหรือมีเหตุผลอื่นทำให้ไม่สามารถเข้าร่วมโครงการต่อได้

การปกป้องรักษาข้อมูลความลับของอาสาสมัคร

ทางผู้วิจัยขอรับรองว่าข้อมูลที่อาจนำไปสู่การเปิดเผยตัวท่าน จะได้รับการปกปิดและจะไม่เปิดเผยแก่สาธารณชน และจะนำมาใช้เฉพาะส่วนของผลการวิจัยและเปิดเผยได้ในรูปแบบของการสรุปผลวิจัยที่เกี่ยวข้องกับการศึกษาเท่านั้น

สิทธิ์ของผู้เข้าร่วมในโครงการวิจัย

ในฐานะที่ท่านเป็นผู้เข้าร่วมในโครงการวิจัย ท่านจะมีสิทธิ์ดังต่อไปนี้

1. ท่านจะได้รับทราบถึงลักษณะและวัตถุประสงค์ของการวิจัยในครั้งนี้
2. ท่านจะได้รับการอธิบายเกี่ยวกับระเบียบวิธีการของการวิจัยและอุปกรณ์ที่ใช้ในการวิจัยครั้งนี้
3. ท่านจะได้รับการอธิบายถึงความเสี่ยงที่อาจจะได้รับจากการวิจัย
4. ท่านจะได้รับการอธิบายถึงประโยชน์ที่ท่านอาจจะได้รับจากการวิจัย
5. ท่านจะได้รับทราบแนวทางในการรักษา ในกรณีที่เกิดอาการผิดปกติที่อาจเกิดขึ้นในระหว่างดำเนินการวิจัย
6. ท่านจะมีโอกาสได้ซักถามเกี่ยวกับงานวิจัยหรือขั้นตอนที่เกี่ยวข้องกับงานวิจัย
7. ท่านจะได้รับทราบว่าการยินยอมเข้าร่วมในโครงการวิจัยนี้ ท่านสามารถขอถอนตัวจากโครงการเมื่อไรก็ได้ โดยผู้เข้าร่วมในโครงการวิจัยสามารถขอถอนตัวจากโครงการโดยไม่ได้รับผลกระทบใด ๆ ทั้งสิ้น
8. ท่านจะได้รับเอกสารข้อมูลคำอธิบายสำหรับผู้เข้าร่วมในโครงการวิจัยและสำเนาเอกสารใบยินยอมที่มีทั้งลายเซ็นและวันที่
9. ท่านมีสิทธิ์ในการตัดสินใจว่าจะเข้าร่วมในโครงการวิจัยหรือไม่ก็ได้ โดยปราศจากการใช้อิทธิพลบังคับข่มขู่ หรือการหลอกลวง

หากท่านไม่ได้รับการชดเชยอันควรต่อการบาดเจ็บหรือเจ็บป่วยที่เกิดขึ้นโดยตรงจากการวิจัย หรือท่านไม่ได้รับการปฏิบัติตามที่ปรากฏในเอกสารข้อมูลคำอธิบายสำหรับผู้เข้าร่วมในการวิจัย ท่านสามารถร้องเรียนได้ที่ คณะกรรมการจริยธรรมการวิจัยในมนุษย์ มหาวิทยาลัยพะเยา หมายเลขโทรศัพท์ 054466666 โทรสาร 054466714 ในเวลาราชการ

ขอขอบคุณในการร่วมมือของท่านมา ณ ที่นี้

.....

.....

รับอาสาสมัครเข้าร่วมโครงการวิจัย

เพื่อทดสอบประสิทธิภาพและการระคายเคืองของผลิตภัณฑ์ความงาม ที่มีส่วนผสมของสารสกัดตาหลา

(รหัสโครงการ PHD59I0032)

คุณสมบัติอาสาสมัคร

- ☑ เพศชายหรือหญิง
- ☑ มีสุขภาพผิวปกติ ร่างกายแข็งแรง
- ☑ อายุระหว่าง 25-55 ปี
- ☑ ไม่อยู่ระหว่างการเข้าร่วมโครงการอื่น

สมัครออนไลน์

วัตถุประสงค์ของการวิจัย

เพื่อทดสอบประสิทธิภาพและความปลอดภัยของผลิตภัณฑ์เครื่องสำอางครีมบำรุงผิว โฟมล้างหน้า และผงขัดผิวจากตาหลาต่อผิวหนังของอาสาสมัคร

สถานที่ทำการวิจัย

ศูนย์ทักษะการเรียนรู้และนวัตกรรมบัณฑิตศึกษา
อาคารแม่โจ้ 60 ปี คณะวิทยาศาสตร์ ม.แม่โจ้

รายละเอียดเพิ่มเติมติดต่อ

นายณัฐวุฒิ หวังสมนึก สาขาวิชาสหวิทยาการเกษตร
อาคารแม่โจ้ 60 ปี คณะวิทยาศาสตร์ ม.แม่โจ้
โทรศัพท์ 087-765-0162
อีเมล: Nattawutt.wh@gmail.com

"เอกสารนี้ได้ผ่านการรับรองของคณะกรรมการจริยธรรมการวิจัยในมนุษย์ มหาวิทยาลัยพะเยาแล้ว"

******รับผลิตภัณฑ์ทดสอบเป็นระยะเวลา 30 วันตลอดโครงการ******

Appendix C

The informed consent form

แบบฟอร์ม UP-HEC 05 / 1

University of Phayao Human Ethics Committee	**หนังสือแสดงความยินยอมเข้าร่วมโครงการวิจัย** **สำหรับอาสาสมัครอายุมากกว่า 20 ปีขึ้นไป** (Informed Consent Form)

การวิจัยเรื่อง การทดสอบประสิทธิภาพและความปลอดภัยของผลิตภัณฑ์ความงามจากดาหลา

วันให้คำยินยอม วันที่.....เดือน..... พ.ศ .....

ข้าพเจ้า นาย/นาง/นางสาว .....

ที่อยู่ .....

ได้อ่านรายละเอียดจากเอกสารข้อมูลสำหรับผู้เข้าร่วมโครงการวิจัยวิจัยที่แนบมาฉบับวันที่ ..... และข้าพเจ้ายินยอมเข้าร่วมโครงการวิจัยโดยสมัครใจ

ข้าพเจ้าได้รับสำเนาเอกสารแสดงความยินยอมเข้าร่วมในโครงการวิจัยที่ข้าพเจ้าได้ลงนามและ วันที่ พร้อมด้วยเอกสารข้อมูลสำหรับผู้เข้าร่วมโครงการวิจัย ทั้งนี้ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย ระยะเวลาของการทำวิจัย วิธีการวิจัย อันตราย หรืออาการที่อาจเกิดขึ้นจากการวิจัย หรือจากยาที่ใช้ รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัย และแนวทางรักษาโดยวิธีอื่นอย่างละเอียด ข้าพเจ้ามีเวลาและโอกาสเพียงพอในการซักถามข้อสงสัยจนมีความเข้าใจอย่างดีแล้ว โดยผู้วิจัยได้ตอบคำถามต่าง ๆ ด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าพอใจ

ข้าพเจ้ารับทราบจากผู้วิจัยว่าหากเกิดอันตรายใด ๆ จากการวิจัยดังกล่าว ข้าพเจ้าจะได้รับการรักษาพยาบาลโดยไม่เสียค่าใช้จ่าย รวมถึงชดเชยรายได้ที่สูญเสียระหว่างการรักษาพยาบาลดังกล่าวภายในวงเงินไม่เกิน 2,000 บาทต่อราย

ข้าพเจ้ามีสิทธิที่จะบอกเลิกเข้าร่วมในโครงการวิจัยเมื่อใดก็ได้ โดยไม่จำเป็นต้องแจ้งเหตุผล และการบอกเลิกการเข้าร่วมการวิจัยนี้ จะไม่มีผลต่อการรักษาโรคหรือสิทธิอื่น ๆ ที่ข้าพเจ้าจะพึงได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลส่วนตัวของข้าพเจ้าเป็นความลับ และจะเปิดเผยได้เฉพาะเมื่อได้รับการยินยอมจากข้าพเจ้าเท่านั้น บุคคลอื่นในนามของบริษัทผู้สนับสนุนการวิจัย คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน สำนักงานคณะกรรมการอาหารและยาอาจได้รับอนุญาตให้เข้ามาตรวจและประมวลข้อมูลของข้าพเจ้า ทั้งนี้จะต้องกระทำไปเพื่อวัตถุประสงค์เพื่อตรวจสอบความถูกต้องของ

ข้อมูลเท่านั้น โดยการตกลงที่จะเข้าร่วมการศึกษานี้ข้าพเจ้าได้ให้คำยินยอมที่จะให้มีการตรวจสอบข้อมูลประวัติทางการแพทย์ของข้าพเจ้าได้

ผู้วิจัยรับรองว่าจะไม่มีการเก็บข้อมูลใด ๆ เพิ่มเติม หลังจากที่ข้าพเจ้าขอยกเลิกการเข้าร่วมโครงการวิจัยและต้องการให้ทำลายเอกสารและ/หรือ ตัวอย่างที่ใช้ตรวจสอบทั้งหมดที่สามารถสืบค้นถึงตัวข้าพเจ้าได้

ข้าพเจ้าเข้าใจว่า ข้าพเจ้ามีสิทธิ์ที่จะตรวจสอบหรือแก้ไขข้อมูลส่วนตัวของข้าพเจ้าและสามารถยกเลิกการให้สิทธิในการใช้ข้อมูลส่วนตัวของข้าพเจ้าได้ โดยต้องแจ้งให้ผู้วิจัยรับทราบ

ข้าพเจ้าได้ตระหนักว่าข้อมูลในการวิจัยรวมถึงข้อมูลทางการแพทย์ของข้าพเจ้าที่ไม่มีการเปิดเผยชื่อ จะผ่านกระบวนการต่าง ๆ เช่น การเก็บข้อมูล การบันทึกข้อมูลในแบบบันทึกและในคอมพิวเตอร์ การตรวจสอบ การวิเคราะห์ และการรายงานข้อมูลเพื่อวัตถุประสงค์ทางวิชาการ รวมทั้งการใช้ข้อมูลทางการแพทย์ในอนาคตหรือการวิจัยทางด้านเภสัชภัณฑ์ เท่านั้น

ข้าพเจ้าได้อ่านข้อความข้างต้นและมีความเข้าใจดีทุกประการแล้ว ยินดีเข้าร่วมในการวิจัยด้วยความเต็มใจ จึงได้ลงนามในเอกสารแสดงความยินยอมนี้

.....ลงนามผู้ให้ความยินยอม

(.....) ชื่อผู้ยินยอมตัวบรรจง

วันที่ .....เดือน.....พ.ศ.....

ข้าพเจ้า ☐ ยินยอม

☐ ไม่ยินยอม

ให้เก็บตัวอย่างชีวภาพที่เหลือไว้เพื่อการวิจัยในอนาคต

.....ลงนามผู้ให้ความยินยอม

(.....) ชื่อผู้ยินยอมตัวบรรจง

วันที่ .....เดือน.....พ.ศ.....

