Cinnamomum porrectum Herbal Tea Production and Its Functional Properties Influenced by Odor Types of Leaves and Blanching Process

Phornthip Saetan

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Food Science and Technology

Prince of Songkla University

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ชื่อวิทยานิพนธ์ การผลิตชาสมุนไพรจากใบเทพทาโร และสมบัติเชิงหน้าที่จากผลของชนิดกลิ่นใบและการลวก

ผู้เขียน นางสาวพรทิพย์ แซ่ตัน

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บทคัดย่อ

เทพทาโร เป็นสมุนไพรและเป็นไม้หอมที่มีการกระจายพันธุ์อยู่ในภาคใต้ของประเทศไทย ในปัจจุบันพบว่ามีปริมาณการลดลงอย่างรวดเร็วเนื่องจากปัญหาการตัดไม้ เพื่อต้องการนำเนื้อไม้และรากไปสกัดเอาน้ำมันหอมระเหยและนำไปทำงานแกะสลัก ทำให้วนอุทยานศูนย์ถ่ายทอดเทคโนโลยีงานวิจัยป่าไม้ ภาคใต้ จังหวัดสงขลา มีแนวคิดที่จะหาแนวทางการใช้ประโยชน์จากใบเทพทาโรเพื่อการส่งเสริมการปลูก ใบเทพทาโรเป็นส่วนที่นำมาใช้น้อยที่สุดเนื่องจากให้น้ำมันหอมระเหยน้อย อย่างไรก็ตาม ใบมีความน่าสนใจเนื่องจากกลิ่นในใบมีความแตกต่างกัน สามารถแบ่งออกได้เป็น 4 กลิ่น คือ กลิ่นจวง กลิ่นเสม็ด กลิ่นตะไคร้ส้ม และกลิ่นดอกไม้ผสมเครื่องเทศ จากการสำรวจและสอบถามจากวิสาหกิจชุมชนพบว่า มีการนำใบเทพทาโรมาผลิตเป็นชาสมุนไพรเพื่อจำหน่ายในชุมชน แต่ยังขาดข้อมูลด้านวิธีการผลิต สมบัติทางชีวภาพรวมทั้งข้อมูลความปลอดภัยของชาเทพทาโร ดังนั้นการศึกษาในครั้งนี้ จึงศึกษากระบวนการผลิตชาเทพทาโรเพื่อหาสภาวะที่เหมาะสมในการผลิตโดยเริ่มศึกษาใบเทพทาโรกลิ่นจวงซึ่งมีปริมาณมากที่สุด จากนั้นจึงนำสภาวะที่ได้ไปประยุกต์ใช้กับใบทั้ง 4 กลิ่น โดยศึกษาองค์ประกอบทางกายภาพและเคมี สารประกอบฟีนอลิก ฤทธิ์การต้านอนุมูลอิสระ การต้านการอักเสบ ความเป็นพิษต่อเซลล์ไต (HEK293) และคุณสมบัติการต้านเซลล์มะเร็งลำไส้ใหญ่ (HT-29 และ Caco-2) ของน้ำชาจากใบเทพทาโร

จากการศึกษาผลของการทำแห้งใบเทพทาโรกลิ่นจวง เพื่อผลิตเป็นชาในสภาวะการตากลมที่อุณหภูมิ 28-30ºC การอบแห้งที่อุณหภูมิ 50 60 70 และ 100 ºC เปรียบเทียบกับการทำแห้งแช่เยือกแข็งแบบระเหิด และระยะเวลาที่ใช้ในการสกัดน้ำชาที่ 5 10 20 และ 30 นาที พบว่าการอบแห้งที่ 60 ºC ให้ปริมาณสารประกอบฟีนอลิก (TPC) และฟลาโวนอยด์ (TFC) สูงสุดเมื่อเทียบกับอุณหภูมิ 70 ºC 50 ºC และ 100 ºC ตามลำดับ จึงเลือกใช้อุณหภูมินี้ในการศึกษาผลของเวลาในการสกัดน้ำชา พบว่าการสกัดน้ำชาโดยการแช่ใบชาเทพทาโรที่เวลามากกว่า 10 นาทีไม่มีผลต่อการเพิ่มขึ้นของ TPC และ TFC (p>0.05) จึงได้มีการศึกษาการเตรียมใบก่อนอบแห้งโดยการลวกและนึ่ง เนื่องจาก (1) การอบแห้งทั้งใบใช้เวลานานทำให้สมบัติการต้านอนุมูลอิสระลดลง (2) แกนของใบ

ทำให้น้ำชาหนืดคล้ายยาง (3) การตัดแกนและลดขนาดใบกลับส่งเสริมการทำงานของเอนไซม์ พบว่าการลวกใบชามีผลทำให้เอนไซม์เปอร์ออกซิเดสและพอลีฟีนอลออกซิเดสลดลงมากกว่าการนึ่งใบชาเมื่อเปรียบเทียบที่เวลา 60 วินาทีเท่ากันและการอบแห้งเพียงอย่างเดียว พบว่าการลวกมีผลทำให้น้ำชามีปริมาณ TPC TFC และ สมบัติการต้านอนุมูลอิสระเพิ่มขึ้นอย่างมีนัยสำคัญ (p<0.05) เมื่อวัดด้วยเทคนิค DPPH และ ABTS นอกจากนี้การลวกยังเพิ่มความสามารถในการสกัด ลดการสูญเสียของคลอโรฟิลด์ ช่วยคงความเป็นสีเขียว (-a*) ในน้ำชาและลดปริมาณของซาฟรอลได้ถึงร้อยละ 89 ในใบกลิ่นจวง

การลวกเป็นเวลา 60 วินาที ถูกนำไปใช้ในใบทั้ง 4 กลิ่นเพื่อเปรียบเทียบกับตัวอย่างที่ไม่ผ่านการลวก พบว่ากลิ่นที่แตกต่างกันมีองค์ประกอบทางเคมีที่เหมือนกัน หากแต่มีปริมาณแตกต่างกัน สารหลักในกลุ่มฟีนอลิกที่พบมี 8 ชนิด คือ ไพโรกาลลอล แกลลิคแอซิด โปรโตคาทิชูอิคแอซิด แคเทชิน คาเฟอิคแอซิด ไซรินจิคแอซิด พีคูมาริคแอซิดและรูทิน ใบเทพทาโรกลิ่นดอกไม้ผสมเครื่องเทศมีปริมาณ TPC สูงสุด จากการศึกษาฤทธิ์ต้านอนุมูลอิสระพบว่าใบชากลิ่นเสม็ดมีสมบัติการต้านอนุมูลอิสระ DPPH, ABTS และ FRAP สูงที่สุด แต่ใบชากลิ่นจวงและตะไคร้ส้มมีปริมาณ TPC TFC DPPH และ FRAP เพิ่มขึ้นในตัวอย่างที่ผ่านการลวกเมื่อเทียบกับตัวอย่างที่ไม่ผ่านการลวก

การศึกษาในเซลล์ไลน์พบว่าน้ำชาจากใบชาทั้ง 4 กลิ่นที่ผ่านและไม่ผ่านการลวกที่ความเข้มข้นน้อยกว่า 50 μg/ml ไม่มีความเป็นพิษต่อเซลล์ RAW264.7 (การรอดชีวิตของเซลล์มากกว่าร้อยละ 80) เมื่อศึกษาคุณสมบัติการยับยั้งการหลั่งไนตริกออกไซด์ (NO) พบว่า การลวกใบชาสามารถเพิ่มความสามารถในการยับยั้งการหลั่งไนตริกออกไซด์ได้ (p<0.05) นอกจากนี้ยังพบว่าใบชากลิ่นดอกไม้ผสมเครื่องเทศและกลิ่นตะไคร้ส้มมีความสามารถในการยับยั้ง NO ที่ดีกว่า แอลไนโตรอาจินีน (สารควบคุม) ซึ่งมีค่า IC_{50}= 30.21±1.48 μg/ml เมื่อศึกษาผลของตัวอย่างน้ำชาต่อเซลล์ไต (HEK293) ในตัวอย่างที่ไม่ลวกพบว่ากลิ่นตะไคร้ส้มมีความเป็นพิษต่อ HEK293 ต่ำที่สุด (CC_{50}= 922.76±50.11 μg/ml) เมื่อเปรียบเทียบกับตัวอย่างที่ลวก พบว่า การลวกลดความเป็นพิษต่อเซลล์ไตอย่างมีนัยสำคัญ ตัวอย่างที่ไม่ลวกกลิ่นเสม็ดมีความสามารถในการยับยั้งเซลล์มะเร็งลำไส้ใหญ่ HT-29 cells สูงสุด (CC_{50}= 438.19±30.36 μg/ml) และ พบว่ากลิ่นดอกไม้ผสมเครื่องเทศและกลิ่นตะไคร้ส้มมีความเป็นพิษต่อเซลล์ Caco-2 มากกว่ากลิ่นจวงและกลิ่นเสม็ด

ดังนั้นบทสรุปที่ได้จากการทดลองคือ ใบชาเทพทาโรกลิ่นจวงควรนำมาลวกก่อนทำชาเพื่อลดความเป็นพิษ ใบชากลิ่นเสม็ดมีฤทธิ์ต้านอนุมูลอิสระและสามารถยับยั้งเซลล์มะเร็ง HT-29 สูงสุด ใบชากลิ่นตะไคร้ส้มและกลิ่นดอกไม้ผสมเครื่องเทศมีความสามารถในการยับยั้งการหลั่ง NO และสามารถยับยั้งการเจริญของเซลล์มะเร็งลำไส้ใหญ่ทั้งสองชนิดได้

Thesis Title *Cinnamomum porrectum* herbal tea production and its functional properties influenced by odor types of leaves and blanching process

Author Miss Phornthip Saetan

Major Program **Food Science and Technology**

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ABSTRACT

Cinnamumum porrectum (Roxb.) Kosterm. with Thai name "Thep tharo", is an aromatic plant grown in Southern of Thailand. Nowadays, this plant is rapidly decrease because the wood and root were used to produce essential oils and handicraft products, so deforestation was occurred. From this problem, the Technology Research Centre of Forestry sector, Songkhla gave the preservative idea for increase the plantation. According to the lowest utilization of leaves since they provided a small amount of essential oils. However, the leaves are still interesting due to their aroma from the chemical constituencies in volatile oils. They were classified as root beer odor, cajuput odor, lemongrass with orange odor and flower with spice odor. The Technology Research Centre of Forestry Sector in Songkhla encouraged the local entrepreneurs to produce Theptharo leaves tea however, there are some problem such as lack of data about herbal tea production and scientific data especially the functional properties and the safety guarantee. So, the main point of this research was to study the process of Thep tharo herbal tea production starting with root beer odor and used the selected process for all 4 odors of Theptharo leaves. The nutritional composition, physiochemical properties, phenolic composition, total extractable phenolic content (TPC), total extractable flavonoid content (TFC), antioxidant activities, nitric oxide inhibition, the cytotoxicity on HEK293 and anti-colon cancer (HT-29 and Caco-2) of Theptharo tea extracts were analyzed.

The effect of drying temperature and steeping time on *C. porrectum* herbal tea were studied (temperature; air-drying at 28-30 ºC, hot air-drying at 50, 60, 70 and 100 ºC compared with freeze-drying and steeping time; 5, 10, 20 and 30 min). The result indicated that drying at 60 ºC provided the highest TPC and TFC when compared with 70 ºC, 50 ºC and 100 ºC, respectively. The steeping time more than 10 min not effect on TPC and TFC ($p>0.05$). So, this drying temperature was selected to

next study. The pre-treatment process was applied to the leaves due to (1) long drying time decreased antioxidant activities (2) the stalk of leaves gave the viscosity like gum in herbal tea infusion (3) cutting process induced enzyme activity. The result indicated that blanching can decrease the peroxidase (POD) and polyphenoloxidase (PPO) more than steaming when compared with the same time for 60 s. The TPC, TFC, DPPH and ABTS were significantly increased. Blanching not only increase the extractability but also decrease chlorophylls loss during drying process, keep the green color (-a*) and can eliminate safrole more than 89%.

Blanching for 60 s was selected and applied into 4 odors of leaves compared with un-blanched herbal tea. The results indicated that the phenolic composition of all samples provided the 8 main compounds including pyrogallol, gallic acid, protocatechuic acid, catechin, caffeic acid, syringic acid, *p*-coumaric acid and rutin. Various odor types of leaves provided the same types of phenolics but different in contents. The highest of TPC was found in flower with spice odor. The cajuput odor provided the highest of DPPH, ABTS and FRAP. The root beer and lemongrass with orange odor gave a higher content of TPC, TFC, DPPH and FRAP in blanched sample when compared with un-blanched sample.

In the cell culture studies, all herbal teas extract at concentration lower than 50 µg/ml had no toxicity on RAW264.7 cells (percentage of cell viability more than 80). The flower with spice odor (both blanched and un-blanched) and lemongrass with orange (blanched) provided a higher ability on NO inhibition more than positive control L-Nω-nitroarginine (L-NA) (IC_{50}=30.21±1.48 µg/ml). In un-blanched group, the lemongrass with orange odor showed the lowest toxicity (CC_{50}=922.76±50.11 µg/ml) on HEK293 cells. The cytotoxicity on HEK293 was decrease in blanched group. In un-blanched group, the cajuput odor provided the highest anti-colon cancer on HT-29 (CC_{50}=438.19±30.36 µg/ml) while the flower with spice odor and lemongrass with orange odor showed a higher cytotoxicity on Caco-2 cells.

The conclusion from this study suggested that the root beer odor should be blanched before making herbal tea. The cajuput odor provided the highest of antioxidant activities and gave the highest anti-colon cancer on HT-29 cells. The lemongrass with orange odor and flower with spice odor provided a high ability on NO inhibition, higher toxicity on both colon cancer cells.

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CHARTER 1

INTRODUCTION AND REVIEW OF LITERATURE

1.1. Introduction

Oxidative stress is the result of the chemical imbalance between antioxidants and pro-oxidants or reactive oxygen species (ROS). This imbalance leads to damage of important biomolecules and cells, with potential impact on the whole organism (Ambriz-Perez *et al.,* 2016). Over production of oxidants have led to significant damage both cell function and structure (Khandrika *et al*., 2009) particularly DNA, protein and lipid (Bergamini *et al.,* 2004; Afonso *et al.,* 2009). Epidemiological and experimental evidence has shown that oxidative stress is closely related to aging and chronic disease, such as inflammation and even cancer (Bergamini *et al.,* 2004).

Inflammation is recognized as a biological process in response to tissue injury (Gunawardena *et al.,* 2014). The inflammation response is a complex self-limiting process precisely regulated to prevent extensive damage to the host. When the self-limiting nature of this protective mechanism is inappropriately regulated, it results in chronic inflammation, which is associated with several chronic inflammatory diseases, including Alzheimer's disease and cancer (Liao *et al.*, 2012).

Cancer is the top second of death worldwide and was responsible for 8.8 million deaths in 2015 inferior to the cardiovascular disease. The worldwide cancer cases and the worldwide cancer deaths are rapidly increase by 50 and 60% from 2012 to 2030. In addition, colorectal cancer is the third most common cause of cancer next to lung and liver cancer (World Health Organization, 2017). In Thailand, colorectal cancer accounts for the most mortality in men (17.21%), while the third in women (8.78%) since 2014 (Information and Technology Division National Cancer Institute, Thailand, 2016). In the last few years, some studied have reported that the diet possesses an important role in the etiology of colorectal cancer especially phenolic compounds from fruit, vegetable, as well as spices and herbs (Lv *et al.,* 2012; Adedapo *et al.,* 2016; Rosa *et al.,* 2016).

In recent year, interest has increased in using natural products such as phenolic compounds present in plant, fruits, vegetables, spices and herbs for pharmacological purpose such as to exert their effect as an antioxidant and anti-inflammatory properties by either quenching the free radicals, increasing the antioxidant defense or by inhibiting the release of pro-inflammatory mediators due to antiproliferative properties (Parry *et al.,* 2011; Chan *et al.,* 2012; Lv *et al.,* 2012; Senawong *et al.,* 2014 and Rosa *et al.,* 2016). Herbal tea is more widely consumed because of health claim, natural and safe as herbs can promote health and assuage illness (Desideri *et al.,* 2011). Though some thermal process such as blanching, steaming and drying is considered as the most effective ways to inactivate or destroy both endogenous and exogenous enzymes leading to quality of products. However, during tea drying process both enzymatic and/ or non-enzymatic processes may still occur leading to significant changes in the phytochemical compositions.

Cinnamomum porrectum (Roxb.) Kosterm. with Thai name "Thep tharo" belonging to the *Cinnamomum* genus, Lauraceae family is an aromatic medicinal plant which mostly distributed in southern of Thailand and grown in Southeast Asia. These leaves, exhibit hot taste and emit a spicy odor when crushed, are used extensively in food and folk medicine in India, China, and Thailand for treating inflammation gastritis, blood circulation, liver and spleen disorders (Lee and Balick, 2005; Palanuvej *et al.,* 2006). Nowadays, this plant is rapidly decrease because the wood and root were used to produce essential oils and handicraft products, so deforestation was occurred. From this problem, the Technology Research Centre of Forestry sector, Songkhla gave the preservative idea to increase the plantation of this plant. According to the lowest utilization of leaves since they provided a small amount of essential oils. However, the leaves are still interesting due to their aroma from the chemical constituencies in volatile oils. The data from the forestry sector Songkhla reported that the *C. porrectum* tree from many locations in the Southern of Thailand were collected to plant in the demonstrate farm at Songkhla province. They reported that the major components in volatile oils from leaves were divided into 4 major odor groups; root beer, cajuput, lemongrass with orange, and flower with spice odors, respectively. From literature review, *C. porrectum* leaves were reported as a promising dietary source of antioxidants such as phenolic and flavonoid compounds (Cai *et al.*, 2004; Lv *et al.,* 2012; Prasad *et*

al., 2009). Although the *C. porrectum* tree with root beer odor is the largest population, it has high toxicity from the main chemical constituents especially safrole. An interesting in safrole because this compound was classified as a group 2 B carcinogen or possible human carcinogen by IARC and was banned in Thailand due to this compound can use to produce in MDMA manufacture. In fact, from marketing survey and personal contact directly with the forestry sector Songkhla, encourage the local entrepreneurs to produce *C. porrectum* leaves as herbal tea product by traditional method or air-drying process. However, there are some problem such as lack of data about herbal tea production and scientific data especially the functional properties and the safety guarantee. Therefore, this work aimed to investigate the effect of some thermal pre-treatments and various odor types of *C. porrectum* on the tea production. Total phenolic, flavonoid contents, antioxidant activities, phenolic profile, nitric oxide inhibition, cytotoxicity on kidney cells (HEK293) and anti-colon cancer (HT-29 and Caco-2) were also determined.

1.2. Review of literature

1.2.1. *Cinnamomum porrectum* (Roxb.) Kosterm.

Botanical Name: *Cinnamomum porrectum* (Roxb.) Kosterm

Family: Lauraceae

Local Thai names: Thep-tha-ro (Central; Chantaburi, Suratthani), Chuang, Chuang hom (Peninsular), Cha khai ton, Cha khai hom (Northern), Phlu ton khoa (Chiang Mai), Mue-dae-ka-ma-nging (Malay-Pattani), Karabun (Nong Khai) (Smitinand, 2001; Uthairatsamee, 2011).

Synonyms *Camphora chinensis* Nees, *Camphora inodora* Blume ex Miq. *Camphora inuncta* Nees, *Camphora parthenoxylon* (Jack) Nees, *Camphora porrecta* (Roxb.) Voigt, *Camphora pseudosassafras* Miq. *Cinnamomum inodorum* (Blume ex Miq.) Meisn, *Cinnamomum inunctum* (Nees) Meisn, *Cinnamomum malaccense* Meisn, *Cinnamomum neesianum* Meisn, *Cinnamomum parthenoxylon* (Jack) Meisn, *Cinnamomum penninervium* Kosterm, *Cinnamomum pseudosassafras* Meisn, *Laurus parthenoxylon* Jack, *Laurus porrecta* Roxb. *Laurus pruinosa* Reinw. ex Blume, *Litsea pruinosa* Nees, *Parthenoxylon porrectum* (Roxb.) Blume, *Phoebe latifolia* Champ. ex Benth, *Sassafras loureiroi* Kostel, *Sassafras parthenoxylon* (Jack) Nees, *Tetranthera camphoracea* Wall. ex Meisn.

1.2.2. Botanical description

In Thailand, *C. porrectum* is mostly distributed throughout the southern part (The forest Association of Thailand, 1984; Chayamarit, 1997; Forest Research and Development Bureau, 2009), but its exact distribution and abundance are not known with any certainty. This plant is an evergreen medium-sized to large tree 10- 45 m tall and up to 105 cm in diameter (Figure 1 (a)). Leaves are sub-opposite to spinal; blade ovate or ovate-oblong, 2.5-8 cm x 5-20 cm; apex blunt, acute or acuminate; base cuneate to rounded; margin entire: glabrous with 3- 8 pairs of lateral veins, main veins prominent above, tertiary venation reticulate, faint on both surfaces, with aromatic when crushed; petiole 1.2- 3.5 cm long (Figure 1 (b)). Flower glabrous or sparingly hairy is creamy white (Figure 1 (c)). Fruit is globose to slightly depressed globose, 0.8-1 cm across, seated on a funnel-shaped perianth cup with an entire margin, one-seeded drupe, and purple-black at maturity (Figure 1 (d)) (Lemmens *et al*., 1995; Chayamarit, 1997). It is widely distributed and locally common in lowland to mountain forest, sometimes in regions with a pronounced dry season, on both fertile and poor soils, usually in well-drained locations, up to 2,000- 3,000 m altitude (Lemmens *et al.*, 1995).

Figure 1 *Cinnamomum porrectum* stem (a), leaves (b), flowers (c) and fruits (d)
Source: Uthairatsamee (2011).

1.2.3. Chemical constituents in volatile oils of *C. porrectum*

Pattanaseree and Anantachoke (2012) studied the volatile oils in leaves, green fruits, ripe fruits and wood of *C. porrectum* form Southern Literature Botanical Garden in Songkhla province, Wat Nirot Rangsi and Tai Muang farm in Pang-Nga province, by water distillation. Chemical compositions of the volatile oils were analyzed by GC-MS with standard library. The results revealed that based on oven dry weight showed that volatile fruit oils yielded 3.50-10.54%, volatile wood oils were 3.58% and volatile leaves oils showed the lowest yield 0.43-0.72%. In addition, the major components in volatile oils from wood was safrole 97.71%.

The chemical constituents in leaves and fruits were divided into 4 groups. The leaves consisted of safrole as major component (90.92- 96.02%) gave root beer odor, volatile oils that had 1, 8-cineole as major component (57.66- 61.61%) gave cajuput odor, volatile oils that had Z-citral (8.43- 36.99%), E-citral (28.88-50.18%), citronellol (1.82- 17.28%) and limonene (0.12- 12.02%) as major component gave lemon grass with orange odor, and volatile oils that had linalool as major component (95.01%) gave flower with spice odor (Figure 2).

