สารต้านอนุมูลอิสระของสารสกัดจากเปลือกเมล็ดมะขาม *Tamarindus indica* และการประยุกต์ในผลิตภัณฑ์สำหรับผิวหนัง

นางสาว เสาวลักษณ์ อุกฤษฎาวิทิต

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ANTIOXIDANT OF *Tamarindus indica* SEED COAT EXTRACTS AND APPLICATION FOR SKIN PRODUCT

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เสาวลักษณ์ อุกฤษฎาวิทิต : สารต้านอนุมูลอิสระของสารสกัดจากเปลือกเมล็ดมะขาม *Tamarindus indica* และการประยุกต์ในผลิตภัณฑ์สำหรับผิวหนัง (ANTIOXIDANT OF *Tamarindus indica* SEED COAT EXTRACTS AND APPLICATION FOR SKIN PRODUCT) อาจารย์ที่ปรึกษาวิทยานิพนธ์หลัก : รศ. ปาริชาต ภู่สว่าง, อาจารย์ที่ปรึกษาวิทยานิพนธ์ร่วม : ผศ. ดร. มณีวรรณ สุขสมทิพย์, 90 หน้า

งานวิจัยนี้ทำการตรวจวิเคราะห์ปริมาณสารประกอบฟีนอลและฤทธิ์ต้านอนุมูลอิสระของสารสกัดในเมทานอลจากเปลือกเมล็ดมะขามชนิดหวาน ได้แก่ "ศรีชมภู" "สีทองหนัก" "สีทองเบา" และ "ขันตี" และชนิดเปรี้ยว ได้แก่ "เปรี้ยวยักษ์" ที่ปลูกในจังหวัดเพชรบูรณ์ ประเทศไทย สำหรับการหาปริมาณสารประกอบฟีนอลได้ทำการหาปริมาณฟีนอลทั้งหมด ปริมาณแทนนินและปริมาณโปรแอนโธไซยานิดิน พบว่าสารสกัดจากเปลือกเมล็ดมะขามชนิดหวาน "สีทองเบา" และ "ขันตี" มีปริมาณฟีนอลทั้งหมดสูงสุดอย่างมีนัยสำคัญ ($p < 0.05$) สารสกัดจากเปลือกเมล็ดมะขามทั้งชนิดหวานและชนิดเปรี้ยวมีปริมาณแทนนินสูง โดย "เปรี้ยวยักษ์" มีปริมาณแทนนินสูงสุด สำหรับโปรแอนโธไซยานิดินพบว่ามีปริมาณน้อยในสารสกัดเรียงตามลำดับดังนี้ "ขันตี" = "สีทองเบา" > "เปรี้ยวยักษ์" = "สีทองหนัก" > "ศรีชมภู" ในการวิเคราะห์หาฤทธิ์ต้านอนุมูลอิสระใช้วิธีต่างๆดังนี้คือ reducing power ฤทธิ์ต้านลิปิดเปอร์ออกซิเดชัน ความสามารถในการจับอนุมูลไฮดรอกซี และความสามารถในการจับอนุมูล 2,2-diphenyl-1-picrylhydrazyl (DPPH) โดยฤทธิ์ reducing power ของสารสกัดจากสายพันธุ์ "เปรี้ยวยักษ์" และ "สีทองเบา" เพิ่มขึ้นเมื่อความเข้มข้นเพิ่มขึ้น ฤทธิ์ต้านลิปิดเปอร์ออกซิเดชันของสารสกัดทุกชนิดมีลักษณะเปลี่ยนแปลงตามความเข้มข้น สารจาก "สีทองเบา" สามารถยับยั้งลิปิดเปอร์ออกซิเดชันได้ดีโดยเฉพาะที่ความเข้มข้น 100-500 ไมโครกรัมต่อมิลลิลิตร และค่า EC_{50} ของสารจาก "สีทองเบา" (62.19±7.13 ไมโครกรัมต่อมิลลิลิตร) ต่ำกว่าวิตามินซี (263.93±8.03 ไมโครกรัมต่อมิลลิลิตร) ที่ใช้เป็นตัวควบคุม อย่างมีนัยสำคัญ ($p < 0.05$) ในขณะที่สารจาก "เปรี้ยวยักษ์" ยับยั้งลิปิดเปอร์ออกซิเดชันน้อยที่สุดที่ความเข้มข้น 10 ถึง 500 ไมโครกรัมต่อมิลลิลิตร มะขามทุกสายพันธุ์มีฤทธิ์จับอนุมูลไฮดรอกซีได้ดีแต่น้อยกว่า Butylated hydroxyanisole ที่ใช้เป็นตัวควบคุมโดยฤทธิ์ดังกล่าวในหน่วยไมโครกรัมต่อมิลลิลิตรที่ความเข้มข้นของสารสกัด 500 ไมโครกรัมต่อมิลลิลิตรสามารถเรียงตามลำดับดังนี้ "Butylated hydroxyanisole" (94.65±1.41) > "สีทองเบา" (77.54±4.81) > "เปรี้ยวยักษ์" (76.47±1.41) > "ขันตี" (73.26±1.93) > "สีทองหนัก" (68.45±0.53) > "ศรีชมภู" (60.96±0.53) ค่า EC_{50} สำหรับฤทธิ์จับอนุมูลไฮดรอกซีของสารสกัดจากเปลือกเมล็ดมะขาม มีความสัมพันธ์แบบผกผันกับปริมาณฟีนอลทั้งหมดและปริมาณโปรแอนโธไซยานิดินอย่างมีนัยสำคัญ ($p < 0.05$) สารสกัดจากเปลือกเมล็ดมะขามทุกชนิดมีฤทธิ์จับอนุมูล DPPH ที่ดี โดยสายพันธุ์ "ขันตี" มีฤทธิ์จับอนุมูล DPPH ดีที่สุดเมื่อเปรียบเทียบกับสารสกัดจากสายพันธุ์อื่น นอกจากนี้ค่า EC_{50} สำหรับฤทธิ์จับอนุมูล DPPH ของ "ขันตี" (70.10±1.75 ไมโครกรัมต่อมิลลิลิตร) และวิตามินซี (138.29±5.54 ไมโครกรัมต่อมิลลิลิตร) ไม่มีความแตกต่างอย่างมีนัยสำคัญ ($p \geq 0.05$) ในส่วนการประยุกต์ได้นำสารสกัดเปลือกเมล็ดมะขาม "สีทองเบา" มาใช้เป็นสารต้านอนุมูลอิสระธรรมชาติในการเตรียมผลิตภัณฑ์เจลสำหรับใช้ภายนอก ซึ่งเตรียมจากสารสกัดเปลือกเมล็ดมะขาม (TSCE) โดยใช้เจลพอลิแซ็กคาไรด์จากเปลือกทุเรียน (PG) หรือพอลิแซ็กคาไรด์จากเนื้อในเมล็ดมะขาม (TSP) เป็นสารก่อเจล จากการศึกษาคุณสมบัติทางกายภาพรวมทั้งความหนืด ความเป็นกรด-ด่างและพฤติกรรมการไหลของผลิตภัณฑ์เจล พบว่าผลิตภัณฑ์เจล TSCE-PG มีคุณสมบัติเป็นที่น่าพอใจมากกว่าผลิตภัณฑ์เจล TSCE-TSP จากการศึกษาฤทธิ์ยับยั้งลิปิดเปอร์ออกซิเดชันของผลิตภัณฑ์เจล TSCE-PG พบว่าฤทธิ์ต้านลิปิดเปอร์ออกซิเดชันของผลิตภัณฑ์เจลเพิ่มขึ้นเมื่อเพิ่มความเข้มข้นของสารสกัดจากเปลือกเมล็ดมะขามในผลิตภัณฑ์ให้สูงขึ้น การทดสอบความคงตัวของผลิตภัณฑ์เจลในสภาวะเร่งพบว่าที่อุณหภูมิ 45°C มีผลทำให้ฤทธิ์ต้านลิปิดเปอร์ออกซิเดชันของผลิตภัณฑ์ลดลง

สาขาวิชา.....เทคโนโลยีชีวภาพ.....ลายมือชื่อนิสิต.....เสาวลักษณ์ อุกฤษฎาวิทิต.....

ปีการศึกษา.....2551.....ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

4972556423: MAJOR BIOTECHNOLOGY
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SAOWALUCK UKRISDAWITHID: ANTIOXIDANT OF *Tamarindus indica* SEED COAT EXTRACTS AND APPLICATION FOR SKIN PRODUCT. ADVISOR: ASSOC. PROF. PARICHART BHUSAWANG, CO-ADVISOR: ASST. PROF. MANEEWAN SUKSOMTIP, Ph.D., 90 pp.

Methanolic extracts from seed coats of certain types of Thai tamarinds including sweet-type: "Srichomphu", "Sithong-nak", "Sithong-bao" and "Khanti" and sour-type: "Priao-yak" cultivated in Petchabun province, Thailand were studied for their phenolic compounds contents and antioxidative properties. Phenolic compounds including total phenols, tannins and proanthocyanidins contents were investigated. The highest total phenol was found in tamarind seed coat extracts (TSCEs) sweet-type such as "Khanti" and "Sithong-bao" ($p < 0.05$). All sweet and sour tamarinds showed high tannins contents while proanthocyanidins were low. The highest tannins content was found in "Priao-yak". Proanthocyanidins were found in the following order: "Khanti" = "Sithong-bao" > "Priao-yak" = "Sithong-nak" > "Srichomphu". Antioxidative activities were evaluated by using different methods including reducing power assay, anti-lipid peroxidation, hydroxyl radical scavenging activity and scavenging activity on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. "Priao-yak" and "Sithong-bao" showed increased reducing powers with increased concentration of TSCEs. All tested cultivars showed anti-lipid peroxidation activity in a dose-dependent manner. "Sithong-bao" exhibited high anti-lipid peroxidation particularly at concentration 100-500 μg/mL and its EC_{50} value (62.19±7.13 μg/mL) was significantly lower ($p < 0.05$) than that of positive control, vitamin C (263.93±8.03 μg/mL). "Priao-Yak" showed the lowest lipid peroxidation inhibition at concentration 10 to 500 μg/mL. All tamarind cultivars exhibited good scavenging activity against hydroxyl radical. But this activity is lower than butylated hydroxyanisole, a positive control. This activity was found in the following order: Butylated hydroxyanisole (94.65±1.41) > "Sithong-bao" (77.54±4.81) > "Priao-yak" (76.47±1.41) > "Khanti" (73.26±1.93) > "Sithong-nak" (68.45±0.53) > "Srichomphu" (60.96±0.53) at 500 μg/mL concentration, with the EC_{50} value inversely correlated with their total phenols and proanthocyanidins contents. All cultivars showed high DPPH radical scavenging activity. Furthermore, "Khanti" showed the strongest scavenging activity against DPPH. However, EC_{50} values of "Khanti" (70.10±1.75 μg/mL) and vitamin C (138.29±5.54 μg/mL) showed no significant difference ($p \geq 0.05$). The TSCE from "Sithong-bao" was used as a natural antioxidant for gel preparation according to its high antioxidative activities. Gel preparation for topical use containing TSCE was prepared by using polysaccharide gel (PG) from durian-rind or tamarind seed polysaccharide (TSP) from tamarind seed kernel as a gelling agent. Physical properties including viscosity, pH and rheology study of TSCE-PG gel products and TSCE-TSP gel preparations were determined. The satisfactory physical properties of TSCE-PG gel preparations were obtained. In contrary, unsatisfied TSCE-TSP gel preparations were found. Lipid peroxidation inhibition activity of TSCE-PG gel preperations at ambient temperature (28±5 °C) was tested *in vitro* by lipid peroxidation assay using lipid in egg yolk as a substrate. It was found that the activity increased with increasing concentrations of TSCE. Accelerated stability test at 45 °C decreased anti-lipid peroxidation activity of TSCE-PG gel preparation.

Field of Study:.....Biotechnology.....Student's Signature.....S. Ukrisdawithid.....
Academic Year:.....2008.....Advisor's Signature.....Parichart Bhusawang.....
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LIST OF ABBREVIATIONS

°C	degree Celsius (centigrade)
DI water	deionized water
et al.	et alii, and others
g	gram (s)
min	minute (s)
mL	millilitre (s)
nm	nanometer (s)
μL	microlilitre (s)
mM	millimolar (s)
μg	microgram (s)
%	percentage
pH	The negative logarithm of hydrogen ion concentration
SEM	standard error of mean
w/v	weight by volume
v/v	volume by volume
rpm	round per minute
TSCE	tamarind seed coat extract
N	normality
TCA	trichloroacetic acid
BHA	butylated hydroxyanisole
PBS	phosphate buffer saline
TBA	2-thiobarbituric acid
TEA	triethanolamine
PG	polysaccharide gel
TSP	tamarind seed polysaccharide
ANOVA	analysis of variance
r	correlation coefficient
SPSS	statistical package for social science

ROS	reactive oxygen specie (s)
ET	electron transfer
MDA	malonaldidehyde
HNE	4-hydroxy-2-nonenal
DNA	deoxyribonucleic acid
ALA	alpha lipoic acid
TKP	tamarind seed kernel powder
DPPH	2,2-diphenyl-1-picrylhydrazyl
TBARS	thiobarbituric acid-reactive substances
FC	folin-Ciocalteu
Pa	pascal (s)
s	second (s)

CHAPTER I

INTRODUCTION

Free radicals and reactive oxygen species (ROS) have been suggested as potentially being important causative agents of aging and several human diseases such as cancer, inflammatory and degenerative diseases, central nervous system injury, diabetes, autoimmune diseases, anemia, ischemia, and reperfusion injury (Valko et al., 2007). Anti-free radical compounds from natural sources are widely studied to provide active and safely agents for medical uses.

Skin is a major sensory organ which possesses the largest surface area in the body and serves as the protective layer for internal organs. Due to its interface function between the body and the environment, skin is constantly exposed to both endogenous and environmental pro-oxidant stimuli (Kohen, 1999; Briganti and Picardo, 2003). Therefore, skin is also a major candidate and target of oxidative stress. Eventhough it is designed to give both physical and biochemical protection as defense mechanisms. Oxidative stress and antioxidant imbalance could play a pivotal role in skin diseases, skin disorder and skin aging. Slowing down the aging processes of the skin will therefore not only help us to keep a more youthful appearance but will most likely have beneficial effects for the whole organism (Giacomoni, 2005). Research on cosmetic product have focused into the efficacy of antioxidants which can better protect and possibly correct the damage by neutralizing free radicals. In addition, an increase understanding of human physiology, disease processes, and chemistry has led to the extraction and synthesis of many medically important substances from botanical remedies. The richness of plant material in the tropical orient led to different plant extracts of great diversity that were used in dermatology and cosmetology (Draelos, 2001).

Tamarind (*Tamarindus indica* Linn.) of family Leguminosae is accepted as food resource and herbal medicine. There are 2 main varieties, sweet and sour, though the genetic diversity. All parts of tamarind are useful especially fruit. The extract of tamarind fruit pulp was developed to provide skin a lighter effect in cosmetic product. However, seed coat or testa which enrich in phytochemical is solely as by-product of tamarind seed processing industry. The previous study has

also reported that seed coat of tamarind composed of phenolic compounds which showed remarkable antioxidant activities (Tsuda et al., 1994; Pumthong, 1999; Sudjaroen et al., 2005; Siddhuraju, 2007) and also inhibitory effect on nitric oxide production (Komutarin et al., 2004), potential on antidiabetic and antihyperlipidemic activities in rat (Maiti, Das and Ghosh, 2004, 2005), synergistic antioxidant action in the edible oil (Tsuda et al., 1995) and inhibited formation of all four products of oxidized low-density lipoprotein and protected supercoiled DNA strand in plasmid pBR322 (Suksomtip and Pongsamart, 2008)

In Thailand, tamarind is a Thai tropical fruit which is in the first group fruit of major economic importance and with a great potential to support the demand of both local and oversea markets. The National Statistical office of Thailand reported that tamarind is produced about 234,835 tons in 2001. Furthermore, much more 40,267 ton per year of tamarind is produced in Petchabun province from 2004 to 2005 reported by The Office of Commercial Affairs, Petchabun province. Hence, there have been an attempt to use a lot of seed coats, the agricultural waste, as a source of valuable material.

In this research, tamarinds from Petchabun province have been chosen because of its famous source of tamarind productions in Thailand. First, we have studied about sample processing and extraction of seed coat from 5 tamarind cultivars including “Srichomphu”, “Sithong-nak”, “Sithong-bao”, “Khanti” and “Priao-Yak”. Secondly, the yields of extraction and contents of phenolic compound have been investigated. Thirdly, the in-vitro antioxidant activity of seed coat extracts have been determined using different assays including reducing power, lipid peroxidation, hydroxyl radical (•OH) scavenging activity and free radical scavenging activity on 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•). Furthermore, the extract of seed coat which provided potent antioxidant activities was used as an active ingredient to formulate gel preparation for skin. Finally, physical appearances, physical properties, stability and also *in vitro* anti-lipid peroxidation efficacy of the finished products were studied.

CHAPTER II

LITERATURE REVIEW

Free radicals and theirs oxidative damage

Free radicals and reactive oxygen species (ROS) have been involved in several human diseases by oxidative damage on various molecular targets in the body. They act as important mediators to oxidative stress, a disturbance in cellular antioxidant defenses which impacts to an imbalance in the dynamic oxidant-antioxidant equilibrium of a cell. Free radicals can be induced in different ways. Basically, they are formed naturally in the body for example, as by products of normal metabolism, by the breakdown of bacteria by white blood cells, or by enzymatic reactions. They are also formed by environment stimuli such as exposure to ionizing radiation, ozone and nitrous oxide (primarily from automobile exhaust), heavy metals (such as mercury, cadmium, and lead), cigarette smoke (both active and passive), alcohol, unsaturated fat, and other chemicals and compounds from food, water and air. ROS can damage to cell structures, nucleic acids, lipids and proteins. They affect directly or indirectly many functions integral to cellular and organ homeostasis (Benedetto, 1998; Valko et al., 2007). Of interesting on ROS relevant in biological system are described below.

Peroxyl radicals (ROO•) Formation of peroxyl radicals is a key step in lipid peroxidation. Peroxyl radicals are formed by

- A direct reaction of oxygen with alkyl radicals (R•), for example, the reaction between lipid radicals and oxygen.
- Decomposition of alkyl peroxides (ROOH) also results in peroxyl and alkoxyl (RO•) radicals.
- Irradiation of UV light or the presence of transition metal ions can cause homolysis or decomposition of peroxides into peroxyl and alkoxyl radicals ($ROOH \rightarrow ROO\bullet + H\bullet$, $ROOH + Fe^{3+} \rightarrow ROO\bullet + Fe^{2+} + H^{+}$).

Peroxyl radicals are good oxidizing agents and can abstract or remove hydrogen from other molecules with lower standard reduction potential. This reaction

is frequently observed in the propagation stage of lipid peroxidation (Halliwell and Gutteridge, 1985).

Lipid oxidation is a free-radical chain reaction, as illustrate in Fig.1 and Fig. 2, and reactive oxygen species can accelerate oxidation of lipid (Boff and Min, 2002). Lipid peroxides and their major break-down products are malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE) (Briganti and Picardo, 2003). MDA is mutagenic in bacterial and mammalian cells and carcinogenic in rats (Valko et al., 2007). Once formed, peroxyl radicals can be rearranged *via* a cyclisation reaction to endoperoxides (precursors of MDA) with the final product of the peroxidation process being MDA. (Fedtke et al., 1990; Fink et al., 1997; Mao et al., 1999; Marnett, 1999; Wang et al., 1996). A high level of lipid oxidation products can be detected in cell degradation after cell injury or disease.