ข้าพเจ้าได้อธิบายถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย อันตราย หรืออาการไม่พึงประสงค์หรือความเสี่ยงที่อาจเกิดขึ้นจากการวิจัย หรือจากยาที่ใช้ รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียด ให้ผู้เข้าร่วมในโครงการวิจัยตามนามข้างต้นได้ทราบและมีความเข้าใจดีแล้ว พร้อมลงนามลงในเอกสารแสดงความยินยอมด้วยความเต็มใจ

.....ลงนามผู้ทำวิจัย

(.....) ชื่อผู้ทำวิจัย ตัวบรรจง

วันที่ .....เดือน.....พ.ศ.....

.....ลงนามพยาน

(.....) ชื่อพยาน ตัวบรรจง

วันที่ .....เดือน.....พ.ศ.....

ข้าพเจ้าไม่สามารถอ่านหนังสือได้ แต่ผู้วิจัยได้อ่านข้อความในแบบคำยินยอมนี้ให้แก่ข้าพเจ้าฟังจนเข้าใจดี ข้าพเจ้าจึงประทับตราลายนิ้วมือขวาของข้าพเจ้าในแบบคำยินยอมนี้ด้วยความเต็มใจ

ประทับลายนิ้วมือขวา

ลายมือชื่อผู้อธิบาย.....

(.....)

พยาน.....(ไม่ใช่ผู้อธิบาย)

(.....)

วันที่.....เดือน.....พ.ศ.....

Appendix D

The satisfaction questionnaire

1

แบบสอบถามประเมินความพึงพอใจในการใช้ผลิตภัณฑ์

การทดสอบประสิทธิภาพและความปลอดภัยของผลิตภัณฑ์ความงามจากดาหลา
(ครีมบำรุงผิวผสมสารสกัดดาหลา)

1 ข้อมูลทั่วไป

1.1. เพศ [ ] ชาย [ ] หญิง

1.2. อายุ .....ปี

- โปรดทำเครื่องหมาย x บนตัวเลขในช่องสี่เหลี่ยมเพื่อประเมินในหัวข้อดังต่อไปนี้

(5) ดีมาก (4) ดี (3) ปานกลาง (2) พอใช้ (1) ควรปรับปรุง

2.ลักษณะทั่วไปของผลิตภัณฑ์	**ครีมบำรุงผิวผสมสารสกัดดาหลา**				
2.1 สี	5	4	3	2	1
2.2 กลิ่น	5	4	3	2	1
2.3 เนื้อของผลิตภัณฑ์	5	4	3	2	1
3. ความพึงพอใจในการใช้ผลิตภัณฑ์	**ครีมบำรุงผิวผสมสารสกัดดาหลา**				
3.1 เนื้อสัมผัสของผลิตภัณฑ์	5	4	3	2	1
3.2 ความหนืดของผลิตภัณฑ์	5	4	3	2	1
3.3 การกระจายบนผิว	5	4	3	2	1
3.4 ความเรียบเนียนของผิว	5	4	3	2	1
3.5 ความกระจ่างใสของผิว	5	4	3	2	1
3.6 ความชุ่มชื้นของผิว	5	4	3	2	1
3.7 ความพึงพอใจโดยรวม	5	4	3	2	1

4. ท่านประสบปัญหาใดๆจากการใช้ผลิตภัณฑ์ในโครงการนี้หรือไม่

[ ] ไม่มี

[ ] มี (โปรดระบุ) .....

5. ข้อเสนอแนะเพิ่มเติม

.....

.....

.....

Version 02

Date 21 Jan. 2019

<u>แบบสอบถามประเมินความพึงพอใจในการใช้ผลิตภัณฑ์</u>

<u>การทดสอบประสิทธิภาพและความปลอดภัยของผลิตภัณฑ์ความงามจากดาหลา</u>

<u>(โฟมล้างหน้าผสมสารสกัดดาหลา)</u>

1. ข้อมูลทั่วไป

1.3. เพศ [ ] ชาย [ ] หญิง

1.4. อายุ .....ปี

- โปรดทำเครื่องหมาย x บนตัวเลขในช่องสี่เหลี่ยมเพื่อประเมินในหัวข้อดังต่อไปนี้

(5) ดีมาก (4) ดี (3) ปานกลาง (2) พอใช้ (1) ควรปรับปรุง

2.ลักษณะทั่วไปของผลิตภัณฑ์	**โฟมล้างหน้าผสมสารสกัดดาหลา**				
2.1 สี	5	4	3	2	1
2.2 กลิ่น	5	4	3	2	1
3. ความพึงพอใจในการใช้ผลิตภัณฑ์	**โฟมล้างหน้าผสมสารสกัดดาหลา**				
3.1 เนื้อสัมผัสของผลิตภัณฑ์	5	4	3	2	1
3.2 การเกิดโฟมของผลิตภัณฑ์	5	4	3	2	1
3.3 ความสะอาดของผิวหลังใช้ผลิตภัณฑ์	5	4	3	2	1
3.4 ความกระจ่างใสของผิว	5	4	3	2	1
3.5 ความพึงพอใจโดยรวม	5	4	3	2	1

4. ท่านประสบปัญหาใดๆจากการใช้ผลิตภัณฑ์ในโครงการนี้หรือไม่

[ ] ไม่มี

[ ] มี (โปรดระบุ) .....

5. ข้อเสนอแนะเพิ่มเติม

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แบบสอบถามประเมินความพึงพอใจในการใช้ผลิตภัณฑ์

การทดสอบประสิทธิภาพและความปลอดภัยของผลิตภัณฑ์ความงามจากดาหลา

(ผงขัดผิวจากดอกดาหลา)

1. ข้อมูลทั่วไป

1.5. เพศ [ ] ชาย [ ] หญิง

1.6. อายุ .....ปี

- โปรดทำเครื่องหมาย x บนตัวเลขในช่องสี่เหลี่ยมเพื่อประเมินในหัวข้อดังต่อไปนี้

(5) ดีมาก (4) ดี (3) ปานกลาง (2) พอใช้ (1) ควรปรับปรุง

2.ลักษณะทั่วไปของผลิตภัณฑ์	**ผงขัดผิวจากดอกดาหลา**				
2.1 สี	5	4	3	2	1
2.2 กลิ่น	5	4	3	2	1
2.3 เนื้อของผลิตภัณฑ์	5	4	3	2	1
3. ความพึงพอใจในการใช้ผลิตภัณฑ์	**ผงขัดผิวจากดอกดาหลา**				
3.1 เนื้อสัมผัสของผลิตภัณฑ์	5	4	3	2	1
3.2 ความลื่นขณะขัดผิว	5	4	3	2	1
3.3 การกระจายของผงขัดผิว	5	4	3	2	1
3.4 ล้างออกง่าย	5	4	3	2	1
3.5 ความกระจ่างใสของผิว	5	4	3	2	1
3.6 ความชุ่มชื้นของผิวหลังล้างออก	5	4	3	2	1
3.7 ความพึงพอใจโดยรวม	5	4	3	2	1

4. ท่านประสบปัญหาใดๆจากการใช้ผลิตภัณฑ์ในโครงการนี้หรือไม่

[ ] ไม่มี

[ ] มี (โปรดระบุ) .....

5. ข้อเสนอแนะเพิ่มเติม

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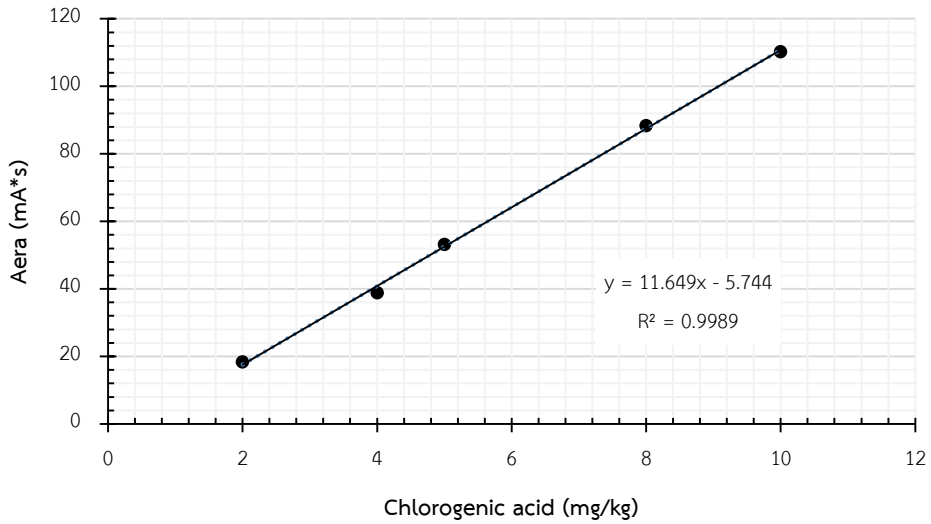
Date 21 Jan. 2019

Appendix E

The calibration curve

Standard curve of chlorogenic acid by HPLC



Appendix F

The satisfaction evaluation of the volunteers

The mean of satisfaction analysis on TG cream, TG foam, and TG scrub

Topic	TG cream
Satisfaction on product	
Color	4.67 ± 0.56
Odor	4.67 ± 0.56
Texture	4.63 ± 0.49
Satisfaction on skin feeling	
Softness of cream	4.67 ± 0.56
Stickiness	4.33 ± 0.64
Spreadability	4.67 ± 0.48
Skin smoothness	4.50 ± 0.59
Skin lightening	4.46 ± 0.78
Skin moisture	4.63 ± 0.58
Overall satisfaction	4.67 ± 0.48

Topic	TG foam
Satisfaction on product	
Color	4.38 ± 0.82
Odor	4.33 ± 0.76
Satisfaction on skin feeling	
Texture of foam	4.50 ± 0.66
Foam forming	4.58 ± 0.65
Cleansing action	4.58 ± 0.58
Skin lightening	4.46 ± 0.66
Overall satisfaction	4.67 ± 0.48

Topic	TG scrub
Satisfaction on product	
Color	4.29 ± 0.81
Odor	4.33 ± 070
Texture	4.25 ± 0.68
Satisfaction on skin feeling	
Texture of scrub	4.33 ± 0.64
Skin slippery	4.42 ± 0.78
Spreadability	4.46 ± 0.66
Wash off easily	4.58 ± 0.65
Skin lightening	4.33 ±0.70
Skin moisture	4.33 ± 0.70
Overall satisfaction	4.63 ± 0.49

LIST OF PRESENTATIONS

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2. **ณัฐวุฒิ หวังสมนึก** ลภัสรดา มุ่งหมาย เกรียงศักดิ์ เม่งอำพัน และดวงพร อมรเลิศพิศาล. 2561. องค์ประกอบฟีนอลิกและฤทธิ์ต้านอนุมูลอิสระของใบดาหลาเพื่อใช้เป็นวัตถุดิบในเครื่องสำอาง. ใน *การประชุมวิชาการระดับชาติประจำปี 2561 มหาวิทยาลัยแม่โจ้*, (น. 174-182). เชียงใหม่: มหาวิทยาลัยแม่โจ้.