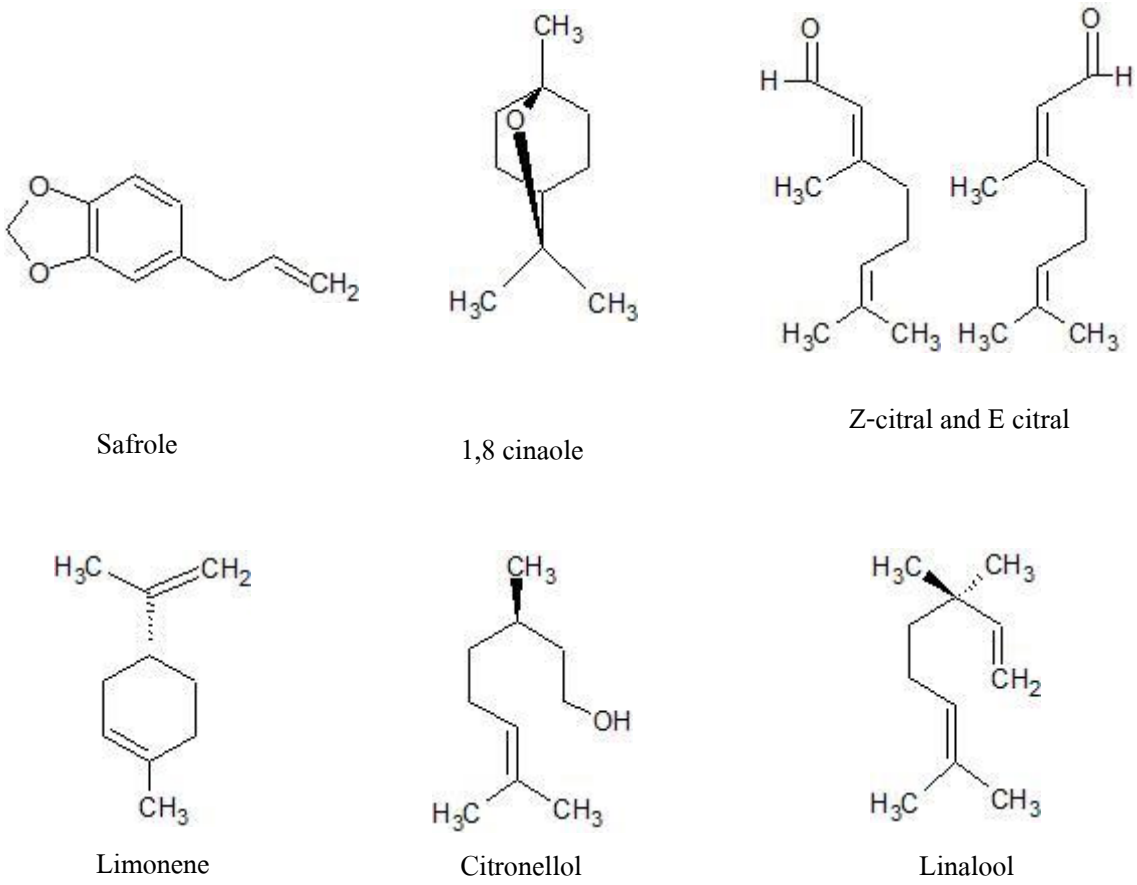


Figure 2 The main chemical constituents in *Cinnamomum porrectum* leaves

1.2.4. Phenolic and flavonoid compounds in *Cinnamomum* species

Cai *et al.* (2006) studied phenolic compounds of *C. cassia* when extracted by aqueous and methanolic extracts. The results showed that total phenolic content of both extracts was 1.87 and 2.83 g/100g DW, respectively. The major types of phenolic compounds of both extracts were phenolic acids (cinnamic acid, protocatechuic acid), coumarin and tannins. Prasad *et al.* (2009) reported that flavonoid compounds from leaves of five *Cinnamomum spp.*, namely *C. burmanni*, *C. cassia*, *C. pauciflorum*, *C. tamala* and *C. zeylanica* extracted by ethanol. Quercetin, quercetrin, and kaempferol were the main flavonoid compounds in all species. The contents of three flavonoids of *C. burmani* was 14.63, 18.76 and 0.04 µg/g, *C. cassia* as 3.39, 19.10 and 0.91 µg/g, *C. pauciflorum* was 5.87, 3.83 and 0.22 µg/g, *C. tamala* as 0.62, 1.07 and 0.03 µg/g and *C. zeylanicum* as 1.22, 2.67 and 0.05 µg/g, respectively.

1.2.5. Pharmacological activities and health benefits of *Cinnamomum* species

In Thailand, the utilization of *C. porrectum* has been reported as providing various benefits to local people (Chayamarit, 1997; Palanuvej *et al.*, 2006; Phongpaichit *et al.*, 2006; Denrungruang, 2007; Plansangkate *et al.*, 2007). In the southern of Thailand, young leaves and flowers are eaten as a vegetable side dish and are a common substitute for the spice. Moreover, dried leaves can be used to make a tea. For medicinal uses, leaves, bark, and wood are used as an ingredient of some Thai traditional medicines for anti-flatulent, treatment for the disorder of heart, liver and kidney. *C. porrectum* bark and leaves are commonly used as an ingredient in spices and their essential oils are used as flavoring agents in the food and beverages industry, a component of perfumes and soap and in many pharmaceutical preparations (Phongpaichit *et al.*, 2006). Seed oil is used as an analgesic. Stem latex has laxative property. In aromatherapy, the oils from fruits and seeds are applied to relieve the muscle pain. In Malaysia, the aromatic bark is used for flavoring food and considered an excellent tonic. The root is used medicinally against fever and applied after childbirth (Lemmens *et al.*, 1995).

1.2.5.1.Antioxidant activities

Previous studied on antioxidant activities of genus *Cinnamomum* were mainly focused on their essential oils (Tung *et al.,* 2008). Jayaprakasha *et al.* (2006) reported volatile oil of the fruit stalks of *C. zeylanicum* showed 55.94 and 66.0% antioxidant activity at 100 and 200 ppm by using in vitro model. Kitazurua (2004) studied on effects of irradiation on natural antioxidants of cinnamon and revealed that ether, ethanol, and aqueous extracts of irradiated *C. zeylanicum* possessed antioxidant activity that can be measured by the β-carotene/ linoleic acid system. Mathew and Abraham (2006a; 2006b) revealed that the antioxidant ability of the methanolic extract of *C. verum* leaves exhibited free radical scavenging activity against the DPPH radical with EC_{50} values of 4.21 and 22.4 µg/ ml, respectively and exhibited reducing power and metal ion chelating activity, along with hydroxyl radical scavenging activity. The relative antioxidant ability to scavenge the radical DPPH and $ABTS^{+\bullet}$ have been compared to the standards Trolox, BHA, ascorbic acid and gallic acid and is an excellent tool for determining the antioxidant activity of hydrogen donating antioxidants and of chain-breaking antioxidants. The reducing power of this extract can act as electron donors and can react with free radicals to convert them to more stable products and thereby terminate radical chain reactions. The reducing power of *C. verum* extract might be due to the di- and mono- hydroxyl substitutions in the aromatic ring, which possess potent hydrogen donating abilities. The metal chelating activity of this extract can chelate iron, which forms σ-bonds with a metal are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Gordon, 1990). Denrungruang (2007) reported the methanolic extract from bark had a good antioxidant activity with an EC_{50} value of 4.03 µg/ ml. In contrast, the essential oil from the root of *C. porrectum* had no ability in antioxidant activity (Itharat *et al.,* 2007). Lin *et al*. (2007) reported that leaves essential oil of *C. osmophloeum* which possessed a high amount (> 50%) of cinnamyl acetate, cinnamaldehyde, β- cubebene and linalool showed a good antioxidant capacity. Chua *et al.,* (2008) reported that the ethanolic extract using liquid-liquid partition to yield n-hexane, ethyl acetate (EtOAc), n-butanol (BuOH) and water fractions of *C. osmophloeum* twigs. The BuOH fraction showed high total phenolic contents (496.7

mg of GAE/g) and good antioxidant activity in DPPH free radical assay, superoxide radical scavenging activity assay, reducing power assay. Prasad *et al.* (2009) reported that the ethanolic extract of *C. zeylanica* leaf exhibited the highest total phenolic content while *C. burmanni* had the highest of flavonoid content (compared with five species of *Cinnamomum* species namely *C. burmanni, C. cassia, C. pauciflorum, C. talama and C. zeylanicum*). *C. zeylanica* showed the highest DPPH radical scavenging activity and reducing power activity. The DPPH radical scavenging activities of all the extract of *Cinnamomum* leaves increased with increasing concentration (compared 50 and 100 µg/mL of methanolic extract of *C. verum* leaf). Pukdeekumjorn *et al.* (2012) reported that the *C. porrectum* wood extracted by boiling water, ethanolic extract by maceration in 95%, 50% ethanol and oil part by water distillation. The 95% ethanolic and oil showed higher antioxidant activity than BHT (EC_{50} values as 7.92±0.20, 13.18±3.95 and 16.91 µg/ ml, respectively). The data from this report indicated that the antioxidant activities of water extract related to the total phenolic content values (264.09±5.77 mg GAE/100 g).

1.2.5.2.Anti-inflammation properties

Lee *et al.* (2006) studied in vitro anti-inflammatory from *C. camphora* by different solvents, the hexane and EtOAc extracts also inhibited nitric oxide (NO) production in LPS/interferon (IFN)-γ-activated macrophages. The methanol (MeOH) extract, as well as two fractions prepared by solvent partition with BuOH and EtOAc, strongly suppressed the prostaglandin E_2 (PGE_2) production in LPS/IFN- γ -activated macrophages up to 70%. These data suggest that the anti-inflammatory actions of *C. camphora* may be due to the modulation of cytokine, NO and PGE_2 production and oxidative stress. Lin *et al.* (2008) studied that the fruit essential oil of *C. insularimontanum* by using water distillation on nitric oxide (NO) inhibitory activity assay, crude essential oil and its dominant compound (citral) presented the significant NO production inhibitory activity, IC_{50} of crude essential oil and citral were 18.68 and 13.18 µg/mL, respectively. Tung *et al.* (2008) studied that the effects of *C. osmophloeum* essential oil on nitric oxide (NO) and prostaglandin E_2 (PGE_2) production in lipopolysaccharide (LPS)-activated RAW264.7 macrophages were also examined. Results of nitric oxide tests indicated that twig essential oil and its major constituents such as trans-cinnamaldehyde, caryophyllene oxide, L-borneol, L-bornyl

acetate, eugenol, β-caryophyllene, E-nerolidol, and cinnamyl acetate have excellent activities. These findings demonstrated that essential oil of *C. osmophloeum* twigs have excellent anti-inflammatory activities and thus have great potential to be used as a source for natural health products. Peterson *et al*. (2009) reported that the water-soluble extract of *C. zeylanicum* can improve inflammation related intestinal dyslipidemia and inhibit an aggregation and filament formation, hallmarks of Alzheimer's disease. The ethanolic extract of *C. zeylanicum* showed suppression of intracellular release of TNF-α in murine neutrophils as well as leukocytes in pleural fluid. The extract was found to inhibit TNF-α gene expression in LPS-stimulated human blood mononuclear cells (PBMSs) at 20 μg/ ml concentration (Joshi *et al.,* 2010). Lin *et al.* (2012a) reported that the ethanolic extract of twigs from *C. osmophloeum* on inhibitory effect against the LPS-induced production of nitric oxide in RAW264.7 macrophages with an IC_{50} value of 41.2 μM. It also slightly reduced PGE_2 accumulation by 26% at the concentration of 50 μM. Pukdeekumjorn *et al.* (2012) reported that the *C. porrectum* wood extracted by water extract by boiling water, ethanolic extract by maceration in 95%, 50% ethanol and oil part by water distillation. The 50%, 95% ethanolic extract and oil showed high anti-inflammatory activity on RAW264.7 macrophage cells (% inhibition as 90.50, 85.86 and 76.87, respectively).

1.2.5.3. Anticancer properties

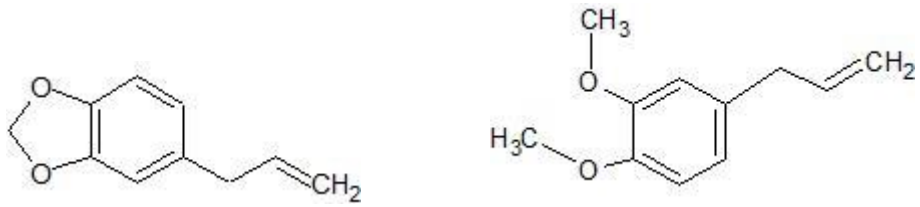
Plants may be an alternative to currently used anticancer agents, because they are a rich source of bioactive chemicals. Since many of them are largely free from adverse effects and have excellent pharmacological actions, they could lead to the development of new classes of possibly safer anticancer agents (Lee *et al.,* 2003). The genus of *Cinnamomum* has been studied on the pharmacological effect use on an antiproliferation and antitumor activity (Yeh *et al.,* 2009). Based on these reviews many species of *Cinnamomum* have potential to be further developed as an anticancer agent. In general, the anticancer properties of *Cinnamomum* species are related with their essential oils especially the cinnamaldehyde (Piantadosi *et al.,* 1964; Lee *et al.,* 2004). The bark from *C. cassia* exhibited potent cytotoxic activity on six model tumor cell lines (HeLa epithelioid cervix, A549 lung, SK-MEL-2 melanoma, SKOV-3 ovarian, XF-498 central nerve system, and HCT-15 colon tumor). The main active compound from the *Cinnamomum* bark was identified as trans-cinnamaldehyde. It has been reported that a number of aliphatic and aromatic aldehydes and their derivatives have cytotoxicity against tumor cell lines (Piantadosi *et al.,* 1964; Lee *et al.,* 2004) and some of them were used as anticancer agents. Not only the essential oils but also the aqueous extract of the *Cinnamomum* species can provide the anticancer property. Singh *et al*. (2009) studied the effect of aqueous cinnamon extract (ACE) from *C. zeylanicum* bark on various cancerous cell lines compared with commercial cinnamaldehyde. The ACE as concentration above 0.16 mg/ ml (containing 1.28 µM cinnamaldehyde) provided cancerous cells cytotoxicity while commercial cinnamaldehyde as concentration 1.6 µM had no cytotoxic effect. A higher cancerous cytotoxicity might be related with the effect of polyphenol in the extract (380.83 µg GAE/ ml or 0.76%). The effect of water soluble polymeric polyphenols from cinnamon can inhibit proliferation and after cell cycle through their potential to interact with phosphorylation/ dephosphorylation signaling activity. Thus, polyphenols may be acting synergistically with the cinnamaldehyde present in the ACE and thereby inducing the increased cytotoxic activity compared to the commercial cinnamaldehyde.

1.2.6. Toxicity of *Cinnamomum* species

Some species of *Cinnamomum* are known to contain safrole (Wang *et al.,* 2008) such as *C. carolinense* (Reynertson *et al.,* 2005), *C. mollissimum* and *C. porrectum* (Subki *et al*., 2013). According to the International Agency for Research on Cancer (IARC), the part of the World Health Organization (WHO). One of its major goals is to identify causes of cancer. The most widely used system for classifying carcinogens comes from the IARC. In the past 30 years, the IARC has evaluated the cancer-causing potential of more than 900 likely candidates, placing them into one of the following groups:

- Group 1: Carcinogenic to humans
- Group 2A: Probably carcinogenic to humans
- Group 2B: Possibly carcinogenic to humans
- Group 3: Unclassifiable as to carcinogenicity in humans
- Group 4: Probably not carcinogenic to humans

The classification of carcinogenic to humans based on how hard it can be tested these candidate carcinogens, most are listed as being of probable, possible, or unknown risk. In 1976, the International Agency for Research on Cancer classified safrole as a Group 2B carcinogen (possible human carcinogen) (Reynertson *et al.,* 2005; IARC, 1987). Not only safrole but also methyleugenol (4-allyl- 1, 2-dimethoxybenzene) (Figure 3 (a, b) were found in *Cinnamomum* leaves, this compound has been demonstrated to be carcinogenic and genotoxic (European Food Safety Authority, 2009). The safety level intake of safrole from food and spices was 1 mg/person/day and methyl eugenol was 13 mg/person/day (European Food Safety Authority, 2009). The solubility of safrole is a few soluble in water 121 mg/l and methyl eugenol is soluble in water 500 mg/l but both compounds very soluble in alcohol (Reynertson *et al.,* 2005).



a b

Figure 3 Chemical structures of safrole (a), methyleugenol (b)

Source: Chen *et al.* (2009).

1.2.7. Tea and herbal tea

1.2.7.1. Tea

Tea is produced from the processed leaf of *Camellia sinensis*, a plant cultivated across the world in tropical and subtropical regions. Tea plants belong to the Theaceae family and come from two main varieties: *C. sinensis* var. *assamica*, a large-leaved tree discovered in the Assam region of India and introduced in several countries enjoying a semitropical climate and *C. sinensis* var. *sinensis*, a small-leaved, bush-like plant from China, grown in several countries of Southeast Asia experiencing a cold climate. The *assamica* variety contains large amounts of tannins and catechins and is particularly used for black tea production, whereas *sinensis* tea accounts for most of the green and oolong tea production (de Mejia *et al.*, 2009).

1.2.7.2. Herbal tea and classifications

Herbal tea (herbal infusion or herbal tisane) is a commonly consumed beverage brewed from the leaves, flowers, seeds, fruits, stems or roots of plant species rather than *Camellia sinensis* L., and it has been used for health care and diseases prevention for worldwide (Zhao *et al.*, 2013). Therefore, quality control is crucial for ensuring the safety and efficacy of herbal tea. Unfortunately, there is no review related to herbal tea to date. From the reviews, the development of phytochemical analysis for medicine and food dual purposes plants used in China in 2011 (Zhao *et al.*, 2011). In this review can summarize and discuss the recent development of phytochemical analysis for herbal tea commonly used in China, but the materials related to medicine and food dual purposes plants were not considered except the updated publications.

Herbal tea is herbal or plant infusion and usually not made from the leaves of the tea bush (*C. sinensis*) (Gill *et al.,* 2011). Like brews made of the tea bush, such infusions are prepared by combining hot water and fruits, leaves, roots, grains or bark. The herbal tea can divide into three groups. First is the herbal tea given only good taste and nice aroma with some health benefits. This tea is usually not harmful and then people could drink it as a casual beverage. Thai herbal teas in this group include Ginger (*Zingiber officinale* Roscoe), Baibuabok (*Centella asiatica* (Linn.) Urban), and other tea made of flowers such as Sarapee (*Mammea siamensis* Kosterm) and Pigul (*Mimusops elengi* Linn.) (Singhawisai 2004, Puddhanon *et al.* 2012).Second is the herbal tea with a minor medicative action such as Rang-jued (*Trunbergia laurifolia*). Herbal tea in this group can be taken twice a day, one in the morning and another at night, for several days without adverse effects. The last group of herbal tea is the beverage which aimed for medicative action such as the laxatives i.e. Chumheadted (*Cassia alata* (L.) Roxb.) and Makhamkeak (*Senna alexandrina* P. Miller.). Tea in this group should be taken under supervision and prolonged consumption is more harmful than good. Both of mentioned tea samples urge the intestine to work, so a prolonged consumption could interface or even stop a normal cycle of our body (Singhawisai, 2004, Puddhanon *et al.* 2012).

1.2.8. Factor affecting on tea and herbal tea products

1.2.8.1.Effect of drying temperature

Drying or dehydration of foods is an extremely important food processing operation used to preserve foods for extended periods of time (Ratti, 2001). The basic objective in drying food products is the removal of water in the solids to a level at which microbial spoilage and deterioration resulting from chemical reaction are greatly minimized (Korus, 2011).

Until now, different drying methods have been applied to different raw materials and each method process its own characteristics. The effect of a particular drying method on the retention of raw quality is not predictable and depends on the involved compounds and the specific plant concerned. However, the unsuitable drying process can provide the negative effects on the taste, color, and nutritional content of

product. Drying with high temperature (more than 60 ºC) can decrease the pharmaceutical properties on banana leaves (*Musa acuminata*) (Sagrin and Chong, 2013).

Katsube *et al.* (2009) reported effects of an air-drying process on the antioxidant capacity and stability of antioxidant polyphenolic compounds in mulberry leaves. The DPPH radical scavenging activity in mulberry leaves was little changed when drying at 60 ºC or under (compared with freeze dry and dry at 40 ºC). Moreover, reducing the level of the polyphenolic compounds found in the mulberry leaves is correlated well to the antioxidant activity, an indication that the decrease of antioxidant activity in leaves when dried at 70 ºC and over resulted from the degradation of the polyphenolic compounds.

1.2.8.1.1. Effect of preliminary processing

Peroxidase (POD) and polyphenoloxidase (PPO) are widely distributed in fruits and vegetables (Lin *et al.*, 2012b). Both enzymes associated with oxidative deterioration reactions that can cause browning of these products when not properly controlled. The POD (EC 1.11.1.7., H_2O_2 donor, oxidoreductase) can oxidize the phenolics with hydrogen peroxide and forms phenoxyl radical which oxidized chlorophyll to colorless low molecular weight compounds (Rudra *et al.*, 2008). The PPO can catalyze the oxidation of phenolic substrates using oxygen as a hydrogen acceptor in two different types of reactions. The PPO (EC 1.14.18.1, monophenol, L-dopa: oxygen oxidoreductase) is involved in the hydroxylation of monophenols in order to originate o-diphenols and the PPO (EC 1.10.3.1, 1,2-benzenodiol: oxygen oxidoreductase), which catalyzes the removal of hydrogen from o-diphenols to produce an o-quinone (Ramírez *et al.*, 2003; Lopes *et al.*, 2014). In general, the POD is usually chosen as an enzymatic indicator for blanching in food industry due to its high thermal resistance and high concentration in most fruits and vegetables (Zhu *et al.*, 2010). However, the heat denaturation in some plant such as Jubileu clingstone peach was highly effective above 80 °C for PPO and above 60 °C. This result can indicate that the thermal stability of PPO was higher than that of POD (Lopes *et al.*, 2014).

Blanching (or steaming) is a common treatment for enzyme inactivation and for retention of the initial quality of the fresh plant. However, several thermolabile

compounds, such as phenolics, may lose their activities due to oxidation or diffusion (or leaching) into the water during blanching (Komes *et al.*, 2010; Lin *et al.*, 2012b). Blanching is required prior to dehydration of many commodities, since the temperatures associated with dehydration are insufficient to inactivate enzyme within the products, and enzyme activity is not controlled by reduced moisture content. In the general, blanching of various food commodities is accomplished using two different types of heating media (1) hot water blanching and (2) steam process. Nantitanon *et al.* (2010) studied the effect of pre-treatment of guava (*Psidium guajava*) leaf by blanching in boiling water. In this study, the TPC of blanching in boiling water for 30 s and after that immersion in ice water for 15 min (BCD) has a high value of TPC than blanching in boiling water for 30 s and then exposure to 30 ºC for 15 min (BD). This result suggested that BCD was suitable for pre-treatment condition of guava leaves. The high temperature was necessary considered to stop certain enzymes that cause degradation of the active antioxidant principle in guava leaves. However, the heat-labile substances may be destroyed by high temperature. The BCD pre-treatment, in which the heat was stopped promptly after blanching by an ice water, prevented that heat-labile antioxidant from degradation caused by prolonged heat.

1.3.Objectives

1. To investigate the effect of drying temperature, steeping time and pre-treatment processes on *C. porrectum* herbal tea production.
2. To investigate the effect of blanching process on nutritional composition, microstructure, chlorophylls, carotenoid contents and safrole elimination.
3. To study the effect of odor types of leaves and blanching process on nutritional composition, physiochemical properties, phenolic composition and antioxidant activities of *C. porrectum* herbal tea.
4. To study the effect of *C. porrectum* herbal tea on nitric oxide inhibition, cytotoxicity on normal cells (RAW264.7 and HEK293) and anti-colon cancer cells (HT-29 and Caco-2).

