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DEVELOPMENT OF FACIAL MASK PRODUCTS CONTAINING GARDENIA FRUIT EXTRACT

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ผลพุดซ้อนถูกนำมาใช้อย่างกว้างขวางในทางการแพทย์ มีสารเจนิโพไซด์ และสารโครซินส์เป็นสารประกอบหลักสำคัญที่มีในผลพุดซ้อนสุก การวิจัยนี้เน้นการศึกษาสารเจนิโพไซด์เนื่องจากสารเจนิโพไซด์มีคุณสมบัติในการต้านอักเสบ โดยมีวัตถุประสงค์เพื่อศึกษาผลของตัวทำละลายต่อการสกัดผลพุดซ้อนแห้ง ตัวทำละลายที่นำมาศึกษาคือ น้ำ 50 เปอร์เซ็นต์เอทานอล และ 70 เปอร์เซ็นต์เอทานอล สารสกัดหยาบแต่ละส่วนถูกสกัด 2 ครั้งอย่างต่อเนื่องกันด้วยตัวทำละลาย 2 ชนิด สารสกัดแห้งที่ได้หลังจากการทำไลโอฟิไลเซชัน มีลักษณะเป็นเกล็ดสีเหลืองส้ม การประเมินปริมาณสารเจนิโพไซด์ในสารสกัดผลพุดซ้อน พบว่าปริมาณของสารสกัดผลพุดซ้อนแห้งสูงสุด (13.06±0.75 เปอร์เซ็นต์โดยน้ำหนัก) และปริมาณสารเจนิโพไซด์ในสารสกัดผลพุดซ้อนแห้งสูงสุด (18.92±0.02 เปอร์เซ็นต์โดยน้ำหนัก) เป็นผลจากการสกัดผลพุดซ้อนแห้งด้วยน้ำ และสกัดต่อด้วย 50 เปอร์เซ็นต์เอทานอล พัฒนาตำรับผลิตภัณฑ์ผงพอกหน้า และผลิตภัณฑ์เจลพอกหน้าชนิดลอกออก เพื่อหาตำรับที่เหมาะสม จากนั้นเติมสารสกัดผลพุดซ้อนในตำรับ เพื่อใช้เป็นผลิตภัณฑ์สำหรับลดการอักเสบของผิวหนังหลังการสัมผัสแสงแดด ตำรับผลิตภัณฑ์ผงพอกหน้าที่เหมาะสมประกอบด้วยสารแซนแทนกัม 1 เปอร์เซ็นต์โดยน้ำหนัก มีความสามารถในการกระจายที่ดี ระยะเวลาในการแห้งเหมาะสม สำหรับผลิตภัณฑ์เจลพอกหน้าชนิดลอกออกที่มีคุณสมบัติเหมาะสมประกอบด้วยสารทรากาแคนท์ 0.5 เปอร์เซ็นต์โดยน้ำหนัก ร่วมกับใช้กลีเซอรีนเป็นสารเพิ่มความยืดหยุ่นในตำรับ ความสามารถในการลอกออกดี ระยะเวลาในการแห้งเหมาะสม เมื่อความคงตัวผลิตภัณฑ์พอกหน้าทั้ง 2 ชนิดที่ประกอบด้วยสารสกัดผลพุดซ้อน โดยเก็บที่อุณหภูมิ 4, 30 และ 40 องศาเซลเซียส เป็นระยะเวลา 3 เดือน พบว่าตำรับผลิตภัณฑ์ผงพอกหน้า และผลิตภัณฑ์เจลพอกหน้าชนิดลอกออกมีความคงตัวทางกายภาพ แต่สารเจนิโพไซด์ยังคงสภาพไม่เปลี่ยนแปลงในตำรับผลิตภัณฑ์เจลพอกหน้าชนิดลอกออกเท่านั้น อาจเป็นผลมาจากสภาวะที่เป็นกรดที่มากกว่า และสารเพิ่มความคงตัวในตำรับผลิตภัณฑ์พอกหน้าชนิดลอกออก

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SUJIMA SUNATWANICHKUL: DEVELOPMENT OF FACIAL MASK PRODUCTS CONTAINING GARDENIA FRUIT EXTRACT. ADVISOR: ASSOC. PROF. PORNPEN WERAWATGANONE, Ph.D., CO-ADVISOR: ASST. PROF. WALAISIRI MUANGSIRI, Ph.D., 98 pp.

Gardenia fruit is widely used in medical treatment. Geniposide and crocins are the major active compounds in gardenia dried ripe fruit. This research focused on geniposide due to its anti-inflammatory activity. This study aims to study solvent effect on the extraction of gardenia dried fruit. The solvents were water, 50% ethanol and 70% ethanol. Each crude dried fruit portion was extracted twice consecutively using 2 solvents. Yellow orange color flake was obtained after the gardenia extract was lyophilized and geniposide content in the gardenia extract flake was evaluated. The highest extraction yield (13.06±0.75% by weight) and geniposide content (18.92±0.02% by weight) were obtained when water and 50% ethanol were consecutively used to extract gardenia dried fruit. Powder facial mask and peel-off gel facial mask were developed and the gardenia extract was added to the mask bases for after sun exposure use to reduce skin inflammation. The powder facial mask base containing 1% w/w of xanthan gum provided satisfied appearance and a suitable peel-off mask film was obtained when 0.5% w/w of tragacanth and glycerine were added in the peel-off gel mask base. Both facial masks containing gardenia extract were kept at 4, 30 and 40 ^{o}C for 3 months. The result showed that the powder facial mask and the peel-off gel facial mask were physically stable but geniposide remained unchanged only in the peel-off gel facial mask. It could be due to more acidic condition and stabilizing agent in the peel-off gel facial mask formula.

Department:	Pharmaceutics and Industrial Pharmacy	Student's Signature .....
		Advisor's Signature .....
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LIST OF FIUGRES

LIST OF ABBREVIATIOS

cP	Centipoise
COX-1	Cyclooxygenase I
COX-2	Cyclooxygenase II
℃	Degree Celsius (centigrade)
iNOS	Enzyme nitric oxide synthase
IL-1	Interlukin 1
IL-1β	Interlukin 1 beta
IL-6	Interlukin 6
IL-8	Interlukin 8
mg	Micrograms
μM	Micromolar
ml	Milliliter
min	Minute (s)
nm	Micromolar
NO	Nitric oxide
ppm	Part per million
PVA	Polyvinyl alcohol
PVP K30	Polyvinyl pyrrolidone K 30
PGE2	Prostaglandin E2
TNF-α	Tumor necrosis factor alpha
UV	Ultraviolet
UVA	Ultraviolet A
UVB	Ultraviolet B
UVC	Ultraviolet C
w/w	Weight per weight

CHAPTER I
INTRODUCTION

Gardenia Jasminoides Ellis known as evergreen flower is a small shrub of the Rubiaceae family. It is commonly found in the tropical zone and growing wide spread in the subtropical climate around the world. The fruit of *Gardenia Jasminoides Ellis* is known as “Gardenia fruit” or “Zhi Zi” in Chinese language. The pigments of gardenia fruit have been used as a natural food colorant in noodles, candies, jellies, juice and beverages, in many Asia countries like China, Taiwan, India, Vietnam, Japan and Korea (Bergonzi et al., 2012; Chen et al., 2008). Moreover, it is also used in medical treatment. According to the record of China Pharmacopeia, it has been used as a traditional Chinese medicine. (Wang et al., 2012). Gardenia fruit extract has been topically used for the treatment of inflammatory, edema, allergy and ulcers (Koo et al., 2004; Sung et al., 2014; Bergonzi et al., 2012). Moreover, it is used as a antioxidant substances in neuroprotection, preventing cardiovascular diseases, and protecting hepatocytes (Chen et al., 2010). In previous studies, the isolated Iridoid glycosides were geniposide, genipin and gardenoside. The main colorant from gardenia extract was crocins (Chen et al., 2010; He et al., 2010). The major compounds in the gardenia fruit are geniposide and crocins. Previous studies have found that geniposide can reduce the inflammation by blocking the pro-inflammatory cytokine production such as nitric oxide,

tumor necrosis factor-alpha, Interleukin-6 and histamine (Sung et al., 2014; Koo et al., 2006). Crocins, yellow substances, are antioxidants which have ability to block the operation of cyclooxygenase-1, cyclooxygenase -2 and reducing the production of prostaglandins (Xu et al., 2009). Furthermore, the Gardenia fruit extract could reduce the paw edema in rat. (Koo et al., 2006)

The extraction of gardenia fruit using solvents is easy and inexpensive. The solvent selection depends on type of the active substances. From the previous studies, various solvents have been used to extract geniposide and crocins from the gardenia fruit such as water, methanol, ethanol, n-butanol and ethyl acetate (Wang et al., 2012). Water, methanol and ethanol, hydrophilic solvents, could extract the geniposide from the gardenia fruit better than using n-butanol and ethyl acetate, hydrophobic solvents. Moreover, different ratios of water and alcohol were also used to extract gardenia fruit (Chen et al., 2010; Debnath et al., 2011; Palakajornsak, 2004).

Every year, the damaging of the ozone layer in the atmosphere is allowed ultraviolet radiation (UV) to pass through the earth more than it ever was. Ultraviolet from sun radiates on human skin, causing disadvantages more than benefits. The ultraviolet radiation is included UVA, UVB, UVC divided by wavelength ranges. The longest ray is UVA, which can penetrate into the skin deeper than UVB ray, and cause many damages towards the skin structure including skin aging, wrinkle and black spot. The UVB can pass through the epidermis layer causing the skin reddening, sunburn,

dermatitis and damage the superficial epidermal layers. The UVC is filtered by the atmosphere (Silva et al., 2014; Nicolaou et al., 2011).

According to inflammation problems caused by the ultraviolet radiation and the properties in pharmacology effects of the gardenia fruit extract as mentioned. The researchers aim to study, the influence of hydroalcoholic solvent on the solvent extraction of gardenia fruit in order to obtain high percentage of geniposide in the extract. Then facial mask products containing gardenia extract were developed in order to reduce inflammatory of skin after sunlight exposure.

Research objectives

1. To study the appropriate solvent extraction procedure for the gardenia fruit.
2. To develop the appropriate formula for preparing facial mask products containing gardenia fruit extract.
3. To investigate the stability of active compound in the facial mask products containing gardenia fruit extract.

CHAPTER II
LITERLATURE REVIEWS

1. Botanical of Gardenia jasminoides Ellis

Gardenia (*Gardenia jasminoides Ellis*) originated in Asia countries and is most commonly found growing wild in China, Japan, Vietnam, Korea and India. It was found at altitudes of 400-1200 meters. It is widely grown in warm temperate and subtropical climates as a houseplant. It thrives best in warm temperatures and humid environments. It requires good drainage and a sunny location and prefers an acidic soil with a pH between 5 to 7 (Lim, 2014; Phatak, 2015; Xiao et al., 2017).

Gardenia is a small shrub growing to the height of 1-2 meters (Figure 1). Gardenia leaves are dark green and spear shape. Its flower is solitary, big, white and has layered petals. It also has soft texture and light fragrance. The fruit of gardenia is oval and grows upside-down. When it is ripe, the color of the fruit turns gold-yellow or orange red. It is 1-1.5 centimeters in diameter and 3-7 centimeters long. Each fruit has about 5-7 ridges along the length and inside contains 3-6 seeds with red arils. The fruit can be harvested from September to November (Lim, 2014; Liu et al., 2013; Tang and Eisenbrand, 1992; Wagner et al., 2011; Liu et al., 2015).

2. Chemical constituents of *Gardenia jasminoides Ellis*

Gardenia contains many natural chemical compounds, such as, iridoids glycoside, terpenoids, crocins, quinic acid derivative, flavonoids, and essential oil (Zhou et al., 2007; Xiao et al., 2017; Wu et al., 2014; Lim, 2014; Liu et al., 2015; Wagner et al., 2011; Tang and Eisenbrand, 1992; Wang et al., 2004). Each compound can be extracted from various parts of gardenia. Natural chemical compounds which can be extracted from the fruits are geniposide, genipin, geniposidic acid, shanzhiside, deacetyl-asperulosidic acid methyl ester, genipin-1-β-D–gentiobioside, gardoside, crocetin, crocin-1, crocin-2, crocin-3, neocrocin A, chlorogenic acid, 4, 5-dicaffeoylquinic acid, 3-O-caffeoyl-5-O-sinapoylquinic acid, quercetin, isoquercitrin, ruin, umuhengerin, nicotiflorin and 5,7-dihydroxyflavone. (Table 1 and Figure 2)

Figure 1 Flower, leaves, bud and fruit of *Gardenia jasminoides Ellis* (Lim, 2014)

Table 1 Phytochemicals isolated from gardenia fruit (Wu et al., 2014; Liu et al., 2013).

Phytochemicals	Molecular formula	Molecular weight	λ max (nm)
Geniposide	$C_{17}H_{24}O_{10}$	388.14	238
Genipin	$C_{11}H_{14}O_5$	226.08	240
Geniposidic acid	$C_{16}H_{22}O_{10}$	374.11	235
Genipin-1-b-gentiobioside	$C_{23}H_{34}O_{15}$	550.19	238
Shanzhiside	$C_{16}H_{24}O_{11}$	392.13	235.5
Gardoside	$C_{16}H_{22}O_{10}$	374.11	235
Deacetyl-asperulosidic acid methyl ester	$C_{17}H_{24}O_{11}$	404.37	237.5
Crocin I	$C_{44}H_{64}O_{24}$	979.96	440
Crocin II	$C_{44}H_{64}O_{24}$	814.38	440
Crocin III	$C_{44}H_{64}O_{24}$	814.38	440
Rutin	$C_{27}H_{30}O_{16}$	610.52	254
chlorogenic acid	$C_{16}H_{18}O_9$	354.31	330

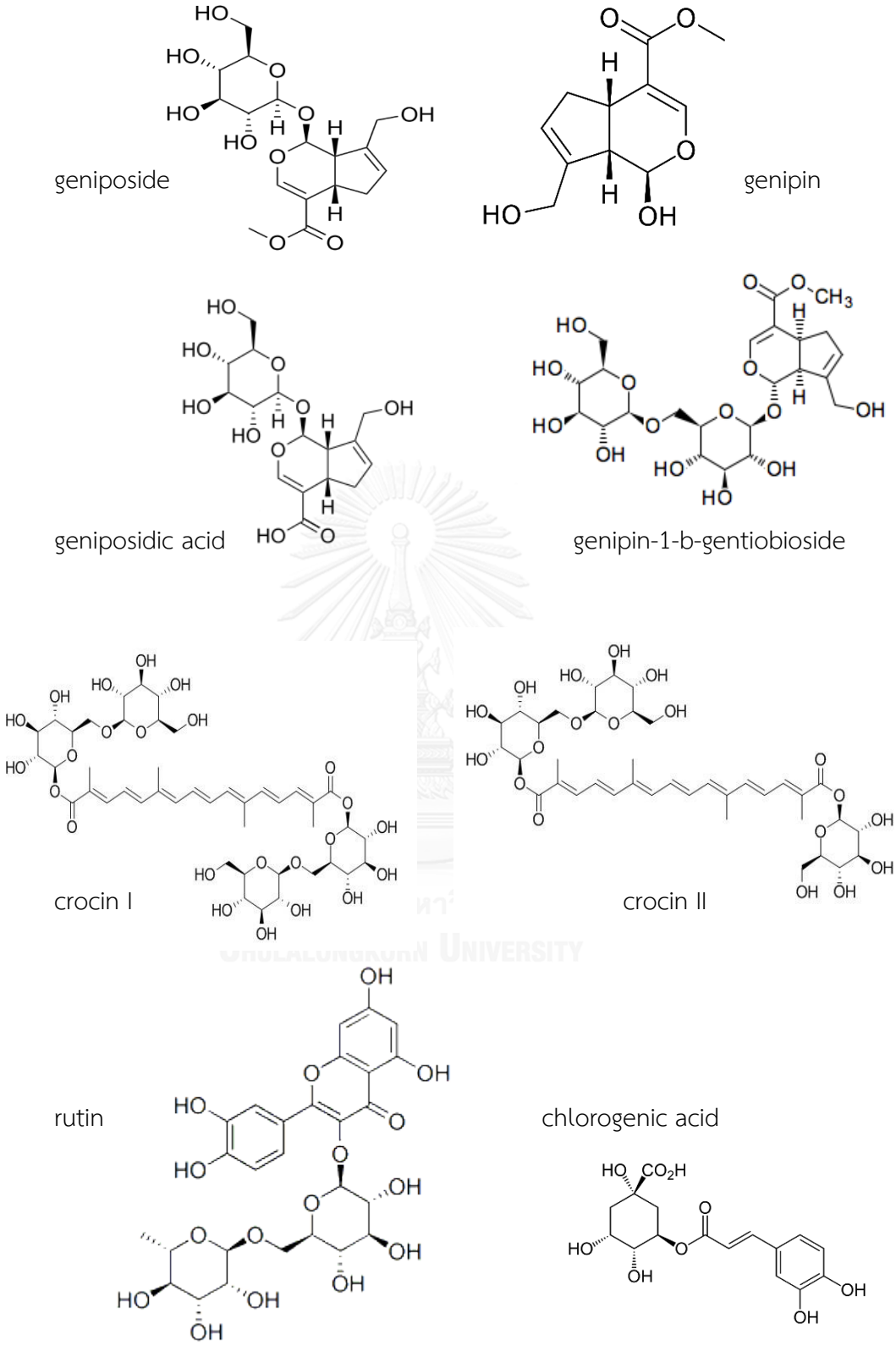


Figure 2 Structures of chemical constituents in gardenia fruit

(Wu et al., 2014; Liu et al., 2015)