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1. **Whangsomnuek, N.**, Mungmai, L., Mangumphan, K. and Amornlerdpison, D. Bioactive compounds and its biological activity from leaves of torch ginger for value added as cosmetic product. *The 9th International Graduate Research Conference*. May 17th-18th, 2018. The Empress International Convention Center, The Empress Hotel, Chiang Mai, Thailand.

2. **ณัฐวุฒิ หวังสมนึก** ลภัสรดา มุ่งหมาย เกรียงศักดิ์ เม่งอำพัน และดวงพร อมรเลิศพิศาล. องค์ประกอบฟีนอลิกและฤทธิ์ต้านอนุมูลอิสระของใบดาหลาเพื่อใช้เป็นวัตถุดิบในเครื่องสำอาง. *การประชุมวิชาการระดับชาติประจำปี 2561 มหาวิทยาลัยแม่โจ้*, 11-13 ธันวาคม 2561. อาคารเฉลิมพระเกียรติสมเด็จพระเทพรัตนราชสุดา, มหาวิทยาลัยแม่โจ้ เชียงใหม่.

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3. รัตนาภรณ์ จันทร์ทิพย์ อุเทน จำใจ **ณัฐวุฒิ หวังสมนึก** และดวงพร อมรเลิศพิศาล. 2560. เครื่องดื่มสุขภาพชนิดเจลจากสาหร่ายน้ำจืด. การประชุมวิชาการ และการประกวดนวัตกรรมบัณฑิตศึกษาแห่งชาติ ครั้งที่ 1. 17-18 สิงหาคม 2560. ศูนย์ประชุมนานาชาติดิเอ็มเพลส โรงแรมดิเอ็มเพลส จังหวัดเชียงใหม่. [รางวัลเหรียญเงิน]

Patents:

Patent in title “สารผสมเครื่องสำอางจากดาหลา” Application No. (*in press*)

PUBLICATION ARTICLE

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Article

Efficiency of Skin Whitening Cream Containing *Etlingera elatior* Flower and Leaf Extracts in Volunteers

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Abstract: Our previous research demonstrated that *Etlingera elatior* possesses whitening and anti-aging properties and also contains bioactive ingredients for cosmeceuticals. Therefore, this research work aimed to evaluate the efficiency of whitening cream containing both the flower and leaf extracts of *E. elatior* in human volunteers and their degree of skin irritation. Both the flower and leaf extracts were formulated as a cosmetic called "FL1 cream", which was assessed for its physical properties and underwent an accelerated stability test. The FL1 cream was also evaluated for skin irritation and its skin whitening effect among 24 healthy volunteers who used it for four weeks. The FL1 cream demonstrated good physical stability under the various conditions for three months, along with six cycles of heating/cooling. The irritation analysis showed that irritation reactions were absent in all volunteers. The efficiency of FL1 cream in improving the appearance of skin whitening was demonstrated by a significant ($p < 0.05$) and continuous decrease in melanin content compared with the initial value. Additionally, the L^* value was significantly and continuously increased after application of the FL1 cream. The highest melanin reduction was 6.67%. The FL1 cream containing *E. elatior* extracts can be used as a whitening cream in cosmetics.

Keywords: clinical evaluation; *Etlingera elatior*; skin irritation; whitening cream

1. Introduction

Melanin is a pigment that plays an important role in skin protection against UV damage and is involved in pigmentary changes in skin color. It is formed through oxidation and by the amino acid tyrosine through cyclization. Tyrosinase is a melanogenic enzyme that catalyzes the rate-limiting synthetic for melanin production. Melanogenesis initially occurs through hydroxylation of L-tyrosine by tyrosinase converted to L-3,4-dihydroxyphenylalanine (L-DOPA) and by the oxidation of L-DOPA to DOPA-quinone, and eventually to melanin pigments [1,2]. Moreover, in another mechanism, a processes of protein glycosylation, Neu5Acα(2-6)Gal- and possibly sialyl(α2-3)gal-terminated glycans play an important role in melanogenesis and melanosome transfer to keratinocytes [3]. Overproduction and accumulation of melanin results in several skin pigmentation disorders, including solar lentigos (age spots), melasma, freckles, and post-inflammatory hyperpigmentation [4]. Therefore, whitening ingredients that result in inhibited tyrosinase activity, including inhibitory effects on melanogenesis and the melanosome transfer process, are essentially significant for reducing melanin synthesis. Tyrosinase inhibitors are obtained from both natural and synthetic sources, such as hydroquinone, arbutin, kojic

acid, L-ascorbic acid, tranexamic acid, ellagic acid, and thiamidol [5]. Lately, Baswan et al. [6–8] reported that cytidine, though not a tyrosinase inhibitor, inhibits melanin synthesis and the melanosome transfer process by interfering with glycosylation processes. In current times, the desire to lighten the complexion has become popular in Asian people seeking beautiful-looking skin. Skin whitening products that propose to lighten the skin's appearance or treat hyper-pigmentation have become highly desirable in the cosmeceutical industries [9]. The use of skin whitening cosmetics has an important role in achieving skin lightening, as well as in diminishing dark spots on the skin [10,11]. Bioactive compounds from plants are gaining popularity for use as cosmetic ingredients in contemporary formulations, as they also contain vitamins, antioxidants, essential oils, proteins, phenolic compounds, and other active compounds [12,13]. Diverse bioactive compounds, including several phenolic compounds, have been reported to contain natural antioxidant compounds, along with having anti-aging, anti-microbial, anti-inflammatory, and tyrosinase-inhibiting actions. Further, these compounds in cosmetic products tend to be safer, biodegradable, more environmentally friendly, and more biologically active when compared with synthetic ingredients [14–16].

Etlingera elatior is a plant in the Zingiberaceae family that is widely cultivated in Southeast Asia as an ornamental flower or is locally consumed as food. The biological activities of *E. elatior* flowers and leaves have been reported over the past few decades. The presence of cosmeceutical properties that are proposed to improve skin appearance, such as antioxidant activity, has been suggested by ferric reducing antioxidant power (FRAP) assay, lipid peroxidation assay, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, and inhibition of tyrosinase activity, as well as non-toxic properties, as shown by brine shrimp lethality assay [17–20].

In our previous study, *E. elatior* flower and leaf extracts were screened for their amino acid content, phenolic content, and biological activities for cosmetic properties, such as collagenase and tyrosinase inhibition, as well as antioxidant properties, via assay on 2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), DPPH, and superoxide radical scavenging activities. Results indicated that the leaf extract exhibited the strongest antioxidant inhibitor property, higher than those of trolox and gallic acid, and showed the highest phenolic contents of isoquercetin, catechin, and gallic acid, as well as moderate anti-collagenase and amino acid contents. The flower extract showed greater potential to inhibit collagenase activity and more amino acids than the leaf extract, while also showing moderate phenolic compound levels and antioxidant activity. In addition, both the flower and leaf extracts were shown to be capable of suppressing melanogenesis through inhibiting tyrosinase activity [21].

On the basis of the previous study, the research team proposed developing a cosmetic cream for the purpose of skin whitening and with other beneficial properties. The cosmetic cream that was developed contained both the flower and leaf extracts of *E. elatior* together, in a formulation called "FL1 cream". The objective of this study was to evaluate the efficiency of the skin whitening cream containing both the flower and leaf extracts of *E. elatior* in human volunteers and to undertake an irritation test as well.

2. Materials and Methods

2.1. Plant Sample

E. elatior was purchased from a cultivator in the Reso District of Narathiwat Province, Thailand. The flowers and leaves were rinsed several times with distilled water, cut into small pieces, and subsequently shade-dried with a hot-air oven at 50 °C. Dried samples were ground into a fine powder using a high-speed disintegrator machine.

2.2. Plant Extraction

An aqueous solution (1000 mL) including 100 g of powdered flowers or leaves was heated at 50 °C for 8 h, then centrifuged at 4000 rpm for 5 min at ambient temperature and filtered through

Whatman No.1 filter paper. The filtrate extracts were concentrated by a rotary evaporator (KNF RC 900, KNF Neuberger, Trenton, NJ, USA) under vacuum pressure and then lyophilized using a freeze-dryer (Labogene CS 55-4, LaboGene A/S, Allerød, Denmark) to obtain the dried flower and leaf extracts.

2.3. Tyrosinase Inhibition

Tyrosinase inhibition was evaluated using the dopachrome method described by Masuda et al. [22], with some modifications. Solutions of 200 U/mL of tyrosinase from mushrooms and 2.5 mM of L-DOPA (3, 4-dihydroxy-L-phenylalanine) were prepared with 20 mM of phosphate buffer (pH 6.8). The different concentrations and ratios of both flower and leaf extracts, comprising 0.5% and 1% in ratios of 1:1, 1:2, and 1:3 of flower/leaf extracts, were tested. Tyrosinase reactions were performed in a 96-well plate, with each well containing 20 µL of different samples of the extracts, 40 µL of tyrosinase solution, and 140 µL of phosphate buffer. The 96-well plate was allowed to stand for 10 min, and then the reaction was started by adding 40 µL of L-DOPA solution (with phosphate buffer used as a control reaction). The 96-well plate was then incubated at an ambient temperature for 20 min, and the absorbance of tyrosinase activity was measured at 492 nm using a microplate reader (Biochrom EZ read 400) with kojic acid used as a reference substance. Each sample was completed with a blank plate of the sample test, except for the tyrosinase solution. The percentage of tyrosinase inhibition activity was obtained using the following equation:

$$\text{Tyrosinase inhibition (\%)} = [\{(A - B) - (C - D)\} / (A - B)] \times 100.$$

In this equation, A is the absorbance of the control reaction without a sample, B is the absorbance of the blank of the control reaction without a sample, C is the absorbance of the sample test with the presence of sample extracts, and D is the absorbance of the blank of the sample with the presence of sample extracts.

2.4. Formulation of the Whitening Cream

The formulations of FL1 cream containing *E. elatior* flower and leaf extracts are shown in Table 1. The process of preparation for the formulation included the addition of oil phase to the water phase, after which both phases were heated to 80 °C before mixing. Stirring was continued using a mixer homogenizer until the temperature was approximately 50 °C, then sodium hydroxide and preservative were added. Finally, the stirring was continued until the emulsion cooled to an ambient temperature.

Table 1. The formulations of FL1 cream containing *Etlingera elatior* flower and leaf extracts.

Phase	Trade Name	INCI Name	%
A (Oil Phase)	Nikkomulese LH	Glycerin (and) Hydrogenated lecithin (and) Hydroxypropyl methylcellulose stearoxy Ether (and) Squalane (and) Sodium methyl stearoyl taurate	4.00
	DC 350	Dimethicone	3.00
	Squalane	Squalane	5.00
	Cetyl alcohol	Cetyl alcohol	0.30
	Stearyl alcohol	Stearyl alcohol	0.30
B (Water Phase)	Na_2EDTA	Disodium EDTA	0.10
	Glycerin	Glycerin	3.00
	Butylene glycol	Butylene glycol	4.00
	Carbopol ultrez 21 polymer	Acrylates/C10-30 alkyl acrylate crosspolymer	0.50
	1Flower/1leaf extract	*Etlingera elatior*	1.00
	DI water	Aqua	q.s. 100
C	NaOH (18% w/v)	Sodium hydroxide (for adjusting pH to 5.5)	0.30
D	Spectrastat BHL	Caprylhydroxamic acid (and) 1,2-hexanediol (and) butylene glycol	2.00

2.5. Stability Testing

The stability was evaluated by the centrifugation method at 4000 rpm and at 20 °C for 30 min to determine the mechanical stress [23]. Accelerated stability testing was also performed in various conditions under room temperature, 4 °C, and 45 °C for three months and six cycles of the heating/cooling method (45 °C, 48 h alternated with 4 °C, 48 h for 1 cycle) [24]. During the stability testing, the physiochemical appearance of the cream, including any change in color or odor, pH, viscosity, phase separation, and precipitation, was investigated.