CHAPTER 2

INFLUENCE OF DRYING TEMPERATURES AND PRE-TREATMENT PROCESSES ON *Cinnamomum porrectum* HERBAL TEA PRODUCTION

2.1. Abstract

This research aimed to study (1) the effect of different drying temperatures (50, 60, 70 and 100 °C) compared with air-drying (28-30 °C, represent traditional drying process from personal contact) and freeze-drying process (control) on the total extractable phenolic content (TPC) and total extractable flavonoid content (TFC) of *C. porrectum* herbal tea infusion. The result showed that drying at 60 °C provided the highest of TPC ($p<0.05$) when compared with other thermal drying processes. However, this herbal tea gave a lower TPC when compared with freeze-dried leaves related with the effect of enzyme activity. Thus, (2) the effect of pre-treatment process and time (blanching and steaming processes for 30, 60, 90 and 120 s) on peroxidase (POD) and polyphenol oxidase (PPO) inactivation and on TPC, TFC and antioxidant activities were studied. The steeping time for 10 min showed not significantly different on TPC and TFC when compared with 20 and 30 min. Blanching of tea for 60 s gave a higher ability to reduce both POD and PPO in before and after dried samples. These results indicated that blanching with hot water blanching for 60 s was a better way to provide more TPC, TFC and antioxidant activities determined as DPPH and ABTS assay when compared with steaming process.

2.2.Introduction

Herbal tea is herbal or plant infusion and usually not made from the leaves of the tea bush (*Camellia sinensis*) (Gill *et al.*, 2011). Due to the health benefits, both tea and herbal tea are more widely commercialized and consumed worldwide (Gill *et al.*, 2011). Many consumers believe that herbal tea is natural and safe as herbs can promote health and assuage illness (Desideri *et al.*, 2011). Drying process is an important preservation process for plant materials because it inhibits the enzymatic degradation and can decreases microbial growth (Pinela *et al.* 2012). The air-drying is still used worldwide because of their lower cost but this process is hard to control in the large quantities and to achieve consistent quality products. Freeze drying was considered due to this method can preserve the quality of plant during processing (Annegowda *et al.* 2014) but this process is an expensive method (Ratti, 2001). The hot air drying shows some benefits such as low energy efficiency and greatly reduce the drying time when compared with air-drying. Not only drying process but also pre-treatment process is the necessary process for inactivate or destroy both endogenous and exogenous enzymes leading to quality of products especially peroxidase (POD) and polyphenol oxidase (PPO) (Rudra *et al.*, 2008). Blanching (or steaming) is a basis treatment for enzyme inactivation and for retention of the fresh plant quality. However, the thermolabile compounds, such as phenolics, can lose their activities due to oxidation or diffusion/ leaching into the water during blanching process (Komes *et al.*, 2010; Lin *et al.*, 2012b).

Cinnamomum porrectum (Roxb.) Kosterm. with Thai name "Thep tharo" is a native plant grown in Southeast Asia. From marketing survey and personal contact, Thep-taro is locally available as herbal tea product. However, there are no scientific data about the *C. porrectum* herbal tea production. Therefore, this experiment aimed to study the effect of drying temperature, steeping time and pre-treatment process (blanching and steaming process) on POD and PPO in leaves before and after drying. In addition, the TPC, TFC and antioxidant activities of *C. porrectum* leaves were monitored in herbal tea infusion.

2.3.Materials and Methods

2.3.1. Material preparation

10 kgs of the *C. porrectum* leaves with root beer odor in developing/ intermediate stage with light green-green color and flexible stalk from 10 marked plants were collected during May to August 2015 from Technology Research Centre of Forestry sector, Songkhla. To preserve their original quality, leaves were stored in a refrigerator at 4 ºC and used within 1 day.

2.3.2. Effect of drying processes and temperature on total extractable phenolic (TPC) and total extractable flavonoid content (TFC)

The fresh leaves of *C. porrectum* were washed, spin dry and cut into size 1×4 cm. The leaves were divided into 3 groups; (1) dried by freeze dryer (2) dried by normal air with temperature 28-30 ºC and (3) dried by hot air dryer with various drying temperature (50, 60, 70 and 100 ºC). All the groups dried until the moisture content between 5-7%. All dried leaves were made to herbal tea powder by ground and sieved though a 60-mesh and kept the powder in aluminium foil ziplock bag until used. The herbal tea infusion was prepared by weighted 0.5 g powder into 100 ml of DI water (in a water bath with control temperature 95 ºC for 10 min. Filtered and cooled down to room temperature (28-30 ºC) within 5 min analyzed TPC and TFC.

2.3.3. Effect of steeping time on total extractable phenolic (TPC) and total extractable flavonoid content (TFC)

The selected tea powder from 2.3.2 was extracted by DI water (in a water bath with control temperature at 95 ºC) for 5, 10, 20 and 30 min. Filtered and cooled down to room temperature (28-30 ºC) within 5 min and then analyzed the TPC and TFC.

2.3.4. Effect of the pre-treatment process on total extractable phenolic (TPC), total extractable flavonoid content (TFC) and antioxidant activities

The fresh leaves were divided into 3 groups including control (un-treated) and pre-treated by blanching and steaming for 30, 60, 90 and 120 s before drying with selected drying temperature from 2.3.2. The dried leaves were prepared to

herbal tea infusion followed by 2.3.2. The herbal tea infusions were adjusted to analyze TPC, TFC, antioxidant activity as DPPH, ABTS, FRAP and ferrous metal chelating activity (FIC).

2.3.5. Effect of pre-treatment process on peroxidase (POD) and polyphenoloxidase (PPO) enzymes

a) Preparation of enzyme extracts

Ten grams of all *C. porrectum* leaves (before and after drying process) were blended with 100 ml of 1 M sodium chloride (4 ºC) in a blender at room temperature for 30 s. The suspensions were filtered by vacuum filter and kept in a refrigerator at 4 ºC and used for analysis within 1 h.

b) Peroxidase (POD) analysis

POD activity was determined based on a method of Gonçalves *et al.* (2010) and Lin *et al.* (2012). Briefly, the solution was a mixture of 0.1 ml of guaiacol and 0.1 ml of H_2O_2, made up to 100 ml with 100 mM NaH_2PO_4–Na_2HPO_4 buffer (pH 6.0). The solution (2 ml) was added to a tube, and the mixture was pre-incubated for 5 min at 37 ºC in a water bath (Memert, D-91126, Schwabach, Germany). Finally, 0.2 ml of sample was added. The reaction was carried out at a constant temperature of 37 ºC. An increase in absorbance from 0 to 5 min was measured at 470 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan).

c) Polyphenol oxidase (PPO) analysis

PPO residue was determined based on a method of Lin *et al*. (2012). The 0.1 mM catechin (substrate solution) 1 ml was added into a tube and then, 0.5 ml of 100 mM NaH_2PO_4–Na_2HPO_4 buffer (pH 6.8) was transferred into the same tube before the mixture was taken to pre-incubate for 5 min at 37 ºC. Thereafter, 0.2 ml of extracted sample was added at to the mixture. An increase in absorbance from 0 to 5 min was measured at 420 nm with the controlled temperature at 37 ºC.

The initial POD and PPO activities in fresh leaves were defined as percentage relative residue enzyme.

2.3.6. Determination of total extractable phenolic content (TPC)

TPC of the infusions was determined by using the Folin-Ciocalteau reagent following method of Chan *et al*. (2009). Briefly, sample, 50 µl, was introduced into 96-well plate followed by adding of 150 µl of Folin–Ciocalteu's reagent (10 times dilution) and 120 µl of sodium carbonate (7.5% w/v). The plates were allowed to stand for 30 min in the dark before subjected to determine absorbance at 765 nm. Gallic acid was used as the reference standard and the results were expressed as mg GAE /g sample.

2.3.7. Determination of total extractable flavonoid content (TFC)

TFC of the infusions was determined by using Aluminum chloride colorimetric method following method of Lobo *et al*. (2011). Briefly, 25 µl of the extracts were added to the 96-well plate containing 100 µl of water. At zero time, 10 µl of 5% $NaNO_2$ was added then, 5 min later, 15 µl of 10% $AlCl_3$ was followed. After that, 50 µl of 1 M NaOH was added to the mixture and the volume was made up to 250 µl with water. An absorbance was measured at 510 nm. Catechin was used as the reference standard and the results were expressed as mg CE /g sample.

2.3.8. Determination of antioxidant activities of *C. porrectum* tea infusion

a) DPPH radical-scavenging activity

DPPH radical scavenging capacity assay was evaluated with the following method of Udayaprakash *et al.* (2014). 0.15 mM DPPH was prepared using methanol to obtain an absorbance of 0.8 ± 0.1 units at 517 nm. Briefly, various concentrations of test samples (100 µl) was mixed with DPPH solution. After 30 min of incubation at room temperature in the dark, the absorbance was measured against a blank (methanol) at 517 nm using a spectrophotometer. Sample blank was prepared in the same manner except methanol was used instead of DPPH solution. A standard curve was made by using Trolox at 5-25 µg/ml and the results were expressed as mg of Trolox equivalent (TE)/g sample.

b) ABTS radical-scavenging activity

ABTS radical-scavenging activity was evaluated with the following method of Arnao *et al.* (2001). 7.4 mM ABTS solution and 2.6 mM potassium persulphate solution were prepared as stock solutions. The working solution was prepared by mixing the two stock solutions in equal quantities. The mixture was

allowed to react for 12 h at ambient temperature in the dark. The mixed solution was diluted by mixing 1 ml of ABTS solution with 50 ml of water in order to obtain an absorbance of 1.1± 0.02 units at 734 nm. Briefly, sample, 15 µl, as mixed with 285 µl of ABTS solution and the mixture was left at ambient temperature for 2 h in the dark. The absorbance was measured at 734 nm using a microplate spectrophotometer. Sample blank was prepared in the same manner by using water instead of ABTS solution. A standard curve was made by using Trolox at 50- 125 µg/ ml and the results were expressed as mg of Trolox equivalent (TE)/g sample.

c) FRAP (ferric reducing antioxidant power)

FRAP (ferric reducing antioxidant power) was assayed according to Benzie and Strain, (1996). 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2, 4, 6-tripyridyl- *s*- triazine) solution dissolved in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution were made for stock solutions. A working solution was prepared freshly by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ solution and 2.5 ml of $FeCl_3.6H_2O$ solution. The mixed solution was incubated at 37 °C for 30 min in the incubator and referred as FRAP solution. Later, sample, 15 µl, was mixed with 285 µl of FRAP solution and kept at ambient temperature for 30 min in dark. The ferrous tripyridyltriazine complex (blue colored product) was measured by reading the absorbance at 593 nm. Sample blank was prepared by omitting $FeCl_3$ from FRAP solution and distilled water was used instead. A standard curve was made by using Trolox at 50-125 µg/ml. The results were expressed as mg of Trolox equivalent (TE)/g sample.

d) Ferrous ion chelating activity (FIC)

The ferrous chelating activity was measured by the method of Boyer and McCleary, (1987). Briefly, 1.0 ml of each sample substance was mixed with 0.1 ml of 0.2 mM $FeCl_2$. The reaction mixture was allowed to stand for 10 minutes at ambient temperature and then 0.2 ml of 5 mM ferrozine was added. The mixture was stand for more than 10 minutes at ambient temperature. The absorbance was then read at 562 nm. A blank sample was prepared in the same manner using distilled water instead of the sample and blank samples of each of the substances under examination with the $FeCl_2$ solution excluded and distilled water used instead were also prepared. The standard curve was constructed using the ethylenediaminetetraacetic acid (EDTA)

ranking from 10-50 µg/ml. The activity was expressed as mg EDTA equivalent/ g sample.

2.3.9. Statistical analysis

All the results were expressed as the mean ± SD (n=3). The data were subjected to analysis of variance (ANOVA) to detect potential differences. The significant differences among the means were established by Duncan's test and *p*-values < 0.05 were considered to be significant.

2.4.Results and Discussion

2.4.1. Effect of drying processes and temperature on total extractable phenolic content (TPC) and total extractable flavonoid content (TFC) of *C. porrectum* herbal tea

The freeze-drying sample showed the highest of both TPC and TFC when compared with the thermal treatment (air-drying and hot air-drying). As known that living cells have biochemical changed, all time, therefore to stop the biochemical reaction freezing process is applied to inactivate enzyme and stabilize cell structure which cannot obtain from heating process. The traditional method or air-drying showed a lower content of both TPC and TFC when compared with freeze-dried sample. The percentage of TPC and TFC reduction when compared with freeze-drying was 23.63 and 22.57 %. In hot air-drying, the highest TPC was obtained when drying at 60 °C followed by 70, 50 and 100 °C. The percentage decrease of TPC when compared with freeze drying was 14.13, 19.27, 22.90 and 60.95 while the TFC was 13.49, 14.12, 17.72 and 45.86, respectively. This result indicated that both TPC and TFC of air-drying was lower than hot air-drying at 60 and 70 °C but not significantly different when compared with drying at 50 °C.

The reduction of both TPC and TFC of air-drying and drying at 50 °C might be due to the initial enzymatic degradation of phenolics where the slow heat transfer resulted in inefficient denaturation of the antioxidant degradative enzymes during the initial drying process (Lim and Murtijava, 2007). The higher drying temperature at 100 °C showed the highest losing on both TPC and TFC might be related to (1) initial enzymatic degradation of phenolics and (2) thermal degradation of phenolics. According to the case of *Vitex trifolia* showed a much larger percentage

decrease in antioxidant activity at 70 °C and 100 °C because of the most heat labile antioxidants had been degraded at 70 °C (Choung and Lim, 2011). Thus, drying at 60 °C by hot air-drying was selected to next study.

Table 1 Effect of drying processes and temperature on total extractable phenolic content (TPC) and total extractable flavonoid content (TFC).

Process	Temp. (ºC)	Time (h)	TPC (mg GAE/g)	TFC (mg CE/g)	% of decreased*	
					TPC	TFC
FT	-50 to -20	24	34.19±1.04[a]	11.12±0.28[a]	0	0
AT	28 to 30	48	26.11±0.52[c]	8.61±0.24[c]	23.63	22.57
HT	50	10-12	26.36±0.43[c]	9.15±0.16[b]	22.90	17.72
	60	7-8	29.36±0.49[b]	9.62±0.11[b]	14.13	13.49
	70	4-5	27.60±0.54[c]	9.55±0.17[b]	19.27	14.12
	100	1-2	13.35±0.82[d]	6.02±0.36[d]	60.95	45.86

Values are presented as mean ± SD (n=3).
Different lowercase letters in the same column indicate significant difference ($p<0.05$).
* Means % decreased calculated by TPC and TFC of (FT– AT or HT) / FT x 100
FT: freeze-drying process, AT: air-drying process and HT: hot air-drying process.

2.4.2. Effect of steeping time on total extractable phenolic content (TPC) and total extractable flavonoid content (TFC) of *C. porrectum* herbal tea infusion

The *C. porrectum* herbal tea infusion was prepared from hot air-dried herbal tea at 60 °C. The effect of steeping time was shown in Table 2. The TPC and TFC from steeping for 10 min was higher than that of 5 min but not significantly from others. Actually, extraction time also affected to TPC and TFC in case of bagged and loose leaf, however, in case of powder tea extraction does not have a significant effect on its TPC, TFC as well as its antioxidant activities (Komes *et al.*, 2010).

Table 2 Effect of steeping time on total extractable phenolic content (TPC) and total extractable flavonoid content (TFC) of *C. porrectum* herbal tea infusion

Steeping time (min)	TPC (mg GAE/g)	TFC (mg CE/g)
5	26.85±1.01[b]	8.45±0.92[b]
10	29.41±1.12[a]	9.59±0.49[a]
20	28.39±1.17[a]	9.59±0.78[a]
30	28.36±1.04[a]	9.54±0.45[a]

Values are presented as mean ± SD (n=3).
Different lowercase letters in the same column indicate significant difference ($p<0.05$).

2.4.3. Effect of pre-treatment process on peroxidase (POD) and polyphenoloxidase (PPO) inactivation

In the experiment, the leaves were cut into the small size for increase the leaves surface. The stalk of the leaves was removed because the rubber from stalk can make the gumminess in herbal tea infusion. The cutting can induce the releasing of the enzymes and phenolics from food matrix. The peroxidase (POD) and polyphenol oxidase (PPO) are the main enzymes involved the phenolic oxidation, their activities have attracted much attention. In this experiment, the pre-treatment process including blanching and steaming process with various process time 30, 60, 90 and 120 s were selected to study the effect on both POD and PPO enzymes compared with the hot air-dried at 60 °C in before and after drying process as showed in Table 3.

The residue enzymes were calculated as relative residues compared with control sample (hot air-dried at 60 ºC) as initial residues or 100% residue (Table 3). In control sample, it was found that both POD and PPO after drying also decreased from 100% residue to 3.80 and 14.58%, respectively. A decrease of both enzymes during drying with hot air dryer at 60 ºC (for 7-9 h) might be explained by 2 main mechanisms; (1) no substrate (phenolics) no end products and (2) enzyme degradation (Zhang and Shao, 2015). According to the report of Zhang and Shao (2015), the PPO activity of loquat (*Eriobotrya japonica*) after heated at 40, 50, 60 and 70 ºC for 10 min was 65, 38, 36 and 30%, respectively. That result indicated that PPO was stable at a temperature of 40 ºC, whereas it was partly denatured in the temperature range of 50-70 ºC.

A POD residue was less than 3.10 and 3.47% when pre-treated with blanching and steaming processes for 60 s. On the other hand, a PPO residue of both pre-treatment was higher than POD residue (14.58% and 40.63% in blanched and steamed leaves) when pre-treated with the same time for 60 s. It pointed out that the PPO of this plant leaves exhibited higher heat resistant compared with the POD. This result agreed with the finding of Lopes *et al.* (2014) who addressed that PPO of Jubileu clingstone peach was more thermal stable compared with the POD. In addition, it pointed out that hot water blanching was efficient for POD and PPO inhibition, when compared with the same time, may be due to heat penetration and distribution coefficient. As well known that liquid (hot water) is a better convection media compared with air (steaming) (Lin *et al.,* 2012a). Zhang and Shao, (2015) addressed that activity of enzyme depended on optimal conditions including pH of test experiment. In this experiment, the pH extracts were 4.50-4.75. This pointed out that residue PPO activity of this plant sample which was higher than POD may be due to proper pH condition, higher acidic compared with POD condition.

Table 3 Effect of blanching and steaming process on peroxidase (POD) and polyphenoloxidase (PPO) residues before and after drying process of *C. porrectum* leaves

Treatment	Process time (s)	Residue POD (%)		Residue PPO (%)	
		Before drying	After drying	Before drying	After drying
Control	un-treated	100.00^{a}	3.80±0.40^{b}	100.00^{a}	14.58±0.04^{b}
Blanching	30	3.54±0.31^{Aa}	3.10±0.16^{Aa}	43.75±3.02^{Ba}	10.42±0.44^{Bb}
	60	3.10±0.29^{Aa}	2.24±0.11^{Ba}	14.58±2.48^{Ba}	7.29±0.41^{Bb}
	90	2.65±0.25^{Aa}	2.03±0.11^{Ba}	6.25±1.82^{Ba}	2.91±0.25^{Bb}
	120	1.24±0.11^{Ba}	1.04±0.21^{Ba}	2.08±1.17^{Ba}	1.04±0.34^{Bb}
Steaming	30	4.15±0.39^{Aa}	3.77±0.51^{Aa}	62.50±4.10^{Aa}	12.50±0.65^{Ab}
	60	3.47±0.27^{Aa}	3.02±0.43^{Aa}	40.63±3.20^{Aa}	9.37±0.30^{Ab}
	90	3.11±0.20^{Aa}	2.46±0.11^{Ab}	17.71±1.79^{Aa}	5.29±0.15^{Ab}
	120	2.54±0.21^{Aa}	1.89±0.11^{Ab}	14.58±1.25^{Aa}	2.38±0.15^{Ab}

Values are presented as mean ± SD (n=3).
Different uppercase letters in the same column within the same process time indicate significant difference ($p<0.05$)
Different lowercase letters in the same column within the same enzyme (compared before and after drying) indicate significant difference ($p<0.05$).

2.4.4. Effect of pre- treatment process on total extractable phenolic content (TPC), total extractable flavonoid content (TFC) and antioxidant activities of herbal tea infusion

The effect of pre-treatment process from control (un-treated), blanched, and steamed teas on TPC, TFC and antioxidant activities of all herbal tea infusion were presented in Table 4. The TPC of the herbal tea extracts ranged from 24.52 to 33.78 mg GAE/ g. TPC of control was the lowest may be due to highest degradation of the phenolic compound by the enzyme as explained above. PPO in plant is located in the chloroplasts while their phenolic substrates are mainly located in the vacuoles, therefore the more physical damage the more enzymatic reaction, for example, cutting process

makes the plant cell damage leading to exposure of PPO to substrate as such phenols to be hydrolyzed and oxidized (Chazarra *et al.*, 2001; Zhang and Shao, 2015). In addition, phenolic compounds degradation may more occur particularly in the initial stage that the temperature did not high enough to destroy enzyme activity which normally occurs more than 100 ºC for 1-2 min (98% loss of activity (Fante *et al.*, 2012). The blanched tea provided TPC ranged from 31.87-33.78 mg GAE/ g and it was found that blanching for 60 s provided the highest of TPC. This may due to PPO was quite destroyed by that heat condition then phenolic compounds were prevented from enzymatic oxidation. In addition, it pointed out that the BT60 not only prevented the enzyme activity but also, broken down the bound or insoluble phenolic structure which generally forms of covalently bound to cell wall structural components such as cellulose, hemicellulose, lignin, pectin and rod-shaped of structural proteins (Wong, 2006) and released more free form of phenolic compound (Lin *et al.*, 2012). However, TPC increased as heating time increased and reached the peak at 60 s before started to decline after blanching for 90 s may be due to 2 hypotheses; (1) some thermo-labile phytochemicals such as small phenolic acids may deteriorate and (2) leaching effect into the water occurred (Pietta, 2000; Cai *et al.*, 2004; Lin *et al.*, 2012a). TPC of steaming process ranged from 27.87-31.49 mg GAE/ g. The TPC of steaming process seemed lower than blanching process may be due to heat transfer coefficient of steam was lower than boiling water thus, the POD/ PPO were less destroyed, and the phenolic compound was not much deteriorated (Pietta, 2000; Cai *et al.*, 2004; Lin *et al.*, 2012a). Lin *et al*. (2012a) reported that the stability of phytochemical compounds of each plant was unique plant structure determinant. In addition, the result showed that when the leaves were blanched with hot water for a long time, 90 s, the lower TPC. This may be due to leaching effect which is commonly found in the sample blanched with hot water or boil in water.

TFC of herbal tea extract ranged from 7.17 to 11.52 mg CE/ g. The trend of TFC was similar to TPC meant that using blanching process yielded TFC higher than steaming. In nature flavonoids presented as glycosides with a single or multiple sugar moieties linked through an OH group (*O*-glycosides) or through carbon-carbon bonds (*C*- glycosides) (Acosta- Estrada *et al.*, 2014). Significant increasing of TFC by blanching leaves for 60 s compared with control tea was explained by the broken down

of the cell walls and glycosides structure for releasing more aglycone forms (Chiremba *et al.,* 2010).