3. Extraction of Gardenia jasminoides Ellis

There are several extraction methods depending on substrates. Factors affecting types and quantity of gardenia extract are types of solvent, methods of extraction, ratio of substrate and solvent, duration and temperature of the processes. (Palakajornsak, 2004)

The solvent used in the extraction process is a major factor affecting the extract. Based on previous studies, five different types of solvent, including methanol, ethanol, n-butanol, and ethyl acetate were used in gardenia extraction process and amount of geniposide was mornitored. It was found that water was the most effective solvent when compared to other solvents with lower polarity than water. It should be noted that, it is preferable to use a high polar solvent to extract geniposide from gardenia fruit (Wang et al., 2012). Dry matter content from water extraction was higher than from 70% ethanol extraction (5.90 and 5.04% yield, respectively). Moreover total phenolics and total flavonoids from water extraction was also superior to that from 70% ethanol extraction (Debnath et al., 2011). Water, ethanol and methanol were selected in a previous study (Wu et al., 2014). Various ratios of water to alcohol (100: 0, 80:20, 70:30, 50:50 and 30:70) were utilized to extract substances from gardenia fruit. It was found the ratio of 50:50 (water: methanol) effectively extracted geniposide, genipin, geniposidic acid, genipin-1-b-D-gentiobioside, crocin I, crocin II, rutin and chlorogenic acid. Chen et al. (2010) effectively used 50% ethanol to extract geniposide

and crocins from gardenia fruit while Sung et al. (2014) used 70% ethanol in their study. Ketmaro (2009) used water to extract yellow substance from gardenia fruit and pectin was precipitated out using 95% ethanol with 9.59±2.52% yield. According to the studies, water, methanol, ethanol and water mixed with alcohol were the appropriate solvents to extract the substances from gardenia fruit. However, water extraction produced pectin in large amount. Methanol extracted well but is highly toxic. Therefore, water and water mixed with ethanol are good candidates for further study.

Reflux and cold percolation methods were used to extract geniposide, while cold percolation was a preferable process for crocins extraction (Xiao et al., 2017). Some active substance could be degraded during the extraction with high temperature. The cold percolation is proper for both geniposide and crocins.

4. Biological activities of Gardenia jasminoides Ellis

The previous studies reported biological activities of substances extracted from *Gardenia jasminoides Ellis* as shown in Table 2.

Table 2 Biological activities of major compound in *Gardenia jasminoides Ellis.*

Phytochemicals	Bioactivities	
Geniposide	Anti-inflammatory Antithrombotic effect Antidiabetes Antiarthritis Antithrombotic and antiangiogenic Antidepressant activity	(Koo et al., 2006; Sung et al., 2014; Wu et al., 2009; Tao et al., 2014; Phatak, 2015)
Genipin	Anti-inflammatory Antiangiogenic activity Antidepressant activity Inhibit gastric lesions Antithrombotic effect	(Koo et al., 2004; Lee et al., 2009; Xiao et al., 2017)
Crocin	Antioxidant Anti-inflammatory Protective of the injured liver Antihyperlipidemic Neuroprotective activity	(Pham TQ et al., 2000; Kim et al., 2014; He et al., 2005)
Crocetin	Anti-inflammatory Antihypertensive effects Antithrombotic effects	(Hong and Yang, 2013; Xiang et al., 2006; Phatak, 2015)

4.1. Anti-inflammatory activity

Numerous studies have been reported that gardenia fruit extract exhibited a broad spectrum anti-inflammatory effect. It could inhibit production of pro-inflammatory cytokines, which are signaling protein involved in systemic inflammation such as nitric oxide (NO), tumor necrosis factor alpha (TNF-α), Interlukin 6 (IL-6) and Interlukin 1 beta (IL-1β). Park et al. (2014) revealed that gardenia extract does not only reduce TNF-α and IL-1β production in keratinocytes by inhibiting the mRNA expressions but also shows antioxidant activity in keratinocytes cells stimulated by UVB. According to Zheng et al. (2010), a major compound extracted from gardenia fruit for instance, geniposide also significantly protected sepsis model mice. Moreover, geniposide inhibited lipopolysaccharide (LPS), TNF-α, IL-6 and IL-1β production induced by the LPS both *in vitro* and *in vivo*. Furthermore, geniposide could neutralize LPS, directly bind LPS and down regulation of the release of TNF-α, IL-6 and IL-1β by regulating TLR4 expression, which affected the downstream nuclear factor-Kreppa B (NF-KB) and mitogen-activated protein kinase (MAPK) signaling pathways (Song et al., 2014). Fu et al. (2012) indicated that geniposide markedly inhibited the LPS-induced TNF-α, IL-6 and IL-1β production by blocking the phosphorylation of IKB-α, p65, p38, ERK and JNK in mouse macrophages. *In vivo* study, geniposide attenuated lung histopathologic changes in the mouse models. The total number of neutrophil and macrophage counts decreased while TNF-α, IL-6 and IL-1β secretion significantly

decreased when compared with the control group (Fu et al., 2012). Genipin is another compound extracted from gardenia fruit that markedly reduced the increase in serum aminotransferase activities, lipid peroxidation and cytosolic cytochrome C protein. Additionally, genipin significantly increased level of NF-KB and decline cytosolic level of IKB-α ,protein (Kim et al., 2010). In the anti-inflammatory study on stimulated paw edema by acetic acid, geniposide and genipin could reduce the swelling of the rat feet when compared with the control group by reducing vascular permeability and the level of NO production (Koo et al., 2006). In another study, the inflammation of rat ears induced by croton oil, the edema of ears was inhibited when genipin (0.55-4.42 micromoles/ear) was applied on the ears. As a result, genipin at the dose of 4.42 micromoles/ear showed the highest swelling reduction (57.1 percent of the weight and the thickness) because genipin inhibited the production of NO in macrophage RAW 264.7 cell stimulated by LPS and interferon-gamma (Interferon-γ) (Koo et al., 2004). There were other pathways that gardenia fruit extract reduced the inflammatory response by inhibiting enzyme nitric oxide synthase (iNOS), cyclooxygenase I (COX-1) and cyclooxygenase II (COX-2) (Liu et al., 2013).

Crocins also are main chemical constituents of gardenia pigment, which have an effect on inhibition of inducible nitric oxide synthase (iNOS) expression and nitric oxide production by stimulated RAW 264.7 macrophage (Kim et al., 2014). *In vitro* study, crocins could exhibit an inhibitory activity on COX-1, COX-2 enzymes and inhibit the production of prostaglandin E2 (PGE2) in LPS-challenged RAW 264.7 (Xu et al.,

2009). Hong and Yang (2013) indicated that crocetin provided the most potent anti-inflammatory activity because it inhibited production of NO in LPS stimulated macrophage RAW 264.7 cells and prevented expressions of protein, m-RNA of iNOS and COX-2. Moreover, impediment of the histamine was another way to decrease inflammatory activity of *Gardenia jasminoides*. Sung et al. (2014) reported that 400 μg of gardenia fruit extract could be useful in preventing or treating allergic disorders because it inhibited the development of skin lesions and decreased serum IgE and histamine levels. Moreover, Masso (2014) recommended concentration level of gardenia fruit extract containing cosmetic product were 0.1-4% w/w. In this study selected concentration of gardenia fruit extract in our preparation was 0.5% w/w.

4.2. Antioxidant activity

Active compounds from gardenia fruit such as genipin, crocins, crocetin, flavonoid and phenolic have a potent antioxidant property. Pham TQ et al. (2000) revealed that concentration of crocin at 20 ppm had an antioxidant effect when compared with butylated hydroxyanisole (BHA). Crocins displayed the antioxidative activity up to the concentration of 40 ppm and the activity decreased at higher concentration. Moreover, the antioxidant property of crocins was better than thiobarbituric acid. The NO production in LPS stimulated by macrophages, was inhibited by genipin and crocetin with IC 50 values of 11.14 μM and 5.99 μM,

respectively (Peng et al., 2013). Furthermore, gardenia resin fraction significantly exhibited stronger antioxidant activity than crocins (Chen et al., 2008). Tseng et al. (1995) indicated that crocetin inhibited the formation of superoxide anion and bleached the free radical of 1,1-diphenyl-Z picrylhydrazyl (DPPH). The protective action of crocetin operated via quenching of the superoxide anion or free radical; therefore, it showed protective activity against ROS-induced hepatotoxicity and genotoxicity. The aqueous extract of gardenia fruit revealed higher antioxidant activities than the ethanolic extract. The extracts presented the scavenging activities against DPPH, ABTS, hydroxyl and superoxide radicals. Moreover, it displayed reducing power activity, nitrite-scavenging activity and preventing lipid peroxidation (Debnath et al., 2011).

5. Ultraviolent radiation

Ultraviolet radiation causes a variety of skin diseases (Elwood and Jopson, 1997). When UV from sun radiates on human skin, it causes biological changes in disadvantage more than benefit. Overexposure to UV radiation induces skin inflammation, edema from sunburn reaction, erythema and cancer. UV radiation fall between the wavelengths of X-rays and visible light.

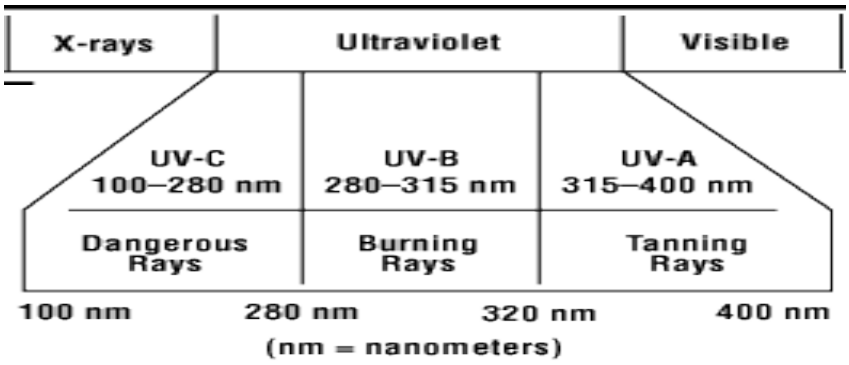


Figure 3 Ultraviolet spectrum (CCOHS, 2016)

Ultraviolet is categorized into three subtypes including UVA, UVB and UVC by wavelength as shown in Figure 3. Sunlight is predominantly UVA (90–95%) and UVB (5–10%) (D'Orazio et al., 2013). UVA radiation, the longest of the three, can penetrate deeply into the dermis. UVA is efficient at generating reactive oxygen species that can damage DNA probably initiating skin cancers. It causes denature of collagen and elastin structure of skin, skin aging, wrinkling and tanning. UVB radiation is almost completely absorbed by the epidermis. UVB causes of sunburn and damage of the superficial epidermal layers. UVC radiation is absorbed by the ozone layer and does not reach on the earth. Long term UV light exposure initiates sunburn and inflammatory response, activation of innate immune cells, neutrophils and macrophages. Both macrophages and neutrophils are the main sources of the reactive oxygen species (ROS) production and induce the release of pro-inflammatory cytokines from the skin cell. (Silva et al., 2014; Nicolaou et al., 2011).

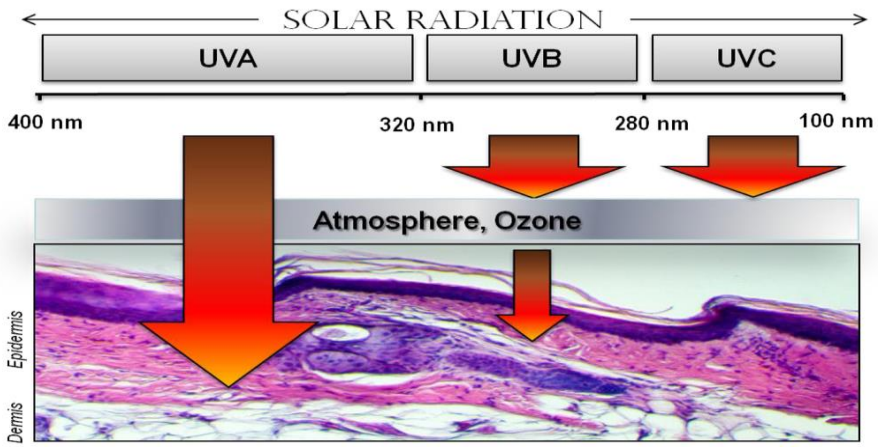


Figure 4 UV radiation and biologic effects on the skin (D'Orazio et al., 2013).

6. Skin respond to ultraviolet radiation. .

The effects of ultraviolet radiation on skin can be both immediate and delayed. One of the most severe effects is inflammation. The contact of ultraviolet radiation on human skin creates sunburn reaction which is the cause of erythema heat edema, pain and blisters. Concurrently the cells under the skin produce and release cytokines such as arachidonic acid, NO, prostaglandin E2 (PGE_2) and histamine. UV stimulates neutrophil and macrophage, and also stimulates the immune system to release TNF-α, IL-6 and IL-1β. Additionally, it generates changes within the cells through DNA damage and ROS (ปราโมท มหคุณากร, 2553; D'Orazio et al., 2013).

The inflammatory process depends on the type of mediator and the type of target cell. Histamine is a proinflammatory mediator which increases the permeability of blood vessels by shrinking the vascular wall cells resulting to widen the gap between the cells. NO is a substance in the inflammatory process. It results in vascular dilation,

increase of vascular permeability, edema of inflammation area (Abramson et al., 2001; Zeilhofer, 2007). Prostaglandin G2 (PGG2), prostaglandin H2 (PGH2), prostaglandin I2 (PGI2), PGE2, and thromboxane A2 (TXA2), are considered as inflammatory mediators. The tumor necrosis factor-alpha is inflammatory cytokines which can stimulate the production of the other cytokine and intermediates for inflammatory processes such as Interleukin 1 (IL-1), interleukin 6 (IL-6) and interleukin 8 (IL-8) (Grewe et al., 1993; ปราโมท มหคุณากร, 2553)

7. Facial mask formulation

A powder facial mask and a peel-off gel mask are semisolid products that are used by applying on facial skin and removed after left on the skin a certain time. Ingredients in the mask preparations contain organic compounds, such as, wax, vinyl resins, rubber, hydrocolloids or earth which create a thin film over the skin (พิมพร ลีลาพรพิสิฐ, 2532).

7.1. Powder facial masks

Intend use of powder facial mask is to hydrate the facial powder with water; the forming paste is applied on skin prior to rinse off after complete dryness. The powder facial mask may increase stability of active compounds that are not stable in aqueous medium. The powder facial mask would absorb dirt or other micro-particles

from the skin, reduce skin inflammation and shed old or dead skin cells. The product contains high level of clay minerals. One of the most popular clay minerals used in powder facial mask is kaolin which reduces excess oil, absorbs dirt or bacteria on the skin and has astringent quality. Gelling agents, such as, xanthan gum, polyvinyl pyrrolidone K 30 (PVP K30) and tragacanth gum are added in this product in order to improve viscosity; therefore, the product can cling to the skin better. Apart from the ingredients previously mentioned, other additives may be added to receive the target properties. For example, zinc is added into the preparation to reduce skin inflammation and irritation and by adding natural organic compounds, the mask will also improve skin moisture (พิมพร ลีลาพรพิสิฐ, 2532).