2.6. Clinical Evaluation in Volunteers

The skin irritation testing and skin whitening efficiency testing on healthy volunteers in this study were approved by the University of Phayao Human Ethics Committee, Thailand (Project identification code: 3/018/61). The efficiency evaluations were performed on 24 healthy volunteers (aged 25–55, n = 24). They were investigated for any skin disease and cosmetics allergy history. Before being enrolled in the study, each volunteer received the information protocol that contained the terms and conditions of the clinical testing and signed an informed consent form.

2.7. Skin Irritation Testing

The skin irritation testing was done using a modified Draize model, as described by Bashir and Maibach [25], using Finn chambers®. Skin irritation was performed on the upper outer arm (left-hand side) of volunteers, with each chamber saturated by FL1 cream containing *E. elatior* extracts, 1% w/v of sodium lauryl sulfate (used as a positive reaction), and deionized water (as a negative reaction), before being covered for 48 h. Subsequently, we observed the erythema and edema at 1, 24, and 48 h after removing the patch. Each of the test substances were evaluated based on the primary dermal irritation index (PDII) using the Draize scoring system.

2.8. Efficiency Testing in Human Volunteers

The skin improvement test of the whitening cream was performed with 24 healthy volunteers. They were tested for reduced melanin content after applying the FL1 cream twice a day, morning and evening, on the skin of their left forearm for four weeks. Following Leelapornpisid et al. [24], an untreated area on each volunteer's right forearm was used as a control. Before the study, the volunteers were rested in a suitable room at 20 °C and 50% relative humidity (RH) for 15 min [26]. The study procedures were conducted on Day 0 for the initial value, then on the 1st, 2nd, 3rd, and 4th weeks. The melanin content and CIE-L^* value were measured using the skin color probe from DermaLab® Combo (Cortex Technology). Finally, the volunteers were asked to fill out a questionnaire regarding their satisfaction with the whitening cream on Day 30. The ability of the FL1 cream to reduce melanin pigment was obtained using the following equation:

$$\text{Reduction activity (\%)} = [(M_{d0} - M_{dm}) / (M_{d0})] \times 100.$$

In this equation, M_{d0} is the melanin content on the initial day and M_{dm} is the melanin content on the day of measuring.

2.9. Statistical Analysis

The statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS), version 17.0 for Windows. Viscosity data were analyzed by the use of a one-way analysis of variance (ANOVA) with Tukey's HSD test. A repeated measures ANOVA with pairwise comparisons by the Bonferroni method was used to analyze the skin whitening effect in volunteers. Statistical significance was determined to be at $p < 0.05$.

3. Results and Discussion

3.1. Tyrosinase Inhibition Activity

Tyrosinase is a crucial rate-limiting enzyme in direct melanin synthesis. Therefore, cosmetic products containing tyrosinase inhibitors are becoming more commonly used for their skin whitening properties [27]. In our previous study, flower and leaf at 10 mg/mL showed tyrosinase inhibition activity of 24.37% ± 0.52% and 31.48% ± 1.28%, respectively [21]. This study aimed to develop a cosmetic cream containing both the flower and leaf extracts of *E. elatior* together for the main purpose of skin whitening. Therefore, a suitable concentration and ratio of mixed flower and leaf extracts were investigated. Mushroom tyrosinase was used to determine the role of *E. elatior* in the process of melanogenesis. As shown in Figure 1, the results demonstrated that all concentrations of mixed flower and leaf extracts inhibited tyrosinase activity. In addition, flower and leaf extracts at a concentration of 1% and in a ratio of 1:1 exhibited the highest tyrosinase inhibition activity at 74.61% ± 0.00%. These results indicate that 1% of mixed flower and leaf extracts of *E. elatior* in a ratio of 1:1 is a suitable concentration for use in formulating the cosmetic "FL1". Furthermore, methanol flower and leaf extracts did not show a cytotoxic effect on WRL-68 (human liver) or Vero (African green monkey kidney), or in an *Artemia salina* lethality bioassay [18,20]. Hence, they might be promising for safe use in cosmetic products.

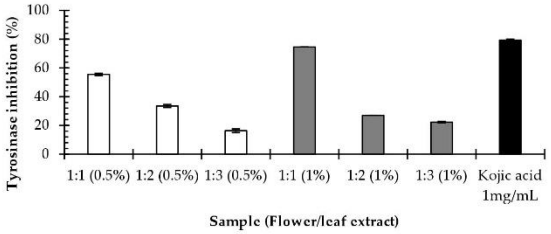

Figure 1. The effect of mixed *E. elatior* flower and leaf extracts on tyrosinase inhibition activity.

3.2. Formulation of the Whitening Cream

In this study, the FL1 cream was evaluated for its physical properties, including pH, color, viscosity (Pa.s), and by centrifugation test, as shown in Table 2. After cosmetic formulation, the physical appearance of the FL1 cream was assessed. The texture was found to be tender, with very good spreadability and a soft feeling on the skin. The FL1 cream was stable and showed no phase separation when centrifuged at 4000 rpm for 30 min.

Table 2. The physical properties of the FL1 cream formulation.

Parameters	pH	Color			Viscosity (Pa.s)	Centrifugation Test
		L^*	a^*	b^*		
FL1 cream	5.5	60.69 ± 0.63	2.47 ± 0.26	20.43 ± 1.08	6.20 ± 0.02	Stable

3.3. Stability Testing

Stability testing under accelerated conditions showed that the pH of the cream kept in all conditions did not change after testing, and separation and precipitation of the FL1 cream were not observed (as shown in Table 3). The viscosity under all conditions compared to the start condition was maintained at room temperature and 4 °C and under heating/cooling cycles was not significantly different ($p > 0.05$). At 45 °C, there was an obvious decrease in viscosity, which may be the effect of heat. Moreover, the

physical properties of color under all conditions were also significantly stable ($p > 0.05$), and odor was stable after the stability tests (data not shown). Therefore, FL1 could be stored long term without any change in the pH, color, or viscosity.

Table 3. The stability testing results of FL1 cream after three months and after heating/cooling for six cycles.

Conditions	pH	Viscosity (Pa.s)	Separation and Precipitation	Color		
				L*	a*	b*
Initial	5.5	6.20 ± 0.02 [a]	x	60.69 ± 0.63 [a]	2.47 ± 0.26 [a]	20.43 ± 1.08 [a]
RT	5.5	6.15 ± 0.02 [a]	x	60.18 ± 0.39 [a]	2.40 ± 0.23 [a]	20.07 ± 0.89 [a]
4 °C	5.5	6.17 ± 0.06 [a]	x	60.09 ± 0.34 [a]	2.49 ± 0.23 [a]	19.75 ± 0.84 [a]
45 °C	5.5	6.01 ± 0.03 [b]	x	60.28 ± 0.95 [a]	2.66 ± 0.64 [a]	21.62 ± 0.45 [a]
H/C	5.5	6.15 ± 0.05 [a]	x	60.78 ± 0.51 [a]	2.51 ± 0.41 [a]	20.13 ± 0.24 [a]

Values are expressed as means ± standard deviation. For the viscosity and color values, not sharing the same letter in each column indicates significant difference at $p < 0.05$. RT = Room temperature; H/C = Heating/cooling conditions.

3.4. Irritation Testing

The dermatological test for irritation and allergy effects on human volunteers was performed to ensure the safety of the FL1 cream. The 24 volunteers were tested with FL1 cream, 1% w/v of sodium lauryl sulfate, and deionized water. The results are shown in Table 4. The FL1 cream was non-irritating, with a low Primary Dermal Irritation Index value (PDII < 0.5), whereas sodium lauryl sulfate (SLS), which was used as a positive control, was revealed to be slightly irritating (PDII range from 0.5 to 2.0).

Table 4. The Primary Dermal Irritation Index (PDII) value and skin irritation reaction observed for FL1 cream.

Test Substances	PDII	Classification of Skin Irritation
FL1 cream	0.00	Non-irritating
1% w/v SLS (positive)	0.80	Slightly irritating
DI water (negative)	0.00	Non-irritating

3.5. Skin Whitening Testing

The whitening effect of FL1 cream containing *E. elatior* extracts was evaluated by comparison before the treatment. This was done by measuring the amount of melanin using DermaLab® Combo (Cortex Technology, Hadsund, Denmark) to confirm the efficiency of this product. As shown in Table 5, the results demonstrated that the melanin content in the skin when using FL1 cream was significantly and continuously decreased after one week, until week three of testing ($p < 0.05$). In week four, the melanin content increased, which may be due to the time of testing (March 2019 to April 2019) in Thailand being in the summer season. This period has a corresponding increase in UV exposure, which is one of the causes of melanin synthesis [28,29]; the UV index is shown in Figure 2. Furthermore, the L^* value was used for indicating skin lightening after applying the FL1 cream, and it was found that the L^* value continuously increased from the 1st to the 3rd week ($p < 0.05$), as did the melanin content. For the untreated areas, the data still showed constant melanin content until week two of testing with no significant difference ($p > 0.05$), but the content increased from the 3rd to the 4th week. However, the L^* value of the untreated area was slightly increased until week three and slightly decreased subsequently until week four of testing. In addition, the melanin reduction activity of the FL1 cream reached its highest level of 6.67% after three weeks of testing (Figure 3). There were no significant differences in melanin reduction observed between the FL1-treated and untreated areas of the volunteers during the testing period. These results indicated that *E. elatior* flower and leaf extracts achieved a decent skin whitening effect on human volunteers.

Table 5. The melanin content (%) and L* value after application of the FL1 cream for four weeks.

Test	Parameter	Baseline	Week 1	Week 2	Week 3	Week 4
FL1 cream	Melanin content (%)	36.00 ± 0.33 ^{a}	35.13 ± 0.38 ^{b}	33.93 ± 0.53 ^{c}	33.60 ± 0.44 ^{c}	35.39 ± 0.55 ^{ab}
	L* value	37.39 ± 0.40 ^{a}	38.54 ± 0.47 ^{b}	39.41 ± 0.47 ^{c}	41.00 ± 0.65 ^{d}	39.13 ± 0.70 ^{bc}
Untreated area	Melanin content (%)	36.44 ± 0.37 ^{a}	36.15 ± 0.34 ^{a}	36.01 ± 0.34 ^{a}	37.13 ± 0.39 ^{ab}	37.52 ± 0.47 ^{b}
	L* value	37.57 ± 0.42 ^{a}	38.07 ± 0.47 ^{ab}	38.33 ± 0.46 ^{b}	37.95 ± 0.42 ^{a}	36.54 ± 0.80 ^{a}

Values are expressed as means ± standard error. For each parameter of each sample test, not sharing the same letter in each row indicates significant difference at $p < 0.05$.