The phenolic antioxidant reacts with DPPH by electron transfer and hydrogen atom transfer (Baumann *et al.,* 1979; Mathew and Abraham, 2006a; Xie and Schaich, 2014). The DPPH activity of the extracts ranged from 10.62 to 17.08 mg TE/ g. The CC sample showed the lowest DPPH activity may due to (1) lowest quantity of phenolic compounds due to the structure of phenolic compound still be bound forms more than free forms and (2) phenolic compounds were reduced by enzymatic activity during the drying process. The blanching for 60 s showed the highest of DPPH activity compared with other samples. The ABTS activities of the extracts ranged from 67.74 to 89.52 mg TE/ g. Again, blanching for 60 s showed the highest of ABTS activity. The FRAP measures the reducing capacity of the compounds in donating electrons by ferric-to-ferrous reduction capacity of water-soluble antioxidants in acidic pH as pH 3.6 (Pulido and Saura-Calixto, 2000; Dai and Mumper, 2010). The pH of *C. porrectum* tea extract ranged from 5.3-5.6, pH was slightly acidic pH and suitable for detecting with FRAP assay. FRAP of the extracts ranged from 27.79 to 41.33 mg TE/ g. The FIC of the extract measures how compounds in the sample can compete with ferrozine for ferrous ions. The data from Table 4 showed that all extracts exhibited a poor metal chelating activity (3.06-3.98 mg EDTA/g) compared with other antioxidant activity assays. However, this result was in agreed of Damiani *et al.* (2014) who reported the metal chelating activity of white tea in hot and cold infusion was quite low (0.3-0.6 mmol/l EDTA). This result indicating the FIC was not correlated with TPC and TFC, suggesting that this activity may in part derive from compounds other than polyphenol (Carloni *et al.,* 2013).

Table 4 Effect of pre-treatment process and time on total extractable phenolic content (TPC), total extractable flavonoid content (TFC) and antioxidant activities

Treatment	Process time (s)	TPC (mg GAE/g)	TFC (mg CE/g)	DPPH (mg TE/g)	ABTS (mg TE/g)	FRAP (mg TE/g)	FIC (mg EDTA/g)
Freeze dry		37.49 ± 2.04^{a}	13.12 ± 0.74^{a}	21.43 ± 1.33^{a}	112.54 ± 3.58^{a}	41.11 ± 1.46^{a}	4.19 ± 0.10^{a}
Control (un-treated)		29.36 ± 2.38^{bc}	9.61 ± 0.54^{c}	12.62 ± 0.94^{d}	67.74 ± 3.11^{d}	30.79 ± 1.65^{cd}	3.46 ± 0.20^{b}
Blanched	30	32.35 ± 1.70^{Ab}	11.01 ± 0.55^{Ab}	14.99 ± 1.01^{Abc}	84.19 ± 3.45^{Abc}	37.66 ± 1.52^{Ab}	3.85 ± 0.20^{Abc}
	60	33.78 ± 1.95^{Aab}	11.52 ± 0.46^{Ab}	17.08 ± 1.10^{Ab}	89.52 ± 3.12^{Ab}	39.73 ± 1.66^{Aab}	3.82 ± 0.20^{Abc}
	90	31.87 ± 1.94^{Ab}	11.02 ± 0.35^{Abc}	14.99 ± 1.21^{Abc}	79.46 ± 3.24^{Bc}	32.21 ± 1.62^{Ac}	3.82 ± 0.10^{Ab}
	120	32.00 ± 1.38^{Ab}	10.31 ± 0.44^{Abc}	14.15 ± 1.02^{Bc}	69.46 ± 2.85^{Bd}	31.21 ± 1.26^{Acd}	3.90 ± 0.10^{Aab}
Steamed	30	27.87 ± 1.52^{Bc}	8.76 ± 0.45^{Bd}	15.06 ± 1.05^{Abc}	74.57 ± 2.51^{Bcd}	28.55 ± 1.41^{Bd}	3.98 ± 0.20^{Aab}
	60	30.15 ± 1.72^{Bbc}	10.29 ± 0.54^{Bbc}	15.16 ± 0.97^{Bbc}	82.42 ± 2.53^{Abc}	41.33 ± 1.54^{Aa}	3.73 ± 0.20^{Abc}
	90	30.92 ± 1.91^{Abc}	10.38 ± 0.39^{Bbc}	16.50 ± 0.78^{Ab}	86.45 ± 2.63^{Ab}	32.44 ± 1.13^{Ac}	3.74 ± 0.20^{Abc}
	120	31.49 ± 1.57^{Abc}	10.64 ± 0.43^{Ab}	15.72 ± 0.67^{Ab}	81.72 ± 2.20^{Ab}	31.12 ± 1.05^{Ac}	3.84 ± 0.17^{Abc}

Values are presented as mean ± SD (n=3).
Different uppercase letters in the same column with in the same process time indicate significant difference ($p<0.05$).
Different lowercase letters in the same column with in the same assays indicate significant difference ($p<0.05$).

2.6. Conclusion

C. porrectum leaves have been used as a medicinal plant and folk medicine in China, India, and Thailand. This work reports the effect of the drying process, steeping time and pre-treatment process on enzyme inactivation, TPC, TFC and antioxidant activities of *C. porrectum* herbal tea infusions. When compared exclude freeze-dried condition, the result indicated that drying at 60 ºC was the optimum temperature that provides the highest of TPC and TFC when compared with air-drying and drying at 50, 70 and 100 ºC. The steeping time at 10 min seems not significantly different when compared with prolongation time. To our knowledge, this report is the first record of using blanching and steaming as pre-treatment for POD and PPO inactivation on this *C. porrectum* (root beer odor) leaves prior making a green tea product. In addition, better TPC, TFC, and antioxidant activities as DPPH, ABTS, FRAP, and FIC were found in sample or tea making from pre-treatment process. Blanching process generally provided betters both TPC, TFC, and antioxidant activity compared with steaming process. In addition, blanching for 60 s was recommended determined from the liberation of TPC and TFC as well as DPPH and ABTS assay. Therefore, it was a high possibility to use *C. porrectum* leaves to produce natural health products due to its antioxidant activities.

CHAPTER 3

EFFECT OF BLANCHING PROCESS ON CHEMICAL AND PHYSICAL PROPERTIES, AND ITS ABILITY ON SAFROLE ELIMINATION OF *Cinnamomum porrectum* HERBAL TEA

4.1. Abstract

This study was conducted to evaluate the effects of blanching on the nutritional composition, microstructure, chlorophylls and carotenoid contents, color values as well as safrole (toxic constituent) elimination in the *C. porrectum* root beer odor leaves when compared with the un-treated herbal tea (control). The result from nutritional composition indicated that blanching process can increase the yield of fat and protein contents than un-treated sample due to the wider pore size confirmed by SEM technique. The chlorophylls and carotenoids were reduced after blanching process but after drying process both compounds still contain higher than untreated sample. Moreover, it was found that blanching process could greater maintain green color in herbal tea infusion. In addition, blanching process was significantly decreased safrole content up to 89 and 82% and methyleugenol up to 68 and 62% when compared with un-blanched and freeze-dried samples, respectively.

3.2. Introduction

Blanching process is an essential operation for many fruits and vegetable processing. Previous studied indicated that water blanching process can reduce the peroxidase (POD) and polyphenol oxidase (PPO) enzymes more than steaming process in fresh *Cinnamomum porrectum* leaves and can increase total extractable phenolic, total extractable flavonoid contents and antioxidant activities in the herbal tea infusion (Saetan *et al.,* 2016). Besides POD and PPO enzymes reduction, increasing of drying rate, removing pesticide residues and toxic constituents was also improved.

Cinnamomum porrectum: Theptharo belonging to the *Cinnamomum* species of the Lauraceae family is an aromatic medicinal plant which mostly distributed throughout southern Thailand (Pukdeekumjorn *et al.*, 2012). These species have been used in traditional systems of medicine or folk medicine and indicated in several pharmacopeias (Subki *et al.*, 2013). However, some species of *Cinnamomum* are known to contain safrole (Singh *et al.,* 2007) such as *C. carolinense* (Reynertson *et al.*, 2005), *C. mollissimum* and *C. porrectum* (Subki *et al.*, 2013) particularly in these plants. In addition, leaves from *C. porrectum* with root beer odor type reported to have safrole as the main chemical constituent (more than 90% when compared with other compositions in volatile oil) (Pattanaseree and Anantachoke, 2012).

In 1976, the International Agency for Research on Cancer classified safrole as a Group 2B carcinogen (possible human carcinogen) (IARC, 1987). Not only safrole but also methyleugenol (4- allyl- 1. 2- dimethoxybenzene which has been demonstrated to be carcinogenic and genotoxic were found in these leaves (European Food Safety Authority, 2009). The safety level intake of safrole from food and spices was set up at 1 mg/person/day and methyleugenol was 13 mg/person/day (European Food Safety Authority, 2009). The solubility of safrole is in water is only 121 mg/l and methyleugenol is not higher than 500 mg/l but both compounds are very alcohol soluble (Reynertson *et al.*, 2005). In fact, from marketing survey and personal contact, *C. porrectum* leaves was made as herbal tea available in some area of Southern Thailand even though there is no scientific data on safety guarantee for this herbal tea. Furthermore, there is no data about the effect of blanching process on some qualities of herbal tea product. Therefore, this experiment aimed to determine the nutritional

composition, microstructure, chlorophylls, carotenoid content as well as safrole content of the leaves and infusion.

3.3. Materials and Methods

3.3.1. Materials and Chemicals

Cinnamomum porrectum with root beer odor wood oil (CWO was kindly provided by Technology Research Centre Forestry sector, Songkhla. In this study, 10 kilograms of the *C. porrectum* leaves with root beer odor from 10 marked plants in developing/ intermediate stage with light green-green color and flexible stalk (collected during May to August 2015) from Technology Research Centre of Forestry sector, Songkhla were selected. In order to preserve their original quality, leaves were stored in a refrigerator at 4 ºC and used within 1 day.

3.3.2. Sample preparation

The leaves were divided into 2 groups. The first groups, leaves were prepared to study the effect of blanching process at 100 ºC for 60 s before dried at 60 ºC compared with un-treated process by hot air dry at 60 ºC. All leaves were dried until the moisture content of all treatments was about 5-7% before taken to grind and sieve though a 60-mesh. The herbal tea infusion was prepared by weight herbal tea powder (0.5 g) then steeped in 100 ml DI water at 95 ºC for 10 min (controlling temperature by using water bath) then filtered and cooled down to reached room temperature 28-30 ºC within 5 min before subjected to freeze-drying (yielded 0.1% w/v). The second group was aimed to analyze the effect of blanching process on safrole elimination which compared with untreated and freeze-dried samples. Therefore, the powders were extracted with methanol as ratio 1:10 (w/v) by using sonicator at 50 ºC for 2 h then filtered through 0.22 µm filter membrane and stored at -20 ºC until used.

3.3.3. Proximate composition determination

Proximate composition of un-treated and blanched groups including moisture, protein, fat, fiber, ash, and carbohydrate contents were analyzed following the AOAC (2000).

3.3.4. Structure of powder determination by scanning electron microscopy (SEM)

The surface morphology of the untreated and blanched *C. porrectum* powders was investigated using a scanning electron microscope, Quanta 400 (FEI, Czech Republic) at high voltage 20.00 kV at 500x magnification.

3.3.5. Total chlorophylls and carotenoid content analysis

Chlorophylls and carotenoid content of un-treated and blanched leaves and powder (dried leaves) were analyzed following the AOAC (2000). Briefly, 0.5-1.0 g of the fresh, pre-treated leaves (before drying process) and powder were separately added with 0.1 g of $CaCO_3$, followed by a pinch of purified acid sand and 20 ml of 80% acetone, then ground in a motor at ambient temperature for 5-10 min before taken to filter through a Whatman No.1 filter paper. The residue was re-extracted with 5 ml of 80% acetone until tissue did not show any greenish color while solvent was colorless. All filtrated solutions of each sample were pooled together and added with Na_2SO_4 anhydrous to remove excess water, and then filtered again before adjusted the final volume to 100 ml with 100% acetone. Total chlorophyll, chlorophyll a, b and carotenoid were determined spectrophotometrically at 470, 649 and 665 nm. The components were calculated as followed equation:

C_a (mg/g of dry weight) = $(12.7A_{665} - 2.69A_{649}) \times ((v/1000)/w)$

C_b (mg/g of dry weight) = $(22.9A_{649} - 4.68A_{665}) \times ((v/1000)/w)$

C_{a+b} (mg/g of dry weight) = $(20.2A_{649} + 8.02A_{665}) \times ((v/1000)/w)$

C_{a+c} (mg/g of dry weight) = $((1000A_{470} - 3.27C_a - 104C_b) / 229) \times (v/1000)/w)$

When;

A= Absorbance (nm), v=Total volume of extract (ml), w=Weight of sample (g), C_a=Chlorophyll a, C_b =Chlorophyll b, C_{a+b} =Total chlorophyll and C_{a+c} =Carotenoid.

3.3.6. Color value

The color herbal tea infusion was measured using a colorimeter (Hunter Lab, Model color Flex, Reston, VIRG, USA). The determination of color was done on five different samples. Standardization of the instrument was done using a black and white Minolta calibration plate. The values were reported in the CIE color profile system as L* - value (lightness) , a* - value (redness/ greenness) , and b* - value (yellowness/blueness). The data on -a and b were also used to calculate the -a/b ratio that expressed as greenness (+) and yellowness (-) color values.

3.3.7. Safrole content analysis by GC/MS techniques

The *C. porrectum* wood oil; CWO obtained from Technology Research Centre Forestry sector, Songkhla was diluted with methanol as initial concentration 1:10 (w/v) and the leaves from un-treated, freeze-dried and blanched before dried powder methanolic extracts called as UM, FM and BM were taken to analyze for chemical compositions by GC-MS technique. The method followed the protocol of Tung *et al.* (2008) with slight modification by using a Gas Chromatograph-Mass Spectrometer, 7890 B GC-5977 A MSD (Agilent, USA), equipped with a 30 m x 0.25 mm VF-WAXms. The GC oven temperature was programmed from 40 ºC for 10 min then raised to 240 ºC at 5 ºC/ min, held for 5 min. The injector temperature was 240 ºC; and the flow rate of carrier gas, helium, was at 1.0 ml/min; the extracts were injected manually in the split mode. The relative concentration of each compound in the CWO and samples were quantified based on the peak area integrated by the analysis program and expressed as a percentage of the total compared with all composition and calculated content compared with CWO calculated by component area of GC-MS data.

3.3.8. Statistical analysis

All the results were expressed as the mean ± SD (n=3) except color values (n=5). The data were subjected to analysis of variance (ANOVA) to detect potential differences. The significant differences among the means were established by Duncan's test and *p*-values < 0.05 were considered to be significant. The T-test was used for analysis between-group comparisons of un-treated and blanched samples.

3.4. Results and Discussion

3.4.1. Proximate composition of *C. porrectum* herbal tea powder

The moisture content of blanched sample was lower than un-treated sample because blanching process induce loose structure to easier water vaporization during drying step. Total ash content was significantly decreased by blanching process ($p < 0.05$) may be due to possible leaching effect into blanching water. Dugo *et al.* (2005) reported the loss of minerals during the boiling of vegetables and tubers including carrot, bamboo shoot, broccoli, potato, and cocoyam due to the leaching effect (Lewu *et al.*, 2010). Though blanched powder was significantly higher in fat and crude fiber content ($p<0.05$. It pointed out that blanching processing significantly facilitated fat and crude fiber content extraction. This may be due to the structure of plant tissue was opened and loosen through β-sheet destruction during blanching process. Ando *et al*. (2016) reported that the pectins in the middle lamella were leached away thereafter, adhesion of the cell walls was weakened, and the tissue was markedly softened after blanching (Sila *et al.*, 2009). Furthermore, the blanching process can denature the proteins in cell walls and make the porosity of membranes. The wider porosity increased the permeability of cell walls and improved solvent diffusivity, resulting in an increase of yield extractability (Deylami *et al.*, 2016, Stamatopoulos *et al.*, 2016).

Table 5 Proximate composition (% dry basis) of un-treated and blanched *C. porrectum* herbal tea powder

Sample	Compositions (%)	Un-treated	Blanched
Powder	Moisture content (%)	5.47±0.02[a]	4.44±0.17[b]
	Ash (%)	4.53±0.11[a]	4.35±0.07[b]
	Protein (%)	7.12±0.00[b]	8.02±0.00 [a]
	Fat (%)	3.76±0.12[b]	5.83±0.07[a]
	Carbohydrate (%)	78.09±0.26[a]	77.35±0.09[a]
	Fiber (%)	10.04±0.09[b]	10.00±0.33[a]

Each value was expressed as the mean ± standard deviation (n=3).
Different little letters (a-b) in the same row indicate significant differences ($p< 0.05$) by compared paired sample.

3.4.2. Structure of *C. porrectum* herbal tea powder determined by scanning electron microscopy

The microstructure of un-treated and blanched at 100 ºC for 60 s was shown in Figure 4. The cross-sectional microstructure of blanched powder at magnification 500x (Figure 4 A 2 and B2) showed a wider porosity than un-treated due to weakening and/ or decreasing of cell wall structure components such as cellulose, hemicellulose, lignin, and structure of protein (Dugo *et al*., 2005; Lewu *et al*., 2010 and Acosta-Estrada *et al.*, 2014) Erbay and Icier (2009) reported that the drying process with high temperature and longtime can induce a case hardening or packing characteristic of fresh leaves surface leading to a decreasing of vaporization in olive leaves. In this present work, the blanched powder had a lower hardening structure compared with un-treated powder (Figure 4 A1 and B1). It pointed out that blanching induced loose structure leading to easier water vaporization during drying step (Ozilgen *et al*., 2001).

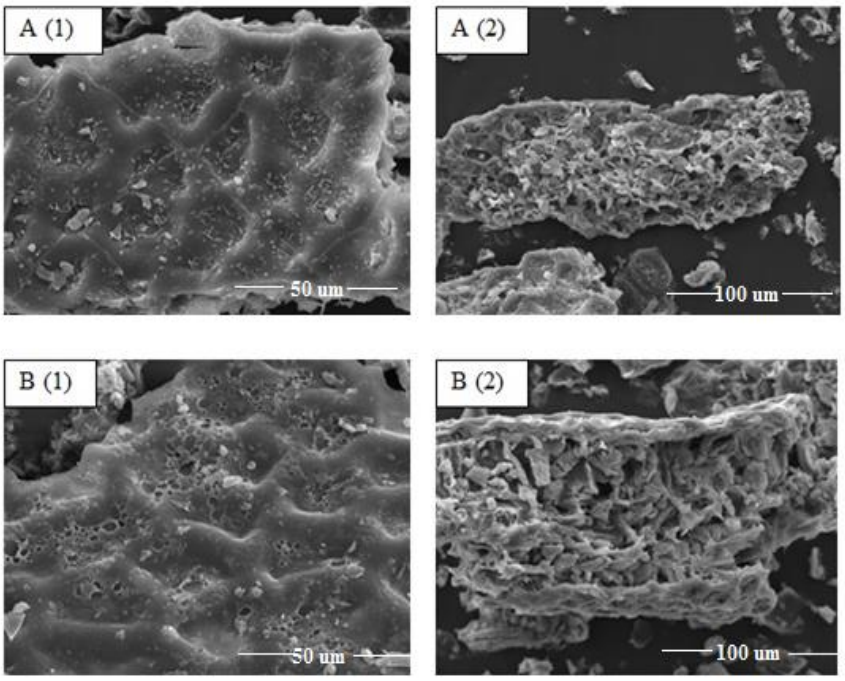


Figure 4 Scanning electron micrographs of un-treated (A) and blanched herbal tea powder (B), remark (1) top view and (2) cross-section of powder.

3.4.3. Total chlorophylls and carotenoid content

It was discovered that the blanching process significantly reduced total chlorophyll, chlorophyll a, b, and carotenoid when compared with un-blanched sample (Table 6). This may due to 2 reasons; (1) blanching treatment degraded chlorophyll and converted to be pheophytin (Tijkens *et al.*, 2001) and (2) during blanching process pronounced more leaching effect particularly chlorophylls (Erge *et al.*, 2008). Percentage loss of chlorophyll a and b from blanched leaves and un-blanched leaves were 5.55±0.19 and 4.86±0.07 respectively. Furthermore, it was found that the retaining of chlorophyll b content was higher than chlorophyll a because of higher thermal stability of chlorophyll b (Schwartz *et al.*, 1991). However, it was also found that percentage loss of chlorophyll a and total chlorophyll in the powder obtaining from the blanched sample was significantly lower than those of un-blanched powder. This possibly explained the more chlorophyll degradation by chlorophyllase enzyme and heating temperature during drying step of the un-blanched sample. In fact, drying process time of blanched and un-treated sample to bring the moisture content down to 5-7% were 7 and 10 h respectively. It confirmed that blanching step reduced drying

process afterward. Erge *et al.* (2008) reported that optimum temperature of chlorophyllase and other enzymes aid to senescence in vegetables ranged between 60 ºC and 82.2 ºC. It pointed out that to preserve chlorophyll content which related to color quality and other functional property, blanching process is still essential to destroy various enzyme including chlorophyllase and polyphenol oxidase. During drying step, chlorophyll b was greater changed to pheophytin b than chlorophyll a changed to pheophytin b Erge *et al.* (2008).

A higher loss of carotenoid content was found in the blanched sample. Cui *et al.* (2004) reported that carotenoid degradation depended on high temperature due to oxidation reaction can cause isomerization and convert trans- form to cis-carotenoid form leading to less intensity of carotenoid color ranging from yellow-orange.

Table 6 Chlorophylls and carotenoid content of un-treated and blanched *C. porrectum* leaves and herbal tea powder

Samples	Treatment	Content (mg/g sample)			
		Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoid
Fresh leaves	Un-treated	2.62±0.01[a]	2.11±0.01[a]	4.83±0.01[a]	0.99±0.01[a]
	Blanched	2.47±0.01[b]	2.01±0.01[b]	4.57±0.01[b]	0.89±0.01[b]
Loss by blanching (%)		5.55±0.19	4.86±0.07	5.25±0.13	10.04±0.36
Powder	Un-treated	0.54±0.01[b]	0.58±0.01[a]	1.15±0.01[b]	0.45±0.03[a]
	Blanched	0.99±0.02[a]	0.51±0.04[a]	1.53±0.04[a]	0.31±0.01[b]
Loss by drying process (%)					
	Un-treated	79.31±0.51[a]	72.38±0.70[a]	75.88±0.04[a]	54.99±2.76[b]
	Blanched	59.92±0.70[b]	74.56±1.90[a]	66.13±1.00[b]	65.55±0.90[a]

Each value was expressed as the mean ± standard deviation (n=3). Different little letters (a-b) in the same row (fresh leaves and powder) indicate significant differences ($p < 0.05$).

3.4.4. Color values

a* value of blanched leaves was higher than that of untreated leaves (Table 7). It indicated that green chlorophyll was converted to olive green pheophytins via the loss of magnesium and replaced with two hydrogen ions (Erge *et al.*, 2008). Additionally, the -a*/b* ratio which expressed the conversion of green color to the yellow color of un-treated was higher (0.31±3.23) than that of the blanched sample (0.22±3.05).

The powder obtained from the un-treated sample showed a lower -a* value than blanched might be due to chlorophyllide compound because of chlorophyllase activity (deMan, 1990). On the other hand, the powder of the blanched sample exhibited olive green-yellow color of pheophytin because of magnesium lost in chlorophyll molecule during blanching process (Gupte *et al.*, 1964 and Erge *et al.*, 2008). The -a*/b* ratio of un-treated powder was higher than that of blanched powder (Table 7).

Although the color of blanched leaves and powder of them seemed to be more yellow or less green, the herbal tea infusion was more greenness compared with

the un-blanched sample. It pointed out that higher chlorophyll content containing in the blanched powder played a key role for greenness in the tea infusion. Additionally, the result also showed that using the only color value of a*, b* and -a*/b* of dried or powder herbal tea may not provide a good reflection of entire tea infusion.