7.2. Peel off gel facial masks

The peel-off gel mask is usually made of gelling agent, wax, latex, plastic, and resin which are thick and viscous in consistency. When the peel-off gel mask is applied on skin, it turns into a thin film over the skin and active ingredients are slowly released from the film to the skin. Once it is completely dry, the mask can be peeled off the skin. From the review on peel-off gel mask, it is found that the most utilized ingredient in creating film-like texture is polyvinyl alcohol (PVA), a film former, because PVA has proper viscosity, tensile stress, and water resistant creating consistent film. Plasticizer is added to improve film flexibility by reducing repulsive forces between molecules. The film is strengthened and hard to rip during the drying time. The common

plasticizers are glycerin and propylene glycol. O'Reilly Beringhs et al. (2013) developed the recipe for green clay and aloe vera peel-off facial masks by using PVA and propylene glycol. Silaon et al. (2015) studied the physical and mechanical properties of *Centella asiatica* peel-off masks and used PVA and glycerin. Alcohol is added into the formulation at 10-15% to reduce the drying time with the appropriate time of 30 minutes. Gelling agents are added to create appropriate viscosity and smooth texture such as carbomer, xanthan gum, tragacanth etc. Moreover, preservatives and other ingredients are also added to stabilize the preparations and promote cosmetic effects.

CHAPTER III

MATERIALS AND METHODS

Materials

1. Gardenia fruit (Vejpong Pharmacy Co., Ltd., Thailand)
2. Geniposide (TCI America, USA, Lot No. I5YEO)
3. 95% Ethanol (The liquor Distillery Organization, Thailand, Lot No. BC2010716)
4. Acetonitrile HPLC (RCI Labscan Co., Ltd., Thailand, Lot No. 16110213)
5. Methanol HPLC (RCI Labscan Co., Ltd., Thailand, Lot No. 16110433)
6. Water HPLC (RCI Labscan Co., Ltd., Thailand, Lot No. 16110375)
7. Kaolin (B.L.H Trading Co., Ltd., Thailand, Lot No. 120814)
8. Tragacanth (B.L.H Trading Co., Ltd., Thailand, Lot No.PW-1800)
9. EDTA disodium (S. Tong Chemicals Co., Ltd., Thailand, Lot No.N0916152E)
10. Glycerin (S. Tong Chemicals Co., Ltd., Thailand, Lot No. 151011008)
11. Propylene glycol (S. Tong Chemicals Co., Ltd., Thailand, Lot No. C815E69TD1)
12. Xanthan gum (S. Tong Chemicals Co., Ltd., Thailand, Lot No. 201612A-N20)
13. Zinc oxide (S. Tong Chemicals Co., Ltd., Thailand, Lot No. B2-13-2345)
14. Polyvinyl pyrrolidone K30 (Namsiang Pharmacy Co., Ltd., Thailand, Lot No. 033325117)
15. Methyl paraben (Ueno Fine Chemical Industry, Japan, Lot No. BH1711)
16. Polyvinyl alcohol (Japan vam & Poval Co., Ltd., Japan Lot No. 63705)

17. Propyl paraben (Clariant production UK Ltd, United Kingdom, Lot No. GBG0004232)
18. Citric acid monohydrate (Merck, Germany, Lot No. K91334944)
19. Hydrochloric acid (Merck, Germany, Lot No. K46915817)
20. Sodium hydroxide (Merck, Germany, Lot No. B1233898)
21. Hydrogen peroxide (Merck, Germany, Lot No. K46920387)
22. Syringe filters 13 mm, 0.45 µm (Vertical Chromatrography Ltd.,)

Equipment

1. Evaporator (R-300, BUCHI Rotavapor®, Thailand)
2. UV-visible spectrophotometer (UV-1601, Shimadzu, Japan)
3. Refrigerated incubator (FOC 225i, VELP® Scientifica, Italy)
4. Incubator (KBF 720, Binder® , Germany)
5. Centrifuge machine (Sigma 302K, Bioblock Scientific, USA)
6. Water bath (WB 22 with SV 1422, Memmert, Germany)
7. pH meter (S220 SevenCompact™, Mettler Toledo, Switzerland)
8. Analytical balance 4 digits (Model AG285, Mettler Toledo, Switzerland)
9. Analytical balance 5 digits (AX105 DeltaRange®, Mettler Toledo, Switzerland)
10. Analytical balance 7 digits (UMT2, Mettler Toledo, Switzerland)
11. Lyophilizer (CoolSafe ™, Scanvac, Denmark)
12. High speed refrigerated microcentrifuge (MX-305, Tomy, Japan)

13. Sonicator (GT-2120QTS, GT sonic, China)

14. Vortex (Vortex Genie2, Scientific industries, USA)

15. Digital viscometer (Brookfield, USA)

16. Liquid Chromatography-Mass spectrometry (LC-MS) equipped with

    - A solvent delivery module (717 plus, Waters, USA)
    - A solvent delivery module (600 Controller, Waters, USA)
    - UV detector : (996 Photodiode Array Detector, Waters, USA)
    - Automatic sample injector (717 plus Autosample, Waters, USA)
    - Mass spectrometer (Bruker Daltonics Model HCT Esquire 3000
    - ESI source Mode Positive, USA)

17. High-performance Liquid Chromatography (HPLC) equipped with

    - A solvent delivery module (LC-20AD, Shimadzu, Japan)
    - A variable wavelength UV detector (SPD-M20A Diode Array Detector, Shimadzu, Japan)
    - A data integrating software (LC-20, Shimadzu, Japan)
    - An automatic sample injection (SIL-20AC HT, Shimadzu, Japan)
    - An oven (CTO-20A_3 degasser, Shimadzu, Japan)
    - A communication bus module (CBM-20A, Shimadzu, Japan)

Methods

1. Gardenia extract characterization

1.1. Characterization of gardenia extract by HPLC

Gardenia extract solution (0.2 mg/ml) was prepared in ultrapure water. Separation of geniposide and crocins was performed on a Shimadzu LC20A equipped with a C18 Column (5 µm), 150 x 4 mm (Agilent®). Separation condition was modified from Chen et al. (2010). The elution profile was 10% acetonitrile at 0 min, the linear gradient to 18% acetonitrile from 0 to 15 min, the linear gradient to 28% acetonitrile from 15 to 20 min, the linear gradient to 38% acetonitrile from 20 to 40 min, the linear gradient to 50% acetonitrile from 40 to 50 min, the final elution at 50% acetonitrile from 50 to 55 min, and then the linear gradient to 10% acetonitrile from 55 to 64 min. The flow rate was 1 ml/min and injection volume of sample was 10 µl. The geniposide was detected at 238 nm (0-20 min) and crocin I were detected at 440 nm (20-60 min) (Chen et al., 2010).

1.2. Characterization of gardenia extract by LC-MS

Gardenia extract was characterized using LC-MS system modified from Bergonzi et al. (2012). A HPLC using the Waters 717 plus HPLC system. The separation was performed with the same column, mobile phase, flow rate and elution gradient as 1.1. Mass spectrometry operating condition was optimized in order to achieve maximum

sensitivity value, gas temperature of 350 ^{O}C at a flow rate of 5 l/min, nebulizer pressure of 11 p.s.i., and capillary voltage of 4000 V. Full scan spectra was from m/z 50 to 1000 in the positive ion. Molecular weight of geniposide and crocin I were 388 and 976, respectively. The experiment was done at the Faculty of Science, Mahidol University.

1.3. HPLC method verification

The HPLC analytical method was partially verified as followed.

1.3.1. Standard geniposide

Accuracy

Accuracy of the analyze method was ascertained in three replications (n=3). The concentrations of standard geniposide in solutions were 0.050, 0.075 and 0.100 mg/ml. The solutions were analyzed using HPLC and the percentage of recovery (% recovery) was calculated using Equation 1.

$$\% \text{ recovery} = \frac{(\text{measured value} \times 100)}{\text{true value}} \quad \text{Equation 1}$$

Acceptance criteria for accuracy was 95-105% recovery according to AOAC International, 2016 (International and Latimer, 2016).

Precision

Precision of the method was determined by repeatability (intraday precision) of standard geniposide. In this study, precision was determined in three replicates the concentrations of standard geniposide in solutions were 0.050, 0.075 and 0.100 mg/ml. The results were expressed as % relative standard deviation (% RSD) of the measurements (Equation 2), which should be less than 3.7% according to AOAC International, 2016 (International and Latimer, 2016).

$$\%\ RSD = \frac{SD \times 100}{\bar{x}} \qquad \text{Equation 2}$$

where

SD is sample standard deviation and $\bar{x}$ is mean of the data.

Linearity

A linear correlation plot between the concentrations of standard solutions and peak area obtained from standard solutions was delineated. Briefly, 1.25 mg of standard geniposide was weighed and dissolved in 10 ml of ultrapure water. Five different concentrations of standard solutions were prepared at the concentrations range of 0.005 to 0.100 mg/mL and analyzed using HPLC method mentioned in 1.1.

The linear correlation coefficient (r) was calculated. Acceptance of the correlation coefficient (r) was not less than 0.999.

1.3.2. Raw material and finish product

Specificity

The specificity of the HPLC condition for the analysis of geniposide was determined. Blank and mask bases were prepared and diluted in the same manner as the preparations containing geniposide. Then, the diluted bases were injected to the HPLC system. Degradation studies of geniposide under acid, base and oxidizing conditions were performed. Four grams of gardenia extract solution and the mask samples were accurately weighed and placed in 10 ml volumetric flask. Then, 1 M HCl, 1 M NaOH or 3% H_2O_2 was added to dissolve the sample to the volume and the samples were kept at 80 ^{o}C for 3 hours. Acid and base samples were neutralized to pH 7 to avoid further decomposition. Then, all the samples were diluted using acetonitrile: water (45:55). The expected final concentration of gardenia extract in the diluted samples was 0.4 mg/ml. The samples were analyzed. The presence of interfering peaks eluted at/or near the retention time of geniposide were investigated. The peak purity of the samples was also determined. For thermal degradation, the extract and the mask samples were kept at 80 ^{o}C for 3 hours and analyzed using the same manner

Accuracy

Accuracy of the analyze method was ascertained in three replications (n=3). The quantity equivalent was 80, 100 and 120% of the target concentration. Briefly, 0.5% w/w of geniposide in ultrapure water (gardenia extract) and 0.5% w/w of geniposide in powder mask and peel off gel mask (finish product) were prepared. Each gram of the extract contained 189.20 mg geniposide (from 2. Extraction of gardenia fruit). Then the samples were weighed and diluted in acetonitrile:water (45:55). The final concentrations of the extract in solutions were 0.32, 0.40 and 0.48 mg/ml which were 80, 100 and 120% of the target concentration, respectively. The solutions were analyzed using HPLC and the percentage of recovery (% recovery) was calculated using Equation 3.

$$\% \text{ recovery} = \frac{(\text{measured value X } 100)}{\text{true value}} \qquad \text{Equation 3}$$

Acceptance criteria for accuracy was 95-105% recovery according to AOAC International, 2016 (International and Latimer, 2016).

Precision

Precision of the method was determined by repeatability (intraday precision) and intermediate precision (interday precision) of both finish product and gardenia

extract. In this study, precision was determined in six replicates of both gardenia extract solution (0.4 mg/ml) and finish product solution (0.4 mg/ml). The results were expressed as % relative standard deviation (% RSD) of the measurements (Equation 4), which should be less than 3.7% according to AOAC International, 2016 (International and Latimer, 2016).

$$\%\ RSD = \frac{SD \times 100}{\bar{x}} \qquad \text{Equation 4}$$

where

SD is sample standard deviation and $\bar{x}$ is mean of the data.

2. Extraction of gardenia fruits

2.1. Preparation of gardenia fruits extract

An accurately weighted 10 g of gardenia fruit was mashed and soaked in 50 ml of solvent 1 for 60 min and then the filtrate of gardenia fruit was collected as portion 1. The crude was soaked again in solvent 2 with the same procedure and the filtrate of the crude was collected as portion 2. Solvent 1 and 2 are shown in Table 3. Portion 1 and 2 were evaporated using R-300 BUCHI Rotavapor® and then freeze-dried using CoolSafe™. Percent yield of the extract was calculated using equation 5. The experiment was done in triplicate.

Table 3 Method and solvent for extraction of gardenia fruits.

Method	Solvent 1	Solvent 2
WW	Water	Water
W5E	Water	50% Ethanol
W7E	Water	70% Ethanol
5EW	50% Ethanol	Water
7EW	70% Ethanol	Water

$$\% \text{ Yield} = \frac{(\text{weight of dry extract (g)} \times 100)}{\text{weight of gardenia fruit (g)}} \quad \text{Equation 5}$$

2.2. Evaluation of geniposide of gardenia fruit extract

The content of geniposide in the dried extract was determined using HPLC. Briefly, five concentrations between 0.005 to 0.100 mg/ml were prepared from standard geniposide and injected to the HPLC system. A linear correlation of peak area at 238 nm and geniposide concentration was plotted. The solution of dried extract was prepared by dissolving 50 mg of the extract in 25 mL of ultrapure water. One ml of the solution was diluted to 10 ml using ultrapure water. The solution was filtered through a 0.45 μm syringe filter. The diluted sample solution was injected to the HPLC system and geniposide concentration in the solution was calculated from the linear correlation**.** Percentage of geniposide in the dried extract and percentage of geniposide in the fruit were calculated using equation 6 and 7, respectively. The experiment was done in triplicate.

$$\%\ \text{geniposide in the dried extract} = \frac{\text{weight of geniposide (g)} \times 100}{\text{weight of dried extract (g)}} \quad \text{Equation 6}$$

$$\%\ \text{geniposide in the gardenia fruit} = \frac{\text{weight of geniposide (g)} \times 100}{\text{weight of gardenia fruit (g)}} \quad \text{Equation 7}$$

3. Preparation and evaluation of facial masks

3.1. Powder facial masks

Powder facial mask bases are shown in Table 4. The formulation was modified from a previous work (Vanderbilt Minerals). The powder facial mask was developed by finding appropriate types and concentrations of gelling agents. Types and amount of gelling agents including polyvinyl pyrrolidone K 30, tragacanth and xantan gum were varied. The substances were mixed using geometric dilution technique and the obtained powder was used for the physical evaluation. Briefly, 1 g of each base was added into water at the ratio of 1:2 (base: water). Appearance, spreadability, pH and drying time of the hydrated powder bases were evaluated. After water was added to the powder facial mask, pH value of the preparation was measured. Half gram of the hydrated powder facial mask was spread on skin and evaluated spreadability by an expert panel. Then, 0.5 g of the hydrated powder facial mask was also applied on a glass plate (18.75 cm^2) and the glass plate was submitted to a heated environment (a water bath set at 37.0±2.0 ^{o}C). The glass plate was monitored every 5 min, and the experiment was finished after the surface of the mask had been dried completely. The results were expressed as mean of three measurements. A formulation providing proper spreadability, pH 5-7 and drying time of 25-35 min was selected.

Table 4 Percentage of each substance in powder facial mask bases.

Substance	P1	P2	P3	P4	P5	P6	P7	P8	P9
Kaolin	75.5	73.5	71.5	75.5	73.5	71.5	75.5	73.5	71.5
Zinc oxide	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Citric acid monohydrate	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Xanthan gum	1.0	3.0	5.0	-	-	-	-	-	-
Tragacanth	-	-	-	1.0	3.0	5.0	-	-	-
Polyvinyl pyrrolidone K 30	-	-	-	-	-	-	1.0	3.0	5.0

3.2. Peel-off gel facial masks

Peel-off gel mask bases are shown in Table 5. The original formula was obtained from the work of Silaon et al. (2015). The peel-off gel facial mask formula was developed by finding appropriate types and amount of gelling agents including suitable plasticizers. Glycerin and propylene glycol were used a plasticizers. Types and percentage of gelling agents including tragacanth and xantan gum were varied. To prepare the masks, PVA was hydrated in hot water. The mixture was cooled down to 40 °C and the remaining substances were added. Then the mixture was stirred until

homogenous. Appearance, spreadability, pH, drying time were evaluated as explained in 3.1. Film formation of the peel-off gel bases was determined by peeling the dried facial mask from the glass plate. The selected criteria were similar to the powder facial mask in 3.1 and easily peelable film after drying.

Table 5 Percentage of each substance in peel-off gel mask bases.

Substance	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Polyvinyl alcohol (PVA)	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
95% ethanol	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
EDTA disodium	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Paraben concentrate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Propylene glycol	-	-	-	-	-	6.0	6.0	6.0	6.0	6.0
Glycerine	6.0	6.0	6.0	6.0	6.0	-	-	-	-	-
Xanthan gum	-	0.5	1.0	-	-	-	0.5	1.0	-	-
Tragacanth	-	-	-	0.5	1.0	-	-	-	0.5	1.0
Water	62.4	61.9	61.4	61.9	61.4	62.4	61.9	61.4	61.9	61.4

4. Preparation and evaluation of facial masks containing gardenia fruit extract.

4.1. Powder facial masks

Gardenia fruit extract was added in the selected powder facial mask base to obtain concentration of 0.5% w/w. Then appearance, spreadability, pH and drying time of the mask were determined. The powder facial mask was placed in aluminium bags and purge with nitrogen gas before they were sealed. The samples were kept at 4, 30 and 40 °C. The powder facial mask was collected to analyze at 0, 1, 2 and 3 months.