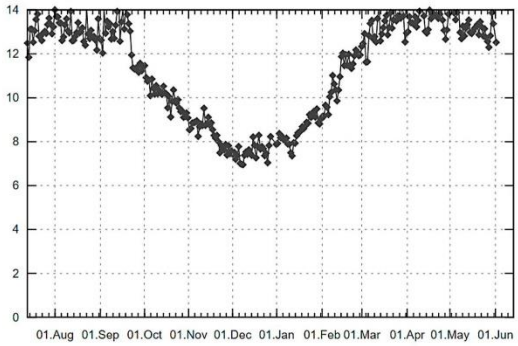

Figure 2. The UV index in Chiang Mai city, Thailand, from August 2018 to June 2019, © weatheronline.co.uk [36].

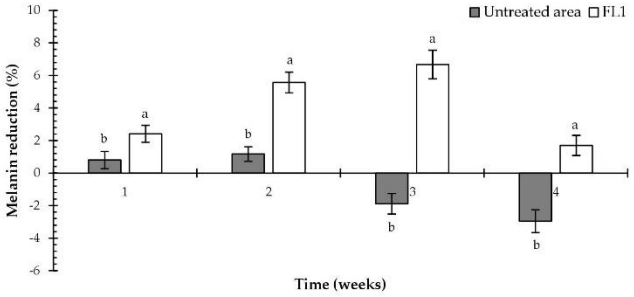

Figure 3. The effect of FL1 cream on melanin reduction. Values are expressed as means ± standard error. For groups in the same week, not sharing the same letter indicates significant difference at $p < 0.05$.

Traditionally, commercial skin whitening agents for suppressing tyrosinase activity have included hydroquinone, arbutin, kojic acid, azelaic acid, ascorbic acid, ellagic acid, and tranexamic acid. These have been widely used in formulations of cosmetic products, with some drawbacks and side effects [27,30]. In the past few decades, research has demonstrated that natural bioactive compounds are increasingly being used in whitening cosmetic formulations. In addition, there are several plant extracts that have been shown to be effective agents to suppress the overproduction of melanin or to regulate melanin synthesis, such as *Cassia fistula* flowers [31], *Asphodelus microcarpus* [32], *Magnolia officinalis* [33], *Dendrobium tosaense* [34], and *Kummerowia striata* [35]. The results of the present study indicated that

extracts of *E. elatior* flowers and leaves achieved a decent skin whitening effect on human volunteers; therefore, these extracts act as the active whitening ingredient in FL1 cream.

3.6. Satisfaction Testing

The volunteers were asked to fill in a questionnaire after using the FL1 cream for 30 days. The satisfaction level was determined using a 5-point scale, in which the point value represented the volunteers' feelings about how well the product worked, from very well (5) to very poorly (1). The results revealed that the volunteers' satisfaction with the FL1 cream was high, with responses between "well" and "very well" for all areas measured, as shown in Figure 4. Further, the most satisfying areas of the FL1 cream were the softness of the cream, its spreadability, and overall satisfaction, which had a mean of 4.67, while the lowest was the cream's glossiness, with a mean of 4.33. Additionally, none of the volunteers suffered skin irritation or allergic reactions during the test period.

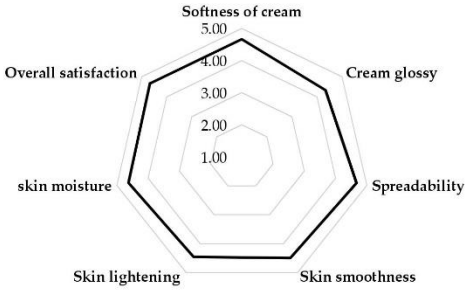

Figure 4. The satisfaction of the volunteers with the FL1 cream.

4. Conclusions

The present study demonstrated that both the flower and leaf extracts of *E. elatior* could potentially be a natural whitening ingredient for commercial cosmetics. In our previous study, aqueous extracts of the flowers and leaves revealed the presence of isoquercetin, catechin, and gallic acid [21], which are capable of inhibiting tyrosinase activity, leading to a skin lightening effect useful in cosmetic applications [37–39]. The results revealed that with the use of the cream, the melanin content decreased and the L^* value increased compared with initial values. The data showed an increased melanin reduction after application of FL1 to the skin when compared to an untreated area. The FL1 cream was found to be safe, with no reported irritation of the skin of the volunteers, and it was also found to be satisfactory by the volunteers. Therefore, FL1 cream containing *E. elatior* flower and leaf extracts might be an effective whitening cosmetic for improving skin appearance.

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Full Paper

Bioactive compounds of aqueous extracts of *Etlingera elatior* (Jack) R.M.Sm. flower and leaves for cosmetic ingredients

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Abstract: *Etlingera elatior* (Jack) R.M.Sm. (*E. elatior)* is a plant of the Zingiberaceae family that possesses medicinal properties and is widely known and cultivated in Thailand as an ornamental flower or locally consumed as food. This research aims to evaluate the bioactive compounds and biological activities of *E. elatior* flowers and leaves for cosmetic application. Aqueous extracts of *E. elatior* were examined for amino acid content and phenolic compounds using GC-MS and LC-MS, respectively. The extracts were evaluated for their antioxidant activities via assays for ABTS, DPPH and superoxide radical-scavenging. The extracts were also assessed for activity of the tyrosinase enzyme and collagenase inhibition to determine possible skin benefits. The total phenolic contents of the flower and leaf extracts were determined to be 38.68 ± 0.45 and 246.52 ± 0.26 mg GAE/g, respectively. The LC-MS analysis showed that the major components of both extracts were isoquercetin, catechin and gallic acid. In addition, the amino acid contents of these extracts, which included lysine and leucine, featured a compound that may be effective in improving the formation of collagen and reducing wrinkle appearance when used on skin. The antioxidant activity established that both extracts can be used as potential sources of natural antioxidants with ABTS, DPPH and superoxide radical-scavenging, as well as trolox and gallic acid. In addition, flower and leaf extracts were shown to inhibit tyrosinase and collagenase activities. The *E. elatior* flower and leaf extracts exhibit potential in their biological activities and bioactive compounds to be used as active ingredients for anti-wrinkle and whitening purposes in cosmetic applications.

Keywords: *Etlingera elatior*, antioxidant, tyrosinase, collagenase, cosmetic ingredients

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INTRODUCTION

Skin aging is a dermatological change influenced by several factors, including age, hormonal changes, environmental exposures and metabolic processes [1]. The prominence of skin-aging effects caused by environmental factors, particularly ultraviolet (UV) radiation exposure, damage skin through the generation of reactive oxygen species (ROSs). ROSs are related to extracellular matrix (ECM) protein degradation in the epidermal and dermal layers, which include collagen and elastin. Matrix metalloproteinases (MMPs) are enzymes involved in ECM degradation and include collagenase and gelatinase. The response of ECM degradation impacts the integrity of the skin structure, resulting in wrinkles, dryness and roughness [2-3]. Further, inhibiting ECM degradation has been determined to combat the skin-aging process through the generation of ROSs [4].

Currently, bioactive compounds from natural plants are becoming popular for their uses as cosmetic ingredients in contemporary formulations. Their phytochemical compounds are comprised of vitamins, essential oils, proteins, phenolic compounds and other properties that engage in several beneficial antioxidant, anti-inflammatory and anti-tyrosinase activities, as well as possessing anti-aging effects [5-6]. Furthermore, the natural ingredients utilised in cosmetic products are gentle, biodegradable, less toxic as well as environmentally friendly when compared with synthetic ingredients [7].

E. elatior is a plant of the Zingiberaceae family that has medicinal properties and is widely cultivated in Southeast Asia [8]. The botanical characteristics of *E. elatior* include growth of up to 5-6 m tall from their underground groves, and the width of the stout rhizome is 3-4 cm in diameter. The leaves are entirely green, feature a lanceolate shape and are up to 81 cm in length. The tall stems, formed by sheaths of leaves, are pseudostems that grow from underground rhizomes. The waxy inflorescences are shaped like spearheads when young, and when blossoming, the bracts are larger and feature prominent red and pink colouration. The fruits are shaped like pineapples and have green to reddish colouration, housing many black seeds inside [9-11].

The phytochemical composition of *E. elatior* flowers and leaves has been reported several times over the past decades. Extracts of both flowers and leaves have revealed the presence of cosmeceutical activities that can improve skin problems [8]. The methanolic extracts of the flower revealed the presence of active compounds containing flavonoids, terpenoids, saponins, tannins and carbohydrates [12]. Meanwhile, the ethanolic extract of leaves uncovered a group of phenolic compounds, including chlorogenic acid and caffeoylquinic acid content that possesses antioxidant capacity, tyrosinase inhibition and antibacterial properties [13]. Regarding pharmacological and biological characteristics, the flower extract exhibits antioxidant activities via scavenging DPPH and chelating ferrous ions, as along with anti-pathogenic bacteria and anticancer cell line (MCF-7 and MDA-MB-231) functioning [14]. Previous studies have revealed that the hydroglycolic extract of flowers have skin whitening and anti-aging properties via tyrosinase and collagenase inhibitory activity [15].

Many studies have been carried out on assessing the biological and pharmaceutical properties of bioactive compounds from flowers or the leaf extract of *E. elatior* by several extractions, such as methanol, ethanol, acetone and water/propylene glycol. The biological activities of *E. elatior* flowers and leaves have been reported now over the past few decades focusing on their medicinal activities, whether anti-oxidant, anti-bacteria or anti-cancer [12-19]. However, investigations of both the aqueous extraction of flowers and leaves for cosmetic ingredients and cosmeceutical applications have not been reported. Therefore, this research seeks to assess the bioactive compounds of the

respective amino acid content and phenolic compounds, and also analyse the biological activities of aqueous extracts of *E. elatior* flowers and leaves, including antioxidant, anti-tyrosinase and anti-collagenase activities, to determine their value as cosmetic ingredients.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu's reagent and sodium carbonate were purchased from BDH Prolabo Chemicals (Paris, France). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), DPPH (2, 2-diphenyl-1-picrylhydrazyl), nitrotetrazolium blue chloride, phenazine methosulfate, NADH ($C_{21}H_{27}N_7Na_2O_{14}P_2 \cdot xH_2O$), potassium persulfate, sodium phosphate monobasic, sodium phosphate dibasic, FALGPA (N-[3-(2-Furyl)acryloyl]-Leu-Gly-Pro-Ala), tyrosinase from mushroom and methanol were purchased from Sigma-Aldrich (St. Louis, USA). ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), gallic acid, kojic acid, and L-DOPA (3, 4-dihydroxy-L-phenylalanine), and ammonium formate were procured from Sigma-Aldrich (Steinheim, Germany). Collagenase type I from *Clostridium histolyticum* and EGCG ((-)-Epigallocatechin gallate) were obtained from Merck (Billerica, USA). Acetonitrile was purchased from Labscan (Ireland). All other reagents were of analytical or chromatographic grade.