MDA from lipid oxidation can react with the free amino group of proteins, phospholipid, and nucleic acids leading to structural modification, which induce dysfunction of immune systems. Another aldehyde product of lipid peroxidation, 4-hydroxy- 2-nonenal (HNE), is weakly mutagenic but appears to be the major toxic product of lipid peroxidation. HNE can react with protein lysine, histidine, and cystein residues (Lee, Koo and Min, 2004; Valko et al., 2007).

Cell membranes are phospholipid bilayers with extrinsic proteins and are the direct target of lipid oxidation. As lipid oxidation of cell membranes increases, the polarity of lipid-phase surface charge and formation of protein oligomers increase; and molecular mobility of lipids, number of SH groups, and resistance to thermodenaturation decrease. As a result, the increased membrane lipid peroxidation may evoke immune and inflammatory response, activate gene expression and cell proliferation, or initiate apoptosis (Lee, Koo and Min, 2004; Briganti and Picardo, 2003).

(Initiation) Lipid + R•/ •OH ⟶ Lipid•

(Propagation) Lipid• + O_2 ⟶ Lipid-OO•

Lipid-OO• + Lipid ⟶ Lipid-OOH + Lipid•

(Termination) Lipid• + Lipid• ⟶ Lipid-Lipid

Lipid-OO• + Lipid• ⟶ Lipid-OO-Lipid

Figure 1. Step of lipid peroxidation

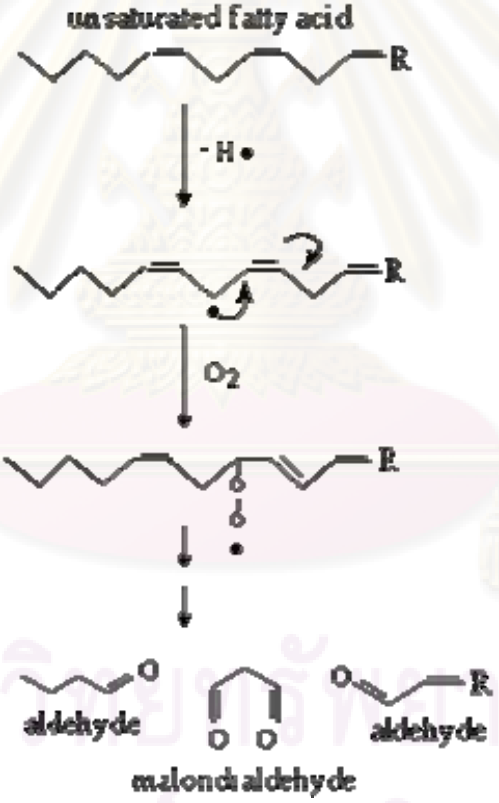


Figure 2. Reactions of lipid peroxidation

Hydroxyl radicals (•OH) Hydroxyl radical is the neutral form of the hydroxide ion. Hydroxyl radicals are strong oxidants and can abstract a hydrogen atom from any carbon–hydrogen bond and oxidize the compound. Hydroxyl radical can oxidize a large variety of biological molecules. It also may undergo three main types of reactions (Kohen, 1999). Reaction of hydroxyl radical with biological molecules is shown in Fig. 3:

(1) Hydroxyl radical may abstract hydrogen atoms (abstraction of hydrogen atoms from unsaturated lipids or proteins). It can be formed from superoxide anion and hydrogen peroxide in the presence of metal ions such as copper or iron ($\bullet O^{2-} + H_2O_2 \xrightarrow{Cu,Fe} \bullet OH + OH^- + O_2$). For example, linoleic acids are mainly located in glycerolipids and phospholipids of cell membranes.

(2) The hydroxyl radical may undergo addition reactions.

(3) The hydroxyl radical can also undergo electron transfer reactions.

The hydrogen atom abstraction of hydroxyl radicals may initiate a chain lipid peroxidation set of reactions that cause irreversible damage to cell membrane integrity.

Hydroxyl radicals or a mixture of hydroxyl/ superoxide radicals involved in the oxidation of proteins in which amino acids, simple peptides and proteins were exposed to ionizing radiations. The side chains of all amino acid residues of proteins, in particular cysteine and methionine residues of proteins are susceptible to oxidation (Stadtman, 2004).

All components of the DNA molecule can react to hydroxyl radical, damaging both the purine and pyrimidine bases and also the deoxyribose backbone (Halliwell and Gutteridge, 1999). There is a variety of DNA damage that may be caused by hydroxyl radicals, including base modifications, breakage of the DNA strands, and inactivation of the repair mechanisms (Kohen, 1999). It oxidizes guanosine or thymine to 8–hydroxy–2–deoxyguanosine and thymine glycol. 8–Hydroxy–2–deoxyguanosine has been used as a biological marker for oxidative stress (Kasai, 1997). Even DNA can be repaired by DNA glycosylase. If oxidative stress is too great and the DNA repair system is not enough, mutagenesis and/or carcinogenesis can be induced (Halliwell, 1997; Lee, Koo and Min, 2004).

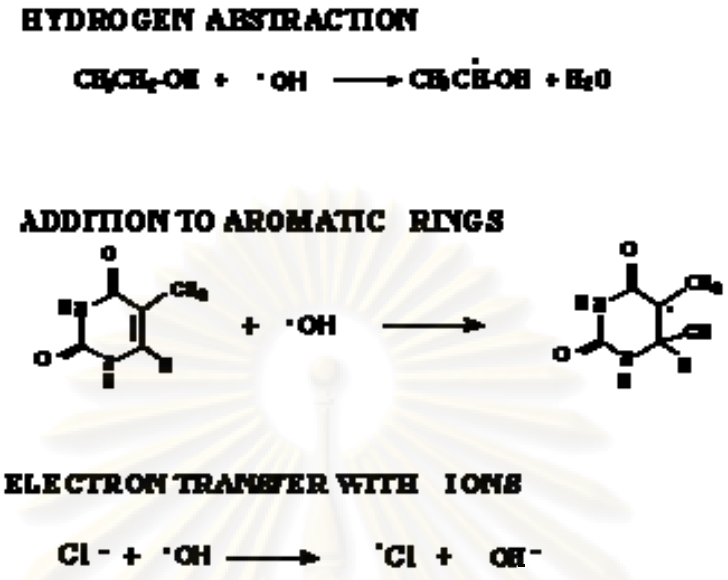


Figure 3. Reaction of hydroxyl radicals with biological molecules

Antioxidants and application for external use in a skin product

Exposure to free radicals from a variety of sources has led organisms to develop a series of defense mechanisms. Defense mechanisms against free radical-induced oxidative stress involved preventative mechanisms, repair mechanisms, physical defenses, and antioxidant defenses (Cadenas, 1997). Halliwell (1990) defined biological antioxidants as "molecules which, when present in small concentrations compared to the biomolecules they are supposed to protect, can prevent or reduce the extent of oxidative destruction of biomolecules". Antioxidants are divided to enzymatic and non-enzymatic antioxidants.

1. Enzymatic antioxidants include superoxide dismutase, glutathione peroxidase and catalase.

2. Non-enzymatic antioxidants are represented by ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), carotenoids, flavonoids, and other antioxidants.

Under normal conditions, antioxidants neutralize damaging free radicals by quenching reactive molecules, thus, protecting cells from both endogenous stress and

exogenous stressors. This is a balance between both the activities and the intracellular levels of these antioxidants. This balance is essential for the survival of organisms and their health (Valko et al., 2007).

Skin is a highly metabolic tissue, it is the body's first line of defence against infectious organisms and physical harm, and it plays a very important role in controlling body temperature. The skin tissue is exposed to a variety of damaging species which originate in the outer environment, in the skin itself, and in various endogenous sources. Skin is rich in lipids, proteins, and DNA, all of which are extremely sensitive to the oxidation process. Fortunately, skin is designed to give both physical and biochemical protection, and is equipped with a large number of defense mechanisms. The structure of skin is quite complex being composed of several layers, each of which plays a specific role and carries out different functions (Giacomoni, 2005; Shindo, Witt and Packer, 1993). Each layer is equipped with its own defense molecules, and the various systems differ from each other based on the layer's susceptibility to oxidative stress and its function. It is generally agreed that one of the major and important contributions to skin from ROS (Rieger and Pains,1993; Lopezl-Torres, Shindo and Packer, 1994). However, oxidative stress and antioxidant imbalance play an important role in skin problem. Oxidative injury may be an important promoter of skin aging and common age-related diseases. To protect the skin against the overload of oxidant species, topical application and cosmetic of antioxidants have been recently suggested as preventive therapy for skin. For example (Benedetto, 1998; Lupo, 2001; Choi and Berson, 2006; Draelos, 2001);

Vitamin A or retinol is an antioxidant member of the retinoid family. Retinol is a scavenger of peroxyl and thyil free radicals. Additionally, Beta carotene, a precursor to vitamin A, protects cell membranes against oxidative damage.

Vitamin B complex includes niacinamide (Vitamin B3) Topical B vitamins have been studied for treatment of acne, wounds and for preventing photocarcinogenesis.

Panthenol (provitamin B5) is a water-soluble cosmeceutical that easily penetrates the stratum corneum and is a humectant. It is a precursor to pantothenic acid, a cofactor in lipid biosynthesis, and it promotes lipid synthesis to improve the barrier function of the skin.

Vitamin C (ascorbic acid) is a water-soluble antioxidant that is the

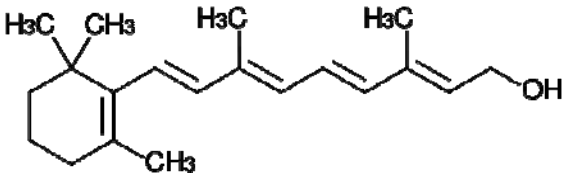
principal and terminal scavenger of various free radicals directly and to regenerate vitamin E. It is important because of its antioxidant actions as well as its function as a cofactor in hydroxylation reactions of collagen production. Humans are unable to synthesize ascorbate, so dietary intake is essential. Vitamin C is also considered an antiaging ingredient because of its potential to stimulate collagen production.

Vitamin E (α-tocopherol) is lipid soluble and the principal chain-breaking antioxidant in cell membranes. Because of this ability to quench free radicals, especially lipid peroxyl radicals, the term "protector" has been used to describe the actions of vitamin E. Clinical improvement in the visible signs of skin aging has been documented with decrease in both skin wrinkling and skin tumor formation.

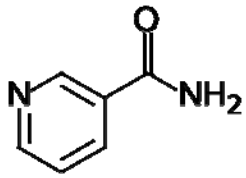
Alpha lipoic acid (ALA) is a lipoamide synthesized in the mitochondria of plants and animals. ALA is both water and lipid soluble, allowing it to penetrate lipophilic cell membranes and enter the aqueous intracellular matrix. It is a scavenger of ROS and a metal chelator. ALA regenerates endogenous antioxidants such as vitamins C and E, glutathione, and ubiquinol.

Coenzyme Q or ubiquinone is a naturally occurring lipid-soluble substance found in high levels in the epidermis. It presences in most cells in the body and it is able to react with ROSs. Like other naturally occurring skin antioxidants, it is depleted by UV exposure.

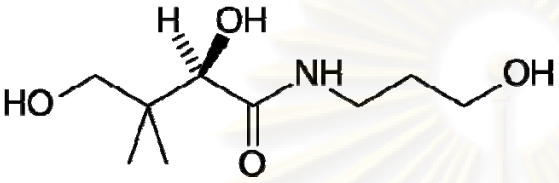
Currently, new antioxidant agents from plants, polyphenolic compounds, have been a remarkable increment in scientific knowledge dealing with the beneficial role of polyphenols during oxidative stress. Plant extracts that are popular additives to skin care products are botanicals. Nowadays, cosmetic formulators have access to plant materials worldwide for incorporation into topical preparations.



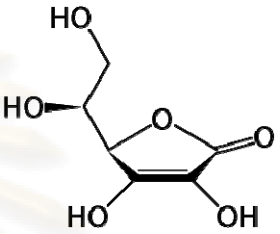
Retinol or vitamin A



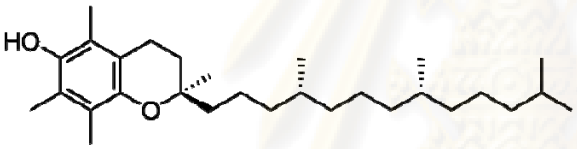
Niacinamide



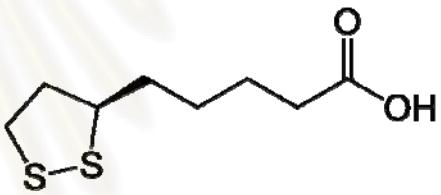
Panthenol



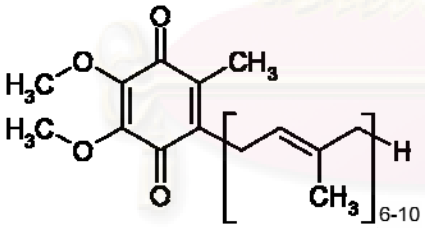
Ascorbic acid



Vitamin E (α-tocopherol)



Alpha lipoic acid



Coenzyme Q or ubiquinone

Figure 4. Structure of antioxidants

Plant polyphenols

Plant accumulates a wide variety of secondary metabolite, including alkaloid, terpene and phenolics. Polyphenols or phenolic compounds constitute one of the most numerous and ubiquitously distributed group of plant secondary metabolites, with more than 8,000 phenolic structures currently known. They may play an important role in protecting plant from herbivory and disease. Polyphenols have been reported in antiinflammatory, photoprotective, and anticarcinogenic properties (Choi and Berson, 2006). Plant extracts have been widely used as topical applications for wound-healing, anti-aging, and disease treatments. Examples of plants are presented in Table 1. These plants share a common character: they all produce flavonoid compounds with phenolic structures (Hsu, 2005).

Phenolics compound Natural polyphenols can range from simple molecules (phenolic acids, phenylpropanoids, flavonoids) to highly polymerised compounds (lignins, melanins, tannins). Since modern chemical and pharmacological methods were first used to investigate traditional medicinal materials, there has been a rapid increase in the number of known natural principles and compounds. Among these compounds, polyphenols including tannins and flavonoids have received increasing attention recently because of some interesting new findings regarding their biological activity studies (Yokozawa et al., 1998).

Tannins Tannins are one of the many types of secondary compounds found in plants. Plant parts containing tannins include bark, wood, fruit, fruitpods, leaves, roots, and plant galls. Tannins or polymeric polyphenolics, may be much more potent antioxidants than simple monomeric phenolics. Furthermore, the tannins had little or no pro-oxidant activity, although many small phenolics are pro-oxidants (Hagerman et al., 1998). On the basis of their structural characteristics it is therefore possible to divide the tannins into four major groups (Khanbabaee and Ree, 2001) as classify in Fig. 5:

(1) Gallotannins are all those tannins in which galloyl units or their meta-depsidic derivatives are bound to diverse polyol-, catechin-, or triterpenoid units.

(2) Ellagitannins are those tannins in which at least two galloyl units are C–C coupled to each other, and do not contain a glycosidically linked catechin unit.

(3) Complex tannins are tannins in which a catechin unit is bound glycosidically to a gallotannin or an ellagitannin unit.

(4) Condensed tannins are all oligomeric and polymeric proanthocyanidins formed by linkage of *C*-4 of one catechin with *C*-8 or *C*-6 of the next monomeric catechin.

Pharmacological properties of tannins have been investigated based on recent advances in the structural study of tannins in medicinal plants and various properties of tannins including antitumor and antiviral effects have been revealed. Chung, Wei and Johnson (1998) had shown that tannins and their precursors can be beneficial as well as detrimental to human health depending upon the concentration of the exposure. Most of these properties are dependent on the chemical structures or molecular shapes of tannins (Madhan et al., 2005). The biological activities of tannins include marked anti-tumor, anti-viral, and anti-HIV activities, inhibition of lipid peroxidation and plasmin activity, mediation of DNA nicking, amelioration of renal failure, and several others (Yokozawa et al., 1998). Moreover, tannins have been used topically to treat acne because of their natural astringent properties. Some of the more commonly reported tannin-containing herbs that may be helpful for the topical treatment of wounds. Tannins used topically are thought to be beneficial in treating dermatitis by coagulating surface proteins of cells, thereby reducing permeability and secretion. The precipitated proteins also form a protective layer on the skin (Bedi and Shenefelt, 2002).

Proanthocyanidins Proanthocyanidins, the oligomer and polymers of flavan-3-ols (catechin), also referred to as “condensed tannins”, are known for contributing astringent flavor to foods and medicinal herbs. Red wine contains many complex proanthocyanidins (extracted from grape skins and seeds); so do blueberries, blackberries, strawberries, elderberries, and other red/blue/purple colored plant parts. Reactivity of condensed tannins with biomolecules is important due to their nutritional and physiological effects. Catechin, gallocatechin and epigallocatechin are all precursors of condensed tannins. The chemical properties of proanthocyanidins in terms of the availability of the phenolic hydrogens as hydrogen donating radical scavengers and singlet oxygen quenchers predicts their antioxidant activity (Chen et al., 1996; Rice-Evans, Miller and Paganda, 1996). Proanthocyanidin can be defined

as an antioxidant, it must satisfy two basic conditions: (1) when present in low concentrations relative to the substrate to be oxidized it can delay, retard or prevent autooxidation or free radical-mediated oxidative injury; and (2) the resulting product formed after scavenging must be stable through intramolecular hydrogen bonding on further oxidation (Shahidi and Wanasundara, 1992).

The biological, pharmacological and medicinal properties of the bioflavonoids and proanthocyanidins have been extensively reviewed. Besides the free radical scavenging and antioxidant activity, proanthocyanidins exhibit vasodilatory, anticarcinogenic, anti-allergic, antiinflammatory, antibacterial, cardioprotective, immune-stimulating, anti-viral and estrogenic activities, as well as being inhibitors of the enzymes phospholipase A2, cyclooxygenase and lipooxygenase (Shahidi and Wanasundara, 1992; Suzuki, 1993; Jovanovic et al., 1994; Salah et al., 1995; Rice-Evans, Miller and Paganda, 1996).

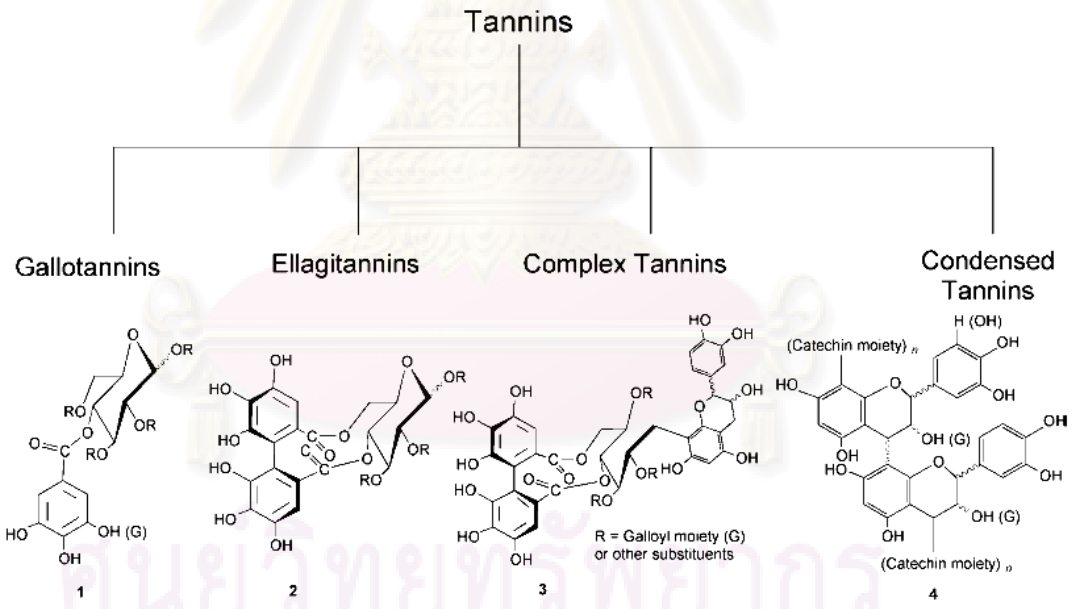


Figure 5. Tannins classification

Table 1. Plant extracts that are popular additives to skin care products (Draelos, 2001).