4.2. Peel-off gel facial masks

Gardenia fruit extract was added in the selected peel-off mask base to obtain concentration of 0.5% w/w. Then appearance, spreadability, pH, drying time and film formation of the peel-off gel mask were determined. The peel-off gel mask was kept in amber glass vial and purged with nitrogen gas before they were sealed with rubber and aluminum caps at 4, 30 and 40 °C and collected to analyze at 0, 1, 2 and 3 months.

5. Evaluation of physical properties of facial masks

5.1. Powder facial masks

The samples taken from different storing time were evaluated. Two grams of water was added to 1 g of the powder facial mask. The pH and drying time of the hydrated powder facial mask were measured as presented in 3.1. Viscosity of the mask was also measured in triplicate using Brookfield viscometer at 25 °C.

5.2. Peel-off gel facial masks

The samples were taken for the storing chambers at different times and directly evaluated their physical properties, pH drying time, viscosity and peel-off film formation, in the same manner as previously explained.

6. Evaluation of chemical stability of facial masks

The powder facial mask and the peel-off gel facial mask were collected at various time points and taken to analyze the amount of the geniposide in the preparations using the HPLC mentioned in 1.1. Five solutions of standard geniposide were prepared in the concentration range of 0.005-0.100 mg/ml to construct a standard curve of geniposide. Two gram of the facial mask diluted to 25 ml using acetonitrile: water (45:55). This solution was filtered and injected to the HPLC system. Concentration of geniposide was calculated from its obtained peak area and the

correlation from the standard curve. Percent remaining of geniposide over storing time was determined using Equation 8.

$$\% \text{ remaining of geniposide} = \frac{\text{concentration of geniposide at time} \times 100}{\text{concentration of geniposide at initial}} \quad \text{Equation 8}$$

7. Statistical analysis

The data was reported as mean ± standard deviation. The observed differences were analysed for statistical significance by one-way analysis of variance with Tukey's multiple comparison as a post hoc test. Differences of P value < 0.05 were considered as significant by SPSS version 22 software.

CHAPTER IV
RESULTS AND DISCUSSION

1. Gardenia extract characterization

1.1. Characterization of gardenia extract by HPLC

HPLC analysis of gardenia extract was developed by the study of Chen et al. (2010). HPLC chromatogram of the gardenia extract displayed 2 major peaks at the retention time of 9.4 min with a wavelength of 238 nm and at the retention time of 23.2 min with a wavelength of 440 nm. HPLC chromatograms and UV spectra of the compounds in the extract are shown in Figure 5 and 6. The HPLC chromatograms of geniposide and crocin I were observed at the retention time of 9.4 min and 23.2 min, respectively. This result is in accordance with the study of Chen et al. (2010) which indicated that geniposide can be found at the retention time between 0 - 20 min with a wavelength of 238 nm and crocin I can be found at the retention time between 20 - 40 min with a wavelength of 440 nm.

1.2. Characterization of gardenia extract by LC-MS

In order to confirm the HPLC result, LC-MS was used to determine the molecular weight of the organic compounds in gardenia extract. ESI positive mode technique was applied to analyze molecular weight of the compounds by scanning

molecular ions (MS) and fragments ions (MS^2). The mass spectra the compounds at retention times of 9.4 and 23.2 min presented at 411.13 and 999.34 m/z, respectively, as shown in Figure 7 and 8. According to the result, the mass spectrum of gardenia extract at 411 m/z represented molecular weight of geniposide + molecular weight of Na^+. These mass spectra were consistent with the previous studies reporting MS^2 spectra of geniposide at 203, 231, 249, and 379 m/z (Cai et al., 2015; Ding et al., 2010). The mass spectrum at 999 m/z represented molecular weight of crocin I + molecular weight of Na^+. Moreover, the MS^2 spectra showed characteristic fragments at 347, 513, and 675 m/z which were in consistence with previous reports for crocin I (Cai et al., 2015; Ketmaro, 2009).

From the analysis of gardenia extract by using HPLC, UV spectra and LC-MS, it can be concluded that the organic compounds of gardenia extract contains geniposide and crocin I, which are main compounds found in gardenia fruits (Zhou et al., 2012; Chen et al., 2010; Liang et al., 2014; Zhou et al., 2005). Due to the main interest of this study, we were interested in geniposide of the extract only since it is the active compound providing anti-inflammatory activity. Crocin I were not further monitored in the rest of this study.

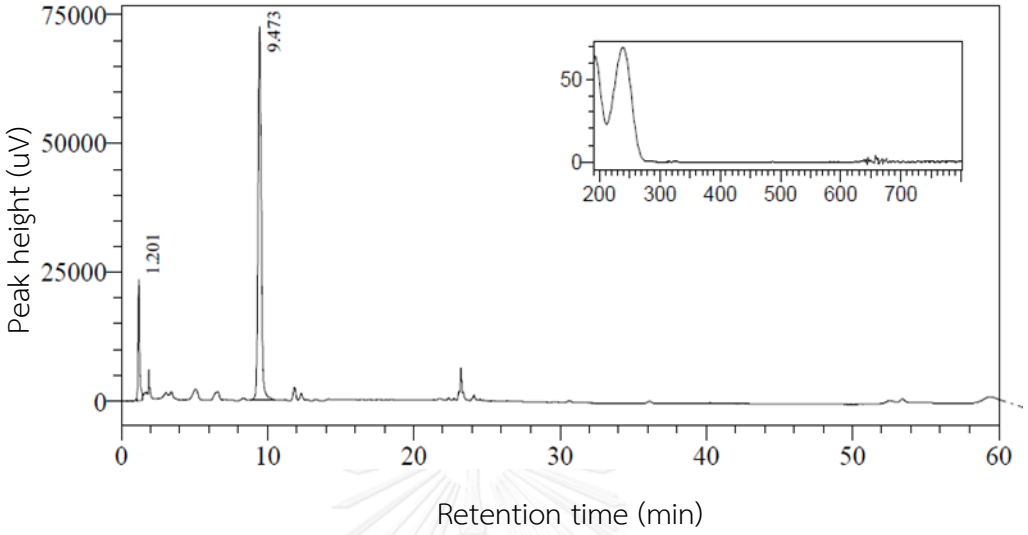


Figure 5 HPLC chromatogram and UV spectrum of geniposide at 238 nm

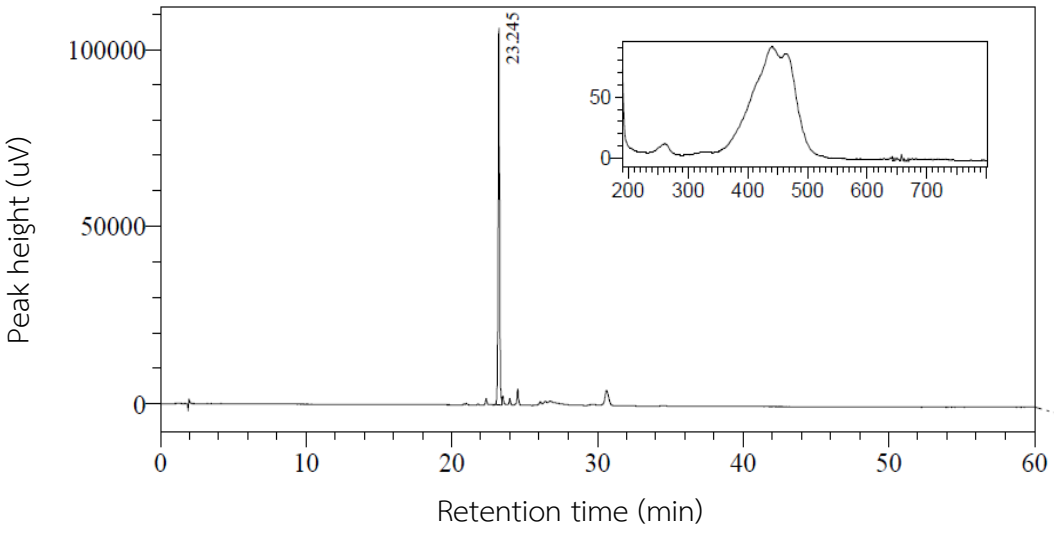
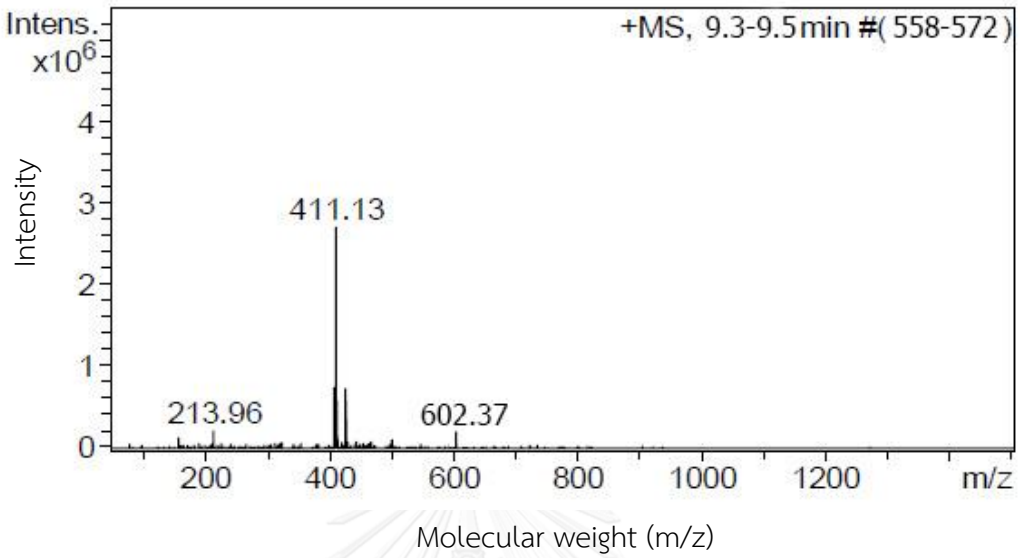


Figure 6 HPLC chromatogram and UV spectrum of crocin I at 440 nm

(a)



(b)

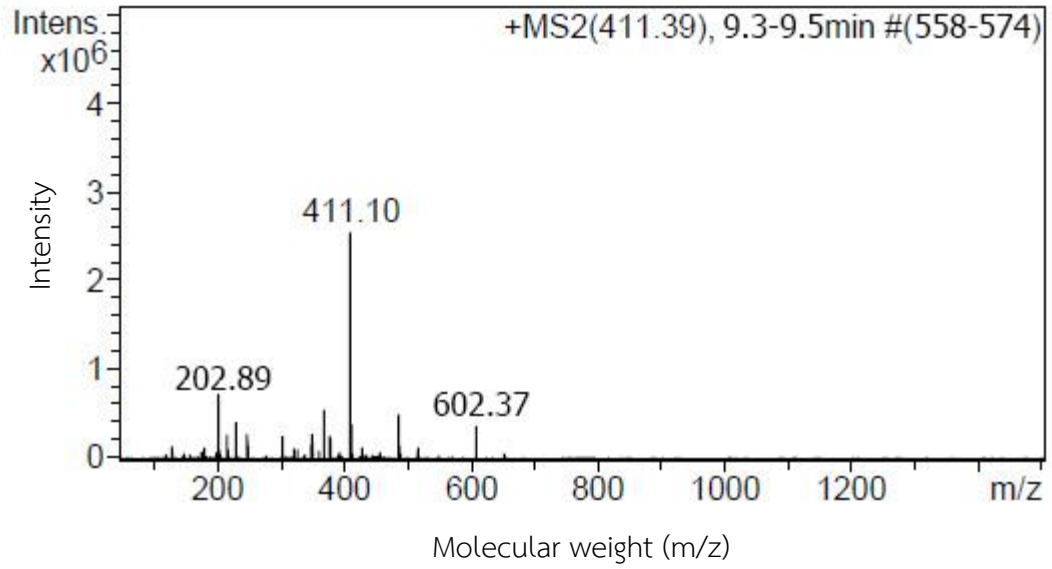
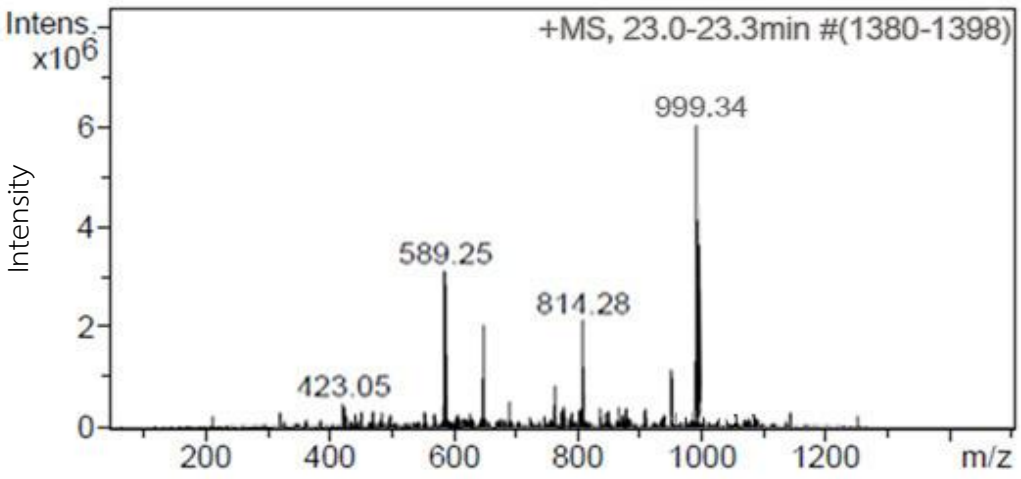


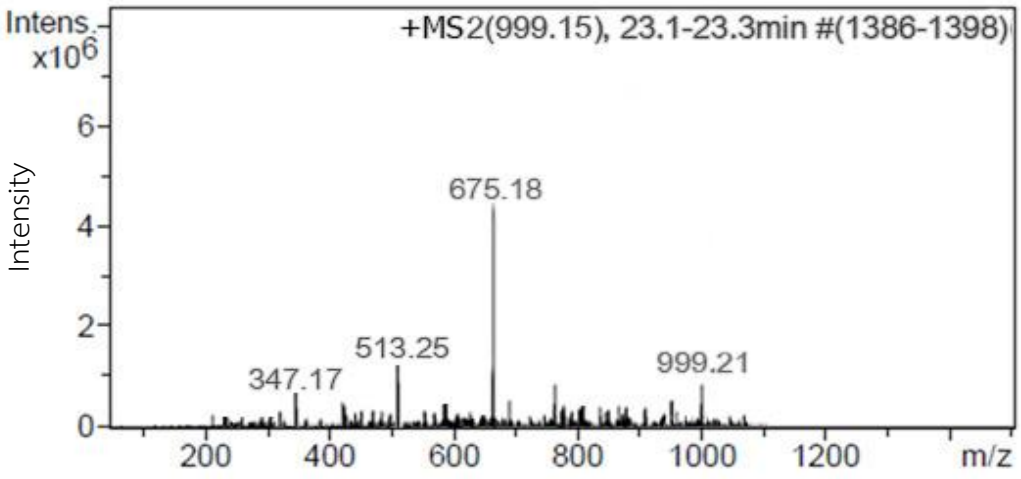
Figure 7 LC-MS of gardenia fruit extract mass specta of the peak at retention time of 9.4 min, molecular ions (MS) (a) fragment ions (MS^2) (b)

(a)



Molecular weight (m/z)

(b)



Molecular weight (m/z)

Figure 8 LC-MS of gardenia fruit extract mass specta of the peak at retention time of 23.2 min, molecular ions (MS) (a) fragment ions (MS^2) (b)

1.3. HPLC method verification

In this study, the HPLC condition modified from Chen et al. (2010) was evaluated using specificity, accuracy, precision and according to Association Of Analytical Communities 2016. The result was concluded as shown in Table 6.

Table 6 HPLC method verification.