Sample preparation

E. elatior flowers and leaves were purchased from a cultivator in the Reso district of Narathiwat Province, Thailand. The samples were rinsed several times with distilled water and cut into small pieces, subsequently being air dried (while being protected from sunlight) with a hot-air oven at 50°C until dry. Dried samples were ground into fine powder using a high-speed disintegrator machine and then stored in amber zip lock bags at room temperature.

Sample extraction

The powdered samples (100 g each) were extracted with 1,000 mL of distilled water at 50°C for 8 h to obtain the aqueous extracts. The samples were centrifuged at 4,000 rpm for 5 min at an ambient temperature and filtered through Whatman No. 1 filter paper to separate the pellets. Then, the aqueous samples were concentrated via evaporation under vacuum pressure. Finally, the samples were lyophilized via freeze drying (Labconco Model 7750020, Allerød, Denmark) to obtain the dried flower and leaf extracts or called aqueous extract *E. elatior* flower and leaf, and then the yields of the extracts were calculated.

Total phenolic content

The total phenolic contents of extracts were measured through the Folin-Ciocalteu method [20]. For this technique, 200 µL of different concentrations of the extracts were added to a test tube, followed by 1,000 µL of 10% (v/v) Folin-Ciocalteu's reagent and 800 µL of sodium carbonate (7.5% w/v). The mixture was incubated for 60 min at an ambient temperature. Next, absorbance was measured at 765 nm using a spectrophotometer (Thermo Scientific™ Evolution 260 Bio, Vantaa, Finland). The total phenolic content was expressed as the gallic acid equivalent (GAE) in milligrams per gram of dried extract (mg GAE/g extract).

Phenolic compound analysis

The phenolic compounds of extracts were determined using a liquid chromatography–mass spectrometry (LC-MS) system (Agilent 1100 series LC/MSD SL, Palo Alto, USA). The following

column was used: LiChroCART (150 x 4.6 mm) Purospher STAR 120 RP-18e (5 µm). Phenolic compounds were determined as described by Peñarrieta *et al.* [21] with some modifications. The mobile phase consisted of acetonitrile (solvent A), and a 10 mM ammonium formate buffer (pH 4) with formic acid (solvent B) was used. The elution flow rate was 1.0 mL/min. The elution of solvent was performed employing the following different conditions: a constant of 100% of B solvent from 0 min to 5 min; 0% to 20% of solvent A from 5 min to 10 min; a constant of 20% of solvent A from 10 min to 20 min; and 20% to 40% of solvent A from 20 min to 60 min. Results were acquired from the mass spectrometer and diode array at wavelengths of 270, 330, 350 and 370 nm. Results from the LC-MS system were compared with apigenin, catechin, eriodictyol, gallic acid, hydroquinin, isoquercetin, kaempferol, quercetin, rutin and tannic acid standard compounds to quantify the amounts of phenolic compounds.

Amino acid analysis

Sample extracts were sent to the Central Laboratory Co., Ltd. (Chiang Mai, Thailand) for amino acid content analysis where an in-house method based on the AOAC official method (2000) was used [22]. The 994.12 method (performic acid oxidation with acid hydrolysis-sodium metabisulfite method) was detected by the GC (Agilent Model 6890N, Wilmington, USA), MS (Agilent Model 5973 inert, Wilmington, USA) and 998.15 method (ion exchange chromatographic method) with the Zebron ZB-AAA capillary column (Phenomenex, Torrance, USA).

Antioxidant activities

1. ABTS radical-scavenging

ABTS (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)) radical-scavenging activity was determined as described by Re *et al.* [23] with particular modifications. The $ABTS^{\bullet+}$ was generated by mixing an equal volume of the 7 mM solution of ABTS with 2.45 mM of potassium persulfate and allowing the mixture to stand for 12 h in the dark at room temperature. Before performing the assay, the $ABTS^{\bullet+}$ solution was diluted to achieve a final absorbance of 0.7 ± 0.5 at 734 nm. A total of 200 µL of different concentrations of each extract was mixed with 1,800 µL of the $ABTS^{\bullet+}$ solution and allowed to stand for 6 min at an ambient temperature (with distilled water used for a control reaction). Then, the absorbance at 734 nm was measured using a spectrophotometer. Trolox was utilised as a reference substance. The results were expressed as trolox equivalent antioxidant capacity (TEAC) in milligrams per gram of dried extract (mg TEAC/g extract).

2. DPPH radical-scavenging

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical-scavenging was evaluated as described by Mensor *et al.* [24] with certain alterations. In this process, 400 µL of different concentrations of each extract was mixed with 2,000 µL of DPPH (0.3 mM) in methanol. After 20 min of incubation at an ambient temperature in darkness (with methanol used as the control reaction), the absorbance at 517 nm was measured using a spectrophotometer. Gallic acid was used as a reference substance. The results were expressed as gallic acid equivalent antioxidant capacity (GEAC) in milligrams per gram of dried extract (mg GEAC/g extract).

3. Superoxide radical-scavenging

Superoxide anion radical-scavenging was measured using the nitrotetrazolium blue chloride (NBT) reduction method described by Nishikimi *et al.* [25] with slight modification. All solutions

were prepared with 100 mM of phosphate buffer (pH 8). The reaction mixture contained 100 µL of the different concentrations of the extracts, 1,000 µL of 156 µM NBT, 1,000 µL of 468 µM NADH and 100 µL of 60 µM phenazine methosulfate (PMS), and it was incubated at an ambient temperature for 5 min (with phosphate buffer used as a control reaction). Subsequently, absorbance at 560 nm was measured using a spectrophotometer. Gallic acid was used as a reference substance. The results were expressed as mg GEAC/g extract.

Enzymatic assays

1. Tyrosinase inhibition

Tyrosinase inhibition was assessed with the dopachrome method as reported by Masuda *et al.* [26] with some modifications. Solutions of 200 U/mL of tyrosinase from mushrooms and 2.5 mM of L-DOPA (3, 4-dihydroxy-L-phenylalanine) were prepared with 20 mM of phosphate buffer (pH 6.8). Tyrosinase reactions were performed in a 96-well plate, with each well containing 20 µL of different concentrations of the extracts, 40 µL of tyrosinase solution and 140 µL of phosphate buffer. The 96-well plate was allowed to stand for 10 min, and then the reaction was started by adding 40 µL of L-DOPA solution (with phosphate buffer employed as a control reaction). The 96-well plate was then incubated at an ambient temperature for 20 min, and the absorbance of tyrosinase activity was measured at 492 nm using a microplate reader (Biochrom EZ Read 400, Cambridge, UK) with kojic acid used as a reference substance. Each sample's testing was completed with a blank plate of the sample test excepting the tyrosinase solution.

2. Collagenase inhibition

The collagenase inhibition activities of the extracts were measured using the method described by Zakiah *et al.* [2] with slight modification. In this process, 50 µL of different concentrations of each extract, 50 µL of 50 mM of tricine buffer (pH 7.5) and 50 µL of 125 U/mL of *Clostridium histolyticum* collagenase (type I) solution were added to a 96-well plate, which was permitted to stand for 15 min. A tricine buffer was used as the control reaction. The experimental reaction was commenced using 50 µL of 0.5 mM of FALGPA (N-[3-(2-Furyl)acryloyl]-Leu-Gly-Pro-Ala), and collagenase inhibition activity was measured immediately with a microplate reader, with the absorbance determined to be 340 nm for 20 min. EGCG was utilised as a reference substance.

The abilities of the extracts to scavenge radicals and inhibit enzyme activity were obtained using the following equation:

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

In this equation, $Abs_{control}$ is the absorbance of the control without sample extracts and Abs_{sample} is the absorbance reading with the presence of sample extracts.

Statistical analysis

Values are expressed as mean ± standard deviation (S.D.). Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS), version 17.0, for Windows (IBM, Chicago, USA) using one-way analysis of variance (ANOVA) with Tukey's HSD (honestly significant difference) test. Statistical significance was determined to be $p < 0.05$.

RESULTS AND DISCUSSION

Yield of extract

The yields of aqueous extracts from the *E. elatior* flower compared to the extracts of its leaf were approximately 20% for the leaf extract (Table 1). The dried sample extracts were crushed to obtain fine powder before they were used for phytochemical and biological analyses.

Table 1. The yield and total phenolic content of *E. elatior* flower and leaf extracts

Part	Yield of extract (% w/w)	Total phenolic content (mg GAE/g extract)
Flowers	23.90 ± 4.09	38.68 ± 0.45
Leaves	24.30 ± 1.98	246.52 ± 0.26

Total phenolic content

Previous research tends to focus on the phenolic compounds of herbal extracts for their biological activity. Therefore, this study determined the total phenolic content of *E. elatior*, which is shown in Table 1. The results indicated the total phenolic content of leaf extracts in comparison to the flower extracts as approximately six times greater, while rhizomes of *E. elatior* were 326 ± 76 mg GAE/100 g in fresh weight [17]. Generally, the phenolic compounds of the plant exhibit significant potential for scavenging free radicals compared to vitamin C, E and carotenoids, and the compound scavenges free radicals by trapping them, along with chelate metals, and donating hydrogen to the free radicals formed through oxidation reactions [7,27]. Consequently, previous reports indicate that phenolic compounds are beneficial in terms of antimicrobial, anti-inflammatory and anti-aging effects, and they can be absorbed through the skin barrier [7].

Phenolic compound analysis

Phenolic compounds are bioactive plant substances that are widely investigated as sources of natural antioxidant compounds, specifically useful as natural additives in the cosmetic and food industries [7]. As a result of this importance, this study analyses 10 phenolic compounds from flower and leaf extracts of *E. elatior* with a focus on apigenin, catechin, eriodictyol, gallic acid, hydroquinin, isoquercetin, kaempferol, quercetin, rutin and tannic acid. The phenolic acid content of both extracts is shown in Table 2, with that of the leaf extract exhibiting higher content than the flower extract. The leaf extract featured high content with respect to isoquercetin, catechin, gallic acid and tannic acid, while the flower extract exhibited high content in terms of isoquercetin, catechin and gallic acid. Chromatograms of the extracts are shown in Figure 1A-B.

Previous studies have reported gallic acid exhibiting significant antioxidant activity, along with oxidative stress damage via ROSs and suppressed cellular melanin synthesis in B16F10 cells through the inhibition of tyrosinase activity [28-29]. Catechin could be considered a metal chelator and may bind with the Zn^{2+} ion inside the collagenase structure, thereby replacing the binding of the substrate, which is also involved in inhibiting the activities of tyrosinase and the elastase enzyme [30-31]. Eriodictyol is also well-known for its antioxidant and anti-inflammatory activities [32], as well as its anti-tyrosinase activity, with an IC_{50} value of approximately 150 μM [33]. Choi and Shin [34] reported that quercetin is a potent tyrosinase inhibitor, in addition to inhibiting melanogenesis, which is shown by many studies through a cellular test of B16F10/B16F1 melanoma cells, human melanoma of the vagina (HMVII) cells and normal human epidermal melanocytes (NHEM). It has also been described as effective in a 20-50 μM concentration of quercetin. Furthermore, quercetin has anti-inflammatory benefits, as well as antioxidant and anti-allergen properties [35-36]. Meanwhile, apigenin has been reported to be a collagenase and hyaluronidase inhibitor, in addition to possessing anti-aging and anti-acne vulgaris bacteria [35, 37]. With this, diverse phenolic compounds, such as tannic acid, rutin and isoquercetin, are regarded to have antioxidant properties [38-39]. The present

study showed that the phenolic compound contained in *E. elatior* flowers and leaves could be included as an ingredient in skin care products that offer a variety of properties for promoting healthy skin, especially for enhancing skin appearance in terms of wrinkles and pigmentation.