Anti-inflammatories	
Ginkgo biloba	The plant leaves are said to contain unique polyphenols such as terpenoids (ginkgolides, bilobalides), flavinoids, and flavonol glycosides that have anti-inflammatory effects. These anti-inflammatory effects have been linked to antiradical and antilipoperoxidant effects in experimental fibroblast models.
Green Tea	The anti-inflammatory effects of topical green tea application on C3H mice. A topically applied green tea extract containing GTP((-) epigallocatechin-3-gallate)) was found to reduce UVB-induced inflammation as measured by double skin-fold swelling.
Echinacea	*Echinacea purpurea* species is currently advocated for its value in treating psoriasis and reducing the inflammation associated with a variety of dermatologic conditions.
Thuja	Thuja, also known as cedarwood or arborvitae, is another American Indian homeopathic remedy for psoriasis. It is found in moisturizing preparations for skin that is sensitive or easily irritated.
Sarsaparilla	Sarsaparilla is also claimed to possess topical benefits due the presence of plant sterols and saponins. It is said to have both antiseptic and antipruritic properties. For this reason, it is incorporated into homeopathic topical preparations to treat both eczema and psoriasis.
Skin Soothing Agents	
Prickly Pear	Mucilages from pad of the prickly pear have a soothing, cooling effect on the skin, due to evaporation of water, when the plant juice is rubbed over the skin.
Sagebrush	The powder was felt to have both antibiotic and skin-soothing effects. Ground sagebrush leaves cooked and mixed with olive oil are still applied to irritated skin by homeopathic healers.

Jojoba	The lightweight oil obtained from its fruit that is highly desirable in the skin care. The oil is now finding its way into botanical moisturizers.
Aloe Vera	The mucilage released from the plant leaves is the most widely used botanical additive to soothe the skin. The reported cutaneous effects of aloe vera include increased blood flow, reduced inflammation, decreased skin bacterial colonization, and enhanced wound healing.
Comfrey	Allantoin from common comfrey root is the basis for sensitive skin claims made for a number of moisturizers marketed to dermatologists.
Skin Cancer Treatments	
Feverwort	The leaves of the plants were collected and made into a poultice that was applied topically for the treatment of skin cancers by the North American Indians.
Bloodroot	The whole plant, from leaves to roots, has been used to make a preparation used by the North American Indians around Lake Superior to treat skin cancer.
Miscellaneous Skin Treatments	
Witch Hazel	The astringent action of witch hazel is probably due to the high tannin content of the plant. In addition, the tannins of the plant also function as venous vasoconstrictors. Witch hazel combined with glycolic acid treat acne by reducing inflammatory papules and pustules.
Papaya	The latex has been shown to have antifungal properties. Proteolytic enzyme valuable in clearing epithelial debris in wound healing.

Tamarind

Tamarind (*Tamaridus indica* Linn.), is a tropical fruit tree which grows in dry/monsoonal climates. It belongs to the family Leguminosae. It is slow growing, but long lived, with an average life span of 80-200 years. Today, tamarind grows widely in most tropical and subtropical regions of the world. It is thought to have originated in tropical East Africa, from where it was carried by seafaring Arabian traders to Asia and Southeast Asia. Tamarind is well adapted to semi-arid tropical conditions, it also grows well in many humid tropical areas with seasonally high rainfall. It grows in well drained, slightly acidic soils, it can tolerate a wide range of physical site characteristics (International Centre for Underutilized Crops, 1999). Tamarind is a multipurpose tree species. It is best known for its fruits.

The blackish-brown sticky pulp is often eaten fresh but has many other culinary uses for example in pickles, jams, candy, juice and drinks. The fruits are reported to have anti-fungal and anti-bacterial properties (Ray and Majumdar, 1976; Guerin and Reveillere, 1984; Bibitha et al., 2002; Metwali, 2003; John, Joy and Abhilash, 2004). The fruit pulp was used as a gentle laxative (El-Siddig, 2006). Fruit extracts have been shown to enhance the bioavailability of ibuprofen in humans to treat fever (Garba et al., 2003). Frequent research on aqueous extracts of seeds has shown a strong anti-diabetic effect in rats (Maiti et al., 2004).

The seed and its extracts can be used in the food processing industry, as an adhesive in the plywood industry. Tamarind seed kernel powder (TKP) is a major industrial product, which is used in the sizing of textile, paper and jute. A substance known as "jellose" can be also be extracted from the seed kernel, this is a polysaccharide with gel forming characteristics. It has both food and industrial applications (Dueñas, Hernández, and Estrella, 2006). In Cambodia and India, it has been reported that powdered seeds have been used to treat burn and dysentery (Rama Rao, 1975; Jayaweera, 1981). Seed powder has also been externally applied on eye diseases and ulcers. Boiled, pounded seeds are reported to treat ulcers and bladder stones (Rama Rao, 1975).

The seed coat or testa is very hard, shiny, reddish, or purplish brown. The seed coat when suitably blended is used for tanning leather and imparting color-fast shades to wool. In leather tanning tests, tamarind tannin gives a harsh and highly colored

leather (El-Siddig, 2006). Because of its function which acts as a protective barrier for the cotyledon, it plays a major role in the physical and chemical defense system of the seeds which are exposed to oxidative damage, for example, oxygen, UV light, or other environmental factors. Thus the seeds coat contains numerous bioactive compounds including polyphenols, which play an important protecting role against oxidative damage and may contribute to antioxidative activity (Osawa et al., 1985). It was founded that seed coat extract of tamarind cultivated in India showed potential antioxidant activity in different solvents. On the other hand, germ was less activity. The isolation and characterization of antioxidative compounds extracted from the seed coat by ethyl acetate were reported. The four compounds consisted of dihydroxybenzene derivatives and epicatechin (Tsuda et al., 1994). The antioxidant activity of extracts of tamarind pericarp from local market in Bangkok, was reported the presence of mainly polymeric tannins and oligomeric procyanidins but these were not definitively identified or quantitated (Pumthong, 1999). The extract is composed of flavonoids including tannin, polyphenols, and procyanidin (Sudjaroen et al., 2005). Supercritical fluid extraction of antioxidants from sweet Thai tamarind seed coat purchased from a local market in Bangkok, Thailand in 2001 produce very low yields. The addition of an ethanol co-solvent significantly improves the extraction of antioxidants. Solvent extraction using ethanol results in high rates of extraction and very low peroxide values (Luengthanaphol et al., 2004). Methanolic extracts from both raw and dry heated seed coat samples contained higher level of total phenolics and tannins than the aqueous acetone extracts. The DPPH radical scavenging activities were well proved with the ferric reducing antioxidant capacity of the extracts. Interestingly, among the extracts, methanol and aqueous acetone extracts of dry heated sample showed the highest hydroxyl radical scavenging activity of 56.6 and 45.7%, respectively. All extracts, exhibited good antioxidant activity (64.5–71.7%) against the linoleic acid emulsion system (Siddhuraju, 2007). The inhibitory effect of seed coat extract on nitric oxide production in vitro and in vivo was also reported (Komutarin et al., 2004). Furthermore, aqueous extract of tamarind seeds was found to have potent antidiabetic and antihyperlipidemic activities that reduce blood sugar level and total cholesterol and triglycerides in streptozotocin (STZ)-induced diabetic male rat, respectively (Maiti, Das and Ghosh, 2005; Maiti, Jana, Das and Ghosh, 2004). The addition of citric acid with the seed coat extract results in

synergistic antioxidant action in the edible oil (Tsuda et al., 1995). The seed coat extract from Thai tamarind shows antioxidant effects against in vitro Cu^{2+}-mediated LDL oxidation and hydroxyl radical induced supercoiled DNA damage (Suksomtip and Pongsamart, 2008). The extract inhibited formation of all four products of oxidized LDL in a dose-dependent manner over 25-1000 ng/mL extracts. Moreover, 1 mg/mL of extract also protected supercoiled DNA strand in plasmid pBR322 against scission induced by Fenton-mediated hydroxyl radical similar to that found with reference antioxidant, 0.5 mg/ml Trolox.

The chemistry of antioxidant assays

Total phenolics assay Folin-Ciocalteu (FC) assay has been used to measure of total phenolics in natural products. It is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo(VI):

$$\text{Mo(VI) (yellow)} + e^- \longrightarrow \text{Mo(V) (blue)}$$

Obviously, the FC reagent is nonspecific to phenolic compounds as it can be reduced by many nonphenolic compounds (e.g. vitamin C, Cu(I), etc.). Phenolic compounds react with FC reagent only under basic conditions (adjusted by a sodium carbonate solution to pH ~10). Dissociation of a phenolic proton leads to a phenolate anion, which is capable of reducing FC reagent. This supports the notion that the reaction occurs through electron transfer mechanism. The blue compounds formed between phenolate and FC reagent are independent of the structure of phenolic compounds, therefore ruling out the possibility of coordination complexes formed between the metal center and the phenolic compounds (Huang, Ou and Prior, 2005; Prior, Wu and Schaich, 2005).

Tannins content Tannins determination also involves adding hide powder to a fraction of the extract that is binding the tannins. This method was used to evaluate the tannins and non-tannins content in a liquid or a material in solution by measuring the amount of matter bound by the hide powder. The tanning effect of tannins is caused by an interaction with the collagen fibres in the hide. Only molecules with a molecular weight between 500 and 3000 have tanning properties.

The polyphenols remaining in the solution undergo photometric determination analogously and tannin content is calculated from the difference between the total phenol content and the content of phenols, which is not adsorbed by hide powder.

Proanthocyanidins estimation The butanol-HCl-iron method is widely used for measurement of extractable condensed tannins in foods and feeds. As the method is based on acid catalysed oxidative depolymerization of condensed tannins into anthocyanidins (Fig. 6), which are subsequently quantitated at 550 nm (Makkara, Gambleb and Becker, 1999).

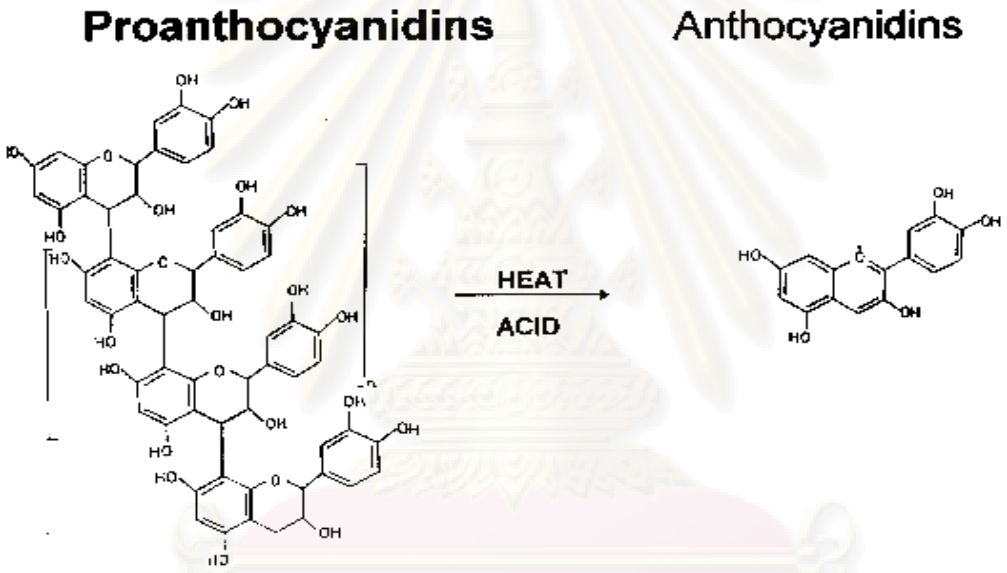


Figure 6. Proanthocyanidins estimation by acid catalysed oxidative depolymerisation method (Porter, Hrstich and Chans, 1986)

Reducing power The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. In this assay, depending on the reducing power of antioxidant samples the yellow color of test solution changes into various shades of green and blue colors of the complex $Fe^{3+}{}_4\,[Fe^{2+}(CN^-)_6\,]_3$ formed. The $Fe^{3+}(CN^-)_6$ ion are reduced to $Fe^{2+}(CN^-)_6$ ions when an antioxidant is added. The ferric chloride solution react with this ion to form the complex $Fe^{3+}{}_4\,[Fe^{2+}(CN^-)_6\,]_3$. The Perl's Prussian blue of Fe^{2+} in the complex is detected at 700 nm (Chung et al., 2002). The reducing properties are generally associated with the presence of reductones (Pin-Der-Duh, 1998). It is presumed that the phenolic

compounds may act in a similar fashion as reductones by donating electrons and reacting with free radicals to convert them to more stable products and terminating the free radical chain reaction (Ramalakshmi, Kubra and Rao, 2008).

Reaction of hydroxyl radical (•OH) For studying reaction with hydroxyl radical, a detector for hydroxyl radical is the sugar 2-deoxy-D-ribose. An EDTA–Fe^{2+} complex was employed to generate hydroxyl radical by the decomposition of H_2O_2, using ascorbic acid as the reducing agent for EDTA–Fe^{3+}. Hydroxyl radical attacks deoxyribose, degrading it into fragments that give a chromogen upon heating with thiobarbituric acid at low pH. If an hydroxyl radical scavenger is added, it competes with deoxyribose for hydroxyl radical and inhibits chromogen formation (Hsu, Coupar and Ng, 2006).

Measuring lipid peroxidation Thiobarbituric acid (TBA) test is widely used to test lipid antioxidant activity. Formation of peroxyl radicals (LOO•) is a key step in lipid peroxidation. Lipid peroxidation is often started by adding metal ions, e.g. Fe^{2+}. Decomposition produces carbon-centred radicals, which react fast with O_2 to give peroxyl radicals that then attack a lipid to cause peroxidation. MDA produced by peroxidation can react with heated 2-thiobarbituric acid (TBA) at low pH. A pink chromogen of TBARS is the indicator of lipid peroxidation (Halliwell, 1995). The reaction is shown in Fig. 7.

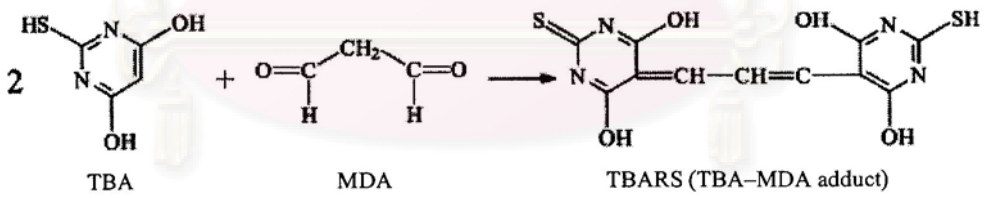


Figure 7. TBARS production of lipid peroxidation reaction

2,2-Diphenyl-1-picrylhydrazyl (DPPH•) assay This assay is based on the measurement of the reducing ability of antioxidants toward DPPH radical. The ability can be evaluated by measuring the decrease of its absorbance (Prior, Wu and Schaich, 2005). The electron transfer (ET) mechanism becomes importance in polar solvents such as methanol or ethanol. DPPH radicals are capable of forming strong hydrogen bonds with the phenols (ArOH) molecules. An ET process from phenols or its phenoxide anion (ArO^-) to DPPH• (Villaño, 2007). A freshly prepared DPPH

solution exhibits a deep purple colour with absorption maximum at 515 nm. This purple colour generally fades when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals and convert them to a colourless/bleached product (for example, 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine) (Ferreira et al., 2007). The reaction is shown in Fig. 8.

ET reactions: $ArO\bullet + DPPH\bullet \rightarrow$ products

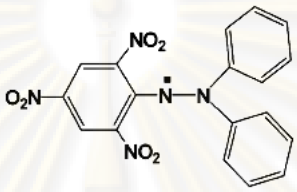


Figure 8. Structure of 2,2-diphenyl-1-picrylhydrazyl (DPPH•)

Preparation of product for external use

Phytopreparations are widely used for the treatment of various diseases of skin. The rational combination of phytopreparations with appropriate auxiliary substances and the use of optimum technological schemes in the production of soft medicinal forms for external use (ointments, gels, creams, and liniments) provide for a significant increase in the quality and therapeutic efficacy of phytopreparations intended for the treatment and prophylaxis of various disorders in dermatology, gynecology, dentistry, and cosmetology (Semkina, 2005). Normally, the constituents that have been used in preparation for skin are following:

Gelling agent One of the most versatile delivery systems that can be compounded is the pharmaceutical gel. Gels are an excellent drug delivery system for various routes of administration and are compatible with many different drug substances. Gels containing penetration enhancers are especially popular for administering anti-inflammatory and antinouseant medications. They are easily prepare and quite efficacy.

Fruit-rinds of durian are agricultural waste can be as a source of valuable material of commercial importance. Polysaccharide gel (PG) isolated

from fruit-rinds of durian have been found to be useful ingredient in the preparation of food product as jelly and has also been used as pharmaceutical excipients in tablet, suspension and emulsion (Pongsamart, Dhumma-upakorn and Panmaung, 1989).

Humectants

Propylene glycol has been used in a wide variety of pharmaceutical formulations and it is generally regarded as a nontoxic material. In topical preparations, propylene glycol is regarded as minimally irritant although it is more irritant than glycerin (Paphattarapong, 2005).

Glycerin is used primarily for its humectant and emollient properties,in topical pharmaceutical formulations and cosmetics. Glycerin may also be used in topical at concentrations up to 30% (Aoshima et al., 2005 and Rowe, 2003).

Emulsifier

Cremophor RH 40 or PEC-40 dehydrogenates caster oil , a non ionic emulsifier used for solubilizing essential oil in oil-in-water formulas.

Amerchol L-101 or Mineral oil and Lanolin alcohol is an oily liquid used in topical pharmaceutical formulations and cosmetics as an emulsifying agent with emollient properties.

pH adjuster

Triethanolamine (TEA) is an alkalizing agent in cosmetics. It is one of the best recognized alcoholamines. Complexation of triethanolamine with anionic polymers decreases its pH (Musial and Kubis, 2004).

Rheology (Rao, 1999)

Rheology is the study of how a material deforms during and after a force is applied. Rheology directly affects product handling and flow characteristics. To study rheological properties, it is convenient to learn the principle of rheological behaviour of fluid. Viscosity is best determined using geometric in which the shear rate can be calculated from experimental data. Shear rate (γ) is the velocity gradient established in a fluid as a result of an applied shear stress (σ). It is expressed in units of reciprocal seconds, s^{-1}. Shear stress is the stress component applied tangentially. It is equal to the force factor (a vector has both magnitude and direction) divided by the area of application and is expressed in force per unit area (Pa). Viscosity is the

internal friction of a fluid or its tendency to resist flow. It is denoted by the symbol η for Newtonian fluids, whose viscosity does not depend on the shear rate, and for non-Newtonian fluids to indicate shear rate dependence by η_a. Depending on the flow system and choice of shear rate and shear stress, there are several equations to calculation. Here, it is defined by the equation:

$$\text{Viscosity}\,(\eta_a) = \frac{\text{shear stress}\,(\sigma)}{\text{shear rate}\,(\gamma)}$$

The preferred units of viscosity are Pa s or mPa s. The fluids flow behaviour can be described by mean of shear rate versus shear stress. There are two types of fluids behaviour: Newtonian and Non-Newtonian.

Newtonian behaviour With Newtonian Fluids, the shear rate is directly proportional to the shear stress. The Newtonian Fluids which means as the shear rate changes the viscosity remains constant such as water, oils, and solvents.