Table 7 Color value of un-treated and blanched *C. porrectum* herbal tea powder and infusion

Samples	Treatment	Parameters			
		L*	**a***	**b***	**-a*/b***
Fresh leaves	**Un-treated**	27.59±2.79[a]	-5.51±2.86[a]	17.31±3.61[a]	0.31±3.23[a]
	Blanched	21.53±3.46[b]	-3.77±2.50[a]	17.31±3.61[a]	0.22±3.05[a]
Powder	**Un-treated**	46.16±0.12[b]	-1.65±0.10[b]	25.57±0.18[b]	0.06±0.14[a]
	Blanched	49.77±0.79[a]	1.42±0.10[a]	30.93±0.22[a]	-0.04±0.16[b]
Infusion	**Un-treated**	33.59±0.35[a]	1.10±0.07[a]	22.25±0.09[a]	-0.05±0.06[b]
	Blanched	35.80±0.04[a]	-0.85±0.05[b]	13.81±0.09[b]	0.06±0.05[a]

Each value was expressed as the mean ± standard deviation (n=5).
Different little letters (a-b) in the same row (fresh leaves, powder, and infusion) indicate significant differences ($p < 0.05$).

3.4.5. Chemical constituents analyzed by GC-MS techniques

The chemical constituents of CWO quantified by GC-MS chromatogram were represented in Figure 5. The results clearly showed that safrole was the main constituent which was confirmed using the CAS# database with a GC-MS data matching factor of 97.90%. Besides safrole which made up 92.94%, elemicin (3.58%) and methyleugenol (1.51%) were found. This data was in agreement with the findings of Pattanaseree and Anatachoke, (2012), who reported that the volatile oils from the wood of *C. porrectum* from the Southern Literature Botanical Garden in Songkhla province obtained by water distillation were safrole, elemicin and methyleugenol the main chemical constituents. The GC-MS chromatogram of un-treated, freeze dried and blanched methanolic extracts were identical safrole and methyleugenol as major component. However, the methanol extracted samples showed a higher intensity of methyleugenol than safrole compared to CWO, which might be because safrole can be lost during the drying process by being vaporized to the air, while methyleugenol is stable to air, heat, and light (Tomlin, 2003). Based on the result from the GC-MS, all extracted samples had similar chemical constituents including caryophyllene, safrole, and methyleugenol. The results also indicated that un-treated extract contained the highest methyleugenol and safrole contents (4.66 and 2.36%) followed by freeze-dried extract (3.92 and 2.06%) and blanched extract (1.50 and 0.26%). The lowest concentrations of both compounds in blanched extract indicated that the blanching process can decrease the methyleugenol and safrole contents compared to un-treated and freeze-dried extracts, which may due to hydrolysis, leaching and thermal degradation effects (Charles *et al.*, 1990 and Okoh *et al.*, 2011). This result was in agreement with the finding of Diaz-Maroto *et al*. (2003) who reported that parsley dried through an air-drying process contained more volatile compounds including monoterpene than the product from a freeze-drying process. However, some compounds including acetol were more sensitive to air drying compared to freeze-drying. Moreover, Diaz-Maroto and Cabezudo, (2003) also reported that freeze-drying resulted in substantial losses of oxygenated monoterpenes in spearmint, which explains why better food quality is dependent on a proper drying method.

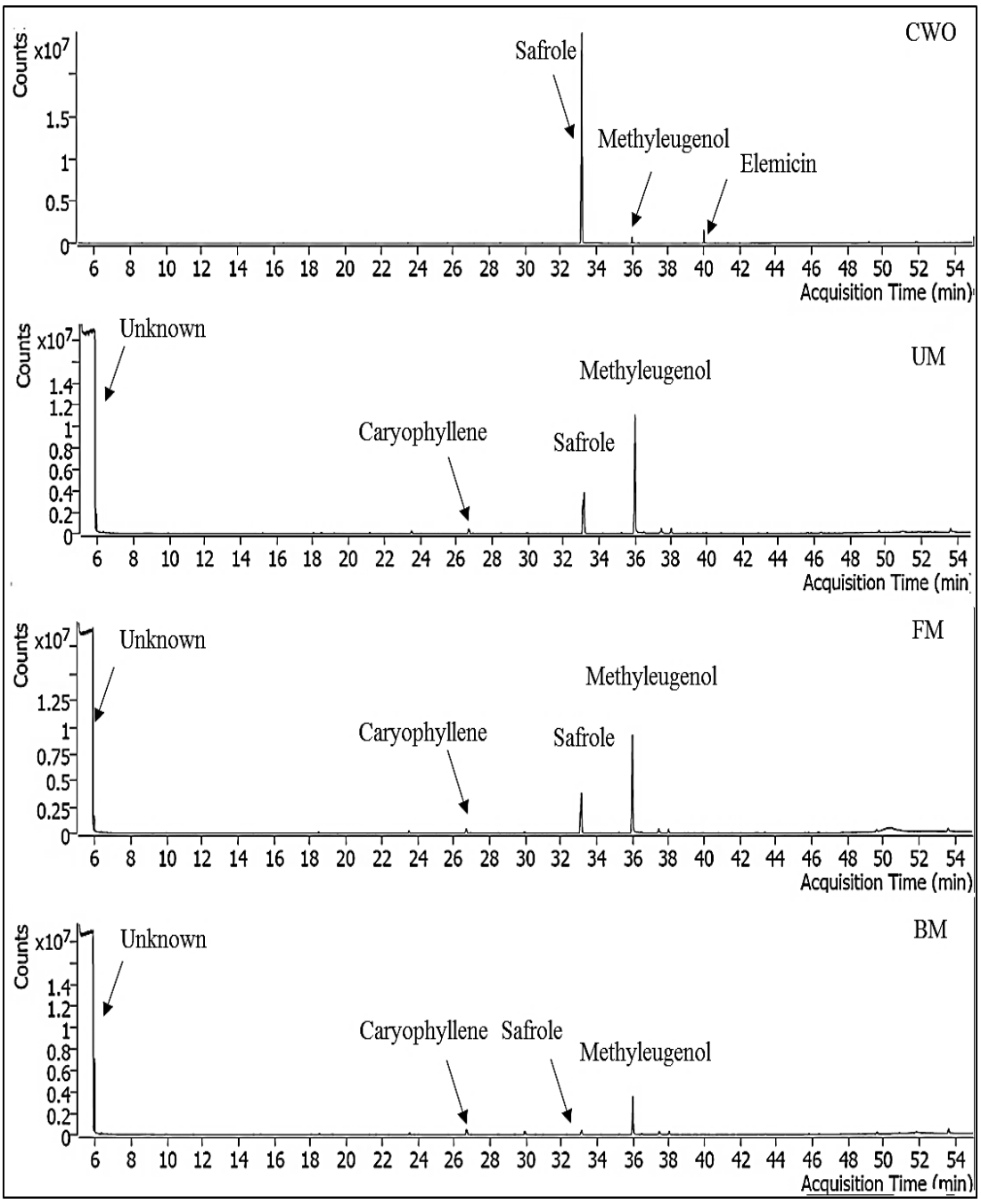


Figure 5 GC/ MS chromatogram of *C. porrectum* wood oil (CWO), un-treated methanolic extract (UM), freeze-dried methanolic extract (FM) and blanched methanolic extract (BM).

3.5. Conclusion

Blanching process can increase structure extractability. Therefore, the moisture content of blanched powder was lower than un-treated sample because of wider pore size in powder determined by SEM-technique. Green color in herbal tea infusion and lower safrole, and methyleugenol (obtained from blanched sample) was observed.

CHAPTER 4

PHENOLIC COMPOSITION AND ANTIOXIDANT ACTIVITIES OF *Cinnamomum porrectum* HERBAL TEA

5.1.Abstract

Cinnamomum porrectum leaves composed of 4 odor types such as root beer (R), cajuput (C), lemongrass with orange (L) and unidentified flower with some spice odor (F) were taken to blanch with hot water then dry at 60 ºC to reach moisture content at 5-7% and called as RB, CB, LB and FB herbal teas. Meanwhile, a control (un-blanched) sample from each odor type and dried at 60 ºC was dried as a control sample and named as RC, CC, LC and FC herbal teas. Both blanched and dried un-blanched samples were taken to ground then brought to determine the nutritional composition. The herbal tea infusions were analyzed physiochemical properties, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities including DPPH, ABTS, FRAP and ferrous ion chelating ability (FIC). The phenolic composition of herbal tea extracts was analyzed by HPLC-technique. The main nutritional composition of *C. porrectum* herbal tea powder was carbohydrate followed by protein, fat and ash contents. The moisture, ash, carbohydrate and fiber contents of the control group were higher than blanched group ($p<0.05$). The R sample showed the highest of carbohydrate and fiber contents. Without blanching process showed the highest of total solid content (TS) and total acidity (TA) with lowest of pH value. The infusion making from blanching process had a greener color than the control group ($p<0.05$). The predominant phenolics and flavonoids of all extracts consisted of seven phenolics and one flavonoid including pyrogallol, gallic acid, protocatechuic acid, catechin, caffeic acid, syringic acid, *p*-coumaric acid, and rutin. Without blanching step, the peak intensity of phenolics and flavonoids seemed to lower than blanching. Based on TPC, all herbal teas were classified as moderate with 2500 to 3800 mg GAE/ 100g. The CC sample gave the highest of all antioxidant activities while the LC sample provided the lowest activities. Only root beer odor, the TPC and TFC and antioxidant activities were significantly increased by blanching process.

5.2.Introduction

Oxidative stress is the result of the chemical imbalance between antioxidants and pro-oxidants or oxidants or reactive oxygen species (ROS). Under a sustained environmental stress if ROS are produced over a long time then significant damage may occur to cell structure and function (Khandrika *et al.*, 2009) and can damage DNA, protein and lipid (Bergamini *et al.*, 2004; Afonso *et al.*, 2007; Chan *et al.*, 2016). This imbalance leads to damage of important biomolecules and cells as well as a further whole organism (Durackova, 2010; Ambriz-Pérez *et al.*, 2016). Epidemiological and experimental evidence has shown that oxidative stress is closely related to aging and chronic diseases such as inflammation, cancer, diabetes, Alzheimer's and Parkinson's diseases (Bergamini *et al.*, 2004; Chan *et al.*, 2016).

Cinnamomum porrectum (Roxb.) Kosterm. belongs to *Cinnamomum* species with Thai name as Theptharo, is a medicinal and aromatic tree mostly distributed throughout Thailand (Pukdeekumjorn *et al.*, 2012). Pattanaseree and Anatachoke, (2012) studied on volatile oils using water distillation of *C. porrectum* leaves from Southern Literature Botanical Garden in Songkhla province, Wat Nirot Rangsi and Tai Muang farm in Pang-Nga province. Four odor types were classified including root beer, cajuput, lemongrass with orange fragrance and unidentified flower type with some spice odor. In general, the major volatile compound of each type was safrole (more than 95%), 1,8-cineole (57%), E-citral (28%) and linalool (95%), respectively.

Plants, the rich source of natural antioxidants particularly polyphenolic compounds (Tiwari *et al.*, 2010) have been found to exert their effect as an antioxidant and anti-inflammatory property via quenching the free radicals, increasing the antioxidant defense or inhibiting the release of pro-inflammatory mediators (Chan *et al.*, 2012). For centuries, herbal tea remedies have been used to treat infections, ailments, and disease (European Pharmacopoeia, 2008). Many consumers believe that herbal tea is quite safe as a natural product having a health benefit and illness solving (Chan *et al.*, 2012).

Green tea, a non-fermented tea is rapidly increasing consumption around the world (Ozturk *et al.*, 2016). The crucial step of green tea production is the heat

treatment to inhibit enzymatic browning reactions. Saetan *et al.* (2016) addressed that blanching process can decrease peroxidase (POD) and polyphenol oxidase (PPO) enzymes as well as maintained color quality related to high chlorophyll contents in root beer odor leaves. Using blanching process not only increased pore size of dried leaves powder but also all TPC, TFC extractability, and antioxidant activities, including ABTS, FRAP, and ferrous ion chelating activities (FIC) (Saetan *et al*., 2016). Moreover, Saetan *et al.* (2017) reported that blanching by hot water for 60 s can reduce toxic constituents such as safrole content (1, 3-benzodioxole, 5-(2-propenyl) and methyleugenol (4-allyl- 1,2-dimethoxybenzene) more than 89 and 68%, respectively when compared with an untreated sample. From marketing survey and personal contact, the herbal tea making from *C. porrectum* plant leaves is now famous and available in some area of Southern Thailand. However, there is no scientific data on nutritional composition, physiochemical properties, phenolic composition as well as antioxidant activities for this herbal tea. Furthermore, there is no scientific data dealing with the effect of blanching process on the tea powder and its herbal tea infusion.

5.3.Materials and methods

5.3.1. Plant material and herbal tea powder preparation

Four odor types of *C. porrectum* leaves consisted of root beer odor (R), cajuput odor (C), lemongrass with orange odor (L) and flower with spice odor (F) from Technology Research Centre of Forestry sector, Songkhla were marked with expert person, the 10% of each plant odor type was selected in the developing leaves or intermediate stage with light green- green color, flexible stalk during May to August 2015 from Technology Research Centre of Forestry sector, Songkhla. The 10 kgs of the *C. porrectum* leaves with root beer odor from 10 marked plants and 5 kgs of others odor types from 10 marked plants. The samples from each odor were pooled together in order to preserve their initial quality, leaves were stored in a refrigerator at 4 ºC and used within 1 day. The four odor types were divided into 2 groups as control (C group) which was prepared by dried at 60 ºC in hot air oven (FD 115, Binder, USA) until moisture content between 5-7% while second group was taken to blanching for 60 s before dried (B group) with the same drying temperature. All dried leaves were ground and sieved through a 60-mesh to get the fine powder before taken to keep in aluminium

foil ziplock bag at room temperature (28-30 ºC) until used within 1 month. The herbal tea powders were adjusted to analyze the nutritional composition and prepared herbal tea infusion.

5.3.2. Herbal tea infusion preparation

Each sample of herbal tea powder, 0.5 g was steeped in 100 ml DI water in a water bath (Memmert, D-91126, Schwabach, Germany) which controlled the temperature at 95 ºC for 10 min, then filtered and cooled down to reach room temperature (28-30 ºC) within 5 min to protect the heat accommodation. The filtered samples were used to analyze physiochemical properties, color value, TPC, TFC and antioxidant activities. These infusions were centrifuged at 6,000 xg for 5 min which controlled the temperature at 4 ºC (Hitachi, CR 22 G III, Tokyo, Japan). The supernatant was collected and dried by freeze dryer (called herbal tea extract) then kept in -20 ºC until used to check phenolic composition.

5.3.3. Nutritional composition analyses

Nutritional composition of each herbal tea powder including moisture, protein, fat, fiber, ash and carbohydrate contents were analyzed following the AOAC (2000).

5.3.4. Determination of physicochemical properties and color value

The physicochemical of the herbal tea infusions were analyzed including total solid (TS), total acidity was expressed as mg of citric acid/ g sample, pH value (AOAC, 2000), total and reducing sugar were expressed as mg glucose/ g sample (Nelson, 1944) and total protein content was expressed as mg BSA/ g sample (Lowry *et al.*, 1951). The color value was measured using a colorimeter (Hunter Lab, Model color Flex, Reston, VIRG, USA). Standardization of the instrument was done using a black and white Minolta calibration plate. The values were reported in the CIE color profile system as L*-value (lightness), a*-value (redness/greenness), and b*-value (yellowness/blueness). The data on -a and b were also used to calculate the -a/b ratio to express as greenness (+) or yellowness (-) color values.

5.3.5. Phenolics and flavonoids composition analysis

Determination of phenolic and flavonoid compounds of four odor types of *C. porrectum* herbal tea were performed using HPLC (High-Performance Liquid

Chromatography, Waters 717 Autosampler- Pump 600- PDA996) equipped with photodiode array detector (PDA) set at 280 nm. Briefly, 1.0 mg of the freeze-dried form of the tea extracts were hydrolyzed by 6 N HCl at ratio 1:5 (w/v) at 70 ºC for 3 h for better peak separation. The hydrolyzed sample was filtered through syringe filter nylon with 0.22 µm pore size before 10 µl of the filtered was injected into HPLC. Separation of phenolic and flavonoid compounds were performed using commercially available reverse-phase Purosher ® STAR RP-18 end-capped 5 µm LiChroCART ® 250 x 4.6 and gradient mobile phase consisted of 1.0% trifluoroacetic acid (TFA) in water (v/v), pH 1.8 (eluent A) and acetonitrile (ACN) (eluent B) following the protocol of Saetan *et al.*, 2017. All standards including pyrogallol, gallic acid, protocatechuic acid, catechin, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, rutin, ferulic acid, quercitrin, rosmarinic acid, tannic acid, quercetin, cinnamic acid, apigenin and kaempferol were purchased from Sigma Chemical Co. (St. Louis, MO, USA) before taken to determine the relationship between the concentration (X-axis) and the peak height of the standard (Y-axis).

5.3.6. Total extractable phenolic content (TPC)

TPC of the infusions was determined by using the Folin-Ciocalteau reagent following method of Chan *et al*. (2009). Briefly, sample, 50 µl, was introduced into 96-well plate followed by adding of 150 µl of Folin–Ciocalteu's reagent (10 times dilution) and 120 µl of sodium carbonate (7.5% w/v). The plates were allowed to stand for 30 min in the dark before subjected to determine absorbance at 765 nm. Gallic acid was used as the reference standard and the results were expressed as mg GAE /g sample.

5.3.7. Total extractable flavonoid content (TFC)

TFC of the infusions was determined by using Aluminum chloride colorimetric method following method of Lobo *et al.* (2011). Briefly, 25 µl of the extracts were added to the 96-well plate containing 100 µl of water. At zero time, 10 µl of 5% $NaNO_2$ was added then, 5 min later, 15 µl of 10% $AlCl_3$ was followed. After that, 50 µl of 1 M NaOH was added to the mixture and the volume was made up to 250 µl with water. An absorbance was measured at 510 nm. Catechin was used as the reference standard and the results were expressed as mg CE /g sample.

5.3.8. Antioxidant activities of *C. porrectum* herbal tea

a) DPPH radical-scavenging activity

DPPH radical scavenging capacity assay was evaluated with the following method of Udayaprakash *et al.* (2014). 0.15 mM DPPH was prepared using methanol to obtain an absorbance of 0.8± 0. 1 units at 517 nm. Briefly, various concentrations of test samples (100 µl) was mixed with DPPH solution. After 30 min of incubation at room temperature in the dark, the absorbance was measured against a blank (methanol) at 517 nm using a spectrophotometer. Sample blank was prepared in the same manner except methanol was used instead of DPPH solution. A standard curve was made by using Trolox at 5-25 µg/ml and the results were expressed as mg of Trolox equivalent (TE)/g sample.

b) ABTS radical-scavenging activity

ABTS radical- scavenging activity was evaluated with the following method of Arnao *et al.* (2001). 7. 4 mM ABTS solution and 2. 6 mM potassium persulphate solution were prepared as stock solutions. The working solution was prepared by mixing the two stock solutions in equal quantities. The mixture was allowed to react for 12 h at ambient temperature in the dark. The mixed solution was diluted by mixing 1 ml of ABTS solution with 50 ml of water in order to obtain an absorbance of 1. 1± 0. 02 units at 734 nm. Briefly, sample, 15 µl, as mixed with 285 µl of ABTS solution and the mixture was left at ambient temperature for 2 h in the dark. The absorbance was measured at 734 nm using a microplate spectrophotometer. Sample blank was prepared in the same manner by using water instead of ABTS solution. A standard curve was made by using Trolox at 50- 125 µg/ ml and the results were expressed as mg of Trolox equivalent (TE)/g sample.

c) FRAP (ferric reducing antioxidant power)

FRAP (ferric reducing antioxidant power) was assayed according to Benzie and Strain, (1996). 300 mM acetate buffer (pH 3. 6), 10 mM TPTZ (2, 4, 6-tripyridyl- *s*- triazine) solution dissolved in 40 mM HCl, and 20 mM $FeCl_3. 6H_2O$ solution were made for stock solutions. A working solution was prepared freshly by mixing 25 ml of acetate buffer, 2. 5 ml of TPTZ solution and 2. 5 ml of $FeCl_3. 6H_2O$ solution. The mixed solution was incubated at 37 °C for 30 min in the incubator and

referred as FRAP solution. Later, sample, 15 µl, was mixed with 285 µl of FRAP solution and kept at ambient temperature for 30 min in dark. The ferrous tripyridyltriazine complex (blue colored product) was measured by reading the absorbance at 593 nm. Sample blank was prepared by omitting $FeCl_3$ from FRAP solution and distilled water was used instead. A standard curve was made by using Trolox at 50-125 µg/ml. The results were expressed as mg of Trolox equivalent (TE)/g sample.

d) Ferrous ion chelating activity (FIC)

The ferrous chelating activity was measured by the method of Boyer and McCleary, (1987). Briefly, 1.0 ml of each sample substance was mixed with 0.1 ml of 0.2 mM $FeCl_2$. The reaction mixture was allowed to stand for 10 minutes at ambient temperature and then 0.2 ml of 5 mM ferrozine was added. The mixture was stand for more than 10 minutes at ambient temperature. The absorbance was then read at 562 nm. A blank sample was prepared in the same manner using distilled water instead of the sample and blank samples of each of the substances under examination with the $FeCl_2$ solution excluded and distilled water used instead were also prepared. The standard curve was constructed using the ethylenediaminetetraacetic acid (EDTA) ranking from 10-50 µg/ml. The activity was expressed as mg EDTA equivalent/ g sample.

5.3.9. Statistical analysis

All the results were expressed as the mean ± SD (n=3) except color values (n=5). The data were subjected to analysis of variance (ANOVA) to detect potential differences. The significant differences among the means were established by Duncan's test and *p*-values < 0.05 were considered to be significant. The T-test was used for analysis between-group comparisons of C group and B group.

5.4.Results and discussion

5.4.1. Proximate composition of *C. porrectum* herbal tea powder

The proximate compositions of all herbal tea powder were presented in Table 8. Actually, the initial percentage of moisture content in fresh leaves of R, C, L and F samples were 57.06%, 57.11%, 58.15% and 55.96%, respectively (data did not show). Thereafter, the percentage of moisture content of the powder from control group was around 5.02% to 5.47% while from blanched group was around 4.44 to 4.76%. It indicated that blanching process helped to open the leaf structure then facilitated them easier for water vaporization during the drying step (Ozilgen *et al.,* 2001; Saetan *et al.,* 2016).

Without blanching process, the FC sample showed the highest of the percentage of ash content (6.00%) and RC sample showed the lowest content (4.54%). With blanching process, the ash content was significantly decreased ($p<0.05$) when compared with the un-treated leaves might be due to the leaching effect during hot water blanching process. This result was in agreement with Dugo *et al.* (2005) who reported a loss of minerals during the boiling of vegetables and tubers including carrots, bamboo shoots, broccoli, potatoes, and cocoyam due to water leaching effect (Lewu *et al*., 2010).

The crude protein content ranged 7.12 to 10.88%, the LC showed the highest content while RC showed the lowest content in control group. When compared with blanched group the protein content of all odors seemed to increase except cajuput odor. The crude fat content ranged 3.78 to 6.15%, the flower with spice odor showed the highest content followed by lemongrass with orange odor. The result indicated that blanched group provided a higher content of crude protein and crude fat might be due to the structure of plant tissues was opened and loosened through β-sheet destruction during the blanching process (Sila *et al.,* 2009). Thereafter, having a wider porosity led to increase the permeability of the cell walls and improve solvent diffusivity, resulting in an increase in yield extractability (Deylami *et al.,* 2016; Stamatopoulos *et al.*, 2016).

The carbohydrate content of the samples without blanching process ranged 73.36 to 78.09%. The RC showed the highest content followed by CC, FC, and LC. With blanching process, RB showed the highest content followed by CC, FB, and

LB. In general, the carbohydrate of the sample without blanching seemed to be higher than the blanched group. Actually, there are two major types of carbohydrates found in plant leaves; complex carbohydrates such as cellulose and simple carbohydrates such as monosaccharide like glucose, fructose and galactose and disaccharides like maltose, lactose, and sucrose (Navarro-González *et al.*, 2015). Decreasing of carbohydrate content in the blanched sample may indicate a loss of simple sugars because of higher water-soluble compared with complex compounds.