Table 2. Phenolic content of *E. elatior* flower and leaf extracts (mg/100 g dried extract)

Phenolic	Flowers	Leaves	Phenolic	Flowers	Leaves
Apigenin	ND	4.72	Isoquercetin	55.36	69.37
Catechin	40.69	65.75	Kaempferol	ND	ND
Eriodictyol	1.78	3.39	Quercetin	12.64	31.24
Gallic acid	35.47	50.01	Rutin	11.66	24.16
Hydroquinin	ND	ND	Tannic acid	15.17	49.10

ND: non-detectable

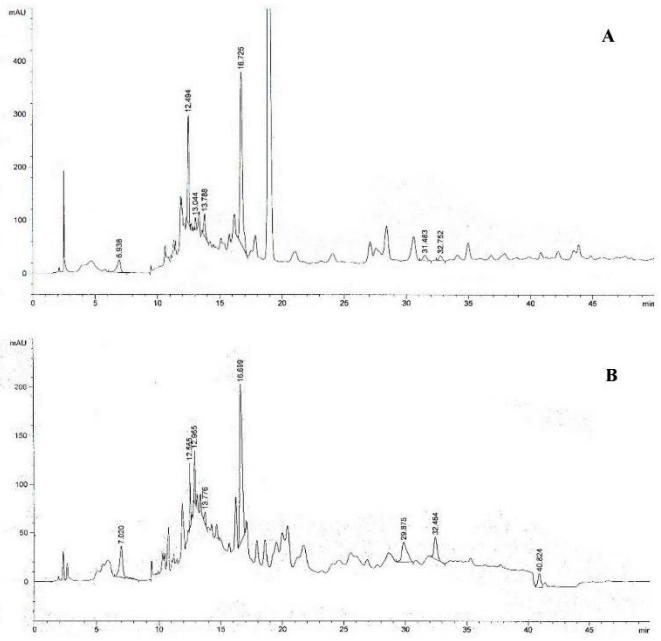


Figure 1. LC-MS chromatogram of *E. elatior* flower (A) and leaf (B) extracts showing the peaks of gallic acid (RT: 6.94 and 7.02 min), catechin (RT: 12.50 and 12.57 min), tannic acid (RT: 13.04 and 12.97 min), rutin (RT: 13.79 and 13.78 min), isoquercetin (RT: 16.73 and 16.70 min), eriodictyol (RT: 31.48 and 29.88 min), quercetin (RT: 32.75 and 32.46 min) and apigenin (RT: 40.82 min)

Amino acid analysis

Amino acids have been demonstrated to play an important role as antioxidants and as cosmetic ingredients engaged in skin improvement functions [40-41]. Therefore, both the flower and leaf sample extracts were analysed for amino acid content - see Table 3. The flower extract exhibited

overall higher amino acid contents than the leaf extract, with the flower extract exhibiting the highest values for lysine, glutamic acid and aspartic acid, in that order. Of the 19 amino acids present in the *E. elatior* extracts, cysteine, glycine, histidine, leucine, lysine, proline and tyrosine have been reported to have strong benefits as cosmetic properties, with cysteine, glycine and histidine exhibiting anti-inflammatory properties [42]. Proline is involved in correcting wrinkles by enhancing the elasticity of the stratum corneum. Further, concerning collagen formation, the combination of proline and branched-chain amino acids has benefits in recovering the biosynthesis of collagen after UV exposure, while lysine and leucine amino acids are significantly involved in the formation of collagen [43-46]. The results of this study suggest that several amino acids present in *E. elatior* exhibit cosmetic properties valuable for restoring and resolving skin-aging problems.

Table 3. Amino acid contents of *E. elatior* flower and leaf extracts (mg/100 g dried extract)

Amino acid	Flowers	Leaves	Amino acid	Flowers	Leaves
Alanine	261	74.35	Lysine	2891	138.50
Aspartic acid	1177	94.67	Methionine	<5.00	<5.00
Cysteine	35	24.65	Phenylalanine	685	141.67
Glutamic acid	1250	202.82	Proline	216	28.02
Glycine	176	41.16	Serine	394	122.65
Histidine	437	19.36	Threonine	146	12.05
Hydroxylysine	<5.00	<5.00	Tryptophan	60	9.99
Hydroxyproline	29	7.79	Tyrosine	372	26.08
Isoleucine	581	83.64	Valine	523	57.41
Leucine	838	112.74			

Antioxidant activity

1. ABTS radical-scavenging activity

The antioxidant activity of extracts was examined via an ABTS assay based on direct reduction of ABTS radicals through electron transfers, which measured the decay of absorbance at 734 nm [47]. The results are expressed as TEAC and presented in Table 4. In this study, both extracts exhibited elevations in antioxidant activity as extract concentration rose. The leaf extract had the highest ABTS radical-scavenging capacity activity with approximately 24-fold greater activity than that of the flower extract. Of note, the ABTS-scavenging activity of the leaf extract closely resembled that of trolox, the reference substance (Figure 2A, Table 4). Further, the flower and leaf extracts featured IC_{50} values of 0.69 ± 0.01 and 0.04 ± 0.00 mg/mL, respectively. This finding indicates that both flower and leaf extracts of *E. elatior* are potent for radical scavenger activities, especially the leaf extract, when compared with trolox.

2. DPPH radical-scavenging activity

The DPPH radical-scavenging assay is based on the extract acting as a hydrogen atom donor in the reduction of DPPH radical-scavenging [48]. The results indicated that radical-scavenging capacity is dependent on the distributed dose of *E. elatior* flower and leaf extracts, with the amounts of activity shown in Figure 2B. In this assay, the leaf extract demonstrated higher antioxidant capacity than the flower extract, as well as featuring greater ABTS radical-scavenging assay results (Table 4). Further, flower and leaf extracts exhibited IC_{50} values of 0.44 ± 0.00 and 0.09 ± 0.00 mg/mL, respectively. In a previous study of the *E. elatior* flower, Lachumy *et al.* [12] determined, using this assay, the IC_{50} value of methanolic extract to be 9.14 mg/mL, with an ethanolic extract exhibiting the

potential range of 0.04-0.14 mg/mL [14]. The aqueous extract of the *E. elatior* flower in the present study could be considered to possess moderate capacity when compared with methanolic and ethanolic extracts, whereas the aqueous leaf extract had larger capacity than the solvent extracts.

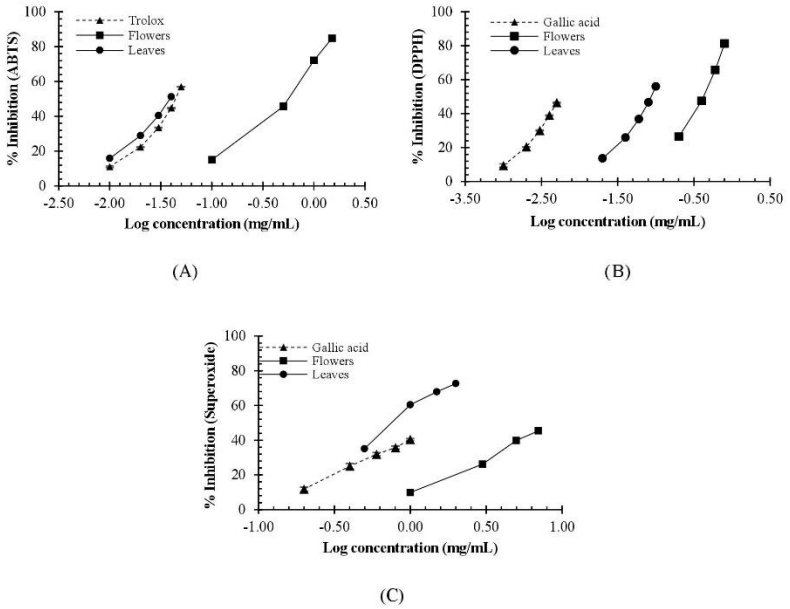


Figure 2. Antioxidant activity: ABTS (A), DPPH (B) and superoxide (C) radical-scavenging activities of *E. elatior* flower and leaf extracts

Table 4. The antioxidant capacities of *E. elatior* flower and leaf extracts

Part	ABTS assay		DPPH assay		Superoxide assay	
	mg TEAC /g extract	IC_{50} (mg/mL)	mg GEAC /g extract	IC_{50} (mg/mL)	mg GEAC /g extract	IC_{50} (mg/mL)
Flowers	43.41±0.56	0.69±0.01[a]	9.85±0.08	0.44±0.00[a]	219.65±4.38	6.39±0.13[a]
Leaves	1030.74±10.82	0.04±0.00[b]	56.86±0.65	0.09±0.00[b]	314.12±12.79	0.88±0.03[c]
Trolox	-	0.04±0.00[b]	-	-	-	-
Gallic acid	-	-	-	0.01±0.00[c]	-	1.37±0.05[b]

Note: Values are expressed as means ± standard deviation (S.D.; n = 3). For each column of IC_{50} values, means not sharing the same letter in each column were significantly different at $p < 0.05$.

3. Superoxide radical-scavenging activity

The superoxide anion radical is well-known as an initial radical that plays an important role in acutely damaging cells and tissues by forming other ROSs, such as singlet oxygen and hydroxyl radicals [49]. As found in Figure 2C, the leaf extract exhibited higher superoxide anion-scavenging capacity than the flower extract, in addition to exhibiting a greater capacity with the ABTS and DPPH methods. Further, the leaf extract exhibited a very different IC_{50} value than that of the flower extract at a rate of approximately seven times greater, just as is presented in Table 4. Skin-aging processes and dermatological conditions are most commonly the result of oxidative stress from many endogenous and exogenous factors, such as metabolism, pollution or exposure to UV radiation [50-

51]. The antioxidant has been reported to be involved in preventing cellular damage from harmful free radicals by inhibiting free-radical formation, interrupting the autoxidation chain reaction and protecting cellular antioxidant defence mechanisms that take place during the oxidation process, which results in slowing the skin-aging process and reducing the severity of dermatological conditions [7, 52-53].

Moreover, cosmetic products containing antioxidants from natural sources have been increasing in popularity over the past few decades and their popularity is expected to grow even further because these products may exhibit less side effects than synthetic compounds while diminishing oxidative stress [53]. Therefore, *E. elatior* flower and leaf extracts, as potent antioxidants, are demonstrated to be effective as cosmetic ingredients to prevent the harm of cellular damage and skin aging while protecting the skin from free radicals.