Non-Newtonian behaviour The fluid which means either that the shear stress-shear rate plot is not linear and/or the plot does not begin at the origin or that the material exhibits time-dependent rheology behaviour as a result of structural changes. Flow behaviour may depend only on shear rate and not on the duration of shear (time independent) or may depend also on the duration of shear (time dependent). These fluids are affected by shear and are divided into shear-thinning behaviour or shear-thickening behaviour and time dependent fluids (Thixotropic). Most polymers are pseudoplastic and thixotropic. Types of rheology behaviour are shown in Fig. 9.

Shear-thinning behavior: With shear-thinning fluids, the curve begins of the stress-shear rate plot, that is an increasing shear rate gives less than proportional increase in shear stress. Shear-thinning fluids are popularly called pseudoplastic. The expression shear-thinning is preferred compared to pseudoplastic because it is an accurate description of the shear rate-shear stress curve. Shear thinning may be thought of being due to breakdown of structural units due to the hydrodynamic forces generated during shear. Many polymeric systems are pseudoplastic, perhaps the most classic example is mayonnaise.

Shear-thickening behaviour: In shear-thickening behaviour, an increasing stress gives a less than proportional increase in shear rate. This type of flow has been encounter in partially gelatinized starch dispersions. The expression

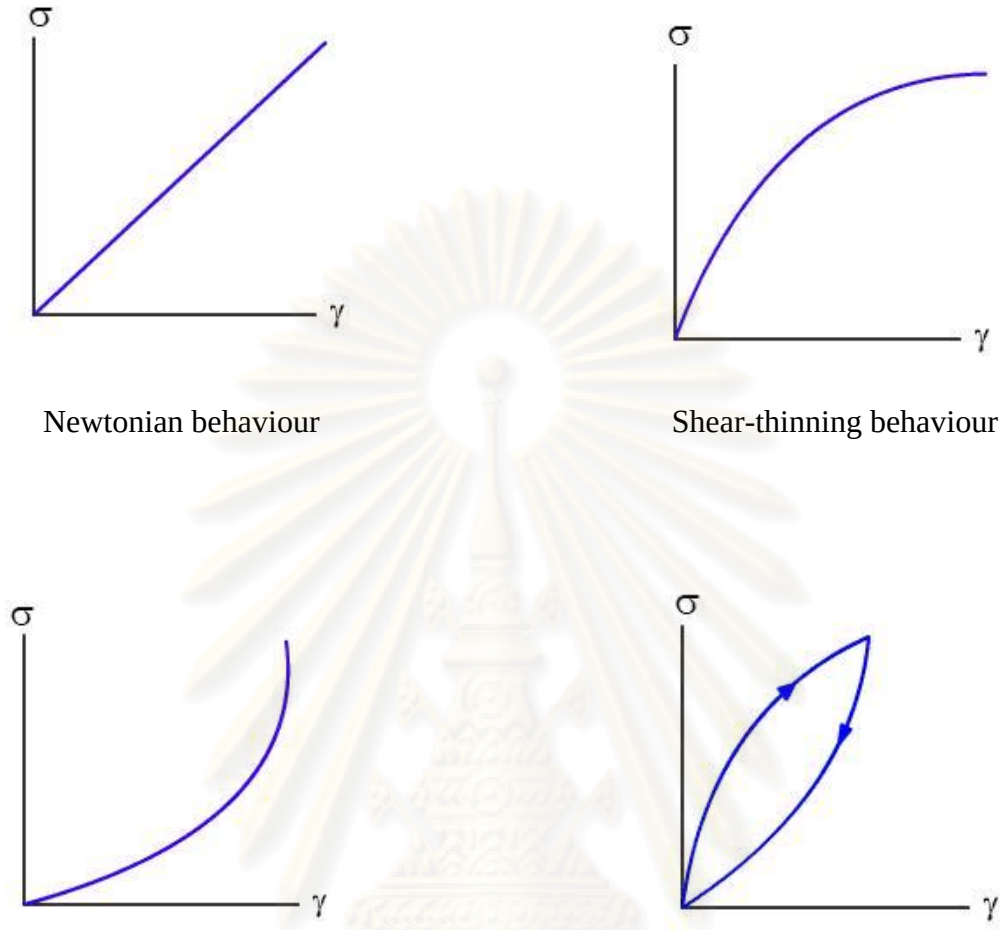


Figure 9. Types of rheology behaviour: σ = shear stress, γ = shear rate

dilatant is popularly and incorrectly used to describe shear-thickening. However, because dilatancy implies an increase in the volume of the sample during the test, it is incorrect to use it to describe shear-thinckening rheological behaviour. Strictly speaking, shear-thickening should be due to increase in size of the structural unit as a result of shear.

Thixotropic behaviour: Fluids exhibit time-dependent shear-thinning behaviour are said to exhibit thixotropic flow behaviour. Most of the foods that exhibit thixotropic behaviour are heterogenous systems containing a dispersed phase that is often very fine, the particles or molecules are linked together by weak forces. When the hydrodynamic forces during shear are sufficiently high, the interparticle linkages are broken, resulting in reduction in the size of structural units that, in turn, offer lower resistance to flow during shear. This type of behaviour is common to food such as salad dressing and soft cheeses where the structural adjustments take place in the food due to shear until an equilibrium is reached.

CHAPTER III

MATERIALS AND METHODS

Materials

1. Chemicals

- Butylated hydroxyanisole (BHA), analytical reagent grade (Sigma – Aldrich Co., MO, U.S.A.)
- Chloroform, analytical reagent grade (Fisher Scientific U.K. Limited, U.K.)
- 2-deoxy-D-ribose, analytical reagent grade (Sigma – Aldrich Co., MO, U.S.A.)
- 2,2-diphenyl-1-picrylhydrazyl, analytical reagent grade (Sigma – Aldrich Co., Ltd., MO, U.S.A.)
- Dipotassium hydrogen phosphate, analytical reagent grade (Ajax Finechem, Australia)
- Disodiumethylenediaminetetracetic acid, analytical reagent grade (Merck KGaA, Germany)
- Disodium hydrogen phosphate anhydrous, analytical reagent grade (Ajax Finechem, Australia)
- Ethanol, manufacturing grade (Government Pharmaceutical Organization, Thailand)
- Ethyl acetate, analytical reagent grade (Fisher Scientific U.K. Limited, U.K.)
- 2N Folin-ciocateu's phenol reagent, analytical reagent grade (Sigma-Aldrich Inc., St. Louis, MO, U.S.A.)
- Ferric ammonium sulfate dodecahydrate, analytical reagent grade (E. Merck, Germany)
- Ferrous Chloride, analytical reagent grade (Merck-Schuchardt, Germany)
- Ferrous sulfate heptahydrate, analytical reagent grade (E. Merck, Germany)
- Gallic acid, analytical reagent grade (Sigma-Aldrich Inc., MO, U.S.A.)
- Hide power, analytical reagent grade (Sigma - Aldrich Chemie GmbH., Germany)
- Hydrochloric acid, analytical reagent grade (Merck KGaA, Germany)

- 3% Hydrogen peroxide, analytical reagent grade (Vidhyasom Co., Ltd., Thailand)
- L-ascorbic acid, analytical reagent grade (Sigma – Aldrich Co., Ltd, MO, U.S.A.)
- Methanol, analytical reagent grade (Millinckrodt Baker, Inc., NJ, U.S.A.)
- n-butanol , analytical reagent grade (Asia Pacific Specialty Chemicals Limited., Australia)
- Potassium dihydrogen phosphate , analytical reagent grade (E. Merck, Germany)
- Potassium hexaferrocyanate, analytical reagent grade (Merck, Germany)
- Pyrogallol, analytical reagent grade (Sigma - Aldrich Chemie GmbH., Germany)
- Sodium carbonate, analytical reagent grade (Ajax Finechem, Australia)
- Sodium chloride, analytical reagent grade (Merck KGaA, Germany)
- 2-thiobarbituric acid, analytical reagent grade (Sigma - Aldrich Chemie GmbH., Germany)
- Trichloroacetic acid, analytical reagent grade (Merck KGaA, Germany)
- Amerchol L101, manufacturing grade (S. Thong Chemicals Co., Ltd., Thailand)
- Cremophor RH-40, manufacturing grade (S. Thong Chemicals Co., Ltd., Thailand)
- Glycerin, manufacturing grade (S. Thong Chemicals Co., Ltd., Thailand)
- Propyl paraben, manufacturing grade (S. Thong Chemicals Co., Ltd., Thailand)
- Sorbitol, manufacturing grade (S. Thong Chemicals Co., Ltd., Thailand)
- Methyl paraben, manufacturing grade (S. Thong Chemicals Co., Ltd., Thailand)
- Propylene glycol, manufacturing grade (S. Thong Chemicals Co., Ltd., Thailand)
- Triethanolamine, manufacturing grade (S. Thong Chemicals Co., Ltd., Thailand)
- Calcium chloride, analytical reagent grade (Merck, Germany)
- Nitrogen gas, industrial grade (Thai Industrial Gases Co., Ltd., Thailand)

2. Equipments

- Analytical balance (Mettler Toledo PL602-5, Switzerland)
- Blender (Model LB20E* (LB 20 EG),Waring commercial., U.S.A.)
- Glassware apparatus (Pyrex, U.S.A.)
- Hot air oven (Mammert, Becthai Co., Ltd., Thailand)
- Magnetic stirrer
- Micropipette (Pipetman, France)
- pH meter (SevenMulti , Mettler Toledo Gmbh., Switzerland)
- Refrigerator (Sharp TH-8903, Thailand)
- Rotary evaporator (Buchi R-200, Switzerland)
- Stirrer (KMO2, Janke and Kenkel GMBC and Co. KG, Germany)
- Suction apparatus (Buchner Funnel, Aspirator, SIBATA circulating aspirator WJ-20, Japan)
- Viscometer (Brookfield, Model LVDV-I+, Brookfield Engineering Laboratories INC., USA)
- Rheometer (Rheowin-RV1 software, HAAKE Rheowin, Germany)
- Vortex mixer (Vortex-2 genie, U.S.A.)
- Centrifuge (EBA 12R, Hettich)
- Water Bath (Memmert, Becthai Co.Ltd.,Thailand)

3. Plant sample

Ripened tamarind pods of *Tamarindus indica* from different cultivars including sweet types “Srichomphu”, “Sithong-nak”, “Sithong-bao” and Khanti; and sour type “Priao-Yak” were collected from Chanika farm, Petchabun province, during January-April in 2006. Tamarind seeds of each cultivar were separated from pulps and kept at -20 ^{o}C until used. Tamarind seeds from different cultivars are shown in Figure 10.

“Srichomphu”

“Sithong-nak”

“Sithong-bao”

“Khanti”

“Priao-Yak”

Figure 10. Seeds of different cultivars of *Tamarindus indica*

Methodology

1. Extraction of tamarind seed coat

Tamarind seeds (100 g) were heated in hot air oven at 140 ^{o}C for 45 min. Seed coats were removed mechanically from kernels. Then, they were dried in hot air oven at 60 °C to constant weight. The seed coats were blended by using blender into powder and stored in screw cap bottles in desiccators. The yield of seed coat powder obtained from 5 different cultivars was recorded.

Extraction of tamarind seed coats was carried out as described by Komutarin et al. (2004) with minor modification. Two grams of seed coat powder were vigorouosly shaked in 70% ethanol for 10-15 min and filtered through WHATMAN no.4 filter paper, repeated extraction until the filtrate became colorless (about 40 mL of 70% ethanol was used), repeated filtration until clear filtrate was obtained, then the filtrate (20 mL) was partitioned with 20 mL chloroform in separating funnel. The 8 mL of the upper layer was extracted by 20 mL of ethyl acetate in separating funnel. The upper layer was concentrated by using rotary evaporator at 35 °C and then purged with nitrogen gas until dried. The dried extract was dissolved in 70% methanol to make 100 mg/mL of tamarind seed coat extract (TSCE) and kept in 4 °C, for further studied.

2. Determination of phenolic compounds

2.1 Total phenolic contents

Total phenolic contents of TSCEs were determined as described by Spanos and Wrolstad (1990) with some modifications. Beginning with 0.1 mL of 1 mg/ml TSCEs, was pipetted into each test tube and made up to the volume of 8.4 mL with distilled water. Then 0.5 mL of 2 N Folin-Ciocalteu reagent was added and mixed. All sample mixtures were standed at room temperature for 3 minute. Then added 1.0 mL of 20 % sodium carbonate solution in each tube, the reaction mixtures were mixed, and then kept at room temperature for 1 hour. The absorbance was recorded at 765 nm against the reagent blank. The amount of total phenol was calculated as gallic acid equivalents from the calibration curve.

2.2 Tannins contents

Tannins contents of TSCEs were determined by the reaction of phosphomolybdotungstic reagent, as described by European pharmacopoeia 4th edition.

2.2.1 Sample preparation

The experiment was followed by diluting 1 mg/mL of TSCEs with 250 mL of distilled water then filtering and discarding the first 50 mL of all TSCE filtrates.

2.2.2 Total phenols

Each of TSCE filtrate (5 mL) was diluted to 25 mL with distilled water. The 2 mL of this diluted solution was mixed with 1 mL of 2N Folin-ciocateu's phenol reagent and 10 mL of distilled water. The 29 %w/v solution of sodium carbonate was added to make up to 25 mL solution. After mixing thoroughly and standing for 30 minutes, the absorbance was measured at 760 nm using water as the blank. The total phenol was expressed as A_1

2.2.3 Phenols not absorbed by hide powder

Each TSCE filtrate (10 mL) was added with 0.10 g of hide powder then shake vigorously for 60 minutes. Next, the solution was filtered through WHATMAN No.4 filter paper. The 5 mL of this filtrate was diluted to 25 mL with distilled water. The 2 mL of this diluted solution was mixed with 1 mL of 2N Folin-ciocateu's phenol reagent and 10 mL of distilled water. The sodium carbonate solution (29% w/v) was added to make up to 25 mL solution. After 30 minutes, the absorbance was measured at 760 nm using water as the blank. Phenols not absorbed by hide powder was expressed as A_2.

2.2.4 Standard

Pyrogallol (0.025 mg/mL, 2 mL) was mixed with 1 mL of 2N Folin-ciocateu's phenol reagent and 10 mL of distilled water. The 29% w/v solution of sodium carbonate was added to make up to 25 mL solution. After 30 minutes, the absorbance was measured at 760 nm, using water as the blank. The absorbance of pyrogallol was expressed as A_3

The percentage content of tannins expressed as pyrogallol was calculated from this equation:

$$\% \text{ tannin} = \frac{62.5(A_1 - A_2)m_2}{A_3 \times m_1};$$

m_1 and m_2 are weight of sample and pyrogallol, in grams, respectively.

2.3 Proanthocyanidins contents

The proanthocyanidins contents in TSCEs were determined by the butyl alcohol-HCL-Fe^{3+} method (Rathee, Hassarajani and Chattopadhyay, 2006). First, acid butanol reagent was prepared by mixing 950 mL of n-butanol with 50 mL concentrated HCl. The iron reagent was prepared by adding concentrated HCl in distilled water to make 2 N HCl , then used 25 mL of 2 N HCl to dissolve 0.5 g of ferrous ammonium sulfate dodecahydrate ($FeNH_4(SO_4).12H_2O$. The solution was stored in the bottle and protected from light. Secondly, each of TSCE (1 mg/mL, 1.0 mL) was added in the screw cap tube then added 6 mL of acid butanol reagent in every tube. Subsequently, the 0.2 mL of iron reagent was added and vortexed the mixture. All tubes were capped loosely and boiled in water bath for 50 min. Finally, each mixture was cooled down at room temperature. The absorbance was read at 550 nm. The reading value of sample was subtracted to that of a blank containing sample solvent, acid butanol and iron from the sample absorbance.

3. Antioxidative activity of TSCEs

3.1 Determination of reducing power

The reducing power of various extract was evaluated according to the method of Yen and Chen (1995). Briefly, TSCEs (0-160 μg/mL, 1 mL) were mixed with 2.5 mL of 0.2 M of phosphate buffer pH 6.6 and then added 2.5 mL of 1% w/v potassium hexaferrocyanate [$K_3Fe(CN)_6$]. The mixtures were incubated at 50 °C for 20 min. The 2.5 mL of 10% w/v trichloroacetic acid (TCA) were added to the mixture, then standed for 10 min. The 2.5 mL of these solutions were mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% w/v of $FeCl_3$. The absorbance was measured at 700 nm, against blank. Vitamin C and BHA were used as positive control. The increased absorbance of the reaction mixture represented the increased reducing power. The effective concentration at which the absorbance was 0.5 (EC_{50}) was analyzed by using program CurveExpert version 1.38.

3.2 Assay of lipid peroxidation

The efficacy of the extract from each tamarind cultivar on in-vitro inhibiting lipid peroxidation was estimated according to the procedure described by Jiang et al. (2005). Lipids in egg yolk were used in the assay study. An egg yolk was mixed with the same volume of phosphate buffer saline (PBS), pH 7.45 and stirred vigorously by using magnetic stirrer, then diluted with 40 times volume of PBS to prepare a yolk emulsion. The 0.5 mL of yolk emulsion was incubated at 37 °C for 15 min. with TSCEs (0-500 μg/mL, 1 mL) and 0.5 mL of 24 mM $FeSO_4$ in PBS. The reaction was stopped by adding 0.5 mL of 20 %w/v TCA and added with 1 mL of 0.8% w/v in 0.05 M NaOH of 2-thiobarbituric acid (TBA), and then heated at 100 °C for 15 min. The reaction mixture was centrifuged at 3500 rpm for 20 min, the color of supernatant was measured its absorbance at 532 nm by spectrophotometer. The inhibition of lipid peroxidation was calculated as follows:

$$\text{Inhibition of lipid peroxidation (\%)} = \frac{(A - B)}{A} \times 100,$$

Where A was the absorbance of the reaction mixture without TSCE and B was the absorbance of the reaction mixture with TSCE. Vitamin C was used as positive control. EC_{50} value (μg/mL) is the concentration at which the anti-lipid peroxidation activity was 50 % which was analyzed by using program CurveExpert version 1.38.

3.3 Hydroxyl radical (•OH) scavenging activity

Hydroxyl radical scavenging activity was performed by measuring the formation of thiobarbituric acid-reactive substances (TBARS) using 2- deoxy-d-ribose as substrate, as described by Hsu et al. (2006) with minor modification. The reaction mixture (1 mL) contained 250 μL of TSCE (0-500 μg/mL), 250 μL of potassium phosphate buffer, pH 7.4 (100 mM), 100 μL of 2-Deoxy-D-ribose (28 mM), 200 μL of Fe^{3+}-EDTA (a mixture of 100 μM $FeCl_3$ + 104 μM Na_2 EDTA in 1:1 ratio, v/v), 100 μl of H_2O_2 (1 mM) and 100 μL of ascorbic acid (1 mM). H_2O_2 and ascorbic acid were added last in that order to initiate the generation of hydroxyl radical. The control tube contained all the reaction reagents except for TSCE. The reaction mixture was mixed by vortex and then incubated for 1 h at 37 °C. To terminate the reaction and form TBARS chromogen, 1 mL of trichloroacetic acid (2.8%, w/v in water) and then 1 mL of 2-thiobarbituric acid (1% w/v in 0.05 M NaOH) were added, and the mixture was heated in a boiling bath for 20 min. The color of the reaction mixture was read at

532 nm against blank. The percentage scavenging effect was calculated from the decreased in absorbance against control. BHA was used as a positive control. EC_{50} value for hydroxyl radical scavenging activity of each cultivar was also analyzed by using program CurveExpert version 1.38.