Table 8 Proximate composition of *C. porrectum* herbal tea powder influenced by odor types and blanching process

Sample	Nutritional composition of *C. porrectum* power (% dried basic)					
	moisture content (%)	ash (%)	protein (%)	fat (%)	carbohydrate (%)	fiber (%)
RC	5.47 ± 0.02^{Aa}	4.54 ± 0.11^{Da}	7.12 ± 0.00^{Cb}	3.78 ± 0.12^{Db}	79.09 ± 0.26^{Aa}	10.04 ± 0.09^{Aa}
CC	5.46 ± 0.25^{Aa}	4.93 ± 0.15^{Ca}	8.35 ± 0.29^{Ba}	4.21 ± 0.12^{Cb}	77.05 ± 0.44^{Ba}	8.24 ± 0.16^{Ba}
LC	5.02 ± 0.25^{Aa}	5.19 ± 0.03^{Ba}	10.88 ± 0.03^{Aa}	5.55 ± 0.03^{Bb}	73.36 ± 0.05^{Da}	8.28 ± 0.15^{Ba}
FC	5.36 ± 0.32^{Aa}	6.00 ± 0.10^{Aa}	8.50 ± 0.17^{Ba}	6.11 ± 0.02^{Aa}	74.03 ± 0.07^{Ca}	7.71 ± 0.31^{Ca}
RB	4.44 ± 0.17^{Bb}	4.35 ± 0.07^{Db}	8.02 ± 0.00^{Da}	5.83 ± 0.07^{Ca}	77.35 ± 0.09^{Ab}	10.00 ± 0.33^{Aa}
CB	4.74 ± 0.18^{Ab}	4.67 ± 0.10^{Cb}	8.12 ± 0.04^{Ca}	5.48 ± 0.07^{Da}	76.99 ± 0.09^{Bb}	8.53 ± 0.26^{Ba}
LB	4.76 ± 0.14^{Aa}	4.99 ± 0.19^{Bb}	10.83 ± 0.08^{Aa}	5.95 ± 0.09^{Aa}	72.47 ± 0.26^{Db}	8.44 ± 0.18^{Ba}
FB	4.67 ± 0.12^{Ab}	5.47 ± 0.20^{Ab}	8.74 ± 0.11^{Ba}	6.15 ± 0.14^{Ba}	74.96 ± 0.09^{Ca}	7.79 ± 0.23^{Ca}

Values are presented as mean ± SD (n=3).

Different uppercase letters in the same column within the same leaves odor indicate significant difference ($p< 0.05$).

Different lowercase letters in the same column within the same process indicate significant difference ($p<0.05$).

The RC, CC, LC and FC samples and the RB, CB, LB and FB samples denote *C. porrectum* leaves with root beer, cajuput, lemongrass with orange and flower with spice odor produced as herbal tea by un-treated (control) and blanched processes, respectively.

5.4.2. Physicochemical properties and color value of *C. porrectum* herbal tea infusion

The herbal tea infusions were prepared by steeping the powder in hot water before the filtered infusion were taken to analyze the physiochemical properties. The CC sample revealed the highest of total solid content (TS) with the highest of total acidity and reducing sugar as shown in Table 9. However, the LC sample contained the lowest total protein content even crude protein content in the powder was highest this may be the fact that protein can form complex with other food compounds including polyphenols (Ozdal *et al.*, 2013). Therefore, odor types and preparation including blanching process method may affect some chemical values.

Generally, blanched sample provided a lower pH value which related to higher total acidity and TS when compared with un-blanched in same odor leaves. The increase in the total sugar in the blanched group was presented in Table 9 may due to carbohydrates such as pectin were hydrolyzed and generated galacturonic acid leading to a decrease in pH value (Vergara-Salinas *et al.*, 2015). The total protein content of blanched group was lower than that of un-treated group except LB sample might be due to thermal disruption of the polyphenol-protein complexes and leaching effect (Xiao *et al.*, 2017).

Table 9 Physical properties of *C. porrectum* herbal tea infusion influenced by odor types and blanching process

Samples	pH	Total acidity (mg citric acid/ g)	Total solid (%)	Total sugar (mg glucose / g)	Reducing sugar (mg glucose / g)	Total protein (mg BSA / g)
RC	5.62 ± 0.01^{Ca}	0.70 ± 0.00^{Bb}	0.24 ± 0.00^{Bb}	181.48 ± 1.71^{Cb}	63.11 ± 0.17^{Ca}	315.23 ± 1.83^{Ba}
CC	5.57 ± 0.01^{Db}	0.73 ± 0.00^{Aa}	0.27 ± 0.00^{Aa}	224.17 ± 1.09^{Ba}	89.70 ± 0.68^{Ab}	308.91 ± 0.74^{Ba}
LC	5.86 ± 0.02^{Aa}	0.62 ± 0.00^{Cb}	0.24 ± 0.00^{Bb}	172.81 ± 2.12^{Db}	68.10 ± 0.20^{Ba}	300.19 ± 1.11^{Bb}
FC	5.75 ± 0.01^{Ba}	0.69 ± 0.00^{Ba}	0.25 ± 0.05^{Aa}	231.04 ± 3.28^{Ab}	68.40 ± 0.40^{Ba}	415.44 ± 0.37^{Aa}
RB	5.59 ± 0.01^{Db}	0.72 ± 0.00^{Aa}	0.28 ± 0.02^{Aa}	231.64 ± 1.69^{Ba}	60.81 ± 0.42^{Cb}	265.62 ± 2.17^{Cb}
CB	$5.61\pm.001^{Ca}$	0.70 ± 0.00^{Ba}	0.28 ± 0.00^{Aa}	204.12 ± 1.97^{Cb}	97.68 ± 0.35^{Aa}	$231.79\pm.74^{Db}$
LB	5.71 ± 0.01^{Ab}	0.68 ± 0.00^{Cb}	0.28 ± 0.01^{Aa}	200.00 ± 3.23^{Ca}	64.33 ± 0.35^{Bb}	324.72 ± 1.48^{Aa}
FB	5.58 ± 0.01^{Bb}	0.73 ± 0.00^{Aa}	0.28 ± 0.03^{Aa}	281.56 ± 3.29^{Aa}	65.60 ± 1.44^{Bb}	303.23 ± 0.99^{Bb}

Values are presented as mean ± SD (n=3).

Different uppercase letters in the same column within the same leaves odor indicate significant difference ($p< 0.05$).

Different lowercase letters in the same column within the same process indicate significant difference ($p<0.05$).

The RC, CC, LC and FC samples and the RB, CB, LB and FB samples denote *C. porrectum* leaves with root beer, cajuput, lemongrass with orange and flower with spice odor produced as herbal tea by un-treated (control) and blanched processes, respectively.

The color values of all herbal tea infusions were in yellow-green color, L* ranged 28.41 to 35.24, a* ranged -1.22 to 1.84, b* ranged 9.40 to 26.22 and –a*/b* ranged -0.07 to 0.09 (Table 10). The data from -a*/b* indicated that color from un-blanched tea was a yellow color that might be due to enzymatic activity especially polyphenol oxidase (PPO) and chlorophyllase induction during the drying process (Erge *et al.*, 2008; Saetan *et al.*, 2016). The blanched infusion had a greener color compared with un-blanched sample indicated the achievement of blanching process to inactivate the chlorophyllase enzyme leading to a higher chlorophyll content in the powder. It pointed out that chlorophyll content played a key role in the greenness of the tea infusion which was in agreement with the finding of Seatan *et al.* (2016).

Table 10 Color values of *C. porrectum* herbal tea infusion influenced by odor types of leaves and blanching process

Samples	Color values			
	L*	**a***	**b***	**-a/b**
RC	32.00 ± 0.06^{Cb}	1.39 ± 0.07^{Ba}	23.35 ± 0.08^{Ca}	-0.06 ± 0.00^{Bb}
CC	33.14 ± 0.08^{Aa}	0.28 ± 0.04^{Da}	22.38 ± 0.15^{Da}	-0.01 ± 0.00^{Ab}
LC	31.81 ± 0.09^{Db}	1.84 ± 0.12^{Aa}	26.22 ± 0.08^{Aa}	-0.07 ± 0.00^{Cb}
FC	32.13 ± 0.03^{Ba}	1.41 ± 0.05^{Ba}	24.79 ± 0.12^{Ba}	-0.06 ± 0.00^{Bb}
RB	35.24 ± 0.04^{Aa}	-0.90 ± 0.05^{Bb}	15.52 ± 0.15^{Bb}	0.06 ± 0.00^{Ca}
CB	29.21 ± 0.02^{Cb}	-0.81 ± 0.03^{Bb}	9.40 ± 0.04^{Db}	0.09 ± 0.00^{Aa}
LB	35.05 ± 0.13^{Ba}	-1.22 ± 0.07^{Cb}	18.38 ± 0.14^{Ab}	0.07 ± 0.00^{Ba}
FB	28.41 ± 0.02^{Db}	-0.30 ± 0.06^{Ab}	10.13 ± 0.09^{Cb}	0.03 ± 0.01^{Da}

Values are presented as mean ± SD (n=3). Different uppercase letters in the same column within the same leaves odor indicate significant difference ($p< 0.05$). Different lowercase letters in the same column within the same process indicate significant difference ($p<0.05$). The RC, CC, LC and FC samples and the RB, CB, LB and FB samples denote *C. porrectum* leaves with root beer, cajuput, lemongrass with orange and flower with spice odor produced as herbal tea by un-treated (control) and blanched processes, respectively.

5.4.3. Phenolic composition of *C. porrectum* herbal tea hydrolyzed extract

Thirteen phenolics and five flavonoids including pyrogallol, gallic acid, protocatechuic acid, catechin, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, rutin, ferulic acid, quercetrin, rosmarinic acid, tannic acid, quercitrin, cinnamic acid, apigenin, and kaempferol were used as standards. Typical chromatograms of phenolics and flavonoids standards of four odor herbal tea extracts were expressed in Figure 6 and 7 (a and b). According to the HPLC technique in this experiment, the peaks were eluted based on their polarity and molecule size (Haghi *et al.*, 2008). The phenolics and flavonoids of all hydrolyzed extracts were quite similar but different in peak intensity. The predominant phenolics and flavonoids (Table 11) of un-blanched samples (control group) including RC were protocatechuic acid and catechin; CC was gallic acid and syringic acid; LC was pyrogallol, caffeic acid, *p*-coumaric acid and rutin and FC were pyrogallol, *p*-coumaric acid, and rutin. While the predominant phenolics and flavonoids of the blanched group including RB was catechin, *p*-coumaric acid, and rutin; CB was protocatechuic acid, syringic acid and rutin; LB was pyrogallol and syringic acid and rutin and FB was pyrogallol, caffeic acid and rutin. The data from HPLC technique indicated that the main phenolics and flavonoids in all odor types of leaves were pyrogallol, catechin, caffeic acid, syringic acid, *p*-coumaric acid and rutin (Table 11). From the result, it was found that blanched group provided a higher intensity of main predominant phenolics and flavonoids than those control group might be due to more extractable ability as discussed earlier in physiochemical properties.

These results were in agreement with the report of Cai *et al*. (2004) who stated that phenolic compounds in the aqueous extract of *C. cassia* bark mainly contained cinnamic acid, protocatechuic acid, courmarin and tannins. Prasad *et al*. (2009) reported that five species of *Cinnamomum* leaves including *C. burmanni, C. cassia, C. pauciflorum, C. tamala* and *C. zeylanica* extracted by 50% ethanol contained 3 main flavonoids including quercetin, kaempferol, and quercitrin. Moreover, Li *et al.* (2008) and Yang *et al.* (2012) reported that rutin was the main flavonoid compound found in *C. zeylanicum* and *C. cassia*. According to HPLC profile results, the total

extractable phenolic and flavonoid contents of the blanched group appeared to be greater than that of the control group. Among all of the identified phenolic acid and flavonoid constituents, it pointed out that some of the phenolics seemed to increase, while some phenolics and flavonoids decreased as presented in Table 10. To confirm phenolics and flavonoids, sample peaks were rechecked by liquid chromatography-mass spectrometry analysis (LC/MS) (data not are shown). In general, blanching process not only increased the predominant of phenolic acids such as protocatechuic acid but also generated two higher intensity of unknown compounds compared with the un-blanched sample. In addition, the unknown peak (1 and 2) in all samples represented as time 17 and 20 min quite closed to the retention time of protocatechuic acid peak (R_t at 19 min).

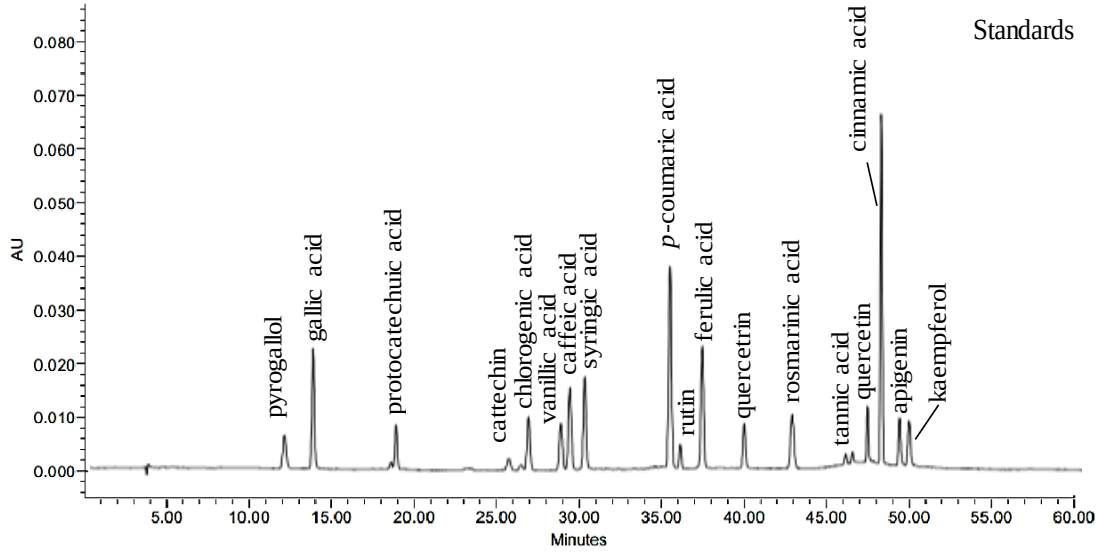


Figure 6 HPLC chromatogram of phenolics and flavonoid standards.

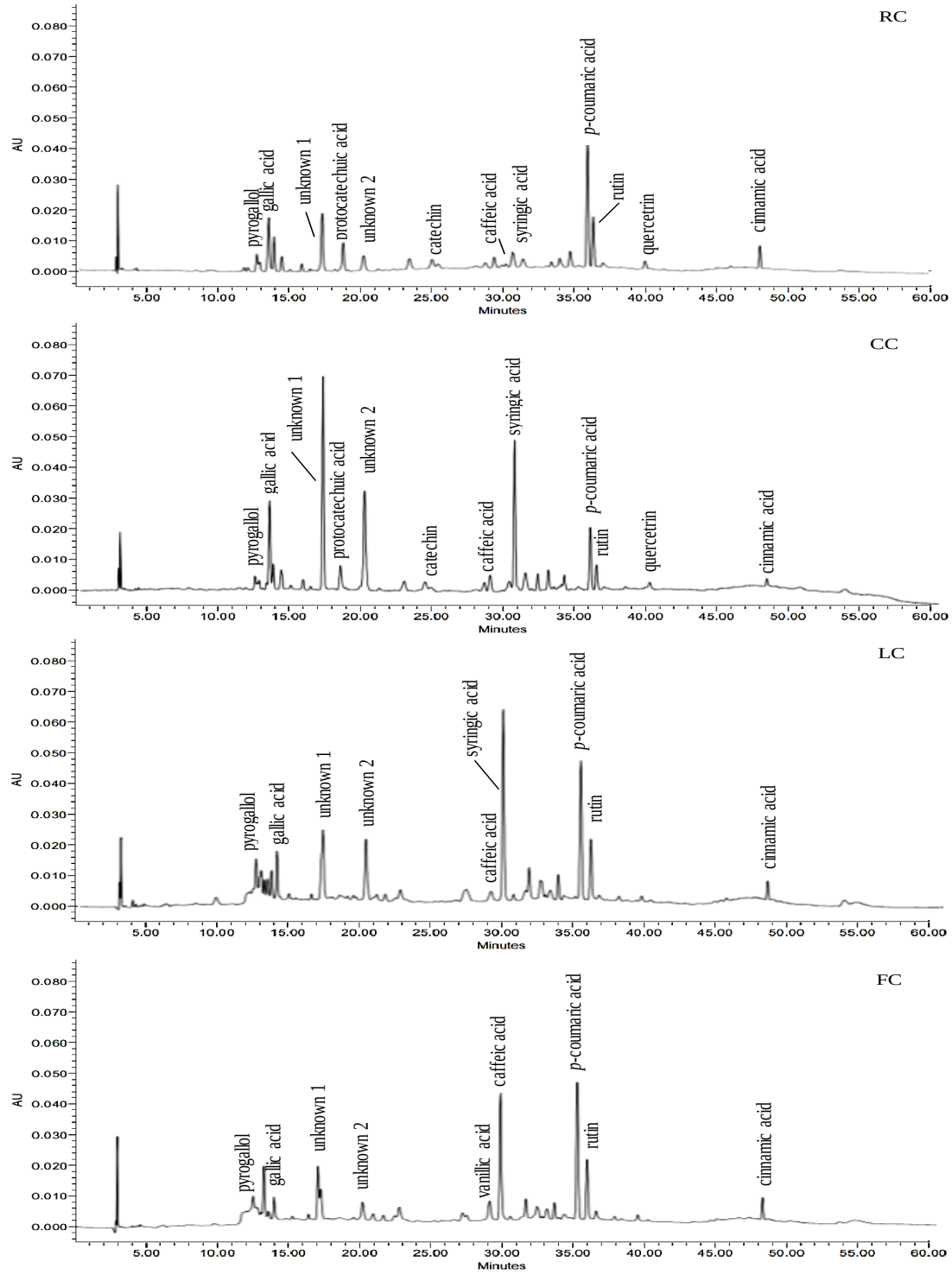


Figure 7 (A) HPLC chromatogram of *C. porrectum* herbal tea hydrolyzed extracts. The RC, CC, LC and FC samples denote un-treated samples from *C. porrectum* leaves with root beer, cajuput, lemongrass with orange and flower with spice odor, respectively.

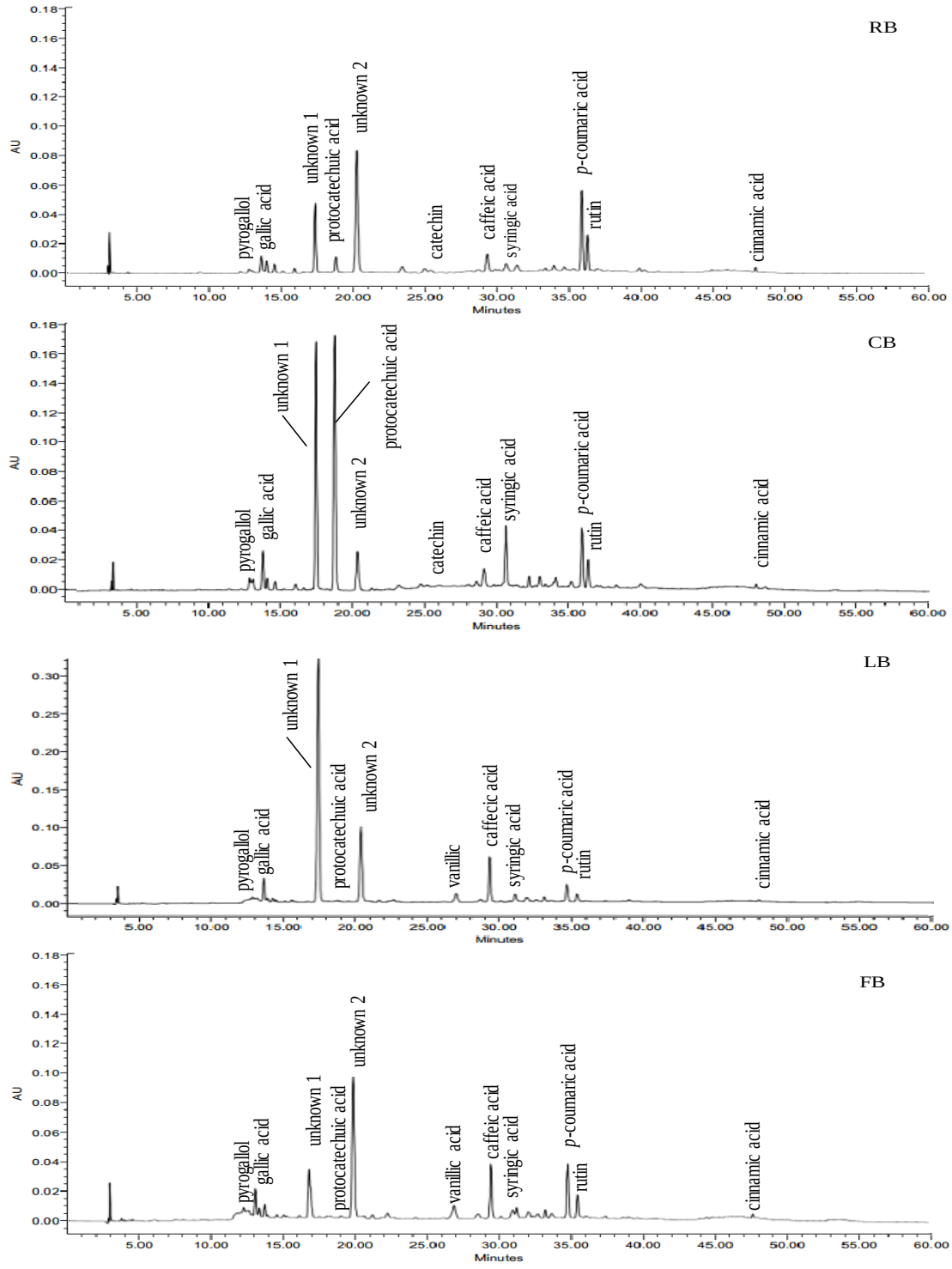


Figure 7 (B) HPLC chromatogram of *C. porrectum* herbal tea hydrolyzed extracts. The RB, CB, LB and FB samples denote blanched samples from *C. porrectum* leaves with root beer, cajuput, lemongrass with orange and flower with spice odor, respectively.