Enzymatic assay

1. Tyrosinase inhibition activity

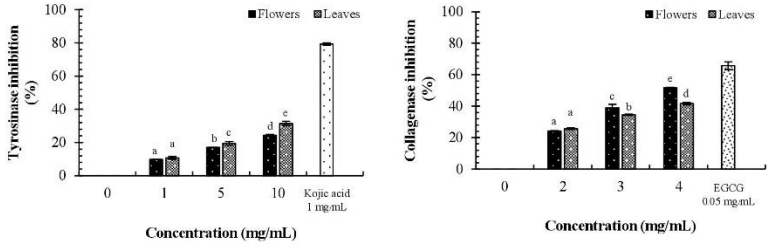
Tyrosinase is a crucial rate-limiting enzyme in direct melanin synthesis. However, overproduction of melanin pigment may cause hyperpigmentation or production of a skin melanoma. Tyrosinase is involved in catalysing the hydroxylation of L-tyrosine to L-DOPA (3, 4-Dihydroxy-L-Phenylalanine) and the oxidation of L-DOPA to DOPA quinone in the biosynthesis pathway of melanin [54]. While melanin pigmentation is normally responsible for preventing skin damage caused by ROSs or UV radiation exposure, overproduction and overdistribution of melanin pigment is a primary cause of melasma, age spots, skin darkening and other issues [55]. Therefore, cosmetic products containing tyrosinase inhibitors are becoming more common for their skin-whitening properties. In this study, mushroom tyrosinase was used to determine the role of *E. elatior* in the process of melanogenesis. Different concentrations of flower and leaf extracts (consisting of 1, 5 and 10 mg/mL) and kojic acid (1 mg/mL) were incubated with the tyrosinase solution, and the reactions were stopped by adding L-DOPA. The results established that all concentrations of flower and leaf extracts inhibited tyrosinase activity. Furthermore, flower and leaf extracts at 10 mg/mL concentrations exhibited tyrosinase inhibition activity at 24.37 ± 0.52% and 31.48 ± 1.28%, respectively. Kojic acid demonstrated more pronounced inhibition activity than both flower and leaf extracts. The flower and leaf extracts presented IC_{50} values of 25.77 ± 0.88 and 18.08 ± 0.74 mg/mL, respectively. These results indicate that both extracts of *E. elatior* significantly inhibit tyrosinase activity in a dose-dependent manner (Figure 3A). According to these findings, the properties of *E. elatior* are capable of suppressing tyrosinase activity, therefore acting as whitening ingredients in cosmetic uses.

2. Collagenase inhibition assay

Collagenase is a proteolytic enzyme belonging to the MMP group and is responsible for breaking down collagen [56]. Thus, inhibiting collagenase activity delays the skin-aging process that produces wrinkles, leading to the appearance of youthful, healthy and glowing skin [57-58]. Different concentrations of *E. elatior* flower and leaf extracts (consisting of 2, 3 and 4 mg/mL) were assessed, along with the reference substance, EGCG (0.05 mg/mL). Collagenase from *C. histolyticum* was employed to measure the anti-aging properties of *E. elatior*. The results demonstrate that both flower and leaf extracts significantly inhibited collagenase activity in a dose-dependent manner, as shown in Figure 3B. The collagenase inhibition activities of the flower and leaf extracts at 4 mg/mL were 51.73 ± 0.20% and 41.54 ± 0.75%, respectively. At 0.05 mg/mL, the reference substance, EGCG, exhibited inhibition of 65.67 ± 2.52%. Finally, the IC_{50} values of flower and leaf extracts were 3.89 ± 0.17 and 5.02 ± 0.47 mg/mL, respectively.

Our findings indicate the leaf extract inhibits tyrosinase activity more than the flower extract, while the flower extract demonstrates more anti-collagenase activity. Previous have suggested that plant extracts possess anti-aging, wrinkle-prevention properties through various mechanisms

depending on their phenolic compounds, antioxidant properties, anti-collagenase activities and anti-elastase traits [2, 59]. Therefore, the results presented herein provide evidence that *E. elatior* could potentially be a natural source for active ingredients for anti-aging and anti-wrinkle benefits in cosmetic products.



(A) (B)

Figure 3. Effects of *E. elatior* flower and leaf extracts on tyrosinase (A) and collagenase (B) inhibition activities

(Note: Values are expressed as means ± standard deviation (S.D.; n = 3). For each concentration, values not sharing the same letter (a-e) in each column were significantly different at $p < 0.05$.

CONCLUSIONS

In this study, both the aqueous extracts of *E. elatior* flowers and leaves exhibited potential as bioactive compounds with effective amino acid and phenolic compounds, as well as beneficial biological properties for cosmetic and cosmeceutical applications. It is worth noting that the aqueous extract of *E. elatior* leaves demonstrated greater potential as an antioxidant than that of the reference substances, including trolox and gallic acid. The flower extract, on the other hand, exhibited more significant collagenase activity than that of the leaf extract. These findings reveal that both the flower and leaf extracts of *E. elatior* possess anti-aging properties, especially the flower extract, which features significant anti-collagenase activity and amino acid content while also possessing a moderate amount of phenolic compounds and antioxidant activity. The leaf extract was found to have the greatest amount of antioxidant activity and phenolic compounds, as well as moderate anti-collagenase and amino acid contents. In addition, the several amino acids present in aqueous solution of *E. elatior* flower and leaf extract were bioactive compounds that engaged in resolving skin aging. In conclusion, *E. elatior* has strong potential for improving and restoring skin damage, especially in addressing skin-aging issues and preventing wrinkles, through the inhibition of free radicals and collagenase. Additionally, these extracts can suppress the melanogenesis process to bestow a skin-lightening effect useful in cosmetic and whitening applications. Besides that, this paper may serve as support for further animal studies to confirm these valuable findings, thus animal testing models should be continued to be pursued. Moreover, to explore the possible benefits and safety concerns related to *E. elatior* extracts in terms of human cosmetic applications, human studies should be also be carried out in the future.

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Bioactive Compounds and Its Biological Activity from Leaves of Torch Ginger for Value Added as Cosmetic Product

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Abstract

Torch ginger (*Etlingera elatior*) is the medicinal plant in family Zingibaraceae and widely cultivated in Southeast Asia. All parts of torch ginger such as inflorescences, leaves, fruits, and rhizome, especially leaves demonstrated the high phytochemical content and biological activity. The research work aimed to evaluate the bioactive compound and antioxidant activity from leaves extract of torch ginger for cosmetic applications. In the present study, dried leaves of torch ginger were extracted with distilled water to obtain the aqueous extract. The leaves extract was evaluated for total phenolic content using the Folin-Ciocalteu method whereas the amino acid profile was examined using GC-MS technique. The extract was determined its biological activity by ABTS radical scavenging and tyrosinase inhibition assays. The results found that the yield of leave aqueous extract was 24.30±1.98% and total phenolic content comparative with gallic acid was 246.52±0.26 mg GAE/g of extract. Furthermore, chlorogenic acid content, a main phenolic compound in leave extract, was 27.58 mg/kg. Amino acid profile consisted of glycine, histidine, leucine and lycine, their amino acid can improve formation of collagen and reduce the wrinkle appearance. The antioxidant activity of the extract showed the IC_{50} value was 0.039±0.00 mg/mL as well as closely resemble with vitamin E derivative (IC_{50} = 0.044±0.00 mg/mL). Beside that leaves extract showed inhibit tyrosinase activity. The finding suggests that the leaves of torch ginger extract exhibited high bioactive compounds and their biological activities illustrate the potential to be formulated in skin care products.

Keywords: antioxidant activity, anti-tyrosinase activity, cosmetic product, phenolic content, torch ginger

INTRODUCTION

Nowadays, bioactive compounds from natural plant are becoming more attractive to be used as cosmeceutical ingredients. Their bioactive compounds are rich source of vitamins, essential oils, proteins, phenolic compounds and antioxidants etc. Antioxidants play an important role in health care as a radical preventer and scavenger in degenerative ailments i.e. cancer, autoimmune disorders, hypertension and also in delaying the ageing process. Cosmetic products containing natural ingredient are gentle, biodegradable, less toxic properties and environmentally-friendly connotations when compared with synthesis cosmetic ingredients [1-5].

Torch ginger (*Etlingera elatior*) is the medicinal plant in largest family of Zingiberaceae and widely cultivated in Southeast Asia as spices for food flavoring or selling inflorescence as ornamental

4. การประชุมวิชาการระดับชาติประจำปี 2561 มหาวิทยาลัยแม่โจ้

องค์ประกอบฟีนอลิกและฤทธิ์ต้านอนุมูลอิสระของใบดาหลาเพื่อใช้เป็นวัตถุดิบในเครื่องสำอาง
Phenolic Contents and Antioxidant Activities of Torch Ginger Leaves for Cosmetic Ingredient

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บทคัดย่อ

ดาหลาเป็นพืชสมุนไพรในตระกูลขิงข่าที่มีการเพาะปลูกอย่างแพร่หลายทางภาคใต้ของประเทศไทย แต่ละส่วนของดาหลาโดยเฉพาะส่วนใบประกอบด้วยสารพฤกษเคมีและฤทธิ์ทางชีวภาพที่สำคัญ งานวิจัยนี้มีวัตถุประสงค์เพื่อตรวจวัดสารออกฤทธิ์ทางชีวภาพกลุ่มฟีนอลิกและฤทธิ์การต้านอนุมูลอิสระในใบดาหลาเพื่อใช้เป็นส่วนผสมในเครื่องสำอางบำรุงผิว โดยนำใบดาหลามาทำการสกัดด้วยน้ำและทำการตรวจวัดสารกลุ่มฟีนอลิกด้วยวิธี Folin-Ciocalteu method จากนั้นทำการวิเคราะห์องค์ประกอบของสารกลุ่มฟีนอลิกด้วย LC/MS ส่วนการตรวจวัดฤทธิ์ทางชีวภาพทำการวิเคราะห์ฤทธิ์ขจัดอนุมูลเอบีทีเอสและดีพีพีเอช ผลการทดลองพบว่าปริมาณสารกลุ่มฟีนอลิกทั้งหมดมีค่าเท่ากับ 246.52 มิลลิกรัมสมมูลของกรดแกลลิกต่อกรัมของสารสกัด โดยมี isoquercetin, catechin และ gallic acid เป็นชนิดเด่น ส่วนฤทธิ์ทางชีวภาพพบว่าสารสกัดใบดาหลามีประสิทธิภาพขจัดอนุมูลเอบีทีเอสและดีพีพีเอชได้โดยมีค่า IC_{50} เท่ากับ 0.039±0.00 และ 0.087±0.00 มิลลิกรัมต่อมิลลิลิตร ตามลำดับ จากการทดลองแสดงให้เห็นว่าใบดาหลาเป็นแหล่งของสารออกฤทธิ์ทางชีวภาพที่มีศักยภาพในการพัฒนาเป็นผลิตภัณฑ์เครื่องสำอางบำรุงผิว

คำสำคัญ: ดาหลา สารประกอบฟีนอลิก สารต้านอนุมูลอิสระ เครื่องสำอาง

Abstract

Torch ginger is the medicinal plant in family Zingiberaceae and widely cultivated in Southern Thailand. All part of torch ginger especially leaves exhibited the phytochemical content and biological activity. The research work aimed to determine the phenolic compounds and antioxidant activity from torch ginger leaves extract for cosmetic applications. In the present study, dried leaves

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