3.4 Free radical scavenging activity on 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•)

The DPPH radical scavenging activity of each TSCE was estimated according to the procedure described by Sanchez-Moreno *et al.* (1998). Each TSCE (0-500 µg/mL, 0.1 mL) was mixed with 3.9 mL of methanolic solution containing 0.025 mg/mL DPPH. After incubating in the dark for 60 minute, the absorbance was measured at 515 nm against blank in spectrophotometer. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation:

$$\%\text{RSA} = (\frac{A_D - A_C}{A_D}) \times 100 ,$$

Where A_C is the absorbance of the solution when the extract has been added at a particular concentration, and A_D is the absorbance of the solution without extract. The assays were carried out in triplicate and the results are expressed as mean values ± standard error of mean (SEM). Vitamin C was used as positive control. The concentration of extract at which DPPH radicals were scavenged by 50 % (EC_{50}) was analyzed by using program CurveExpert version 1.38.

4. Preparation of external used preparation

TSCE was used as an active ingredient for antioxidative activity, the preparation was formulated by using Polysaccharide Gel (PG) from durian-rind as a gelling agent. PG was supplied by Department of Biochemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

4.1 Formulation

The preparations were prepared in the following :

4.1.1 Preparation of PG base

PG base was prepared according to Lertchaiporn, 2003. PG

powder was dispersed in the deionized water and stirred until homogenous, then gradually poured glycerin and propylene glycol and mixed them to PG dispersion in a mortar. Amerchol L-101 and cremophor RH-40 were measured and mixed before adding to PG in the mortar and mixed well. Calcium chloride solution was added, then triethanolamine (TEA) was dropped into the mixture to adjust pH. The deionized water (DI water) was added to make a total weight of 100 gram. The viscosity and pH of the gel were measured and the formulation was evaluated. Physical appearances, flow property, air bubbles, color, pH and rheology were recorded. Stability test was performed.

Ingredients	***Content (%w/w)***
PG	2.5
Glycerin	10
Propylene glycol	15
Amerchol L-101	0.25
Cremorphor RH-40	10
0.05 M $CaCl_2$ solution	0.02
Triethanolamine to pH 4-4.5	qs
DI water to make	100

4.1.2 Preparation of TSCE in PG gel base

First, PG powder was dispersed in the DI water and stirred until uniform. PG was pour into a mortar and added glycerin, propylene glycol while mixing continuously until homogenous. Next, the oil phase ingredients such as amerchol L-101 and cremophor RH-40 were mixed well together and then slowly added to the PG mixture in the mortar. TSCE was added and mixed thoroughly. Triethanolamine was added to adjust pH between 4-5. Finally, DI water was added to make a total weight of 100 gram and mixed until uniform. Each formulation was prepared in triplicates. The finished product was evaluated. Physical properties such as flow, air bubbles, color, and rheology were recorded.

Ingredients	*Content (%w/w)*
PG	2.5
Glycerin	10
Propylene glycol	15
Amerchol L-101	0.25
Cremorphor RH-40	10
0.05 M $CaCl_2$ solution	0.02
TSCE in 70% ethanol 500 µL	7-28 mg
Triethanolamine to pH 4-4.5	qs
DI water to make	100

4.1.3 Preparation of tamarind seed polysaccharide (TSP) gel base

4.1.3.1 Isolation and viscosity of TSP

Isolation of TSP

Tamarind kernels from Tamarind seed of *Tamarindus indica* "Priao" type sour (Figure 11) from Nakorn Ratchasrima province, was used to isolate TSP for TSP gel preparation. Isolation of TSP from kernel of tamarind seed was processed according to Suttananta, 1986 with minor modification. Dried kernel of tamarind seed was blended. Tamarind kernel powder was extracted in 40 times of hot DI water and continuously stirred in water bath at 90 °C for 2 hour. The mixture was centrifuged at 6800xg for 20 min and collected supernatant. The precipitate was repeatly extracted in 20 times of hot DI water and continuously stirred in water bath at 90 °C for 1 hour, and centrifuged by the same condition. The supernatant was collected and pooled, then concentrated at 70 °C under reduced pressure by using rotary evaporator to reduce 40 times of total volume. The viscous liquid was treated in 1.5 volumes of cold 95% ethanol to precipitate the polysaccharide. TSP precipitate was collected by filtration though a fine nylon sheet. TSP was dried in hot air oven at 50 °C, pulverized to a fine powder of TSP and kept in desiccator for further used.

Figure 11. Seeds of *Tamarindus indica* "Priao"

Viscosity of TSP

- Effect of concentration on viscosity of TSP

TSP powder was dispersed in DI water to make concentrations at 1-4 % w/v of TSP dispersion. Viscosity in triplicates measurement was recorded.

- Effect of some ingredient on viscosity of TSP

TSP powder was dispersed in DI water. Various amounts of glycerin, propylene glycol, cremorphor RH-40 and sorbital were seperately added into 2% w/v TSP dispersion in a mortar. The viscosity of TSP with various amounts of each ingredient was recorded.

4.1.3.2 TSP gel base formulation

TSP powder was dispersed in DI water and stirred until homogenous. Glycerin and propylene glycol were gradually poured into TSP dispersion in a mortar with continuously mixed until uniform. Paraben concentrate was added into the gel mixture. The DI water was added to make total weight of 100 g and mixed. The viscosity and pH were measured and then formulation was evaluated. Physical appearances; flow, air bubbles, color, pH and viscosity were determined. Stability test was performed.

Ingredients		*Content (%w/w)*
TSP gel		3
Glycerin		10
Propylene glycol		10
Paraben concentrate		1
DI water	to make	100

4.1.4 Preparation of TSCE in TSP gel

First, TSP powder was dispersed in the DI water and stirred until uniform. TSP dispersion was poured into a mortar and added glycerin and propylene glycol while mixing continuously until homogenous. Paraben concentrate was added and mixed well. Finally, DI water was added to make a total weight of 100 gram. The final product was evaluated for the physical appearances, pH and viscosity.

Ingredients		*Content (%w/w)*
TSP gel		3
Glycerin		10
Propylene glycol		10
TSCE in 70% ethanol 500 µL		14 mg
Paraben concentrate		1
DI water	to make	100

4.2 Assessment of the stability of the finished products

4.2.1 Storage at ambient temperature

The 50 g of each formulation was filled in a wide mouth glass bottle with a tightly closed cap. The products were stored at ambient temperature $(28\pm5)^{o}C$ for 30 days. Any physical changes including pH and rheology were examined by using rheometer.

4.2.2 Accelerate stability test by temperature cycling test

Each formulation was tested for stability by the method of

heating-cooling cycle. The products were incubated at 45 ^{o}C for 48 hours and then transferred into a refrigerator at temperature 4 ^{o}C for 48 hours to complete 1 cycle. The same procedure was continued for 4 cycles. The products were similarly observed and recorded for any physical changes.

4.3 The efficacy of TSCE-PG gel preparation on inhibition of lipid peroxidation

The efficacy on anti-lipid peroxidation of each preparation was studied *in vitro* by lipid peroxidation inhibition test. Lipids in egg yolk were used in the assay study (Jiang et al., 2005). The gel preparation (3 g) was extracted by 3 mL of 70% ethanol. The extract of gel preparation (1 mL) was used as sample. The method was followed the assay of lipid peroxidation in **3.2**. The inhibition of lipid peroxidation was calculated as follows:

$$\text{Inhibition of lipid peroxidation (\%)} = \frac{(A-B)}{A}\times 100,$$

Where A was the absorbance of the reaction mixture without extract, B was the absorbance of the reaction mixture with extract.

5. Statistical analysis

Mean values and standard error of mean (SEM) were calculated from the results. One way analysis of variance (ANOVA) with Tukey HSD was applied for comparison of the mean values. The independent sample t-test was analyzed for the different of two mean values. The p value < 0.05 was regarded as significant. Correlation coefficients (r) were determined the relationship between two variables (between antioxidant activity and content of phenolic compounds in TSCEs). The Statistical Package for Social Science (SPSS) programme was used for calculation.

CHAPTER IV

RESULTS AND DISCUSSION

1. Extraction of tamarind seed coat

The content of dry Tamarind seed coat from 5 tamarind cultivars is shown in Table 1 in appendix. The yields of dried extract were 36.9, 28.0, 18.7, 19.7 and 27.5 mg/g of seed coat from "Srichomphu", "Sithong-nak", "Sithong-bao", "Khanti" and "Priao-yak", respectively.

2. Determination of phenolic compounds content

The results of total phenolic, tannins and proanthocyanidins contents in 5 TSCEs are summarized in Table 2. It was found that different cultivars exhibited different total phenols. The highest contents were found in sweet-type TSCEs as "Khanti" and "Sithong-bao" ($p < 0.05$). Mean values of total phenol in different cultivars are as follow: "Khanti" > "Sithong-bao" > "Priao-yak" > "Sithong-nak" > "Srichomphu". However, Folin-Ciocalteu method detects all phenolic compound from simple molecule to highly polymerised polyphenol. Tannins, a polymeric polyphenol, were found in all tested TSCEs. According to European pharmacopoeia 4th edition for the determination of tannins contents, all sweet and sour tamarind showed remarkable tannins contents from the highest tannins content of "Priao-yak" to the lowest content of "Srichomphu". The percentages of tannins content were from 10.08±0.51 to 27.16±4.94. "Priao-yak" exhibited higher tannins content than that of "Srichomphu" ($p < 0.05$). The mean values of tannins content (%) were the following order: "Priao-yak" > "Sithong-bao" > "Khanti" > "Sithong-nak" > "Srichomphu". Proanthocyanidins are a group of tannins. It was reported that proanthocyanidins are good antioxidant which were found in seed coat of tamarind (Pumthong, 1999 and Fine, 2000). Proanthocyanidins were determined by acid catalysed oxidative depolymerisation. The absorbance values of anthocyanidin at 550 nm were represented proanthocyanidins in TSCEs. The mean values of proanthocyanidins in TSCE were found as the following order: "Khanti" = "Sithong-bao" > "Priao-yak" = "Sithong-nak" > "Srichomphu". However, the low absorbance data presented that this extraction obtained low yield of proanthocyanidins. It has been

Table 2. Content of phenolic compounds in tamarind seed coat extract (TSCE) from different tamarind cultivars

Seed coat extract	Total phenolics (GAE, g/100g dry extract)	% Tannin (as pyrogallol equivalents)	Proanthocyanidins (A_{550})
Srichomphu	20.74±0.74[a]	10.08±0.51[a]	0.08±0.01[a]
Sithong-nak	24.07±0.37[a]	16.87±0.17[a,b]	0.13±0.01[b]
Sithong-bao	35.93±0.98[c]	21.83±1.05[a,b]	0.18±0.01[c]
Khanti	36.30±0.64[c]	18.87±1.05[a,b]	0.18±0.01[c]
Priao-yak	29.63±0.98[b]	27.16±4.94[b]	0.13±0.01[b]

Data represent mean ± SEM (n = 3).

Values with different letters within a column are significantly different, $p < 0.05$.

GAE = gallic acid equivalents.

recently reported that maximum yield of phenolic compounds was obtained for the methanol extracts of raw and dry-heated seed coat samples. Moreover, the extractable total phenolics in methanol extracts are comparable in both raw and dry heated samples. However, the level of extractable tannin contents is substantially reduced in 70% acetone extracts of dry heated sample than that of the raw sample (Siddhuraju, 2007).

3. Antioxidative activity of TSCEs

3.1 Determination of reducing power

Reducing power of TSCEs at various concentration are presented in Figure 12. In this assay, the yellow colour of the test reaction mixture changes to various shades of green and blue, depending on the reducing power of each sample. The presences of antioxidants in TSCEs caused the reduction of the Fe^{3+}/ferricyanide complex into the ferrous form. Hence, measuring the formation of Perl's Prussian blue at 700 nm can monitor the Fe^{2+} concentration. In the present study, reducing powers of all TSCEs observed at concentration 160 µg/mL were in the following order: "Priao-yak" > "Sithong-bao" > "Srichomphu" > "Khanti" > "Sithong-nak". The reducing abilities of "Priao-yak" and "Sithong-bao" were high and their reducing power in term of absorbance values at 160 µg/mL were 0.564 and 0.507, respectively, where reducing powers of vitamin C and BHA at 160 µg/mL were only 0.345 and 0.316, respectively. The reducing powers of both cultivars were dose-dependences manner. They showed reductive ability more potential than that of the other cultivars. Furthermore, "Priao-yak" and "Sithong-bao" were stronger reducing agent than those of vitamin C and BHA. Increased reducing power with increased concentration was observed on the concentration ranges 20-60 µg/mL of "Srichomphu", "Sithong-nak" and "Khanti" but theirs reducing ability remained constant at the concentration ranges 80-160 µg/mL. From the previous reported of other plants, reducing power of some tea extracts named green tea, pouchong tea and black tea, showed reducing power in term of absorbance value at 0.5 by using tea extracts exceed 200 µg (Yen and Chen, 1995). Methanol extract of low grade coffee beans presented reducing power in term of absorbance value at approximately 0.5 at concentration 200 µg/mL (Ramalakshmi, Kubra, and Rao, 2008). The reducing power of TSCEs might be due to their

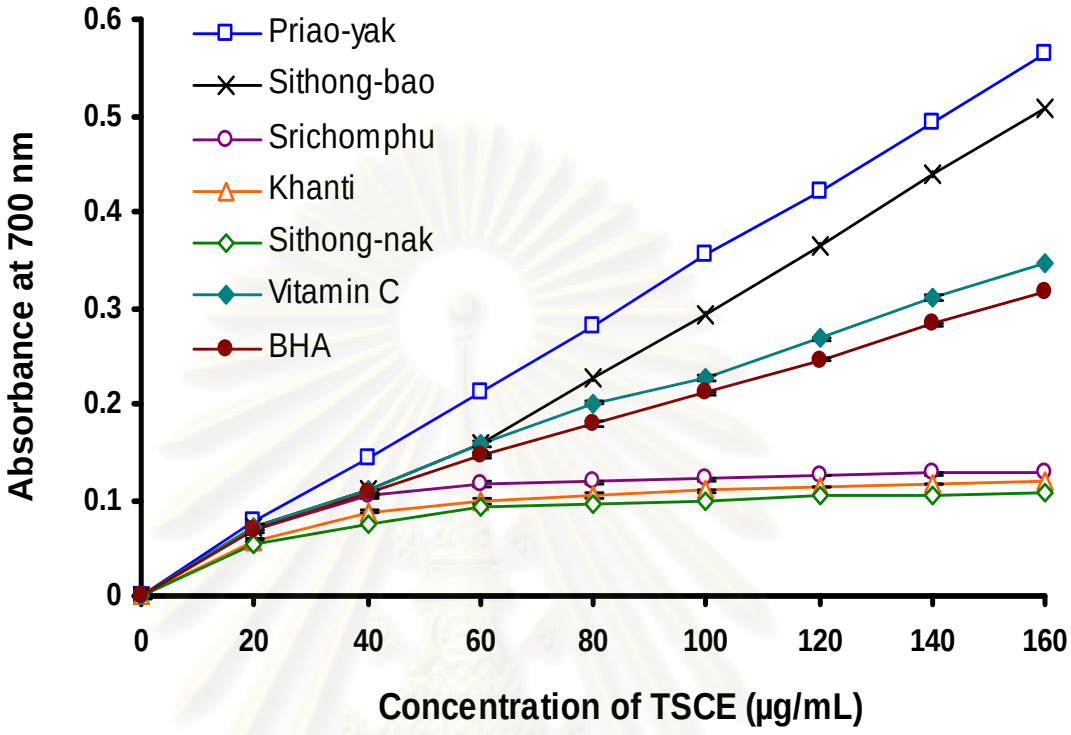


Figure 12. Reducing powers of methanolic extracts from tamarind seed coat (TSCE). Each value expressed as mean ± SEM (n = 3). BHA and vitamin C were used as positive controls.

electron-donating ability. Accordingly, “Priao-yak” and “Sithong-bao” might contain higher amounts of reductone, which could react with free radicals to stabilise and block radical chain reactions. There was a high correlation (r = 0.81) between tannins in the extracts and theirs reducing power at the highest concentration. Tannins, the high molecular weight polyphenols in TSCEs, might be responsible for reducing ability. Pulido, Bravo, and Saura-Calixto (2000) reported that reducing power of polyphenols was higher than those of ascorbic acid and trolox. Antioxidant activity of polyphenols seemed to depend on the extent of hydroxylation and conjugation. However, Siddhuraju (2007) reported that such potential reducing power activity might be attributed to the presence of dihydroxy type of benzene derivatives and (-)-epicatechin present in the seed coat.

3.2 Assay of lipid peroxidation

Lipid peroxidation is a complex process. It involves the formation and propagation of lipid radicals and eventual destruction of membrane lipids, producing the breakdown products such as malondialdehyde (MDA). The anti-lipid peroxidation activity of tamarind seed coat extracts (TSCE) was studied. Anti-lipid peroxidation activity of the TSCE was determined by measuring the inhibitory ability on lipid oxidation of egg yolk. The end-products of the process were measured in terms of the thiobarbituric acid-reactive substances (TBARS) formed. The percentage of inhibitory activity on lipid peroxidation of various concentration of different TSCEs are demonstrated in Figure 13. All cultivars showed continuously increased lipid peroxidation inhibition with increased concentration of TSCEs. “Sithong-bao” exhibited high anti-lipid peroxidation particularly at the concentration between 100-500 μg/mL. From table 3 in appendix, the anti-lipid peroxidation activities of TSCEs were found in the following order : “Sithong-bao” > “Khanti” > “Srichomphu” > “Sithong-nak” > “Priao-yak” with %inhibition of 98.34±1.44, 81.13±0.99, 78.15±2.98, 67.22±0.99 and 62.91±1.33, respectively, at 500 μg/mL. “Sithong-bao” was found to inhibit lipid peroxidation for more than 50 % at concentration of 80 μg/mL where, vitamin C was 22.85±2.94% inhibition. “Srichomphu”, “Sithong-nak”, “Priao-yak” and “Khanti” inhibited lipid peroxidation more than 50 % at the concentration of 200, 200, 400 and 300 μg/mL, respectively. Among various cultivars, “Priao-yak” showed the lowest lipid peroxidation inhibition

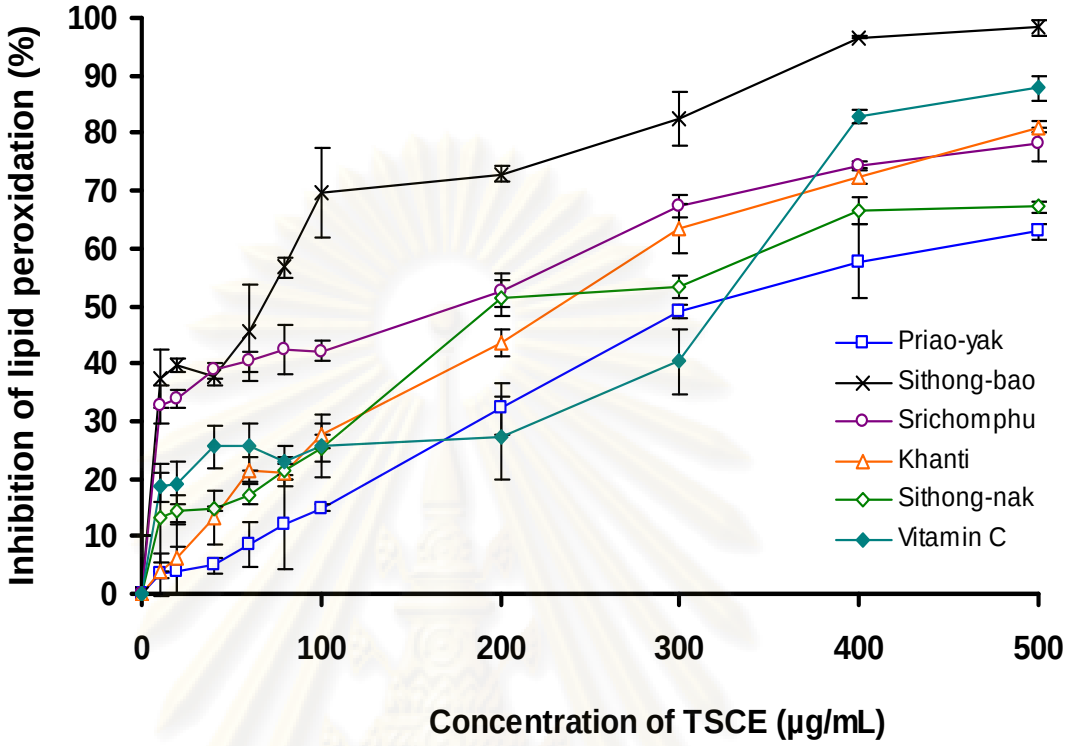


Figure 13. Lipid peroxidation inhibitory activity of methanolic extracts from tamarind seed coat. Each value is expressed as mean ± SEM (n = 3). Vitamin C was used as a positive control.

activity at concentration between 10 to 500 µg/mL concentration. The reference antioxidant, vitamin C, showed lower anti-lipid peroxidation activity than that of "Sithong-bao" in all tested concentrations. Vitamin C at concentration 500 µg/mL showed higher activity than those of tamarind cultivars except for "Sithong-bao". There was no significant correlation between anti-lipid peroxidation activity and the contents of phenolic compounds. In previously published works, Tsuda et al. (1994) found the following compounds, 2-hydroxy-3',4'-dihydroxyacetophenone, methyl 3,4-dihydroxybenzoate, 3, dihydroxyphenyl acetate, and (-)-epicatechin in ethyl acetate extract of Indian tamarind seeds. The dihydroxy type of benzene derivatives have been found to be stronger on anti-lipid peroxidation activity than (-)-epicatechin. Siddhuraju, 2007 reported that defatted raw and dry heated seed coat of Indian TSCEs (1 mg/mL), exhibited 64.5–71.7% anti-lipid peroxidation activity against the linoleic acid emulsion system by using thiocyanate method. However, the aqueous acetone extracts of raw and dry heated seed coat samples were found to be higher lipid peroxidation inhibition than the methanol extracts of both samples.