Table 11 Phenolics and flavonoids composition of *C. porrectum* herbal tea hydrolyzed extracts

	Phenolic acids and flavonoid compounds (mg/ 100 g of hydrolyzed extract)							
Name	pyrogallol	gallic acid	protocatechuic acid	catechin	caffeic acid	syringic acid	*p*-coumaric acid	rutin
RC	114.33±0.04^{Ba}	39.50±1.27^{Ba}	59.30±0.28^{Ab}	33.75±4.31^{Ab}	12.80±0.85^{Bb}	3.40±0.71^{Ca}	56.30±0.14^{Ab}	187.80±1.27^{Bb}
CC	50.05±1.77^{Cb}	62.65±2.76^{Aa}	3.90±0.00^{Bb}	27.75±4.17^{Ba}	1.50±0.00^{Db}	149.03±1.32^{Aa}	28.00±0.14^{Bb}	92.50±1.41^{Cb}
LC	299.20±8.34^{Aa}	33.80±0.42^{Bb}	9.46±0.65^{Ba}	3.75±0.07^{Da}	202.25±6.01^{Aa}	31.15±0.78^{Ba}	59.40±2.12^{Aa}	214.70±2.40^{Aa}
FC	312.15±15.34^{Ab}	30.35±12.52^{Ba}	5.35±2.62^{Bb}	7.80±0.00^{Ca}	135.65±3.32^{Ba}	3.70±0.28^{Ca}	61.15±1.06^{Aa}	217.35±0.35^{Aa}
RB	88.24±3.20^{Cb}	24.04±1.17^{Ca}	64.04±2.16^{Ba}	40.08±0.38^{Aa}	39.57±1.85^{Ba}	3.62±0.83^{Ca}	73.11±0.59^{Aa}	267.08±4.04^{Aa}
CB	58.65±0.74^{Da}	51.72±9.28^{Ba}	1052.75±17.19^{Aa}	9.83±1.99^{Bb}	36.34±1.64^{Ba}	118.84±0.66^{Bb}	53.84±0.71^{Ba}	213.15±7.39^{Ba}
LB	283.90±1.68^{Ba}	68.89±0.12^{Aa}	11.36±0.13^{Ca}	5.72±3.30^{Ca}	10.35±0.23^{Cb}	164.56±1.48^{Aa}	30.11±0.50^{Cb}	115.57±3.68^{Cb}
FB	376.40±8.28^{Aa}	20.22±0.22^{Ca}	9.20±1.39^{Ca}	5.03±0.69^{Ca}	122.83±0.85^{Ab}	4.32±0.18^{Ca}	49.08±0.65^{Bb}	173.30±4.02^{Cb}
	Increasing or decreasing after blanching (%)*							
R	-22.82±2.82	-39.07±4.92	8.00±4.16	19.66±14.16	210.26±34.99	6.09±2.27	29.87±1.38	42.21±1.19
C	17.27±5.63	-17.70±11.20	26893.46±440.76	-64.72±1.88	2322.67±103.37	-20.26±0.26	92.28±1.57	130.40±4.47
L	-5.07±3.21	103.82±2.20	20.42±9.63	53.26±90.95	-94.88±0.27	428.49±17.94	-49.27±2.66	-46.16±2.32
F	20.66±3.28	-27.37±29.23	88.12±66.09	-35.51±8.88	-9.42±2.84	16.91±3.97	-19.74±0.33	-20.27±1.72

Values are presented as mean ± SD (n=3). Different uppercase letters in the same column within the same leaves odor indicate significant difference ($p< 0.05$). Different lowercase letters in the same column within the same process indicate significant difference ($p<0.05$).
*Means % increased calculated by phenolic contents of (blanching-control)/control x 100. The RC, CC, LC and FC samples and the RB, CB, LB and FB samples denote *C. porrectum* leaves with root beer (R), cajuput (C), lemongrass with orange (L) and flower with spice (F) odor produced as herbal tea by un-treated (control) and blanched processes, respectively.

5.4.4. Effect of various odor types of leaves and blanching process on total extractable phenolic content (TPC), total extractable flavonoid content (TFC), and antioxidant activities in *C. porrectum* herbal tea infusion

Several available assays are used to measure the antioxidant capacity of natural products (Galano *et al.*, 2015). The methods of measuring total antioxidant capacity *in vitro* can be classified into three groups: (1) based on hydrogen atom transfer (HAT), (2) based on single electron transfer (SET) and (3) combination of the HAT and SET mechanisms (Liang *et al.*, 2014). Any product containing TPC $\geq$ 5000 mg/ 100 g, $\geq$1000 < 5000 mg/ 100 g and < 1000 mg/100 g is classified as having a high, moderate or low TPC content, respectively (Chan *et al.*, 2012). Therefore, the herbal tea in this experiment was classified as a moderate group, because of both control group and a blanched group containing TPC around 2500 to 3800 mg GAE/ 100 g (Figure 8 (A)). Moreover, the FC showed the highest of TPC followed by CC, RC, and LC, respectively. However, after blanching some odor types including C and F contained lesser TPC. The TPC values of all samples were in agreement with previous experiment reported by Cai *et al.* (2006) and Ademe *et al.* (2015) who stated that the TPC values from the *C. cassia* bark water extract (18.70 mg GAE/g) and that from a *C. zeylanicum* infusion (29.32 mg GAE/g). Moreover, the TPC values of the extracts in the present study were higher than that of *C. porrectum* wood water extracts (26.41 mg GAE/g) (Pukdeekumjorn *et al.*, 2012). The RB showed the highest of TFC while LC and FB showed the lowest. However, the metal chelating activity (Figure 10 (B) of the two groups was not significantly different except FC sample. It pointed out that using only TFC may not be a good indicator of metal chelating activity which is related to the free-form rather than the bound form (Chandrasekara *et al.*, 2010; Symonwicz *et al.*, 2012).

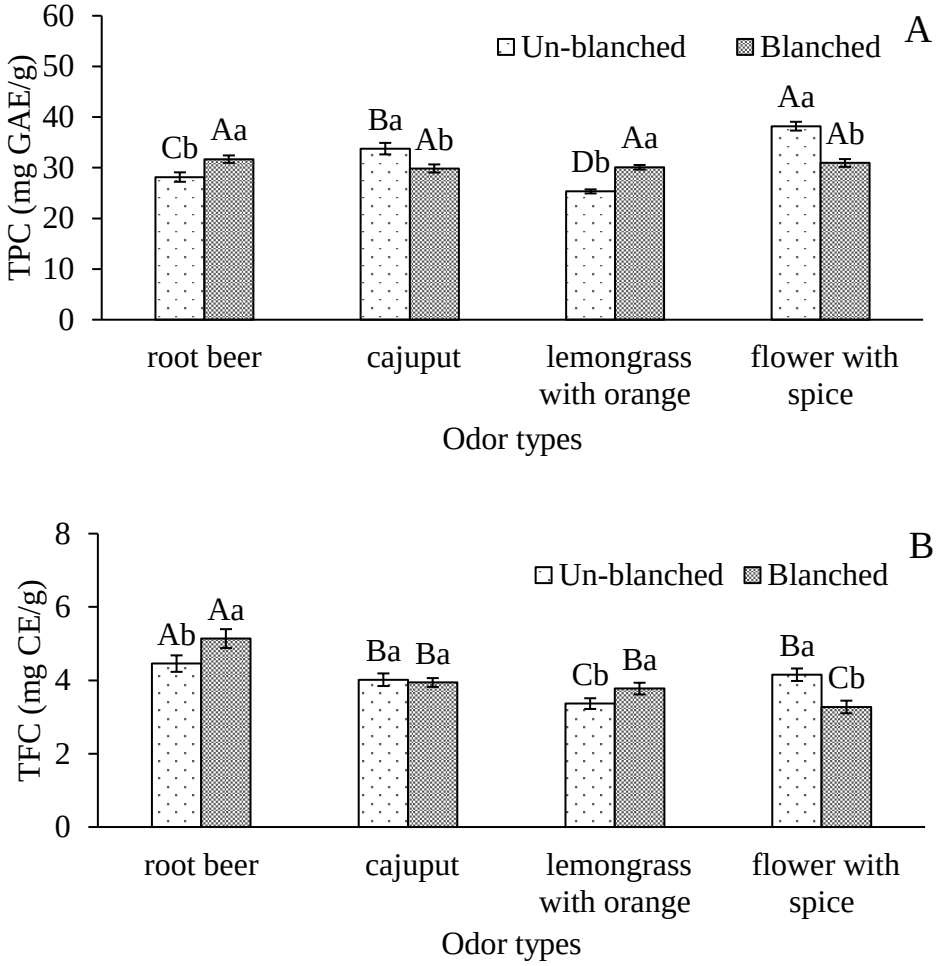


Figure 8 Effect of various odor types of leaves and blanching process of *C. porrectum* herbal tea infusion on total extractable phenolic content (TPC) (A) and total extractable flavonoid content (TFC) (B). Different uppercase letters indicate significant difference ($p < 0.05$). Different lowercase letters indicate significant difference ($p<0.05$).

The results in Figure 9 (A) showed that there was no significant difference in the DPPH assay by blanching effect except the C odor. When compared to different odor leaves, the result showed that the C and the L showed the highest of DPPH followed by the R and the F odors, respectively. The ABTS assay showed the highest activity in CC followed by FC, RC, and LC, respectively while the ABTS activity of blanched group seemed to lower than control group except for the R odor. The FRAP assay indicated that CC showed the highest activity than other samples.

When comparison all antioxidant activities, the results indicated that CC provided the highest of all antioxidant activities. Although CC showed the highest of all antioxidant activities, it was second in TPC next to FC might be due to the Folin-Ciocateu's reagent could also interact with various non-phenolic organic compounds especially aromatic amino acid as well as some inorganic substances to give elevated apparent phenolic content (Lowry, 1951; Pękal *et al.*, 2014).

The results also showed that the R odor provided the highest of TFC while, CC provided the highest of DPPH, ABTS and FRAP activities. In addition, without blanching process, FC contained the highest of TPC while LC showed the lowest of TPC, TFC and ABTS activities. On the other hand, the antioxidant activities of the blanched group seemed to lower activities than control group except the R and the L odors.

The highest of TPC but lowest of DPPH activity found in FC might be related with the data from physiochemical properties that explained by Labuckas *et al*. (2008) who reported that soluble protein might decrease opportunity of phenolics to react with DPPH radicals because of protein-phenolic interaction. Furthermore, phenolics can bind with sugar as glycoside structure leading large structure of phenolics or call as bound phenolics thereafter difficult to react with DPPH radical as a steric effect (Cai *et al.*, 2006).

Based on antioxidant assays using Trolox equivalent, the results showed that the ABTS assay provided the highest content followed by FRAP and DPPH assays. Although the DPPH and ABTS assays were determined by the same mechanisms (HAT and SET), the DPPH radical prefers methanol or ethanol solvents while the ABTS radical can be used for a wide range of solvents including methanol or ethanol or phosphate-buffered and aqueous solvents. In addition, ABTS assay is more flexible and

can be used at different pH levels compared to DPPH, which is sensitive to an acidic pH (Ou *et al.*, 2001; Shalaby *et al.*, 2013). In addition, it was noticed that antioxidants having big molecules or steric effect to difficult was react with DPPH radical.

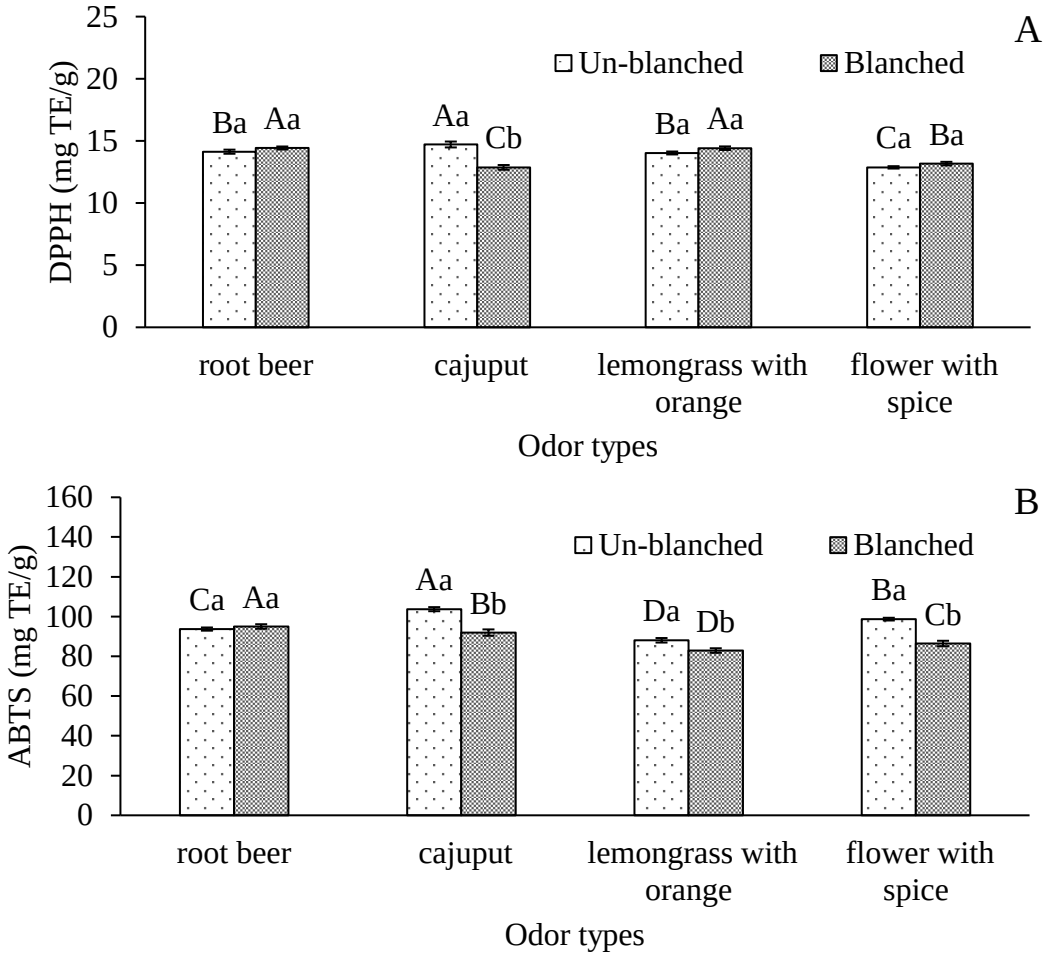


Figure 9 Effect of various odor types of leaves and blanching process of *C. porrectum* herbal tea infusion on DPPH (A) and ABTS (B) activities. Different uppercase letters indicate significant difference ($p < 0.05$). Different lowercase letters indicate significant difference ($p<0.05$).

FRAP is used to measure the reductive ability of antioxidant by the transformation of ferric-TPTZ (Fe(III)-TPTZ) complex to ferrous-TPTZ (Fe(II)-TPTZ) complex. The ability of samples to reduce Fe(III)-TPTZ may be due to the hydrogen donation from phenolic compounds which is also related to the presence of reducing agent (Huda *et al.*, 2009). The FRAP activity of *C. porrectum* herbal tea, when compared with different odor types of leaves and blanching process, were presented in Figure 10 (A). Without blanching step, the CC provided the highest of FRAP activity followed by FC while no significantly different in RC and LC. However, the declined in FRAP activity after blanching was founded in some odor types including C and F might be related to TPC and TFC.

In general, free radical scavenging or antioxidant activity of phenolics mainly depends on the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the phenolic molecules (Cai *et al.*, 2004). The results suggested that different odor types of leaves provided different types and form of phenolics with a high variation of scavenging capacity. As a consequence, different assays should be conducted to verify the antioxidant activity of various compounds, in which mode of action could be different (Maqsood *et al.*, 2010).

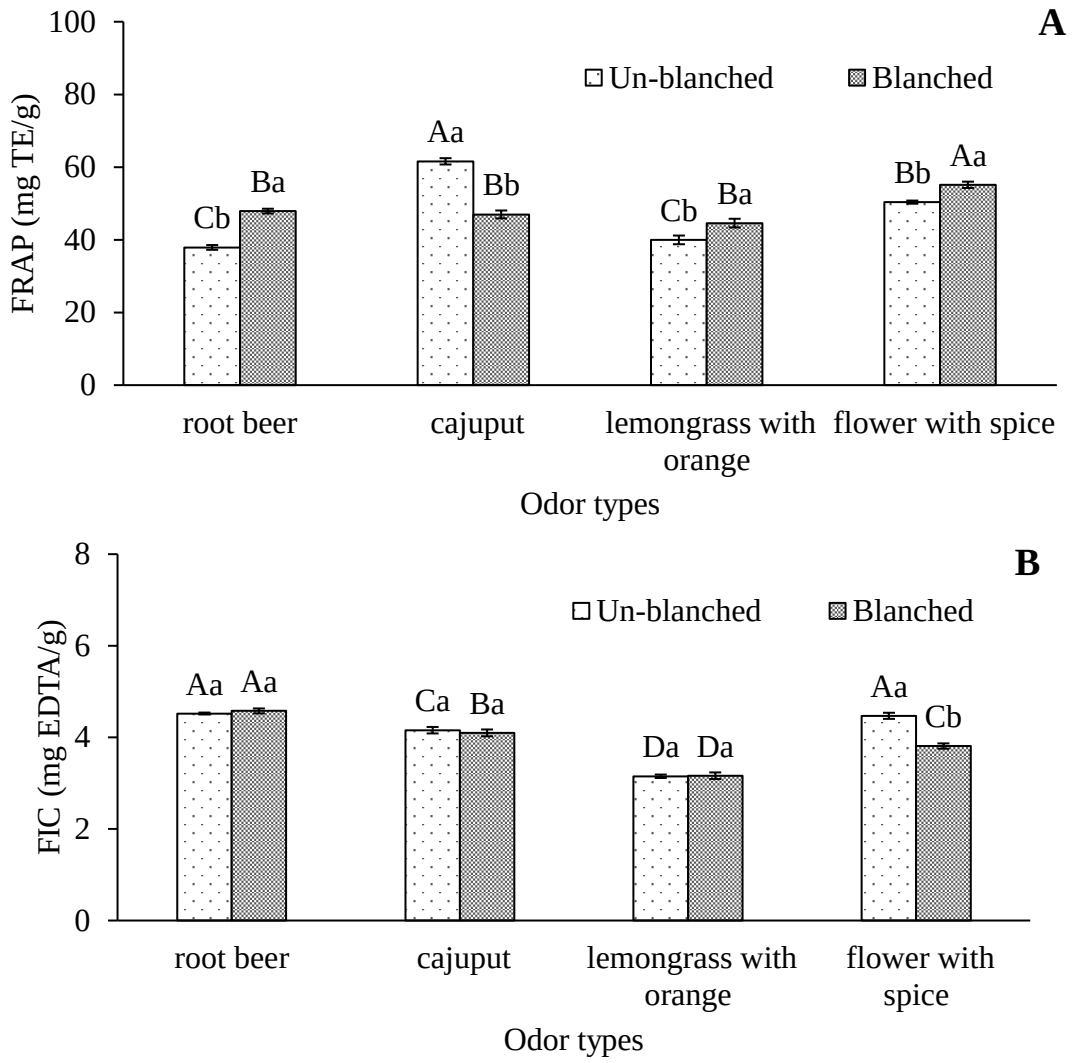


Figure 10 Effect of various odor types of leaves and blanching process of *C. porrectum* herbal tea infusion on FRAP (A) and FIC (B) activities. Different uppercase letters indicate significant difference ($p < 0.05$). Different lowercase letters indicate significant difference ($p<0.05$).

5.5. Conclusion

The 4 odor types of *C. porrectum* samples including root beer, cajuput, lemongrass with orange and unidentified flower with spice odors provided differently of nutritional composition content, physiological properties, phenolic composition as well as antioxidant activities. The main nutritional composition of *C. porrectum* herbal tea powder was carbohydrate followed by protein, fat and ash contents. The moisture, ash, carbohydrate and fiber contents of the control group were higher than blanched group ($p<0.05$). The R sample showed the highest of carbohydrate and fiber contents than others. Without blanching process, the C sample (CC) showed the highest of total solid content (TS) and total acidity (TA) with lowest of pH value while L sample (LC) provided the lowest TA. The infusion making from B group had a greener color than the control group ($p<0.05$). The predominant phenolics and flavonoids of all C. *porrectum* extracts consisted of seven phenolics and one flavonoid including pyrogallol, gallic acid, protocatechuic acid, catechin, caffeic acid, syringic acid, *p*-coumaric acid, and rutin. The peak intensity of phenolics and flavonoids of control sample seemed to lower than in B sample. Based on TPC, both herbal teas producing from blanching process were classified as moderate with 2500 to 3800 mg GAE/ 100g. The CC sample gave the highest of all antioxidant activities in control group while the LC sample provided the lowest activities. Only root beer odor, the TPC and TFC and antioxidant activities were significantly increased by blanching process.

CHAPTER 5

EFFECT OF *Cinnamomum porrectum* HERBAL TEA ON NITRIC OXIDE INHIBITION, CYTOTOXICITY ON NORMAL CELLS (RAW264.7 AND HEK293) AND ANTI-COLON CANCER CELLS (HT-29 AND Caco-2)

6.3.**Abstract**

The 4 odor types of *Cinnamomum porrectum* leaves including root beer (R), cajuput (C), lemongrass with orange (L) and an unknown flower with some spice odor (F) were made to herbal tea by hot air dried at 60 ºC called control group and blanched group using hot water for 60 s before dried with same temperature called blanched group. In this experiment, the cytotoxicity was determined in macrophage cells (RAW264.7) and normal kidney cells (HEK293) and anti-cancer properties on colon cancer cells (HT-29 and Caco-2). In the cell culture studies, all herbal teas at concentration lower than 50 µg/ml had no toxicity on RAW264.7 cells with percentage of cell viability more than 80%). The flower with spice odor (both blanched and un-blanched) and lemongrass with orange (blanched) provided a higher ability on NO inhibition more than positive control L-NA (L-Nω-nitroarginine) (IC_{50}= 30.21±1.48 µg/ml). In un-blanched group, the lemongrass with orange odor showed the lowest toxicity (CC_{50} = 922.76±50.11 µg/ml) on HEK293 cells. The cytotoxicity on HEK293 and HT-29 cells were decreased in blanched group except for Caco-2 cells. In addition, among un-blanched group, the cajuput odor provided the highest anti-colon cancer on HT-29 (CC_{50} = 438.19±30.06 µg/ml) while the flower with spice odor and lemongrass with orange odor (blanched) expressed a higher cytotoxicity on Caco-2 cells.

6.4.**Introduction**

Inflammation is recognized as a biological process in response to tissue injury (Gunawardena *et al.,* 2014). The inflammation response is a complex self-limiting process precisely regulated for preventing extensive damage to the host. When the self-limiting nature of this protective mechanism is inappropriate regulation, it induces to chronic inflammation which is associated with a number of chronic diseases including Alzheimer's disease and cancer (Moncada *et al.,* 1991; Chang *et al.,* 2011). Macrophages are important components of the mammalian immune system and play a key role by protecting an immediate defense against foreign agents prior to leukocyte migration and production of various pro-inflammatory mediators including the short-lived free radical nitric oxide (NO) (Liao *et al.,* 2012). Lipopolysaccharide (LPS), a component from the cell walls of Gram-negative bacteria is one of the most powerful activators of NO production in LPS-stimulated RAW264.7 cells which is used for possible ways to screen various anti-inflammatory drugs (Joo *et al.,* 2014).

Cancer is the top second of death worldwide and was responsible for 8.8 million deaths in 2015 next to the cardiovascular disease (World Health Organization, 2017). In addition, colorectal cancer is the third most common cause of cancer next to lung and liver cancer (World Health Organization, 2017). In Thailand, colorectal cancer accounts for the most mortality in men (17.21%), while the third in women (8.78%) since 2014 (Information and Technology Division National Cancer Institute, Thailand, 2016). The current treatment for colorectal cancer is generally surgical resection combined with chemotherapy by anticancer drugs and radiation (Senawong *et al.,* 2014). This therapy is just moderately successful especially for late stage, however, it is costly and causes negative side effects to normal cancer cells (Herdwiani *et al.,* 2016 and Rosa *et al.,* 2016).

Recently, there are many studies reported that the healthy diet can reduce the risk of inflammatory and colorectal cancer (Rosa *et al.,* 2016). Phenolics are considered to possess anti-inflammatory properties which have been proposed as an alternative natural approach to prevent or treat chronic inflammatory diseases (Sergent *et al.,* 2010). From marketing survey and personal contact, *C. porrectum* leaves was made as herbal tea available in some area of Southern Thailand such as Songkhla and Trang provinces. The herbal tea products are often regarded as low risk

since they have been used by human throughout history. However, some of them may reveal a very strong and even toxic activity in humans. For this reason, it seems very important to conduct screening tests to assess both the beneficial effects and the toxicity of the herbal tea products. Therefore, the ability of *C. porrectum* herbal tea on NO inhibition and the cytotoxicity on normal kidney cells (HEK293) as well as the anti-colon cancer cells (HT-29 and Caco-2) were investigated.