Parameters	Result values	Limitation
Standard geniposide		
1. Accuracy (% Recovery)	99.87, 99.49 and 100.97	95-105
2. Precision (% RSD)	0.6027, 0.1575 and 0.6773	< 3.70
3. Linearity	0.9999	≥ 0.999
Raw material and finish product		
1. Specificity	No peak interfere	No peak interfere
2. Accuracy (% Recovery)		
2.1. Gardenia extract	98.44, 98.22 and 97.99	95-105
2.2. Powder facial masks	97.08, 97.75 and 97.55	95-105
2.3. Peel-off gel facial masks	101.62, 102.43 and 101.94	95-105
3. Precision (% RSD)		
3.1. Intraday		
- Gardenia extract	1.2760	< 3.70
- Powder facial masks	0.7979	< 3.70
- Peel-off gel facial masks	0.5923	< 3.70
3.2. Interday		
- Gardenia extract	1.0452	< 3.70
- Powder facial masks	1.2421	< 3.70
- Peel-off gel facial masks	0.6494	< 3.70

3.2.1. Standard geniposide

Accuracy

The accuracy of the analytical method was determined in term of % recovery of standard geniposide at the concentrations of 0.050, 0.075 and 0.100 mg/ml. The result is shown in Table A1 and concluded in Table 6. The percentages of recovery were in the acceptable range of 95-105%.

Precision

The precision of the HPLC method was evalutated using intraday analyses. The intraday result is shown in Table A2. The % RSD values were less than 3.70 indicating that the analytical method used was consistent to analyze the amount of geniposide

Linearity

The linear relationship of geniposide was determined in the concentration range of 0.0050-0.1000 mg/ml. Analysis for area under the curve (AUC) of geniposide solutions is shown in Table A3. The plot of AUC vs concentration is shown in Figure 9. The linear correlation was y = 14,831,258.0585x + 2,708.7057 and r = 0.9999. The acceptance criteria is $r \geq 0.999$.

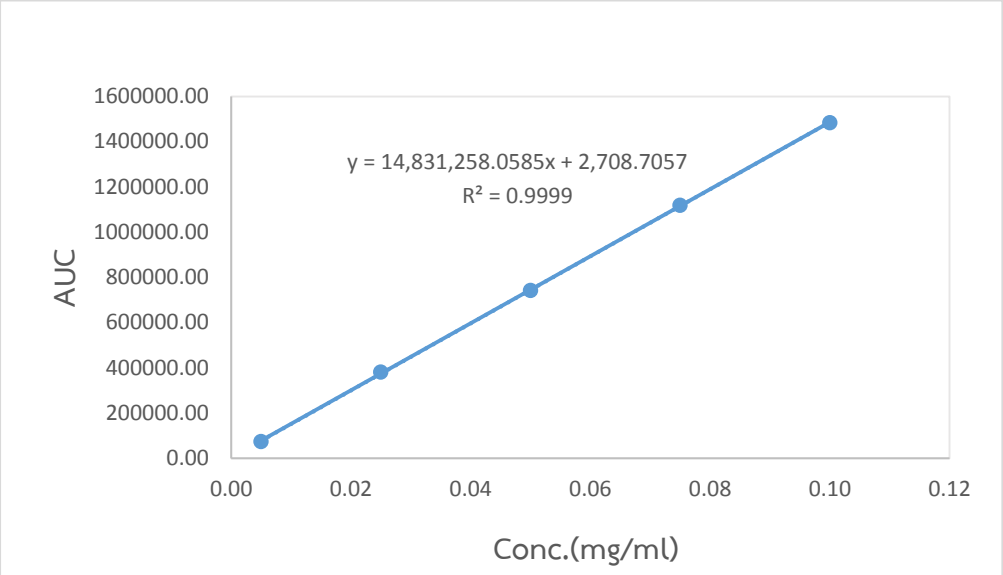


Figure 9 The calibration curve of standard geniposide

3.2.2. Raw material and finish product

Specificity

The HPLC chromatograms of blank and facial mask bases compared to the mask preparations containing gardenia extract are shown in Figures 10, 11 and 12 presenting the chromatograms of powder mask and peel-off gel mask, respectively. At the retention time of 9.4 min, peak of geniposide was only found in the mask preparation containing gardenia extract. The peak purity values were 0.9999, 0.9998 and 0.9999 in blank, powder facial mask and peel off gel facial mask, respectively. This peak purity values were in the acceptable criteria (≥ 0.99). Therefore, the analytical method had specificity to measure the content of geniposide in dried gardenia extract and facial mask products. The degradation study of geniposide caused by hydrolysis,

oxidation and heat was investigated. All forced degradation tests founded that the peak purity values passed the acceptance criteria; i.e. peak purity indexes were greater than 0.99. The results are shown in Figure 13, 14 and 15 and Table A4, A5 and A6.

Accuracy

The accuracy of the analytical method was determined in term of % recovery of gardenia extract, powder facial mask and peel-off gel facial mask at the extract concentrations of 0.32, 0.40 and 0.48 mg/ml. The result is shown in Table A7 and concluded in Table 6. The percentages of recovery were in the acceptable range of 95-105%. Therefore, the modified analytical method was accurate for analyzing geniposide content in the dry extract and the face mask products.

Precision

The precision of the HPLC method was evalutated using intraday and interday analyses. The intraday result is shown in Table A8 and the interday result is shown in Table A9-A11. The % RSD values were less than 3.70 indicating that the analytical method used was consistent to analyze the amount of geniposide. .

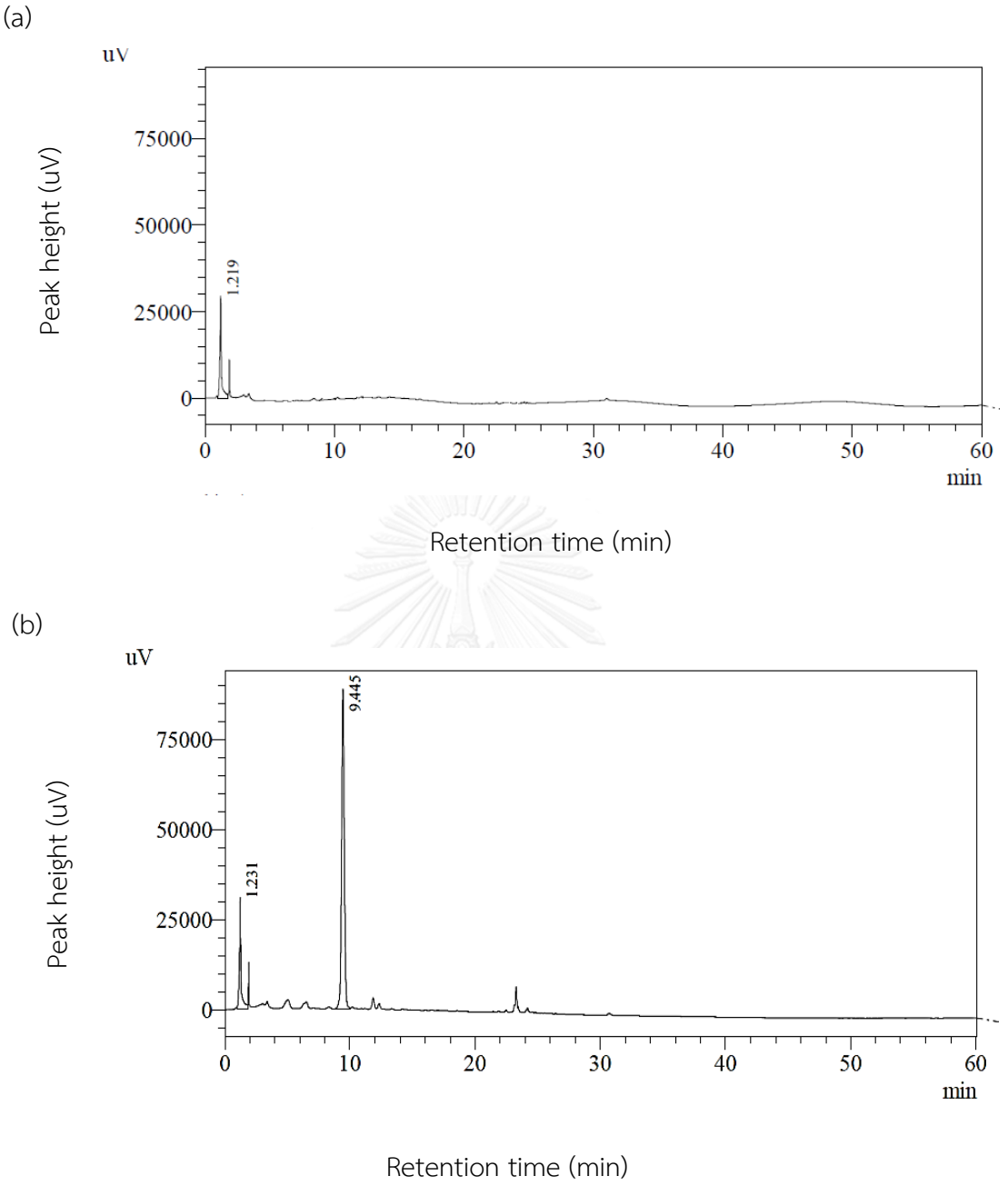
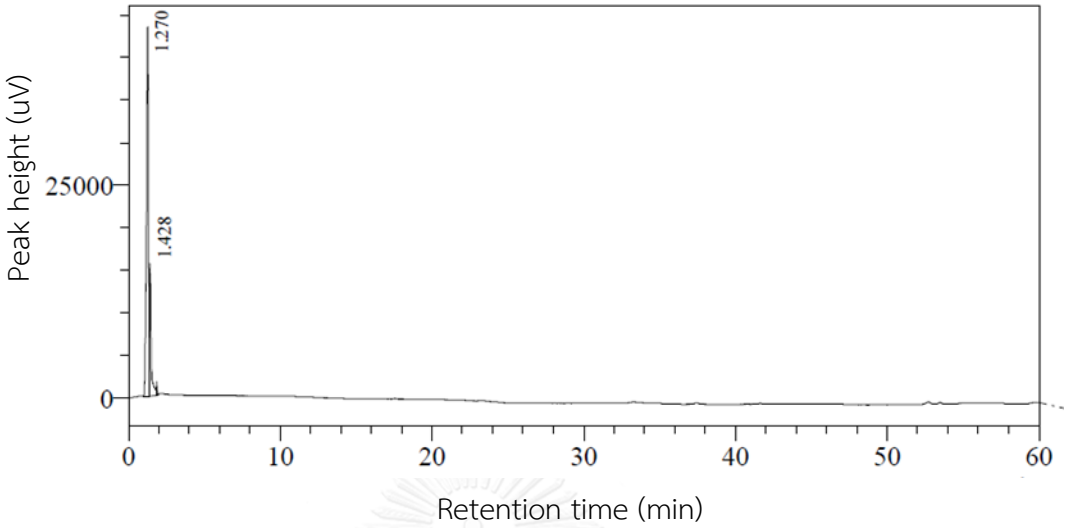


Figure 10 HPLC chromatogram of blank (a) and the blank containing gardenia extract (b)

(a)



(b)

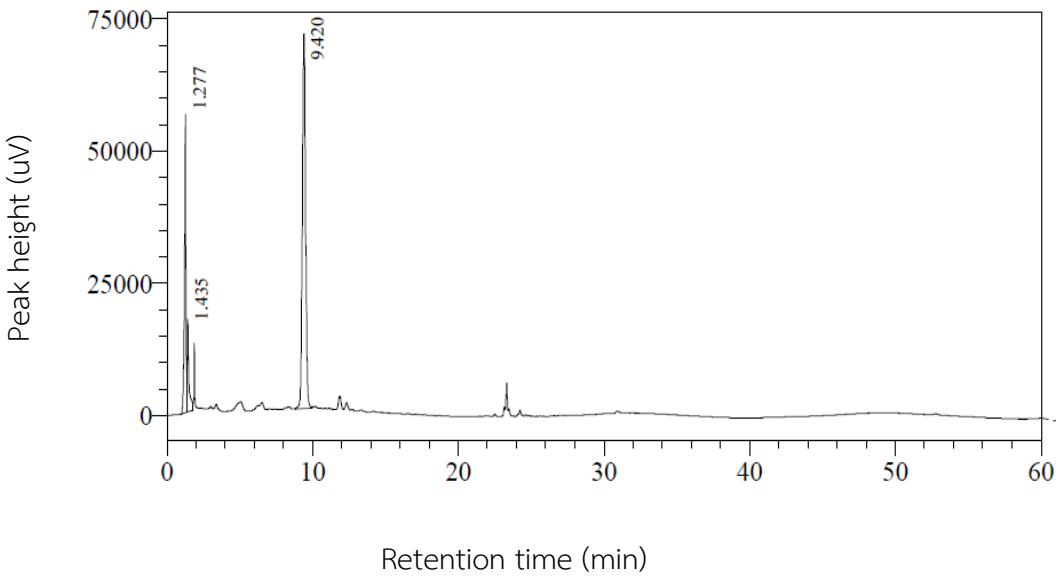
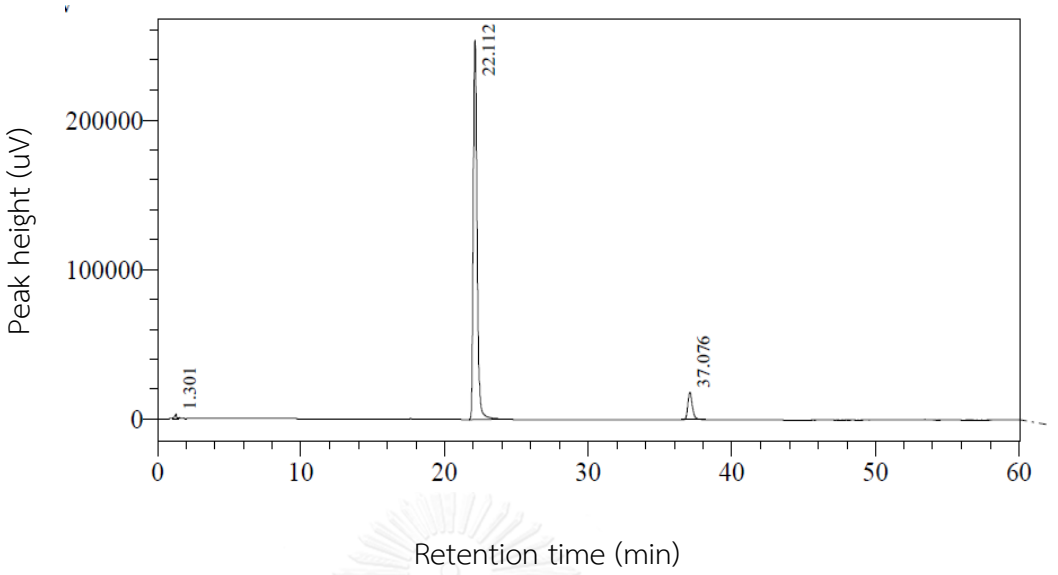


Figure 11 HPLC chromatogram of powder facial mask base (a) and the powder facial masks containing gardenia extract (b)

(a)



(b)

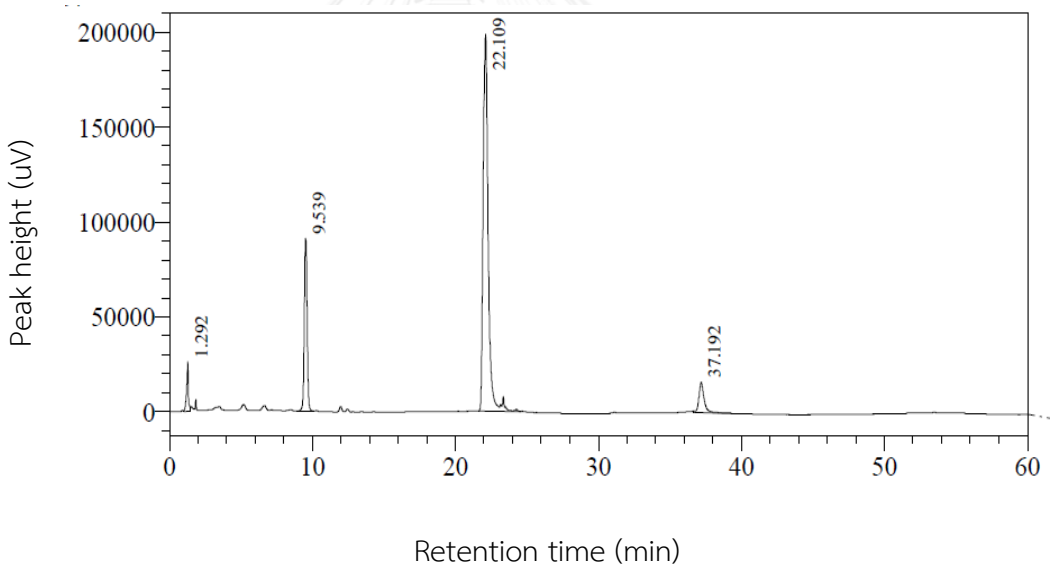


Figure 12 HPLC chromatogram of peel-off gel facial mask base (a) and peel-off gel facial masks containing gardenia extract (b)