3.3 Hydroxyl radical (•OH) scavenging activity

Hydroxyl radical scavenging activity was evaluated by using deoxyribose method. Hydroxyl radical is generated from a reaction mixture of ascorbic acid, H_2O_2 and Fe^{3+}-EDTA. Hydroxyl radical scavenger will compete with deoxyribose for hydroxyl radical and inhibits chromogen formation (Halliwell, 1995). The scavenging activities on hydroxyl radical at various concentrations of TSCEs by using deoxyribose method are presented in Figure 14. All tested TSCEs showed potential scavenging activity against hydroxyl radicals. The graphs of TSCEs showed similar trend with dose response manner. BHA, a synthetic antioxidant, showed obviously different pattern of high scavenging activity compared with TSCEs. BHA at very low concentration (10 µg/mL) gave 94.65±0.53% scavenging activity but TSCEs were gradually increased scavenging activity with increased concentrations. Table 4 in appendix showed the percentage of scavenging activity of TSCEs and BHA at concentration between 0-500 µg/mL. Among different TSCEs, the scavenging activity against hydroxyl radical were found in the following order: "Sithong-bao"(77.54±4.81%) > "Priao-yak"(76.47±1.41%) > "Khanti"(73.26±1.93%) > "Sithong-nak" (68.45±0.53%) > "Srichomphu" (60.96±0.53%) at 500 µg/mL

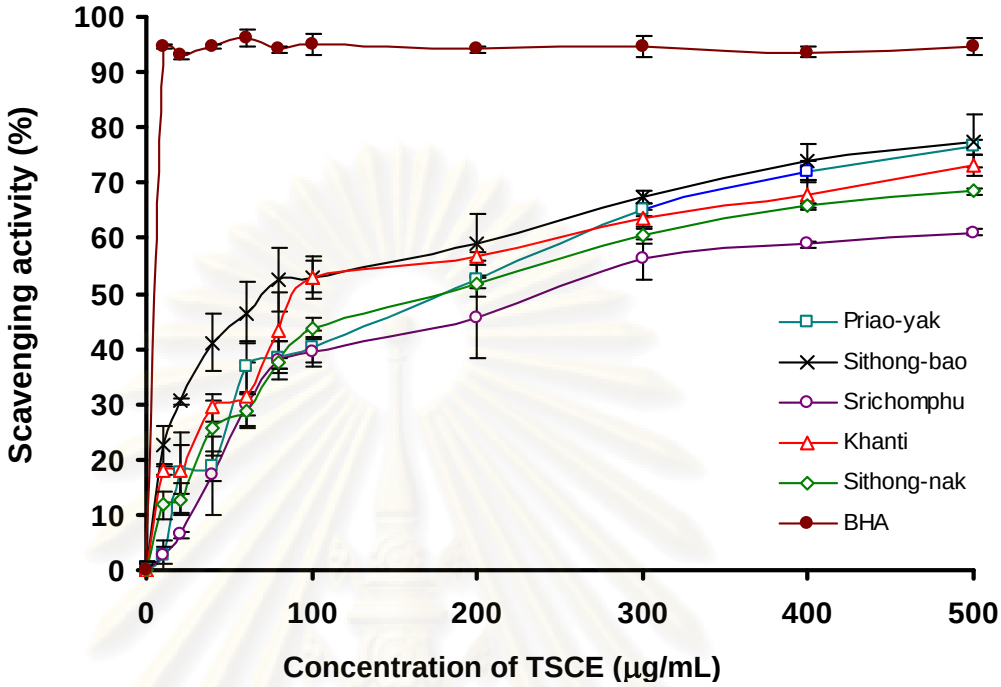


Figure 14. Scavenging activity (%) on hydroxyl radicals of methanol extracts of tamarind seed coat. Each value is expressed as mean ± SEM (n = 3). BHA was used as a positive control.

concentration. At concentration 500 μg/mL, the percentage of scavenging activity against hydroxyl radicals of TSCEs correlated with tannins content (r = 0.92) significantly ($p < 0.05$). The amounts of tannins, the high molecular weight polyphenolic compounds, affected hydroxyl radicals scavenging ability. Nevertheless, BHA was the most effective agent to stabilize hydroxyl radical. Siddhuraju (2007) reported that Indian TSCEs had hydroxyl radical scavenging activities of 35.4–56.6% at concentration of 250 μg/mL. The highest activity was found in methanolic extract of dry heated *T. indica* seed coat. Similarly, the antioxidant activity of tannins in fababean was due to the direct interaction of tannins with hydroxyl radical (Carbonaro, Virgili and Carnovale, 1996).

3.4 Radical scavenging activity on 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•)

Nitrogen centered radicals such as DPPH radicals react with phenols via a mechanism of an electron transfer process in methanol. The spectrophotometric technique employs DPPH radical, which shows a characteristic UV–VIS spectrum with a maximum of absorbance close to 515 nm in methanol. After adding an antioxidant, a decrease in absorbance proportional to concentration of antioxidant was observed (Brand-Williams, Cuvelier, and Berset, 1995). In the present study, the potential DPPH radical scavenging activity of TSCEs was found. In Figure 15, “Khanti” exhibited good scavenging activity at the concentration range between 10-200 μg/mL. It showed higher activity than that of the other 4 cultivars including vitamin C. However, theirs activities remained constant at concentration over 200 μg/mL. According to Table 5 in appendix, all tested cultivars showed high scavenging activity from 70.10±1.92 % to 81.84±2.95 %, which was comparable to the activity of vitamin C (82.18±2.61 %) at 500 μg/mL concentration. High correlation between proanthocyanidins and scavenging activity was found (r = 0.85) at concentration of 500 μg/mL. Villaño et al. (2007) reported that procyanidins B1 and B2 were the most potent scavenging compounds. Chemical structure of flavan-3-ol family showed generally good antioxidant response towards DPPH radical. In addition, Yokozawa et al. (1998) reported that scavenging activity against DPPH radical is closely associated with their chemical structure of antioxidant.

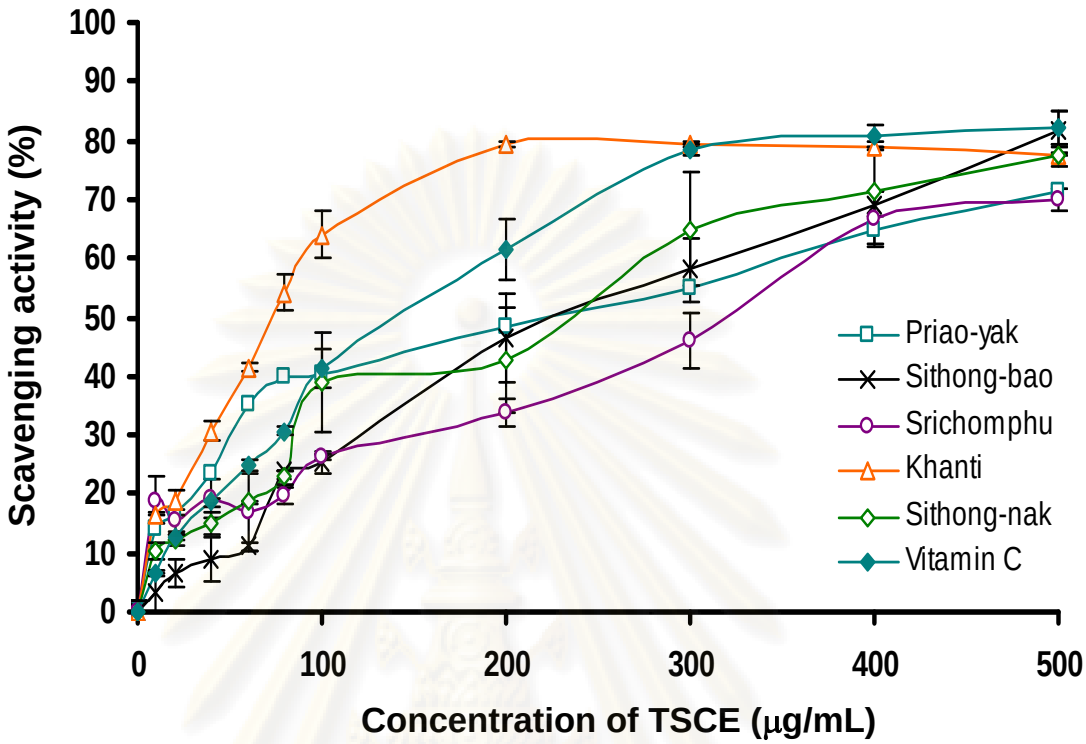


Figure 15. Scavenging activity (%) against DPPH radicals of methanolic extracts from tamarind seed coat. Each value is expressed as mean ± SEM (n = 3). Vitamin C was used as a positive control.

3.5 Estimation of EC_{50} values of antioxidant activity of TSCEs

To facilitate comparison of antioxidant activity of various different tamarind cultivars, the EC_{50} values for different antioxidant activity were estimated. EC_{50} value represented concentration at which antioxidant activity was 50%. Table 3 shows EC_{50} values of the different antioxidant activity of all tested TSCEs including positive control, vitamin C and BHA.

The reducing powers of TSCEs were investigated for their EC_{50} values. The absorbance values represented theirs reductive ability of each extract. EC_{50} value for reducing power was expressed as the concentration of the extract with absorbance value of 0.5. EC_{50} value of "Priao-yak" was significantly lower than "Sithong-bao" ($p < 0.05$). Other tamarind cultivars showed lower reducing power (absorbance < 0.5). So theirs EC_{50} values could not be determined.

The potent activity on inhibition of lipid peroxidation was observed for the extract of "Sithong-bao" with EC_{50} value of 62.19±7.13 μg/mL and was significantly lower ($p < 0.05$) than that of positive control, vitamin C, where the EC_{50} value of vitamin C was 263.93±8.03 μg/mL. "Priao-yak" showed the lowest activity (354.45±14.05 μg/mL). The content of phenolic compounds including tannins and proanthocyanidins in TSCEs did not directly influent the anti-lipid proxidation ability. The correlation (r) between EC_{50} values of lipid peroxidation and content of phenolic compounds show no significantly different ($p \geq 0.05$). It has been reported that the observed values in aqueous acetone extracts of raw and dry heated samples of tamarind seed coats were found to be higher than that of methanol extracts (Siddhuraju, 2007). The dihydroxy type of benzene derivatives had been found to be stronger in lipid peroxidation inhibitory activity than that of (-)-epicatechin (Tsuda, 1994). The polyphenolic compounds as well as polymeric polyphenol may have lesser anti-lipid perxidation activity than that of dihydroxy type of benzene derivatives.

The EC_{50} value of TSCEs on hydroxyl radicals scavenging activity demonstrated that all tested TSCEs were a good scavenger. Among tamarind cultivars, the strongest hydroxyl radical scavenging activity with the lowest EC_{50} value of 62.23±9.36 μg/mL was "Sithong-bao". On the other hand, the weakest scavenging activity with EC_{50} value of 187.95±26.84 μg/mL was "Srichomphu".

Table 3. EC_{50} values (μg/mL) of tamarind seed coat extracts (TSCEs) in reducing power, free radical (DPPH and hydroxyl) scavenging and lipid peroxidation assay

Tamarind cultivars	EC_{50}* (μg/mL)			
	reducing power	lipid peroxidation	hydroxyl radicals	DPPH
Srichomphu	na**	192.94±0.50[b]	187.95±26.84[d]	314.31±11.56[c]
Sithong-nak	na**	226.95±9.53[b,c]	138.05±7.56[c,d]	217.09±56.15[b,c]
Sithong-bao	162.26±0.61[b]	62.19±7.13 [a]	62.23±9.36[a,b]	233.25±14.92[b,c]
Priao-yak	142.06±1.72[a]	354.45±14.05[d]	129.11±4.02[c]	152.95±4.14[a,b]
Khanti	na**	218.19±11.85[b]	102.67±4.02[b,c]	70.10±1.75[a]
Vitamin C	na**	263.93±8.03[c]	nd***	138.29±5.54[a,b]
BHA	na**	nd***	5.28 ± 0.03[a]	nd***

* Each value is expressed as mean ± SEM of triplicates.

a, b, c, d = significantly different ($p < 0.05$) between cultivars.

na** : not applicable (absorbance was stable after increasing concentration and value was less than 0.5.

nd*** : not determined

Nonetheless, BHA was extremely strong scavenger against hydroxyl radicals with EC_{50} value of 5.28±0.03 μg/mL. It was found that EC_{50} value inversely correlated with total phenol (r = 0.91) and proanthocyanidins content (r = 0.95) with significantly difference ($p < 0.05$). Phenolic compounds in the extract were accounted for the hydroxyl radicals scavenging activity of TSCEs. Siddhuraju (2007) found that TSCE with highest tannin content had the highest scavenging activity on hydroxyl radical. Furthermore, Hagerman et al. (1998) reported that one of tannins, polygalloyl glucose, quickly reacted with hydroxyl radicals.

EC_{50} is inversely related to the antioxidant capacity of a compound, as it expresses the amount of antioxidant needed to decrease the radical concentration by 50%. The lower EC_{50} the sample has, the higher antioxidant activity the compound in the sample has. The EC_{50} value of TSCEs exhibited effective scavenging ability against DPPH radical. With EC_{50} value of 70.10±1.75 μg/mL, "Khanti" was the strongest scavenging activity against DPPH radical. Additionally, no significant difference ($p \geq 0.05$) on their EC_{50} values were found on both "Khanti" and vitamin C. There is no correlation between phenolic compound contents and EC_{50} values of each tamarind cultivar ($p \geq 0.05$). Yokozawa et al. (1998) reported that IC_{50} values of monomeric, dimeric, trimeric, tetrameric and polymeric tannins against DPPH radical decreased in order with the increase in molecular weight.

All four methods used for the evaluation of antioxidant activity revealed that the extract of "Sithong-bao" showed effective antioxidant activity. Furthermore, extract of "Priao-yak" and "Khanti" also showed marked reducing power and scavenging activity, respectively. These results were in agreement with higher content of polyphenolic compounds (tannins and proanthocyanidins) found in these tamarind cultivars. Polyphenols are known to exhibit stronger antioxidant activity than that of monophenols. In addition, tannins with the high polyphenolic and high molecular weight compound do not have pro-oxidant activity as observed with monomeric phenol compound as reported by Hagerman et al. (1998). Perchellet et al. (1996) also reported that some foliage tannins had potent antioxidant and anti-inflammatory activities. The presence of high tannins contents in the extracts of "Sithong-bao" and "Priao-yak" could explain the marked antioxidant activity and reducing power as observed in these two cultivars. Furthermore, Bagchi et al. (1997 and 1998) and Takahashi et al. (1998) reported that the proanthocyanidins, a group of

polyphenolic bioflavonoids, had beneficial effects in radical scavenging. The presence of higher proanthocyanidins content in seed coat of "Sithong-bao" and "Khanti" may explain the higher radical scavenging activity observed in these cultivars. Results from the present study suggest that tannins and proanthocyanidins were the main components contribute significantly to antioxidant activities of tamarind seed coat extracts. In addition, Moran et al. (1997) and Rice-Evans et al. (1996) also reported that phenolic compounds are chemically diverse and do not have the same antioxidant activity, some are powerful, others are weak, the molecular structure of phenolic contributes to antioxidant activity. Our results also support that chemical nature of specific phenolic constituents in the extract could be major responsible for different antioxidant activities observed in seed coat extracts of different tamarind cultivars.

4. Preparation of TSCE-PG gel for external used preparation

4.1 Formulation of TSCE-PG gel preparation

The 2.5% w/v polysaccharide gel (PG) from fruit-hull of durian was used as a gelling agent. This concentration was used because of its effective concentration in inhibiting bacterial growth and also compatible with other ingredients used in gel preparation (Lertchaiporn, 2003; Nantawanit, 2001). Propylene glycol is also used in cosmetics and in the food industry as humectant and carrier for emulsifier. It dissolves a wide variety of materials. In the formulation of gel preparation for skin the concentration of 15% propylene glycol was used as humectant, as well as 10% glycerin. The 0.25% amerchol L-101 was used as an emollient. The 10% of cremorphor RH-40 was selected for using in the preparation as solubilizing agent. TSCE of "Sithong-bao" was used as an active ingredient because it showed an excellent efficacy on anti-lipid peroxidation activity. The amount of the extract used was 70, 140 and 280 μg, respectively in one gram preparation according to its anti-lipid peroxidation efficacy (1-4 times EC_{50}). The satisfying gel preparations of the finished products were obtained. Figure 16 shows the preparations of TSCE-PG gel. Products description are summarized in Table 4.

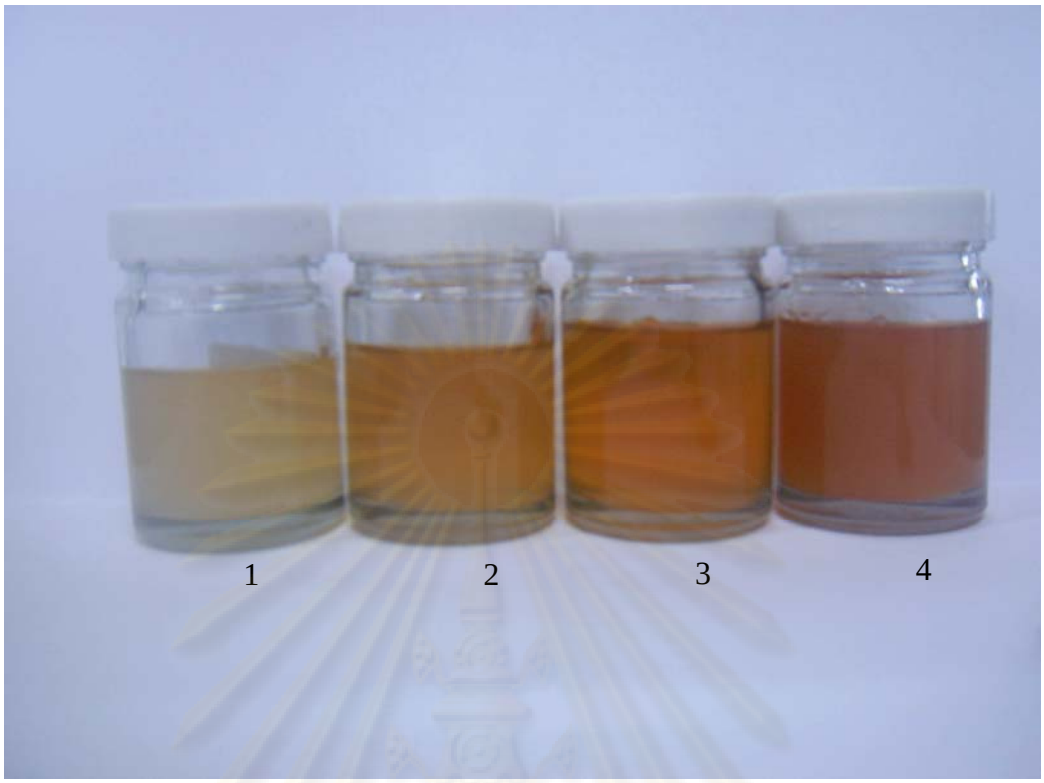


Figure 16. Preparations of TSCE in PG gel: 1 = PG gel without TSCE (Formulation 1), 2 = PG gel with TSCE 70 μg/g (Formulation 2), 3 = PG gel with TSCE 140 μg/g (Formulation 3) and 4 = PG gel with TSCE 280 μg/g (Formulation 4)

Figure 17. PG gel with TSCE 70 μg/g (Formulation 2): 1 = After 30 days at ambient temperature 2 = After 4 cycles of temperature cycling test

Figure 18. PG gel with TSCE 140 μg/g (Formulation 3): 1 = After 30 days at ambient temperature 2 = After 4 cycles of temperature cycling test

Figure 19. PG gel with TSCE 280 μg/g (Formulation 4): 1 = After 30 days at ambient temperature 2 = After 4 cycles of temperature cycling test

Table 4. Characteristics of PG gel and TSCE-PG gel preparations with various concentrations of TSCE

Formulation no. (TSCE concentration, μg/g)	Characteristics of gel preparations			
	Condition	freshly prepared	30 days at ambient temperature	After 4 cycles of heating-cooling
1 (0)	Appearance:	not clear gel	not clear gel	not clear gel
	Air bubble:	less	none	none
	Color:	light yellow	light yellow	light yellow
	Flow:	easy	not easy	not easy
2 (70)	Appearance:	clear gel	clear gel	clear gel
	Air bubble:	less	none	none
	Color:	light yellow-brown	light yellow-brown	light yellow-brown
	Flow:	easy	not flow	not flow
3 (140)	Appearance:	clear gel	clear gel	clear gel
	Air bubble:	less	none	none
	Color:	yellow-brown	yellow-brown	yellow-brown
	Flow:	easy	not flow	not flow
4 (280)	Appearance:	not clear gel	not clear gel	not clear gel
	Air bubble:	less	none	none
	Color:	light brown	light brown	light brown
	Flow:	easy	not flow	not flow

4.2 Assessment of stability of TSCE-PG gel preparation

Stability testing was studied to ensure that a developed product was suitable for use during its shelf life. Products were tested at ambient temperature and by heating-cooling cycles methods for their stability. The successful 4 cycles of heating-cooling cycles are usually indicated that the products will have an adequate shelf-life, the result showed that physical appearance of the gel was not changed. TSCE-PG gel preparations are shown in Figure 16-19. Satisfactory appearances of TSCE-PG gel preparations were obtained. The physical appearances of the preparations are described in Table 4. The color of finished products was varied from light-yellow brown to light brown, with different concentration of TSCE. The preparation of PG gel without TSCE shows light yellow of PG gel natural color. In TSCE-PG gel with 70, 140 and 280 μg/g of TSCE, the color was changed to light yellow-brown, yellow-brown and light brown, respectively, by the amounts of TSCE added in PG gel base. The amounts of TSCE were responsible to the color of preparations. However, the color of preparations did not change after stability testing. Although the air bubble occurred after the preparation was freshly prepared, it disappeared after standing for 24 hrs. During mixing the preparations, mixing of all ingredients until homogeneously mixed could affect the bubble formation. All formulations of TSCE-PG gel easily flowed after freshly prepared. It was observed that the flow of PG gel without TSCE after freshly prepared was better than after stability tests. For PG gels with TSCEs, the gel preparation did not flow after being kept for long time, however, it flowed after shaking. Gel appearance did not change after stability tests.

Figure 20 presents pH values of different gel formulation after freshly prepared of PG gel without TSCE, PG gel with TSCE 70 μg/g, 140 μg/g and 280 μg/g showed pH values of 4.32±0.01, 4.44±0, 4.45±0 and 4.70±0, respectively. The pH values of all preparations in the same condition did not significantly change ($p \geq 0.05$). All data were presented in Table 6 of appendix. The different amounts of TSCE did not affect pH value of preparation.

The viscosity values of different gel formulations after freshly prepared and after stability tests are shown in Figure 21. Viscosity of the preparations was scan at shear rate from 0-6000 1/s by Rheometer using C60/1 Ti as a sensor. The viscosity values of all preparations are presented in Table 7 of appendix. These values were

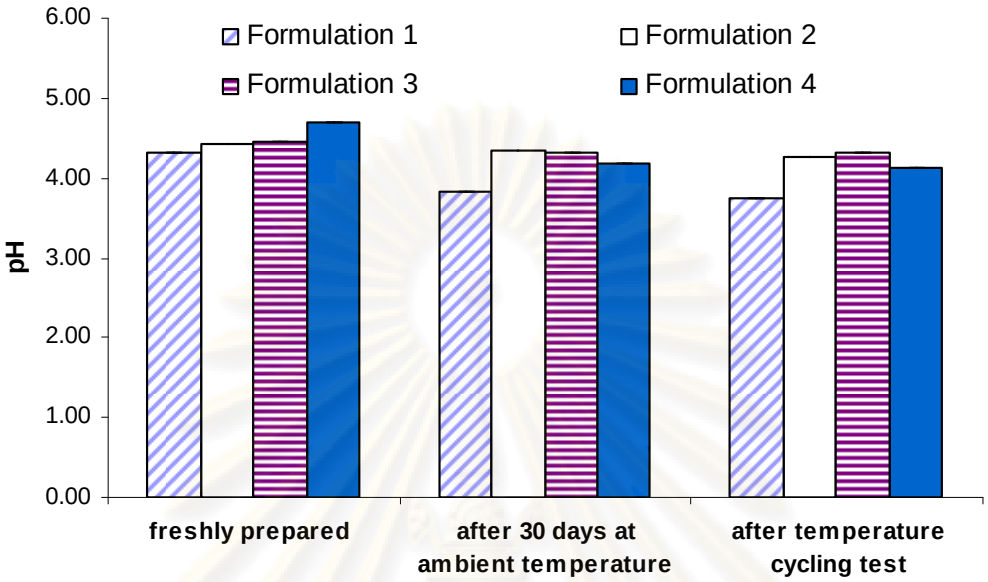


Figure 20. The pH values of the PG gel and TSCE-PG gel preparations after freshly prepared and stability tests. Each value is expressed as mean ± SEM (n = 3). Formulation 1 = PG gel without TSCE, Formulation 2 = PG gel with TSCE 70 μg/g, Formulation 3 = PG gel with TSCE 140 μg/g and Formulation 4 = PG gel with TSCE 280 μg/g

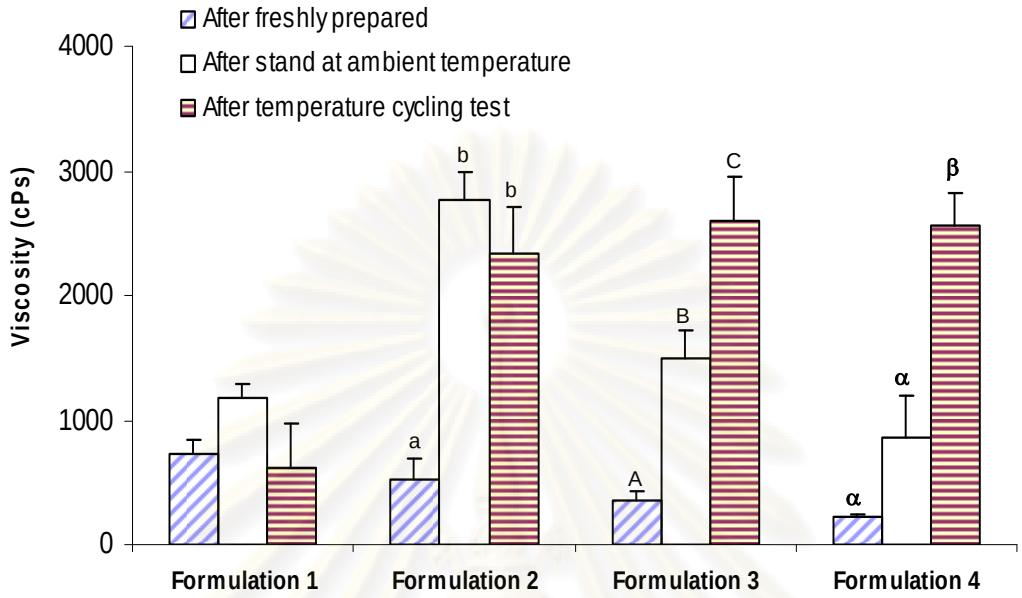


Figure 21. The viscosity of PG gel and TSCE-PG gel preparation, after freshly prepared and stability tests. Each value is expressed as mean ± SEM (n = 3). The different letters within a group of column indicate significant different results ($p < 0.05$). Formulation 1 = PG gel without TSCE, Formulation 2 = PG gel with TSCE 70 μg/g, Formulation 3 = PG gel with TSCE 140 μg/g and Formulation 4 = PG gel with TSCE 280 μg/g

calculated from this equation:

$$\text{Viscosity (cPs)} = \frac{\text{Shears stress (Pa)} \times 1000}{\text{Shear rate}}$$

It was found that the viscosity values of PG gel without TSCE (Formulation 1) between after freshly prepared and after stability tests in Figure 21 showed no significant difference ($p \geq 0.05$). In the PG gel with TSCE (Formulation 2-3), the viscosities after stability tests preparation were higher than that of after freshly prepared ($p < 0.05$). This property of gel viscosity seem to be a normal property of gel with pseudoplastic flow behavior. The viscosity of TSCE-PG gel (Formulation 4) showed significantly lower viscosity than that of PG-gel without TSCE (Formulation 1) after freshly prepared ($p < 0.05$), however, their viscosities were higher after stability tests.

Rheology study of the gel products is shown in figure 22-25, the flow curve of all preparations showed the shear-thinning (pseudoplastic) behavior. The TSCE did not affect shear-thinning behavior of PG gel.

4.3 The efficacy of TSCE-PG gel preparation on inhibition of lipid peroxidation

The inhibition of lipid peroxidation of TSCE-PG gel preparations were tested by using lipids in egg yolk as substrate. The pink chromogen of TBARs production was detected by spectrophotometric technique. The percentage inhibition of lipid peroxidation of the TSCE-PG gel preparations after stability tests are shown in Table 4.

The result of anti-lipid peroxidation activity of the different TSCE-PG gel preparations using the same condition stability test in Table 5 indicated that after keeping at ambient temperature for 30 days, the increased of lipid peroxidation inhibition were related to the increased of TSCE concentrations. The percentage lipid peroxidation inhibition of PG gel 1 g with 280 μg TSCE (Formulation 4) was significantly higher than that of PG gel with 70 μg TSCE ($p < 0.05$). The percentage lipid peroxidation inhibition of the products after temperature cycling test seemed to increase with the increased concentrations of TSCE although those values were not significant difference. The result suggested that high temperature (45 °C) used in temperature cycling test reduce the anti-lipid peroxidation activity of TSCE. The anti-lipid peroxidation activity of the same preparation with different stability tests

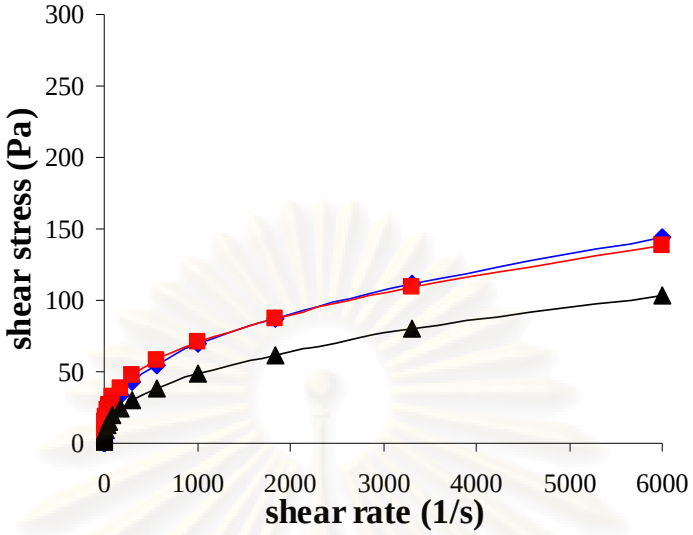


Figure 22. Flow curve of the PG gel without TSCE (Formulation 1). ♦: After freshly prepared, ■ : After 30 days stand at ambient temperature., ▲ : After temperature cycling test.

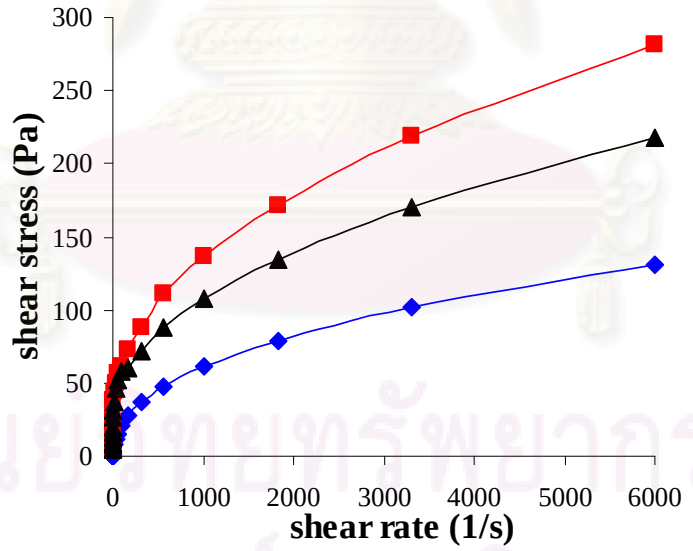


Figure 23. Flow curve of the PG gel with TSCE 70 µg/g (Formulation 2)♦: After freshly prepared, ■ : After 30 days stand at ambient temperature, ▲ : After temperature cycling test

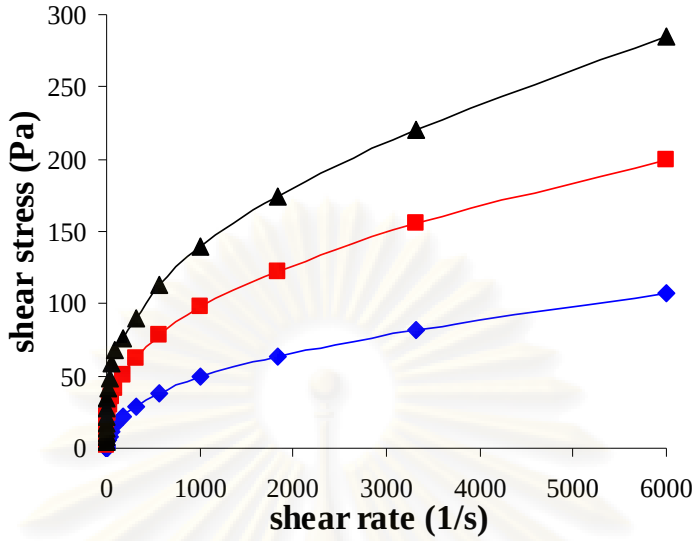


Figure 24. Flow curve of the PG gel with TSCE 140 µg/g (Formulation 3). ♦: After freshly prepared, ■ : After 30 days stand at ambient temperature., ▲ : After temperature cycling test

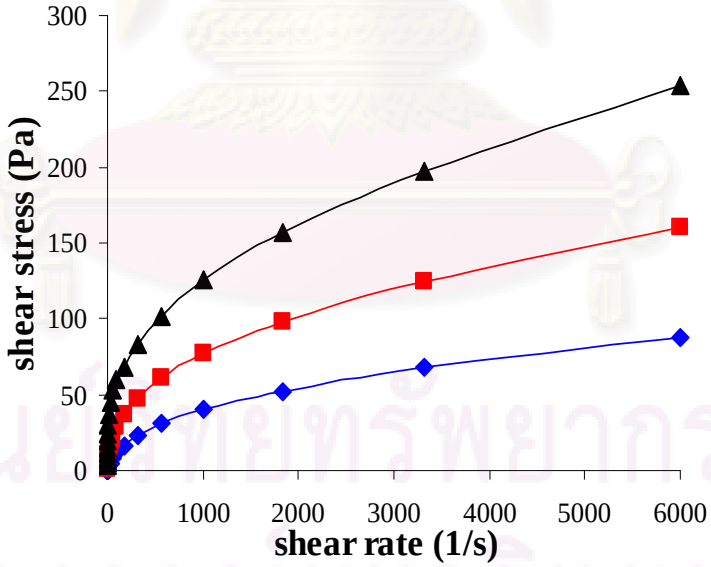


Figure 25. Flow curve of the PG gel with TSCE 280 µg/g (Formulation 4). ♦: After freshly prepared, ■ : After 30 days stand at ambient temperature, ▲ : After temperature cycling test

Table 5. Lipid peroxidation inhibition (%) of TSCE-PG gel products

Formulation no. (μg TSCE in 1g PG gel)	Inhibited lipid peroxidation (%) (mean±SEM)	
	After 30 days stand at ambient temperature	After heating-cooling cycle for 4 cycles
1 (0)	0	0
2 (70)	2.72±1.36[a]	2.67±2.67
3 (140)	15.01±9.22[a,b]	7.21±3.29
4 (280)	41.01±8.35[b]	13.31±6.67

Each value is expressed as mean ± SEM (n=3).

The different letters are significantly different ($p < 0.05$).

was examined. The accelerated stability test by heating-cooling cycle for 4 cycles showed that long exposure to high temperature at 45 °C of TSCE-PG gel product resulted in 2-3 times decreased anti-lipid peroxidation of the product, where keeping this product at ambient temperature (28±5)°C seemed not to affect its activity. However, anti-lipid peroxidation activity of the products after a certain time of storage should be further study.

5. Preparation of TSCE-TSP gel for external used product

5.1 Isolation and physical properties of TSP isolated from tamarind seed

The total yield of TSP powder isolated from tamarind kernels of tamarind seed type sour “Priao” was 59.73±0.75%. TSP powder was grayish white. Effect of TSP concentration on viscosity of TSP gel is shown in Table 6. Increased concentration of TSP resulted in increased viscosity. TSP has been widely used as thickening agent in pharmaceutical preparations.

Table 6. Viscosity (cPs) of TSP gel

TSP (%)	**Viscosity (cPs) (mean ± SEM)**
1	66.3 ± 19.5
2	617.5 ± 196.7
3	3403.3 ± 983.3
4	> 10,000

Each value is expressed as mean ± SEM (n=3).