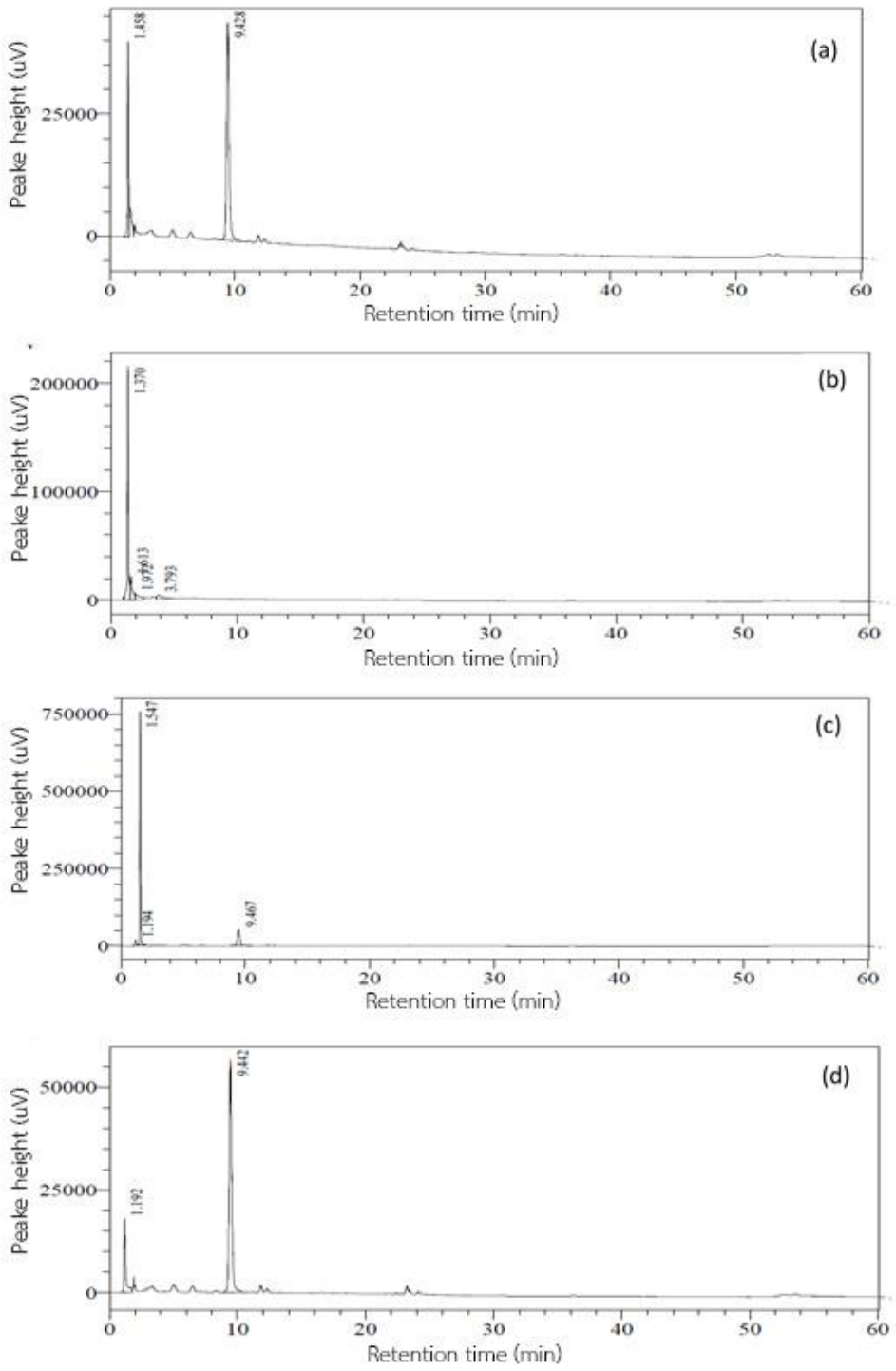


Figure 13 Chromatogram degraded product by 1 M HCl (a),1 M NaOH (b), 3% H_2O_2(c) and 80 °C (d) of gardenia extract

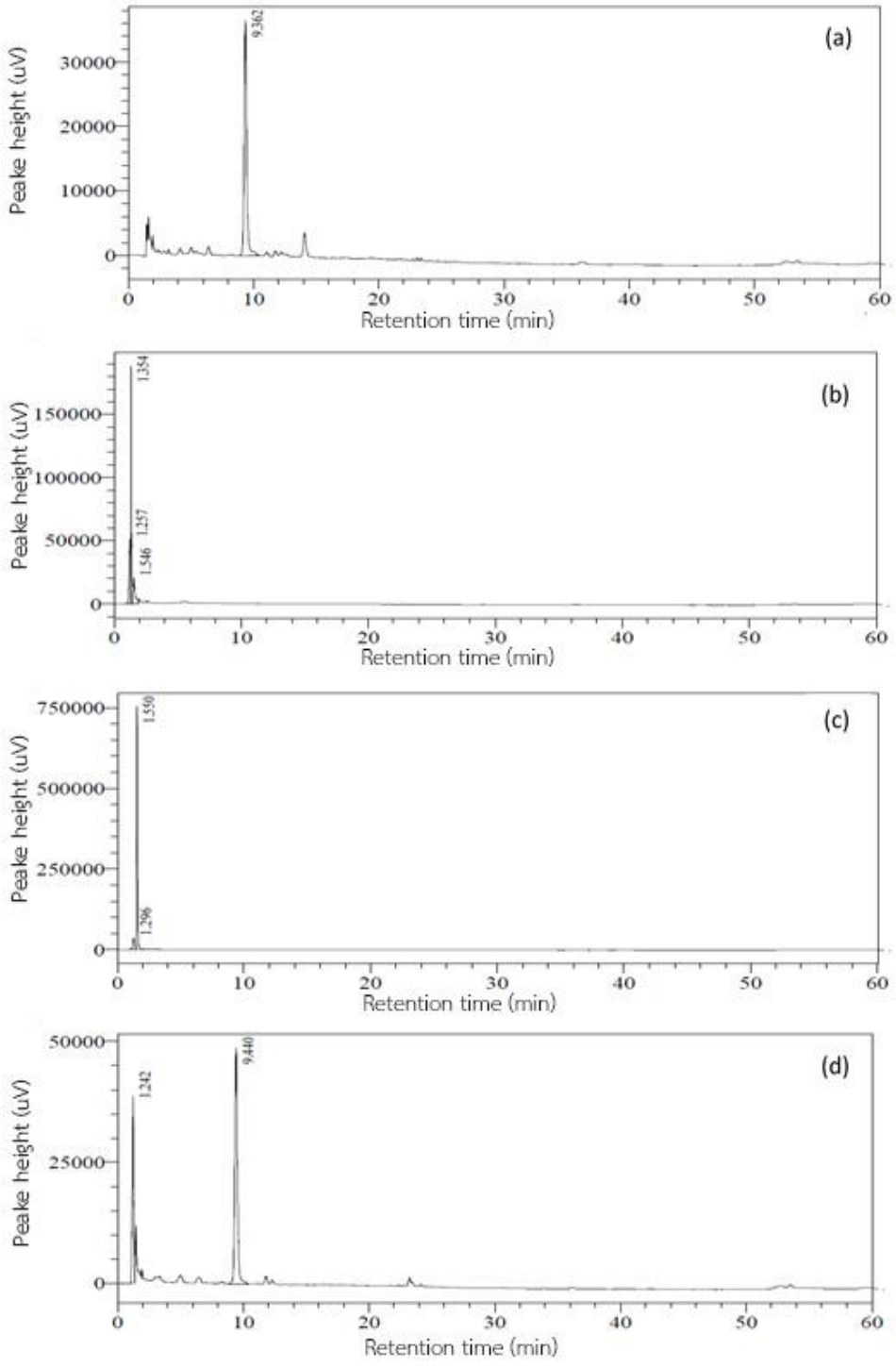


Figure 14 Chromatogram degraded product by 1 M HCl (a),1 M NaOH (b), 3% H_2O_2(c) and 80 °C (d) of powder facial mask.

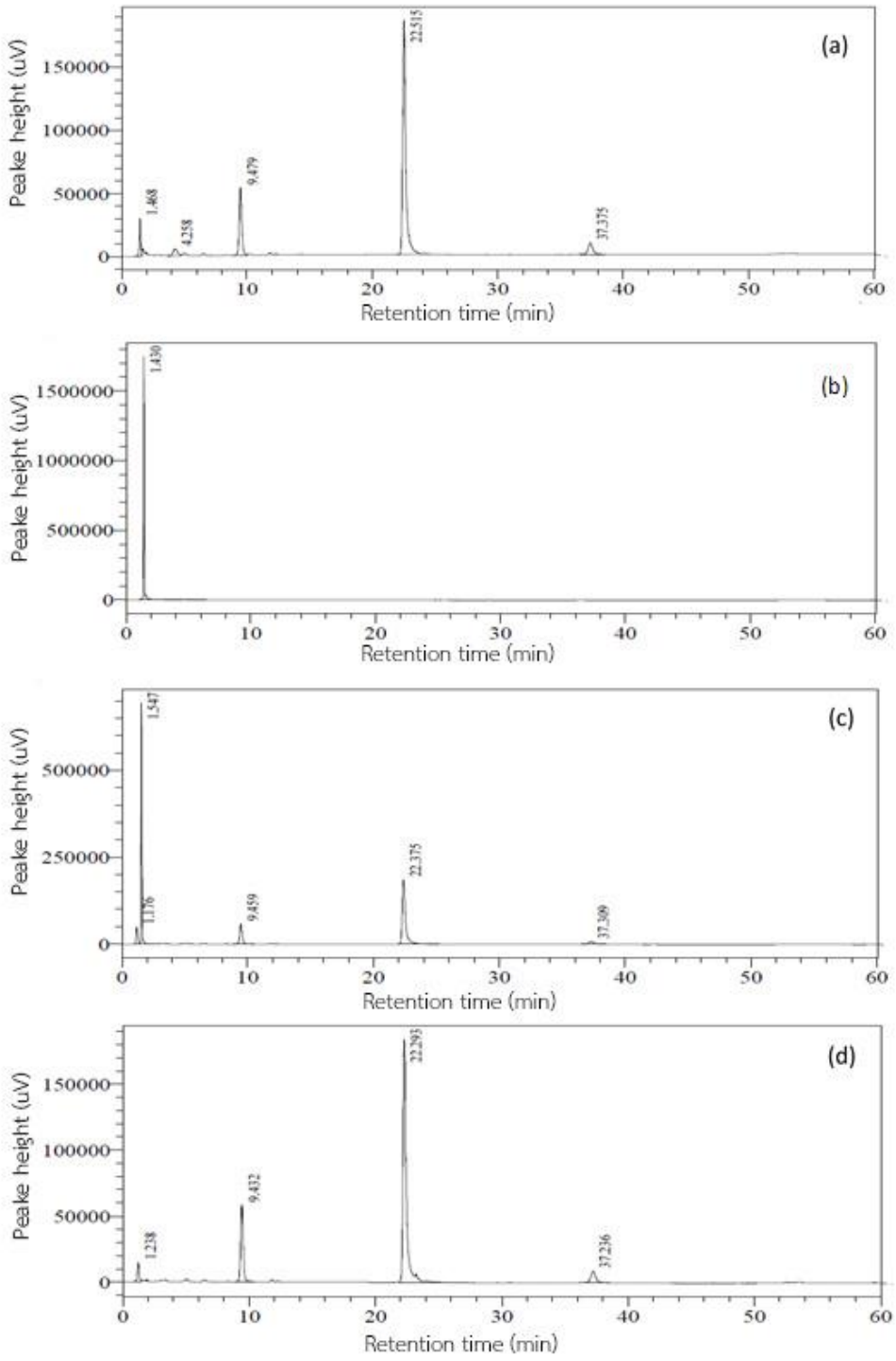


Figure 15 Chromatogram degraded product by 1 M HCl (a), 1 M NaOH (b), 3% H_2O_2(c) and 80 °C (d) of peel-off gel facial mask

The study of degradation in gardenia fruit extract and stability in face mask products containing gardenia extract were evaluated using geniposide content. After gardenia extract and the mask preparations containing the extract were carried out under 4 accelerated conditions, degradation of geniposide was observed. There was no interference of any degradation product at the retention time of 9.4 min. Under alkaine condition, peak of geniposide was not found meaning that the compound was degraded the fastest at high pH.

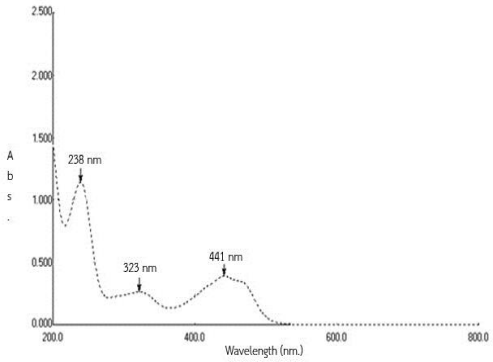
2. Extraction of gardenia fruits

2.1. Preparation of gardenia fruit extract

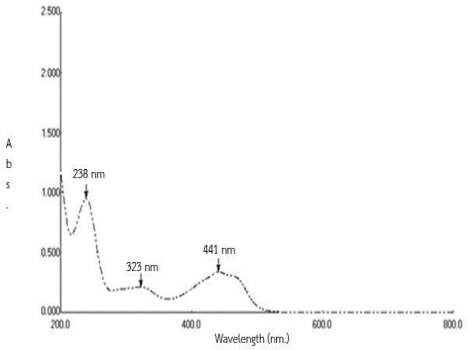
The difference extraction processes were used for the gardenia extraction. Water and water mixed with ethanol were used as solvents. Yellow dry powder of gardenia extract was obtained after the extract solution was lyophilized as shown in Figure 16. Visible-spectrum pattern of the extract did not change when compared between before and after the lyophilization processes, as shown in Figure 17. Therefore, the lyophilization process did not cause degradation of the gardenia extract. In previous studies, it is reported that the UV absorption spectra displayed 3 absorption peaks. The peaks were geniposide, chromogenic acid and crocins with wavelength at 238, 323, and 442 nm respectively (Zhou et al., 2005; Chen et al., 2012; Carmona et al., 2006; Wang et al., 2004; Zhou et al., 2007).

Figure 16 The lyophilized of gardenia fruit

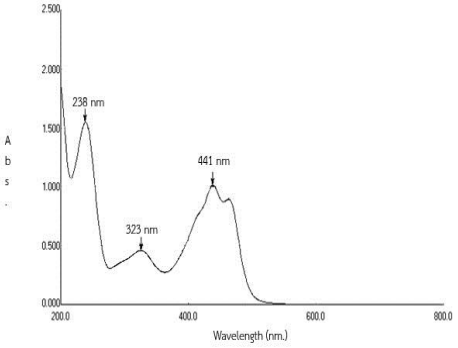
(a) Extract solution



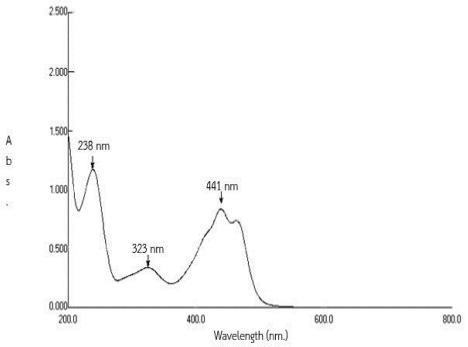
(b) After lyophilization



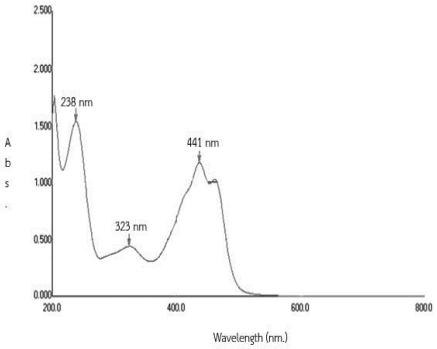
(c) Extract solution



(d) After lyophilization



(e) Extract solution



(f) After lyophilization

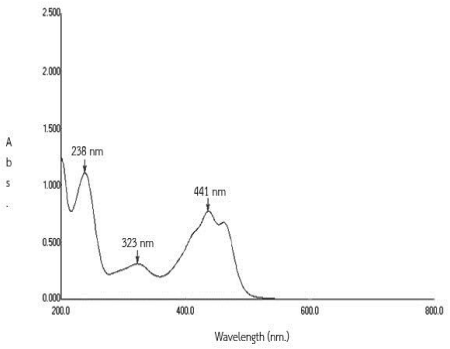


Figure 17 Visible spectra of gardenia fruit dye solutions and after lyophilization in water (a,b), 50% ethanol (c,d) and 70% ethanol (e,f)

When compared the percent yield during the first and the second extractions (shown in Table 7), the first extraction gave higher percent yield than the second extraction. The highest percent yield was obtained from W5E as shown in Table 7 and A12 (not significant; p value > 0.05). It could be explained that water in the first extraction swelled the dry crude and dissolve compounds in the crude very well. In the second extract, 50% ethanol providing suitable polarity could dissolve semipolar compounds from the swollen crude better than other solvents.