5.2 Effect of ingredients on viscosity of TSP

The concentration of 0-2.5% of each ingredient including propylene glycol, glycerin, chremophor and sorbitol were individually added in 2%TSP. Figure 26 showed that viscosity of TSP gel slightly increased with the increased concentration of propylene glycol as well as glycerin. Sorbitol did not change the

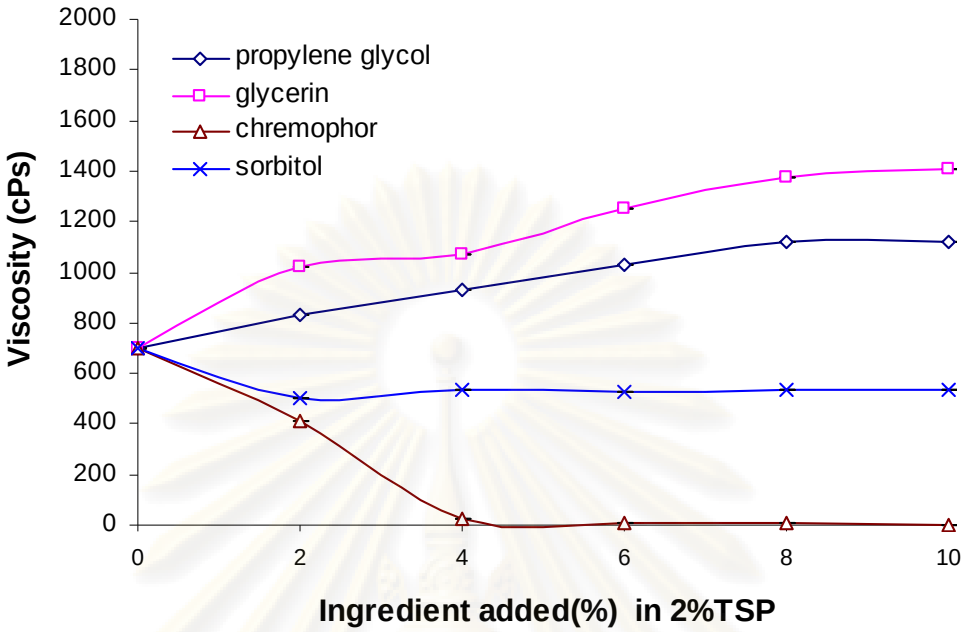


Figure 26. Effect of each ingredient on viscosity of TSP. Each value is expressed as mean ± SEM (n = 3).

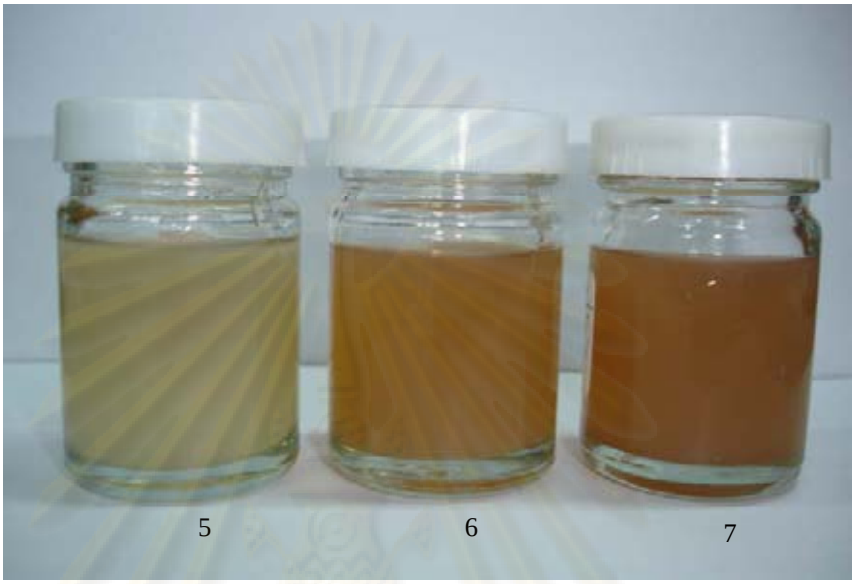


Figure 27. Preparations of TSCE in TSP gel: 5 = TSP gel without TSCE, 6 = TSP gel with TSCE 140 μg/g of after 30 days at ambient temperature, 7 = TSP gel with TSCE 140 μg/g after temperature cycling test

Table 7. Physical appearances and properties of TSP base and TSCE-TSP gel preparations

Formulation no. (TSCE concentration, μg/g)	Description of gel preparations			
	Condition	freshly prepared	30 days at ambient temperature	After 4 cycles of heating-cooling cycle
TSP gel base (0)	Appearance:	not clear gel	non homogeneity	non homogeneity
	Air bubble:	none	none	none
	Color:	white	white	white
	Flow:	not easy	easy	easy
	pH (mean±SEM)	5.86±0	nd*	nd*
	Viscosity (mean±SEM)	1030.5±50.3	nd*	nd*
TSCE-TSP gel (140)	Appearance:	not clear gel	not clear gel	not clear gel
	Air bubble:	none	none	none
	Color:	light brown	light brown	light brown
	Flow:	easy	very easy	very easy
	pH (mean±SEM)	5.79±0.01	nd*	nd*
	Viscosity (mean±SEM)	805.2±117.3	na*	na*

Each value is expressed as mean ± SEM (n=3).

nd* : not determined (Product was not homogenous.)

na* : not applicable (Viscosity was very low.)

viscosity of TSP gel. However, chremophor obviously decreased the viscosity of TSP gel at concentration over 4 %.

5.3 Preparation of TSCE-TSP gel

TSP was used as a gel forming agent in the gel TSCE-TSP preparation. An appearance of TSCE-TSP gel products are shown in Figure 27 and description of TSCE-TSP gel are summarized in Table 7. Products with satisfactory appearance were obtained after freshly prepared.

5.3.1 Assessment of the stability of TSCE-TSP gel preparation

The TSP gel without TSCE showed a white natural of color of TSP gel. The color of TSCE-TSP products were light brown. The color was not change after keeping at ambient temperature for 30 days and after temperature cycling test. The freshly prepared products of TSP gel base and TSCE-TSP gel showed viscosity values (cPs) at 1030.5±50.3 and 805.2±117.3, respectively. Viscosity of TSCE-TSP products was not stable after stability testing. The freshly prepared products of TSP gel base and TSCE-TSP gel showed pH values at 5.86±0 and 5.79±0.01, respectively. However, pH values of product after stability tests were not determined because they were not homogeneous. Unsatisfactory appearances of TSCE-TSP gel products were obtained.

CHAPTER V

CONCLUSION

Antioxidant extracted from seed coat of tamarind is a natural phenolic compounds that composes of mainly polymeric tannins and oligomeric procyanidins (Pumthong, 1999). TSCEs of 5 tamarind cultivars including "Srichomphu", "Sithong-nak", "Sithong-bao", "Khanti" and "Priao-Yak" from Pethabun province, Thailand were studied. The total phenolic content was assay by using Folin-Ciocalteu method. TSCEs in sweet tamarind "Khanti" and "Sithong-bao" showed the highest total phenol contents. Proanthocyanidins contents were not dominant in all of 5 tamarinds.

TSCEs were evaluated for their antioxidant activity by using different assays including reducing power, inhibition of lipid peroxidation, hydroxyl radical scavenging activity, DPPH radical scavenging activity. All of TSCEs showed antioxidant potential.

"Priao-yak" and "Sithong-bao" showed stronger reducing powers than that of positive control, vitamin C and BHA. "Priao-yak" and "Sithong-bao" were dose-dependent characteristic with reducing power assay. Furthermore, the extract from "Priao-yak" exhibited EC_{50} value higher than that of "Sithong-bao".

The lipid peroxidation inhibition of TSCEs was studied by using lipids in egg yolk as substrate, the decreased in pink chromophore of MDA in the reaction mixture represented the increased lipid peroxidation inhibition of TSCEs. All TSCEs of tested cultivars exhibited anti-lipid peroxidation activity with the increased concentration of TSCEs. The extract from "Sithong-bao" showed markedly anti-lipid peroxidation particularly at the concentration 100-500 μg/mL, where the extract from "Priao-yak" showed the lowest lipid peroxidation inhibition. Furthermore, "Sithong-bao" showed higher anti-lipid peroxidation activity than that of positive control antioxidant, vitamin C. The EC_{50} value of the extract from "Sithong-bao" was low as 66.19±7.13 μg/mL compared to the value of 263.93±8.03 μg/mL of vitamin C (positive control).

Hydroxyl radical scavenging activity of TSCEs was determined. TSCEs showed scavenging activity potential against hydroxyl radical with dose dependence manner. However, BHA (positive control) was more effective antioxidant than those

of TSCEs with the lowest concentration at 10 μg/mL. At the highest concentration of TSCEs tested, “Sithong-bao” showed the strongest scavenging activity with the low EC_{50} value of 62.23±9.36 μg/mL. But, BHA (positive control) showed extremely strong scavenging agent against hydroxyl radicals with the lowest EC_{50} value (5.28±0.03 μg/mL). Therefore, TSCEs with the high molecular weight polyphenolic compounds, tannins, exhibited hydroxyl radicals scavenging ability.

A commercial DPPH radical was used to evaluate the free scavenging activity of TSCEs. The extract from “Khanti” showed good DPPH radical scavenger with concentration dependence manner at the concentration range of 10-200 μg/mL. However, the scavenging activities of all TSCEs from different tamarind cultivars remained constant at the concentration over 200 μg/mL. Additionally, EC_{50} value of the extract from “Khanti” and vitamin C (positive control) showed no significantly different. So, polyphenolic compounds in TSCEs seemed to be a good scavenger against DPPH radicals.

TSCE-PG gel preparation showed satisfactory appearance after freshly prepared and also after stability tested. The color of 70, 140 and 280 μg/g of TSCE-PG gel preparations with TSCE from “Sithong-bao” was vary from light yellow-brown, yellow-brown and light brown, respectively. The different amounts of TSCE did not significantly change the pH value of preparations. However, stability tests did not affect the color and pH value of preparations. Shear-thinning behavior of PG gel was not affected by TSCE. The viscosity of TSCE-PG gel products of different formulation showed different viscosity values. Nonetheless, viscosity of PG gel without TSCE after stability tests did not significantly change. Anti-lipid peroxidation activity of the gel preparation at ambient temperature was increased with the increased concentration of TSCE. Temperature cycling test affected lipid peroxidation inhibitory activity of TSCE in PG gel.

TSP powder isolated from tamarind seed kernel type sour “Priao” was grayish white color. Its viscosity increased with increased concentration of TSP. Effect of ingredients in gel preparation including propylene glycol, glycerin, chremophore and sorbitol on viscosity of TSP showed different change. Viscosity of TSP increased with increased the amounts of propylene glycol and glycerin. On the contrary, viscosity of TSP decreased with the increased amounts of chremophore. However, sorbitol did not affect viscosity of TSP. The natural color of TSP gel was white. The

TSCE-TSP gel preparations were light brown. Unsatisfactory product was obtained after stability test, viscosity of TSCE-TSP product was not stable. The formulation used in this study was not suitable for TSCE-TSP gel preparation.

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APPENDIX

Table 1. The content of dry matter from 5 tamarind seed cultivars

Tamarind seed	Dry matter (g/100 g) from		
	Kernel	Seed coat	Seed coat powder
Srichomphu	69.87	27.83	27.72
Sithong-nak	68.57	30.13	29.71
Sithong-bao	71.92	16.95	16.42
Priao-yak	69.14	26.12	25.56
Khanti	66.13	28.76	28.95

Table 2. Reducing power of TSCEs at absorbance 700 nm in the concentration of 0-160 μg/mL

Concentration (μg/mL)	Absorbance(mean±SEM)						
	Srichomphu	Sithong-nak	Sithong-bao	Priao-yak	Khanti	Vitamin C	BHA
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
20	0.069±0.003	0.053±0.003	0.072±0.002	0.078±0.001	0.058±0.002	0.073±0.001	0.070±0.001
40	0.104±0.002	0.074±0.002	0.111±0.001	0.144±0.002	0.087±0.003	0.109±0.002	0.107±0.003
60	0.117±0.001	0.092±0.001	0.159±0.002	0.213±0.002	0.099±0.001	0.158±0.003	0.145±0.002
80	0.119±0.002	0.095±0.000	0.226±0.002	0.281±0.002	0.104±0.002	0.201±0.002	0.178±0.001
100	0.122±0.002	0.099±0.001	0.294±0.002	0.355±0.002	0.109±0.002	0.227±0.002	0.211±0.003
120	0.124±0.001	0.103±0.001	0.365±0.001	0.421±0.002	0.114±0.001	0.268±0.002	0.246±0.001
140	0.127±0.000	0.105±0.001	0.439±0.002	0.492±0.002	0.116±0.001	0.310±0.001	0.283±0.002
160	0.128±0.001	0.107±0.001	0.507±0.002	0.564±0.002	0.118±0.000	0.345±0.002	0.316±0.001

Each value is expressed as mean ± SEM (n=3).

Table 3. Inhibition (%) on lipid peroxidation of TSCEs at absorbance 532 nm in the concentration of 0-500 μg/mL

Concentration (μg/mL)	% Inhibition on lipid peroxidation of TSCEs (mean±SEM)					
	Srichomphu	Sithong-nak	Sithong-bao	Priao-yak	Khanti	Vitamin C
0	0.00±10.26	0.00±10.26	0.00±10.26	0.00±10.26	0.00±10.26	0.00±10.26
10	32.78±3.31	13.25±9.46	37.42±4.97	3.31±3.69	3.97±1.32	18.54±2.63
20	33.77±1.66	14.57±2.63	39.74±1.19	3.97±4.15	6.29±6.13	19.21±3.76
40	38.74±1.32	14.90±0.33	37.75±1.44	4.97±1.44	13.25±4.64	25.50±3.82
60	40.40±1.72	17.22±1.75	45.36±8.33	8.61±3.97	21.52±2.07	25.50±4.23
80	42.38±4.14	21.52±0.99	56.62±1.66	11.92±7.83	21.19±2.59	22.85±2.94
100	42.05±1.75	25.17±2.32	69.54±7.79	14.90±0.66	27.48±2.07	25.83±5.47
200	52.65±2.89	51.32±3.03	72.85±1.32	32.12±4.30	43.71±2.39	27.15±7.12
300	67.22±1.99	53.31±2.07	82.45±4.64	49.01±1.32	63.58±4.30	40.40±5.65
400	74.50±0.66	66.56±2.17	96.69±0.33	57.62±6.45	72.52±1.19	82.78±1.19
500	78.15±2.98	67.22±0.99	98.34±1.44	62.91±1.32	81.13±0.99	87.75±2.17

Each value is expressed as mean ± SEM (n=3).

Table 4. Scavenging activity (%) on hydroxyl radicals of TSCEs at absorbance 532 nm in the concentration of 0-500 μg/mL

Concentration (μg/mL)	% Scavenging activity (mean±SEM)					
	Srichomphu	Sithong-nak	Sithong-bao	Priao-yak	Khanti	BHA
0	0.00±1.41	0.00±1.41	0.00±1.41	0.00±1.41	0.00±1.41	0.00±1.41
10	2.67±2.67	11.76±2.45	22.46±3.51	2.67±1.41	18.18±0.93	94.65±0.53
20	6.42±0.53	12.83±2.98	30.48±0.53	17.65±7.19	18.18±4.24	93.05±0.53
40	17.11±7.07	25.67±5.10	41.18±5.10	18.72±2.67	29.41±2.45	94.65±0.53
60	29.95±1.93	28.88±2.83	46.52±5.43	36.90±4.57	31.55±5.88	96.26±1.60
80	37.97±3.51	37.43±0.93	52.41±5.66	38.50±2.83	43.32±6.83	94.12±0.53
100	39.57±2.14	43.85±1.85	52.94±3.86	40.11±3.25	52.94±2.83	95.19±1.93
200	45.45±7.23	51.87±0.93	58.82±5.66	52.41±2.83	56.68±0.93	94.12±0.53
300	56.15±3.51	60.43±1.41	67.38±1.07	65.24±1.07	63.64±1.41	94.65±1.93
400	58.82±0.53	65.78±0.53	73.80±3.25	72.19±1.93	67.91±2.45	93.58±0.93
500	60.96±0.53	68.45±0.53	77.54±4.81	76.47±1.41	73.26±1.93	94.65±1.41

Each value is expressed as mean ± standard error (n=3).

Table 5. Scavenging activity (%) on DPPH• of TSCEs at absorbance 515 nm in the concentration of 0-500 μg/mL

Concentration (μg/mL)	% Scavenging activity (Mean±SEM)					
	Srichomphu	Sithong-nak	Sithong-bao	Priao-yak	Khanti	Vitamin C
0	0.00±1.73	0.00±1.73	0.00±1.73	0.00±1.73	0.00±1.73	0.00±1.73
10	18.99±3.92	10.36±1.51	3.45±3.33	14.16±1.25	16.57±0.35	6.56±0.60
20	15.54±1.83	12.09±0.91	6.56±2.16	16.23±0.69	18.65±2.10	12.78±0.60
40	19.34±3.29	15.19±1.83	8.98±3.89	23.48±2.26	30.73±1.83	18.65±0.69
60	16.92±6.58	18.65±0.35	11.05±0.91	35.22±0.91	41.44±0.69	24.86±0.91
80	19.68±1.51	22.79±1.25	24.17±0.00	40.06±2.10	54.21±3.16	30.73±0.91
100	26.24±0.60	39.02±8.40	25.21±1.79	40.40±0.91	63.88±3.98	41.44±3.29
200	33.84±2.49	42.82±8.81	46.62±7.53	48.34±2.49	79.42±0.35	61.46±5.21
300	45.93±4.75	64.92±9.50	58.01±5.23	54.90±0.69	79.42±0.35	78.38±0.91
400	66.64±4.52	71.13±8.47	69.06±2.10	64.57±1.58	78.73±0.35	80.80±1.83
500	70.10±1.92	77.69±0.35	81.84±2.95	71.48±3.45	77.35±1.83	82.18±2.61

Each value is expressed as mean ± SEM (n=3).

Table 6. pH values of TSCE-PG gel preparation

Formulation no. (TSCE concentration, μg/g)	pH (Mean±SEM)		
	After freshly prepared	After 30 days stand at ambient temperature	After heating-cooling cycle for 4 cycles
1 (0)	4.32±0.01	3.83±0	3.75±0.01
2 (70)	4.44±0	4.34±0	4.26±0
3 (140)	4.45±0	4.32±0	4.32±0
4 (280)	4.70±0	4.17±0	4.13±0

Each value is expressed as mean ± SEM (n=3).

Table 7. Viscosity values of TSCE-PG gel preparation

Formulation no. (TSCE concentration, μg/g)	Viscosity, cPs (mean±SEM)		
	After freshly prepared	After 30 days stand at ambient temperature	After heating-cooling cycle for 4 cycles
1 (0)	720.3±115.1	1183.9±103.9	609.3±144.5
2 (70)	515.7±168.6	2760.2±227.4	2337.7±375.6
3 (140)	362.1±68.6	1503.3±222.8	2605.0±356.6
4 (280)	216.3±23.9	865.0±337.8	2554.8±268.0

Each value is expressed as mean ± SEM (n=3).

Table 8. Viscosity values of ingredient added in 2% TSP gel

Ingredient added (%)	Viscosity, cPs (mean±SEM)			
	Propylene glycol	Glycerine	Chremophore	Sorbital
0	700.0±0.2	700.0±0.2	700.0±0.2	700.0±0.2
2	829.0±0.0	1023.3±0.7	409.2±0.2	503.4±0.0
4	927.7±0.1	1072.7±0.7	25.5±0.0	538.8±0.0
6	1028.3±0.7	1252.0±0.0	6.9±0.0	530.4±0.0
8	1118.3±0.7	1371.0±0.0	5.7±0.0	534.6±0.0
10	1122.7±0.3	1407.7±0.3	3.8±0.0	535.2±0.0

Each value is expressed as mean ± SEM (n=3).

VITA

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