Table 7 The percent yields of extract from various extraction processes.

Method	Percent yield of extract (mean±SD, n=3) (Solvent 1)	Percent yield of extract (mean±SD, n=3) (Solvent 2)	Percent yield of extract* (mean±SD, n=3)
WW	7.13±1.08	3.70±0.32	10.83±1.02
W5E	7.88±0.32	5.17±0.48	13.06±0.75
W7E	7.56±1.30	4.09±0.65	11.66±1.83
5EW	7.12±0.61	4.49±0.16	11.62±0.72
7EW	6.20±0.83	4.53±0.18	10.73±0.65

* Not significantly different (p value > 0.05)

2.2. Evaluation of geniposide of gardenia fruit extract

Geniposide is an active major compound in gardenia fruit. In this study amount of geniposide in the fruit extract was monitored. The content of geniposide in gardenia extract from various processes are presented in Table 8 and A13. The extraction process using water with following by 50% ethanol showed the most efficiency to extract geniposide from dried gardenia fruit, which was in accordance with the percent yield mentioned. The percent of geniposide in the gardenia fruit and the percentage of geniposide in the dried extract were 2.47±0.14 and 18.92±0.02, respectively. The W5E showed significantly (p value < 0.05) the most effective process than other processes. According to the previous studies, amount of geniposide in gardenia fruit was 1.799-2.366% per dry weight (Gao and Zhu, 2013) and geniposide in the dried extract was 12.55 - 21.33% per dry weight (Bergonzi et al., 2011; Bergonzi et al., 2012). The result of geniposide content in the gardenia fruit and the dried extract were consistence with previous studies.

From the chemical structure of geniposide (Figure 2), it is an iridoid glycoside and rather water soluble. Water seems to be a suitable solvent to extract this compound which is reported in a previous study (Wang et al., 2012). Gardenia fruit also contains pectins (Xu et al., 2016) causing high viscosity during the extraction process. Since pectins were readily hydrated in water, the gardenia extract would contain a large amount of pectins as well when water was used as solvent. With the use of 50%

ethanol to extract the crude after water extraction, 50% ethanol could desorp geniposide from the swollen crude and less amount of pectins came out. Pectins were less hydrated in 50% ethanol while geniposide was still dissolved in this solvent leading to high percent yield of geniposide.

Table 8 The percent geniposide in the gardenia fruit and in the dried extract.

Method	Percent geniposide in the gardenia fruit (mean±SD, n=3)	Percent geniposide in the dried extract ((mean±SD, n=3)
WW	1.97±0.18	18.20±0.05
W5E	2.47±0.14	18.92±0.02
W7E	1.78±0.27	15.25±0.14
5EW	1.82±0.11	15.62±0.01
7EW	1.40±0.09	13.07±0.23

3. Preparation and evaluation of facial masks

3.1. Powder facial masks

Type and concentration of gelling agents in the powder facial mask base were varied. Fine off-white powder was obtained from all formulations and the powder turned to be viscous light yellow paste after water was added. Physical properties regarding the spreadability, pH value, and drying time of the paste were evaluated and the result are shown in Table 9 and A14. All formulations provided pH in the acceptable range (5-7). Less drying time was observed in the paste containing polyvinyl pyrrolidone K30 due to less ability to bind to water. Both xanthan gum and trgacanth are polysaccharides containing a lot of hydroxyl groups which can hold water molecules stronger than polyvinyl pyrrolidone K30 binging to water molecules. At the same concentration of the viscosity enhancers, xanthan gum provides higher viscosity than tragacanth and polyvinyl pyrrolidone K30. More viscous facial masks were obtained in the formulations containing xanthan gum when compared to the formulations containing tragacanth and polyvinyl pyrrolidone K30. P1 containing 1% w/w xanthan gum presented proper viscosity and good spreadability meaning that it was not too watery and not too thick to be spreaded on skin with drying time of 25 min.

5.2. Peel-off gel facial masks

Physical properties of the peel-off gel mask bases were changed by gelling agents and plasticizers as shown in Table 10 and A15. Clear colorless gel was obtained from all formulations and within acceptable pH range (5-7). Both glycerin and propylene were good plasticizers. However, without gelling agent, the peel-off gel mask bases were too liquidity and continuous film was not obtained (F1 and F6). The peel-off gel mask bases with 0.5 and 1% w/w xanthan gum and 1% w/w tragacanth were high viscosity cause of hard spreadability (F2, F3, F5, F7, F8 and F10). Then less amount of xanthan gum was needed to receive proper viscosity. Appropriate spreadability was obtained from F4 (0.5% tragacanth and 6.0% glycerin) and F9 (0.5% tragacanth and 6.0% propylene glycol). Moreover, F4 and F9 also presented proper drying time and peel-off film formation. Since glycerin provides skin moisture effect more than propylene glycol (Leelapornpisid et al., 2014), therefore F4 was selected for further study.

Table 9 Physical evaluation of powder facial mask base.

Properties	P1	P2	P3	P4	P5	P6	P7	P8	P9
1. pH (SD)	6.80 (0.03)	6.78 (0.05)	6.75 (0.02)	6.75 (0.05)	6.75 (0.02)	6.74 (0.01)	6.74 (0.01)	6.70 (0.03)	6.52 (0.01)
2. Spreadability	++++	++	+	+++	++	+	+	+	++
3. Drying time (min)	25	30	30	25	30	30	15	15	20

Note: Spreadability ++++ Excellent +++ Good ++ Fair + Poor.

Table 10 Physical evaluation of peel-off gel mask base.

Properties	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
1. pH (SD)	6.09 (0.03)	6.06 (0.03)	5.89 (0.02)	5.73 (0.06)	5.55 (0.03)	6.19 (0.04)	6.11 (0.06)	5.95 (0.01)	5.83 (0.02)	5.51 (0.01)
2. Spreadability	+++	++	+	+++	++	+++	++	+	+++	++
3. Drying time (min)	25	25	30	30	30	30	35	35	35	35
4. Peel-off film formation	++	++++	+++	++++	++++	++	++++	+++	++++	++++

Note: Film formation ++++ Excellent +++ Good ++ Fair + Poor.

4. Preparation and evaluation of facial masks containing gardenia fruit extract.

4.1. Powder facial masks

P1, the powder facial mask base, was selected from our pervious study (3.1) due to its proper speadability, pH (6.59±0.02) and drying time (25 min). The off-white color of the powder facial mask base turned yellowish after gardenia extract was added (Figure 18). Then physical properties and chemical stability of this powder facial mask base was further evaluated.

4.2 Peel-off gel facial masks

F4, the peel-off gel mask base, provided suitable physical properties including speadability, pH (5.51±0.03), drying time (30 min) and peel-off film formation. The colorless clear gel of the peel-off mask base became bright yellowish clear gel after gardenia extract was added (Figure 19). This peel-off gel mask was studied on physical and chemical stabilities.

5. Evaluation of physical properties of facial masks

The physical properties of powder facial masks and peel-off gel facial masks over 3 months of storage are shown in Tables 11 and 12, respectively. Raw data are present in Table A14-A17. Appearance of the mask preparations are presented in Figure 18, 19, A1 and A2. The result showed that the pH, viscosity and drying time of both facial mask did not change from the beginning especially drying time and peel-off film formation meaning that the preparation were physically stable.

Figure 18 Appearance of powder facial mask.

Figure 19 Appearance of peel-off gel facial mask.

Table 11 Physical evaluation of powder facial mask.

Properties	0 Month			1.25 Months			2 Months			3 Months		
	4 °C	30 °C	40 °C	4 °C	30 °C	40 °C	4 °C	30 °C	40 °C	4 °C	30 °C	40 °C
1. pH (SD)	6.59 (0.02)	6.59 (0.02)	6.59 (0.02)	6.57 (0.02)	6.58 (0.01)	6.57 (0.02)	6.56 (0.03)	6.57 (0.02)	6.57 (0.02)	6.58 (0.02)	6.56 (0.03)	6.59 (0.01)
2. Viscosity (cP) [a]	370.69 ±1.28	370.69 ±1.28	370.69 ±1.28	370.28 ±0.71	370.89 ±0.35	370.69 ±0.94	370.07 ±0.35	371.10 ±1.06	371.71 ±0.61	369.87 ±0.61	371.30 ±1.42	371.92 ±0.35
3. Drying time (min)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00

a Each value is expressed as mean ± standard deviation (n=3)

Table 12 Physical evaluation of peel-off gel facial mask.

Properties	0 Month			1.25 Months			2 Months			3 Months		
	4 °C	30 °C	40 °C	4 °C	30 °C	40 °C	4 °C	30 °C	40 °C	4 °C	30 °C	40 °C
1. pH (SD)	5.51 (0.03)	5.51 (0.03)	5.51 (0.05)	5.53 (0.04)	5.52 (0.05)	5.53 (0.06)	5.51 (0.05)	5.52 (0.06)	5.52 (0.04)	5.52 (0.06)	5.52 (0.06)	5.52 (0.04)
2. Viscosity (cP)[a]	16973.13 ±30.03	16973.13 ±30.03	16973.13 ±30.03	16960.03 ±40.93	16973.13 ±40.93	16992.79 ±30.03	16927.13 ±19.66	16981.65 ±21.20	16992.79 ±63.20	16927.76 ±34.05	16986.24 ±52.02	16999.35 ±22.70
3. Drying time (min)	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
4. Peel-off film formation	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

a Each value is expressed as mean ± standard deviation (n=3)

Note: Peel-off film formation ++++ Excellent +++ Good ++Fair + Poor.

6. Evaluation of chemical stability of facial masks

Gardenia was used as a marker for this chemical stability study since it is a major active compound in the extract. The content of geniposide in both powder facial mask and peel-off gel facial mask were determined. The results were shown in Figures 20, 21 and Table A18. At 4 ^{o}C, geniposide was quite stable in both facial mask preparations. Remaining of geniposide in the powder facial mask became less than 90% after stored for 2 months at 30 and 40 ^{o}C. Content of geniposide in the peel-off gel facial mask remained unchange over 3 months at 4, 30 and 40 ^{o}C. It means that geniposide was more stable in the peel off gel facial mask than in the powder facial mask although the peel-off facial mask contained water more than 60%. It could be due to pH and other ingredient in the peel-off facial mask such as EDTA stabilizing geniposide. The peel-off gel facial masks provided more acidic environment (pH=5.51) than the powder facial mask (pH=6.81). The basic environment of powder facial mask could be caused by the alkaline property of the substances in the formula such as kaolin and zince oxide. These substances could adsorb moisture from environment causing alkaline condition. Oxidation reaction causing the degradation should not be the reason because all samples were purged with nitrogen gas before they were sealed. Moreover, heat accelerated the loss of geniposide.

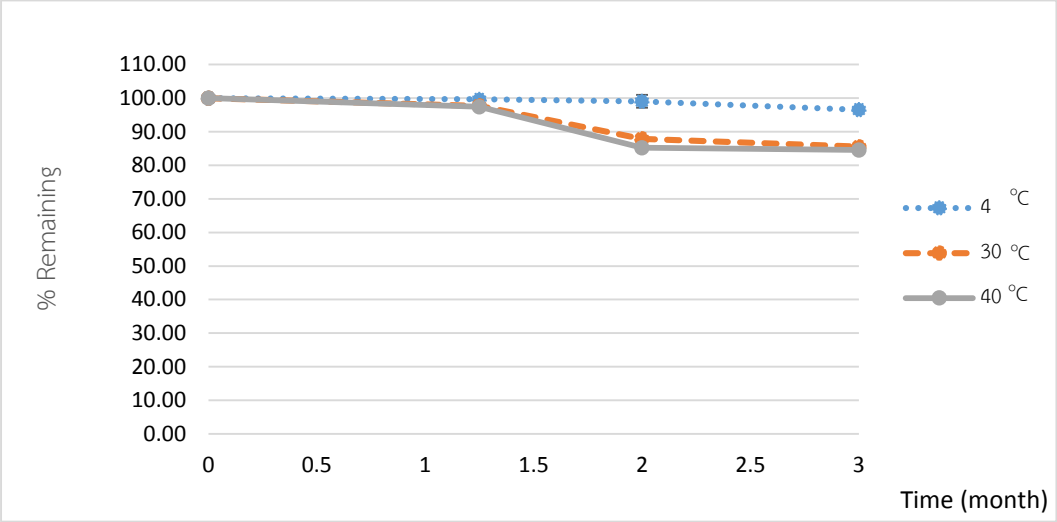


Figure 20 Percent remaining of geniposide in powder facial mask over 3 months

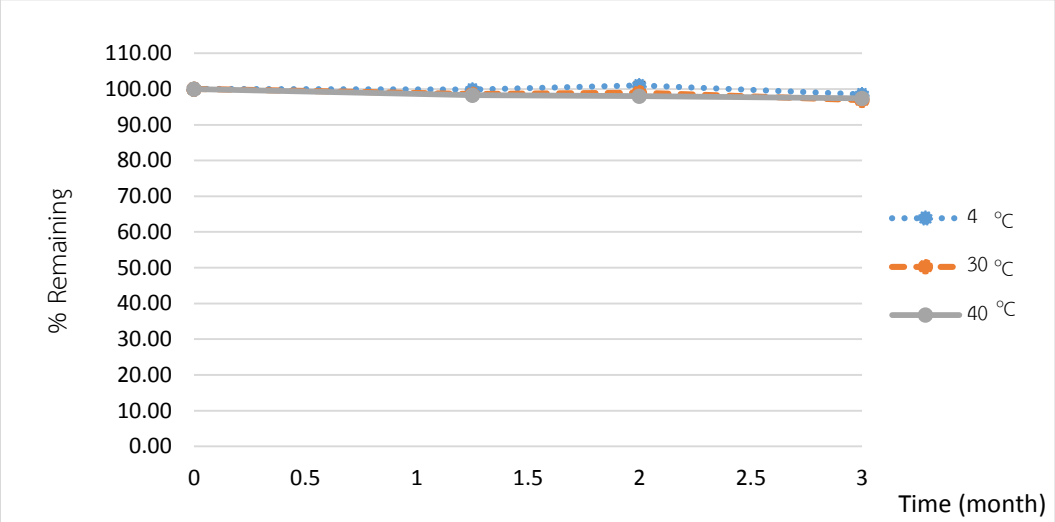


Figure 21 Percent remaining of geniposide in peel-off gel facial mask over 3 months.

CHAPTER V
CONCLUSIONS

With the use of solvent extraction method, the optimum solvent to extract gardenia fruit was water following by 50% v/v of ethanol. Yellow – orange color flake was obtained after gardenia extract was lyophilized. Yield of gardenia extract was 13.06±0.75% by weight from this process and this gardenia extract contained the highest amount of geniposide 18.92±0.02% by weight.

Powder facial mask base containing 1% w/w xanthan gum as a gelling agent provided satisfied appearance. A suitable peel off gel mask base film was obtained when 0.5% w/w tragacanth as a gelling agent and 6% w/w glycerine as a plasticizer were added in the preparation. Facial mask preparations containing 0.5% gardenia extract were prepared from these facial mask bases. The hydrated gardenia powder facial mask provided pH value of 6.59±0.02 and viscosity 370.69±1.28 cP. The hydrated mask was easily spreaded on skin with drying time of 25 min**.** The gardenia peel-off gel mask provided pH value of 5.51±0.03, viscosity of 16973.13±30.03 cP and drying time of 30 min**.**

The physical and chemical properties of facial mask products were determined. Both facial masks containing gardenia extract were physically stable. Geniposide was used as a marker to monitor chemical stability of the gardenia extract in the facial masks. Geniposide was more stable in the peel-off gel facial

mask than in the powder facial mask. The geniposide contents in powder facial mask at 4, 30 and 40 ° C after 3 months storage were 96.54±0.49, 85.55±0.47 and 84.55±0.63% by weight, respectively, while the geniposide contents in peel-off gel facial mask were 98.48±0.42, 96.8±0.33 and 97.40±0.34% by weight, respectively. It implied that increase of temperature induced the degradation of geniposide in the powder facial mask. Lower pH value and ingredients in the peel-off gel facial mask probably helped the preparation to stabilize geniposide. Efficacy and safety of the gardenia facial masks should be further investigated.

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APPENDIX

Table A 1 Accuracy and precision studies of standard geniposide.

Conc. (mg/ml)	AUC	% Recovery	Average % recovery	SD
0.0496	749881	99.4601	99.8651	0.6019
	758157	100.5567		
	750774	99.5785		
0.0743	1127551	99.6687	99.4894	0.1567
	1124747	99.4210		
	1124267	99.3786		
0.0991	1523231	100.9661	100.9679	0.6839
	1533595	101.6527		
	1512950	100.2850		

Table A 2 Intraday precision of geniposide in gardenia extract and facial masks.

Conc. added (mg/ml)	AUC	Conc. recovered (mg/ml)	Average Conc.	% RSD
0.0496	749881	0.0493	0.0495	0.6027
	758157	0.0498		
	750774	0.0493		
0.0743	1127551	0.0741	0.0739	0.1575
	1124747	0.0739		
	1124267	0.0739		
0.0991	1523231	0.1000	0.1001	0.6773
	1533595	0.1007		
	1512950	0.0994		

Table A 3 Area under curve of standard geniposide at various concentrations.

Conc. (mg/ml)	No. of injection	AUC	Average AUC
0.0050	1	72074	72499.67
	2	72229	
	3	73196	
0.0248	1	373310	379602.67
	2	379234	
	3	386264	
0.0496	1	738707	741644.00
	2	740162	
	3	746063	
0.0743	1	1096833	1118559.00
	2	1134889	
	3	1123955	
0.0991	1	1465017	1483209.00
	2	1468842	
	3	1515768	

Table A 4 Degradation study of geniposide in gardenia extract.

Parameter	Conc. added (mg/ml)	Conc. recovered (mg/ml)	Percentage of degradation
Hydrolysis by 1 M HCl (Acidic degradation)	0.0759	0.0427	43.79
Hydrolysis by 1 M NaOH (Alkaline degradation)	0.0759	0.0000	100.00
Oxidation by 3% H_2O_2	0.0759	0.0488	35.82
Thermal degradation (80 °C)	0.0759	0.0531	30.22

Table A 5 Degradation study of geniposide in powder facial masks.

Parameter	Conc. added (mg/ml)	Conc. recovered (mg/ml)	Percentage of degradation
Hydrolysis by 1 M HCl (Acidic degradation)	0.0757	0.0350	53.81
Hydrolysis by 1 M NaOH (Alkaline degradation)	0.0757	0.0000	100.00
Oxidation by 3% H_2O_2	0.0757	0.0000	100.00
Thermal degradation (80 °C)	0.0757	0.0477	37.18

Table A 6 Degradation study of geniposide in peel-off gel facial mask..

Parameter	Conc. added (mg/ml)	Conc. recovered (mg/ml)	Percentage of degradation
Hydrolysis by 1 M HCl (Acidic degradation)	0.0758	0.0507	33.17
Hydrolysis by 1 M NaOH (Alkaline degradation)	0.0758	0.0000	100.00
Oxidation by 3% H_2O_2	0.0758	0.0547	27.88
Thermal degradation (80 °C)	0.0758	0.0553	27.09

Table A 7 Accuracy studies of geniposide in gardenia extract and facial masks.

Type	Conc. (mg/ml)	AUC	% Recovery	Average % recovery	SD
Gardenia extract	0.0606	887009	98.46	98.44	0.09
		885454	98.34		
		887956	98.51		
	0.0757	1108545	98.18	98.22	0.04
		1109970	98.27		
		1110426	98.21		
	0.0909	1331880	98.12	97.99	0.30
		1324799	97.65		
		1332558	98.19		
Powder facial masks	0.0606	873469	97.14	97.08	0.20
		870588	96.85		
		874698	97.24		
	0.0757	1101330	97.61	97.75	0.15
		1106160	97.91		
		1104019	97.73		
	0.0909	1316284	97.01	97.55	0.77
		1319834	97.22		
		1339208	98.43		
Peel-off gel facial masks	0.0608	957349	101.36	101.62	0.35
		962998	102.02		
		958936	101.49		
	0.0760	1201614	101.80	102.43	0.55
		1211820	102.80		
		1215664	102.70		
	0.0912	1381634	101.82	101.94	0.25
		1387872	102.22		
		1381139	101.78		

Table A 8 Intraday precision of geniposide in gardenia extract and facial masks.

Type	Conc. added (mg/ml)	AUC	Conc. recovered (mg/ml)	Average Conc.	% RSD
Gardenia extract	0.0758	1123623	0.0739	0.0750	1.2760
		1186678	0.0766		
		1168410	0.0754		
		1165576	0.0752		
		1160998	0.0749		
		1148722	0.0742		
Powder facial masks	0.0757	1173064	0.0763	0.0758	0.7979
		1177185	0.0766		
		1154198	0.0751		
		1159824	0.0755		
		1155755	0.0752		
		1165563	0.0759		
Peel-off gel facial masks	0.0759	1201871	0.0776	0.0773	0.5923
		1199018	0.0774		
		1204611	0.0777		
		1203411	0.0777		
		1186533	0.0766		
		1191871	0.0769		

Table A 9 Interday precision of geniposide in gardenia extract.

Day	Conc. added (mg/ml)	AUC	Conc. recovered (mg/ml)	Average Conc.	% RSD
1	0.0758	1123623	0.0739	0.0750	1.0452
		1186678	0.0766		
		1168410	0.0754		
		1165576	0.0752		
		1160998	0.0749		
		1148722	0.0742		
2	0.0757	1141001	0.0748		
		1128348	0.0739		
		1130646	0.0741		
		1131163	0.0741		
		1159280	0.0760		
		1129937	0.0740		
3	0.0758	1156043	0.0755		
		1155143	0.0755		
		1158288	0.0757		
		1152946	0.0753		
		1154924	0.0755		
		1149230	0.0751		

Table A 10 Interday precision of geniposide in powder facial masks.

Day	Conc. added (mg/ml)	AUC	Conc. recovered (mg/ml)	Average Conc.	% RSD
1	0.0757	1173064	0.0763	0.0750	1.2421
		1177185	0.0766		
		1154198	0.0751		
		1159824	0.0755		
		1155755	0.0752		
		1165563	0.0759		
2	0.0760	1170137	0.0755		
		1168629	0.0754		
		1164085	0.0751		
		1166877	0.0753		
		1159941	0.0749		
		1167209	0.0753		
3	0.0757	1133641	0.0738		
		1136802	0.0740		
		1131699	0.0737		
		1136597	0.0740		
		1138130	0.0741		
		1127394	0.0734		

Table A 11 Interday precision of geniposide in peel-off gel facial mask..

Day	Conc. added (mg/ml)	AUC	Conc. recovered (mg/ml)	Average Conc.	% RSD
1	0.0759	1201871	0.0776	0.0778	0.6494
		1199018	0.0774		
		1204611	0.0777		
		1203411	0.0777		
		1186533	0.0766		
		1191871	0.0769		
2	0.0760	1211820	0.0782		
		1201614	0.0775		
		1209250	0.0780		
		1207844	0.0779		
		1209797	0.0781		
		1215664	0.0784		
3	0.0759	1206335	0.0787		
		1202104	0.0776		
		1210214	0.0781		
		1205309	0.0778		
		1206541	0.0779		
		1202717	0.0776		

Table A 12 The percent yields of gardenia extract from various extraction processes.

Method		Solvent 1	Solvent 2	Sum of % yield
WW		7.38	3.87	11.26
		7.84	3.32	11.16
		6.16	3.90	10.06
	Average	7.13	3.70	10.83
	SD	0.87	0.33	0.66
W5E		7.64	4.84	12.48
		7.82	5.73	13.54
		8.20	4.95	13.15
	Average	7.88	5.17	13.06
	SD	0.29	0.48	0.54
W7E		6.06	3.54	9.60
		8.28	4.81	13.09
		8.34	3.94	12.29
	Average	7.56	4.10	11.66
	SD	1.30	0.65	1.83
5EW		6.63	4.31	10.93
		6.94	4.61	11.55
		7.80	4.56	12.36
	Average	7.12	4.49	11.62
	SD	0.61	0.16	0.72
7EW		5.97	4.56	10.53
		7.12	4.34	11.46
		5.50	4.71	10.21
	Average	6.20	4.54	10.73
	SD	0.83	0.19	0.65

Table A 13 The percent geniposide in the gardenia dried fruit and percent geniposide in the dried extract.

Method		Percent geniposide in the gardenia fruit (mean±SD)	Percent geniposide in the dried extract (mean±SD)
WW		2.11	18.17
		2.03	18.19
		1.77	18.26
	Average	1.97	18.20
	SD	0.18	0.05
W5E		2.36	18.93
		2.63	18.91
		2.42	18.90
	Average	2.47	18.92
	SD	0.14	0.02
W7E		1.47	15.34
		1.98	15.09
		1.88	15.33
	Average	1.78	15.25
	SD	0.27	0.14
5EW		1.71	15.61
		1.81	15.63
		1.93	15.63
	Average	1.81	15.62
	SD	0.11	0.01
7EW		1.40	13.33
		1.49	12.97
		1.32	12.90
	Average	1.40	13.07
	SD	0.09	0.23

Table A 14 The pH of hydrated powder facial mask base.

Property	P1	P2	P3	P4	P5	P6	P7	P8	P9
	6.84	6.73	6.73	6.70	6.74	6.74	6.74	6.67	6.52
pH	6.78	6.82	6.77	6.81	6.78	6.75	6.76	6.73	6.54
	6.80	6.80	6.75	6.75	6.75	6.75	6.74	6.70	6.52
Average	6.81	6.78	6.75	6.75	6.76	6.75	6.75	6.70	6.53
SD	0.03	0.05	0.02	0.06	0.02	0.01	0.01	0.03	0.01

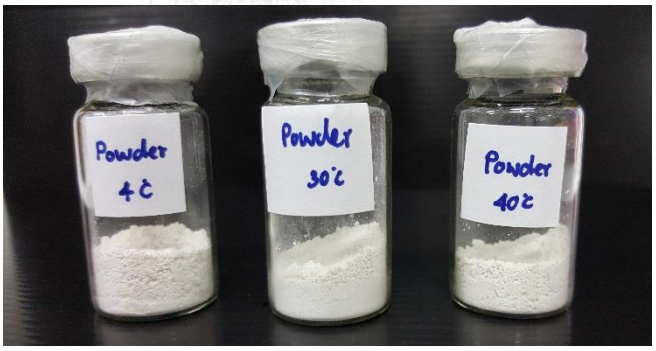
Table A 15 The pH of peel-off gel mask base.

Property	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
	6.12	6.08	5.88	5.70	5.52	6.15	6.17	5.94	5.84	5.52
pH	6.06	6.03	5.91	5.80	5.55	6.22	6.06	5.95	5.81	5.51
	6.08	6.06	5.89	5.70	5.57	6.19	6.11	5.95	5.83	5.51
Average	6.09	6.06	5.89	5.73	5.55	6.19	6.11	5.95	5.83	5.51
SD	0.03	0.03	0.02	0.06	0.03	0.04	0.06	0.01	0.02	0.01

0 Month

1.25 Months

2 Months



3 Months

Figure A 1 Appearance of powder facial mask over storage time.

0 Month

1.25 Months

2 Months

3 Months

Figure A 2 Appearance of peel-off gel facial mask over storage time.

Table A 16 The pH of hydrated powder facial mask containing gardenia extract.

Property		0 Month			1.25 Months			2 Months			3 Months		
		4 °C	30 °C	40 °C	4 °C	30 °C	40 °C	4 °C	30 °C	40 °C	4 °C	30 °C	40 °C
pH		6.59	6.59	6.59	6.59	6.58	6.55	6.57	6.56	6.58	6.55	6.53	6.58
		6.60	6.60	6.60	6.56	6.57	6.57	6.59	6.59	6.54	6.59	6.56	6.60
		6.57	6.57	6.57	6.55	6.58	6.58	6.53	6.57	6.58	6.59	6.58	6.59
	Average	6.59	6.59	6.59	6.57	6.58	6.57	6.56	6.57	6.57	6.58	6.56	6.59
	SD	0.02	0.02	0.02	0.02	0.01	0.02	0.03	0.02	0.02	0.02	0.03	0.01

Table A 17 The viscosity of hydrated powder facial mask containing gardenia extract.

Property	0 Month			1.25 Months			2 Months			3 Months		
	4 °C	30 °C	40 °C	4 °C	30 °C	40 °C	4 °C	30 °C	40 °C	4 °C	30 °C	40 °C
Viscosity	371.71	371.71	371.71	371.10	370.48	369.87	369.87	369.87	372.33	369.87	370.48	371.71
	369.25	369.25	369.25	369.87	371.10	371.71	369.87	371.71	371.71	369.25	370.48	372.33
	371.10	371.10	371.10	369.87	371.10	370.48	370.48	371.71	371.10	370.48	372.94	371.71
Average	370.69	370.69	370.69	370.28	370.89	370.69	370.07	371.10	371.71	369.87	371.30	371.92
SD	1.28	1.28	1.28	0.71	0.35	0.94	0.35	1.06	0.61	0.61	1.42	0.35

Table A 18 The pH of peel-off gel facial mask containing gardenia extract.

Property	0 Month			1.25 Months			2 Months			3 Months		
	4 °C	30 °C	40 °C	4 °C	30 °C	40 °C	4 °C	30 °C	40 °C	4 °C	30 °C	40 °C
pH	5.54	5.54	5.54	5.49	5.47	5.47	5.56	5.58	5.56	5.45	5.46	5.52
	5.51	5.51	5.51	5.56	5.57	5.59	5.52	5.51	5.53	5.56	5.53	5.55
	5.48	5.48	5.48	5.53	5.51	5.52	5.46	5.46	5.48	5.55	5.58	5.48
Average	5.51	5.51	5.51	5.53	5.52	5.53	5.51	5.52	5.52	5.52	5.52	5.52
SD	0.03	0.03	0.03	0.04	0.05	0.06	0.05	0.06	0.04	0.06	0.06	0.04

Table A 19 The viscosity of peel-off gel facial mask containing gardenia extract.

Property	0 Month			1.25 Months			2 Months			3 Months		
	4 °C	30 °C	40 °C	4 °C	30 °C	40 °C	4 °C	30 °C	40 °C	4 °C	30 °C	40 °C
Viscosity	16946.92	16946.92	16946.92	17005.90	16986.24	17025.56	16927.26	17005.90	16946.92	16907.60	17005.90	16986.24
	17005.90	17005.90	17005.90	16927.26	16927.26	16986.24	16907.60	16966.58	17064.88	16907.60	17025.56	17025.56
	16966.58	16966.58	16966.58	16946.92	17005.90	16966.58	16946.92	16972.48	16966.58	16966.58	16927.26	16986.24
Average	16973.13	16973.13	16973.13	16960.03	16973.13	16992.79	16927.26	16981.65	16992.79	16927.26	16986.24	16999.35
SD	30.03	30.03	30.03	40.93	40.93	30.03	19.66	21.20	63.20	34.05	52.02	22.70

Table A 20 Percent remaining of geniposide in the facial masks.

Month	% Remaining of geniposide in the powder facial mask (mean±SD)			% Remaining of geniposide in the peel-off gel facial mask (mean±SD)		
	4 °C	30 °C	40 °C	4 °C	30 °C	40 °C
0	100.00	100.00	100.00	100.00	100.00	100.00
	99.83	97.13	97.38	99.50	97.60	97.94
1.25	99.64	97.26	97.53	99.79	99.04	98.38
	99.52	98.66	97.29	100.17	99.12	98.57
Average	99.66	97.68	97.40	99.82	98.58	98.30
SD	0.16	0.85	0.12	0.33	0.86	0.32
	96.98	88.20	85.13	99.79	100.11	97.87
2	99.31	88.57	85.85	101.43	98.55	98.00
	100.78	86.85	84.62	101.74	98.29	98.29
Average	99.02	87.87	85.20	100.99	98.98	98.06
SD	1.92	0.90	0.62	98.47	96.54	97.08
	97.07	85.28	85.17	98.47	96.54	97.08
3	96.44	86.10	84.58	98.07	96.90	97.37
	96.10	85.28	83.91	98.91	97.20	97.75
Average	96.54	85.55	84.55	98.48	96.88	97.40
SD	0.49	0.47	0.63	0.42	0.33	0.34

VITA

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