6.5.**Materials and methods**

6.5.1. Plant materials and herbal tea powder and infusion preparation

Four odor types of *C. porrectum* leaves consisted of root beer odor (R), cajuput odor (C), lemongrass with orange odor (L) and flower with spice odor (F) from Technology Research Centre of Forestry sector, Songkhla were marked with expert person, the 10% of each plant odor type was selected in the developing leaves or intermediate stage with light green- green color, flexible stalk during May to August 2015 from Technology Research Centre of Forestry sector, Songkhla. The 10 kgs of the *C. porrectum* leaves with root beer odor from 10 marked plants and 5 kgs of others odor types from 5 marked plants. The samples from each odor were pooled together in order to preserve their initial quality, leaves were stored in a refrigerator at 4 ºC and used within 1 day. The leaves were divided into 2 groups. First, control group (C) prepared by dried at 60 ºC in hot air oven (FD 115, Binder, USA) until moisture content between 5-7% and second group was blanching for 60 s before dried (B) with the same drying temperature. All dried leaves were grinded and sieved though a 60-mesh and kept the powder in aluminium foil ziplock bag until used. Each sample of herbal tea powder, 0.5 g was extracted by 100 ml DI water which controlled temperature at 95±2 ºC with water bath (Memmert, D-91126, Schwabach, Germany) for 10 min, then filtered and cooled down to reach room temperature 28-30 ºC within 5 min. The filtered samples were centrifuged at 6,000 xg for 5 min which controlled temperature at 4 ºC (Hitachi, CR 22 G III, Tokyo, Japan). The supernatant was collected and dried by freeze dryer called as herbal tea extracts then kept in -20 ºC until used.

6.5.2. Sample preparation for cell culture

Each herbal tea extracts, some phenolic and flavonoid compounds and positive control compounds (L-NA: L-Nω-nitroarginine, a NO synthase inhibitor and cisplatin: cis-diamminedichloroplatinum (II), an anticancer drug) were initially filtered through a 0.22 µm sterile filter. All the samples were then diluted in a culture medium for adjust to the required concentrations. For the cytotoxicity test, a hundred microliters of sample were added into each well of the microplate to obtain a final concentration of 1-2,000 µg powder/ml. L-Nω-nitroarginine (L-NA) as 1-100 µg/ml was used as a positive control in RAW264.7 cells, while cisplatin as 1-100 µg/ml was used as a positive control in HT-29, Caco-2 and HEK293 cells. The phenolics including pyrogallol, protocatechuic acid, caffeic acid and syringic acid (10-250 µg/ml) were used as a phenolics standards.

6.5.3. Phenolics composition

Determination of phenolics of four odor types of *C. porrectum* herbal tea of control and blanched groups were performed using HPLC technique (Saetan *et al.*, 2016). The amount of individual phenolics in the extracts were determined by a standard curve between the concentration of phenolics standards, the results were reported as mg/ 100 g of hydrolyzed extract.

6.5.4. Cell culture preparation

The RAW264.7 (murine macrophage cells), HEK293 (human embryonic kidney) and colon cancer cells; HT-29 (human colorectal adenocarcinoma; female, 44 years) and Caco-2 (human colorectal adenocarcinoma; male, 72 years) were used for NO inhibition and cytotoxicity studied. The RAW264.7 cells were cultured in Roswell Park Memorial Institute medium (RPMI-1640 at pH 7.0), both HEK293 and HT-29 were cultured in Dulbecco's Modified Eagle Medium (DMEM at pH 7.2) and Caco-2 cells were maintained in MEM with 1% sodium pyruvate, and 0.5% nonessential amino acids and). All of medium were containing 10% fetal bovine serum (FBS) and 1.5% penicillin (100 U/ml), and streptomycin (100 µg/ml). Cells were incubated at 37 ºC in a humidified atmosphere with 5% CO_2.

6.5.5. Determination of nitric oxide (NO) inhibition in RAW264.7 cells.

The test compounds were considered to test NO inhibition when the cell survival of sample-treated group was more than 80 % when compared with control group (Matsuda *et al.,* 2003). Study of this activity in RAW264.7 cells was following the method of Matsuda *et al.,* 2003 and Sae-Wong *et al.,* 2011. In briefly, RAW264.7 cells were washed with phosphate buffer saline (PBS) free of magnesium and calcium (pH 7.2). The PBS was decanted, and cells were harvested with 0.25% trypsin-EDTA and resuspended with 10 ml of fresh RPMI-1640 medium to make a single cell suspension. One hundred microliters per well of this cell suspension were seeded in each well of a 96-well microplate with 1×10^5 cells/well and allowed to adhere for 2 hours at 37 ºC in 5% CO_2. The medium was then replaced with a fresh medium containing 0.5 µg/ml of Lipopolysaccharide (LPS) together with the test samples at various concentration that gave 80% cell viability from previous part were used and then incubated for 24 h. The NO inhibition was determined by Griess's reagent. The inhibition (%) was calculated using the following equation and the half maximal inhibition concentration (IC_{50} values) were determined graphically (n=3).

Inhibition (%) = [(A-B) / (A-C)] × 100

A: LPS (+), Sample (-),

B: LPS (+), Sample (+) and

C: LPS (-), Sample (-)

6.5.6. Determination of cytotoxicity in RAW264.7, HEK293, HT-29 and Caco-2 cells.

The cytotoxicity in this experiment were investigated by MTT assay based on reduction of the tetrazolium salt, MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) by actively growing cells to produce a blue formazan product (Berridge and Tan, 1993). The assessment of cytotoxicity was based on a comparison with un-treated cells and expressed as CC_{50} values (the 50% of cytotoxic concentration) and presented as means of triplicate analyses.

In briefly, all of cells were washed with phosphate buffer saline (PBS) free of magnesium and calcium (pH 7.2). The PBS was decanted, and cells were harvested with 0.25% trypsin-EDTA and resuspended with 10 ml of the specific medium to make a single cell suspension. One hundred microliters per well of this cell suspension were seeded in each well of a 96-well microplate. The cytotoxicity test in RAW264.7 cells, briefly, the cells were seeded in 96-well plates at the density of 1×10^5 cells/well. The cells were allowed to adhere for 2 h before treated with the herbal tea samples. The normal kidney cells (HEK293) and both colon cancer cells (HT-29 and Caco-2) were prepared as cell density of 1×10^5 cell/ml and 1×10^4 cell/ml, respectively (Parry *et al.*, 2011 and Kaisoon *et al.*, 2012). All cells were added into 96 well plates then incubated for 24 h. All of cells were then treated with *C. porrectum* herbal tea extracts as concentration of 1 to 2,000 µg/ml.Twenty-four hours later, after removed 100 µl of the old medium, MTT was added to a final concentration of 0.5 mg/ml, and the cells were incubated with MTT for 2 h. DMSO was used to dissolve the formazan dye, which was detected by a microplate reader at 570 nm.

6.5.7. Statistical analysis

All the results were expressed as the mean ± SD (n=3). The data were subjected to analysis of variance (ANOVA) to detect the potential differences. The significant differences among the means were established by Duncan's test and *p*-values < 0.05 were considered to be significant. The t-test was used for analysis between-group comparisons of control (un-blanched) and blanched groups.

6.6.**Results and Discussion**

6.6.1. Phenolics and flavonoids composition

The predominant of phenolics of all extracts were presented in Table 12 that consisted of pyrogallol, protocatechuic acid, caffeic acid and syringic acid. The results indicated that the increment and decrement of each phenolics and flavonoids in each odor type of leaves were different. The predominant compounds of RC sample were pyrogallol and protocatechic acid. However, some phenolics compounds of root beer odor increased such as protocatechuic acid and caffeic acid after blanching. Caffeic acid and syringic acid were predominant compounds found in CC sample. However, after blanching CB sample provided the different predominant phenolics especially the dramatic increase of protocatechuic acid and caffeic acid. Although, the predominant compounds of LC were pyrogallol and caffeic acid, after blanching, it provided a higher content in syringic acid but lower content of caffeic acid when compared with LC sample. Pyrogallol and caffeic acid were mainly found in FC, in addition, similar pattern, the result showed that the contents of pyrogallol was significantly increased but the content of caffeic acid was significantly decreased after blanching.

This result can be concluded that different odor types of leaves provided same phenolics but different in the content. The effect of blanching process on each leaf might be increase or decrease the phenolics. However, some phenolics were decreased in blanched samples might be due to leaching effect and the degradation of thermo-labile phenolics compound during blanching process (Saetan *et al.,* 2016). It pointed out even using the same preparation, extraction and determination, each odor type may provide the phenolics and flavonoids differently. Therefore, further study needed to carry out.

Table 12 Phenolics and flavonoids composition of *C. porrectum* herbal tea hydrolyzed extracts

	Phenolic acids and flavonoid compounds (mg/ 100 g of hydrolyzed extract)							
Name	pyrogallol	gallic acid	protocatechuic acid	catechin	caffeic acid	syringic acid	*p*-coumaric acid	rutin
RC	114.33 ± 0.04^{Ba}	39.50 ± 1.27^{Ba}	59.30 ± 0.28^{Ab}	33.75 ± 4.31^{Ab}	12.80 ± 0.85^{Bb}	3.40 ± 0.71^{Ca}	56.30 ± 0.14^{Ab}	187.80 ± 1.27^{Bb}
CC	50.05 ± 1.77^{Cb}	62.65 ± 2.76^{Aa}	3.90 ± 0.00^{Bb}	27.75 ± 4.17^{Ba}	1.50 ± 0.00^{Db}	149.03 ± 1.32^{Aa}	28.00 ± 0.14^{Bb}	92.50 ± 1.41^{Cb}
LC	299.20 ± 8.34^{Aa}	33.80 ± 0.42^{Bb}	9.46 ± 0.65^{Ba}	3.75 ± 0.07^{Da}	202.25 ± 6.01^{Aa}	31.15 ± 0.78^{Ba}	59.40 ± 2.12^{Aa}	214.70 ± 2.40^{Aa}
FC	312.15 ± 15.34^{Ab}	30.35 ± 12.52^{Ba}	5.35 ± 2.62^{Bb}	7.80 ± 0.00^{Ca}	135.65 ± 3.32^{Ba}	3.70 ± 0.28^{Ca}	61.15 ± 1.06^{Aa}	217.35 ± 0.35^{Aa}
RB	88.24 ± 3.20^{Cb}	24.04 ± 1.17^{Ca}	64.04 ± 2.16^{Ba}	40.08 ± 0.38^{Aa}	39.57 ± 1.85^{Ba}	3.62 ± 0.83^{Ca}	73.11 ± 0.59^{Aa}	267.08 ± 4.04^{Aa}
CB	58.65 ± 0.74^{Da}	51.72 ± 9.28^{Ba}	1052.75 ± 17.19^{Aa}	9.83 ± 1.99^{Bb}	36.34 ± 1.64^{Ba}	118.84 ± 0.66^{Bb}	53.84 ± 0.71^{Ba}	213.15 ± 7.39^{Ba}
LB	283.90 ± 1.68^{Ba}	68.89 ± 0.12^{Aa}	11.36 ± 0.13^{Ca}	5.72 ± 3.30^{Ca}	10.35 ± 0.23^{Cb}	164.56 ± 1.48^{Aa}	30.11 ± 0.50^{Cb}	115.57 ± 3.68^{Cb}
FB	376.40 ± 8.28^{Aa}	20.22 ± 0.22^{Ca}	9.20 ± 1.39^{Ca}	5.03 ± 0.69^{Ca}	122.83 ± 0.85^{Ab}	4.32 ± 0.18^{Ca}	49.08 ± 0.65^{Bb}	173.30 ± 4.02^{Cb}

Values are presented as mean ± SD (n=3). Different uppercase letters in the same column within the same leaves odor indicate significant difference ($p< 0.05$). Different lowercase letters in the same column within the same process indicate significant difference ($p<0.05$).
The RC, CC, LC and FC samples and the RB, CB, LB and FB samples denote *C. porrectum* leaves with root beer, cajuput, lemongrass with orange and flower with spice odor produced as herbal tea by un-treated (control) and blanched processes, respectively.

6.6.2. Cytotoxic effect of *C. porrectum* herbal tea on RAW264.7 cells viability

In order to avoid possible cytotoxic effects from the *C. porrectum* herbal tea the MTT assay was using to determine the cell viability. Samples at concentrations of 10 to 1,000 µg/ml were added to RAW264.7 cells for 24 hours before the cell viability was measured. The percentage of cell viability of each concentration was showed in Table 13. The concentration that gave 80% cell viability was classified into the non-toxic category (Panichayupakaranan *et al.,* 2010), the results in Table 13 indicated that all samples from both un-treated and blanched groups provided the 80% cell viability (CC_{20} value) at concentration more than 100 µg/ml except RC and RB (50.29 and 90.92 µg/ml, respectively). Therefore, the concentration less than or equal 50 µg/ml was selected for further study selected by the highest concentration that gave cell viability more than 80% in all samples. The un-treated root beer odor provided a higher toxicity when compared with other odor types might be due to this odor leaves contains some toxic chemical constituents especially safrole and methyleugenol. Both safrole and methyleugenol were classified as a group 2B carcinogen (possible human carcinogen) (IARC, 1987). The blanched root beer odor gave a lower cytotoxicity than un-treated sample because blanching process can decrease toxic constituents especially safrole and methyleugenol more than 89 and 68% when compared with un-treated leaves (Saetan *et al.,* 2017).

Table 13 Effect of *C. porrectum* herbal tea on concentration that gave 80% cell viability (CC_{20} value) and 50% cell viability (CC_{50} value) on RAW264.7 cells

Sample	CC_{20} µg/ml	CC_{50} µg/ml
RC	50.29 ± 5.72^{Cb}	654.94 ± 33.78^{Db}
CC	223.73 ± 9.77^{Bb}	754.06 ± 37.14^{Cb}
LC	349.53 ± 13.89^{Aa}	1008.92 ± 68.39^{Aa}
FC	315.67 ± 14.70^{Aa}	831.88 ± 27.09^{Ba}
RB	90.92 ± 3.64^{Ba}	702.43 ± 25.29^{Ba}
CB	326.62 ± 37.00^{Aa}	832.70 ± 37.20^{Aa}
LB	333.01 ± 15.77^{Aa}	826.67 ± 19.92^{Ab}
FB	322.70 ± 18.56^{Aa}	825.83 ± 35.22^{Aa}

Values are presented as mean ± SD (n=3). Different uppercase letters in the same column within the same leaves odor indicate significant difference ($p< 0.05$). Different lowercase letters in the same column within the same process indicate significant difference ($p<0.05$). The RC, CC, LC and FC samples and the RB, CB, LB and FB samples denote *C. porrectum* leaves with root beer, cajuput, lemongrass with orange and flower with spice odor produced as herbal tea by un-treated (control) and blanched processes, respectively.

6.6.3. Nitric oxide inhibition activity

It is known that macrophages respond to the inflammation process via the production of several pro-inflammatory molecules, including nitric oxide (NO). Actually, RAW264.7 cells were treated with LPS to induce over-expression of inducible nitric oxide synthase (iNOS) in order to create inflammation (Hobbs *et al.*, 1999). Therefore, if NO was less created than inflammation may not occur. Some NO-suppression activity can be generated via three different pathways: (1) the blocking of iNOS expression, (2) the inactivation of the iNOS catalytic function and (3) the scavenging of NO (Sheu *et al.*, 2001; Tsai et al., 2007). During inflammation, patient must be advised to take NSIADs. However, there are some adverse side effects of using NSIADs, therefore, traditional medicines and natural products used in folk medicine have been studied as potential alternative drugs.

In the experiment the RAW264.7 cells were stimulated with LPS before tested percentage of cell viability by MTT assay and tested NO inhibition by Greiss's reagent. The results of percentage of cell viability and NO inhibition when treated by *C. porrectum* herbal tea, standard compounds (phenolics and positive control: L-NA) were presented in Table 14. The result from percentage of cell viability can confirm that at concentration lower than 50 µg/ml, all *C. porrectum* herbal tea provided the cell viability more than 80% in LPS-stimulated RAW264.7 cells. The IC_{50} values of NO inhibition was in range 27.28 to 49.79 µg/ml in control group. The FC provided the highest of NO inhibition followed by LC and CC samples might be due to the phenolics including pyrogallol (IC_{50} = 21.50±1.05 µg/ml) and caffeic acid (IC_{50} = 28.76±1.41 µg/ml) in the FC and LC samples were higher than CC sample. In blanched group the IC_{50} values were in range 22.59 to 42.29 µg/ml, the FB provided the highest NO inhibition followed by LB, CB and RB, respectively might be due to the FB consists of pyrogallol higher than LB, RB and CB, respectively. However, the CB provided a higher NO inhibition ability more than RB might be related with the CB consists of a higher content of syringic acid (IC_{50} = 39.94±1.94 µg/ml) and protocatechuic acid (IC_{50} = 79.20±3.88 µg/ml). When compared with L-NA, only the FB, LB and FC provided a higher ability on NO inhibition more than positive control L-NA (IC_{50}= 30.21±1.48 µg/ml).

Table 14 Nitric oxide inhibition of *C. porrectum* herbal tea, phenolics and positive control on LPS-stimulated RAW264.7 cells

Types of sample	**Sample name**	**IC_{50} value (µg/ml)**
C. porrectum herbal tea samples	RC	Not observed
	CC	49.79 ± 3.04^{Aa}
	LC	35.63 ± 2.17^{Ba}
	FC	27.28 ± 1.66^{Ca}
	RB	42.29 ± 2.58^{A}
	CB	34.05 ± 2.08^{Ba}
	LB	24.15 ± 1.47^{Cb}
	FB	22.59 ± 1.38^{Cb}
Phenolics	Pyrogallol	21.50 ± 1.05^{D}
	Protocatechuic acid	79.20 ± 3.88^{A}
	Caffeic acid	28.76 ± 1.41^{C}
	Syringic acid	39.94 ± 1.94^{B}
Positive control	L-NA*	30.21±1.48

*L-NA (L-Nω-nitroarginine): positive control for NO inhibition. Values are presented as mean ± SD (n=3). Different uppercase letters in the same column within the same leaves odor indicate significant difference ($p< 0.05$). Different lowercase letters in the same column within the same process indicate significant difference ($p< 0.05$). The RC, CC, LC and FC samples and the RB, CB, LB and FB samples denote *C. porrectum* leaves with root beer, cajuput, lemongrass with orange and flower with spice odor produced as herbal tea by un-treated (control) and blanched processes, respectively.

6.6.4. The cytotoxicity on normal cells (RAW264.7 and HEK293) and colon cancer cells (HT-29 and Caco-2)

The concentration that gave cell viability 50% (CC_{50}) on normal cells (RAW264.7 and HEK293) and cancer cells (HT-29 and Caco-2) were showed in Table 15. The control sample from root beer odor provided a higher anticancer ability HT-29 and Caco-2 cells, however, also provided a higher toxicity on both normal cells RAW264.7 and HEK293 compared with blanching sample. In addition, the results indicated that the root beer odor provided a higher toxicity on normal kidney cells and macrophage cells more than the anti-cancer properties on both colon cancer cells. Saetan *et al*. (2017) reported that root beer odor leaves contained a higher safrole, methyleugenol and other toxic chemical compounds compared with other odor types. However, those toxic chemical constituents were significantly reduced by blanching process (Saetan *et al*., 2017). Moreover, the RC sample provided a higher toxicity on HT-29 cells more than Caco-2 cells might be due to the permeability of HT-29 was higher than Caco-2 cells (Grajek and Olejnik, 2004). It pointed out that cytotoxicity of each cell may differ from one to other due to its cell component and metabolism.

The CC sample provided the highest anti-cancer property on HT-29 cells when compared with other samples (CC_{50}= 438.19±30.36 µg/ml) while the CB sample showed the lowest property may due to different phenolics composition (Table 12). As mentioned earlier. The predominant phenolics in CC sample (Table 12) was syringic acid while CB sample provided a dramatic content of protocatechuic acid. The data from Table 12 indicated that syringic acid (CC_{50} as 272.94±15.73 µg/ml) showed a higher on anti-cancer on HT-29 cells more than protocatechuic acid (325.22±23.27 µg/ml). Moreover, the CC sample provided a higher toxicity on normal HEK293 cells because the syringic acid (CC_{50} as 457.82±24.13 µg/ml) gave a higher cytotoxicity on HEK293 than protocatechuic acid (CC_{50} as 589.80±36.40 µg/ml).

The data from Table 15 reported the CC_{50} values of normal cells (RAW264.7 and HEK293 cells) and colon cancer cells (HT-29 and Caco-2 cells). The samples from lemongrass with orange and flower with spice odor gave the lowest toxicity on HEK293 and RAW264.7 cells when compared with the samples from root beer and cajuput odor. The LC and LB provided a higher toxicity on Caco-2 followed by HT-29 might be related to the main phenolics in LC was pyrogallol and caffeic acid while LB was pyrogallol and syringic acid. The FB sample provided a higher toxicity on Caco-2 than FC might be related with the pyrogallol content in FB was higher than FC due to the pyrogallol provided the highest cytotoxicity when compared with other tested phenolics standard (Table 15). In un-blanched group, the lemongrass with orange odor (LC) showed the lowest toxicity (CC_{50} = 922.76±50.11 μg/ml) on HEK293 cells. The cytotoxicity on HEK293 was decrease in blanched group. In un-blanched group, the cajuput odor provided the highest anti-colon cancer on HT-29 (CC_{50} = 438.19±30.36 μg/ml). The blanched lemongrass with orange odor (LB) and flower with spice odor (FB) showed a higher cytotoxicity on Caco-2 cells (327.15±16.43 and 433.93±17.00 μg/ml, respectively).

In conclusion, from all results, the root beer odor should be blanched before making herbal tea. The cajuput odor provided the highest of antioxidant activities and gave the highest of anti-colon cancer on HT-29 cells. The lemongrass with orange odor and flower with spice odor provided a high ability on NO inhibition, higher toxicity on both colon cancer cells.

Table 15 Effect of *C. porrectum* herbal tea, phenolics and positive control on normal cells (RAW264.7 and HEK293) toxicity and anti-colon cancer (HT-29 and Caco-2) activity

Samples/ phenolics/ positive control	Cytotoxicity; CC_{50} (µg/ml)			
	Normal cells		Colon cancer cells	
	RAW264.7	HEK293	HT-29	Caco-2
RC	654.94 ± 23.78^{Db}	585.18 ± 42.41^{Cb}	516.65 ± 34.25^{Bb}	1074.17 ± 76.06^{Aa}
CC	754.06 ± 37.14^{Cb}	595.16 ± 40.15^{Cb}	438.19 ± 30.36^{Cb}	737.33 ± 60.13^{Ba}
LC	1008.92 ± 68.39^{Aa}	922.76 ± 50.11^{Aa}	598.08 ± 41.02^{Ab}	624.21 ± 50.57^{Ca}
FC	831.88 ± 27.09^{Ba}	752.39 ± 41.25^{Bb}	503.86 ± 39.15^{Ba}	629.22 ± 53.60^{Ca}
RB	702.43 ± 25.29^{Ba}	752.39 ± 21.21^{Ca}	811.69 ± 24.08^{Ba}	1250.78 ± 34.07^{Aa}
CB	832.70 ± 37.20^{Aa}	912.47 ± 23.84^{Ba}	1141.52 ± 32.46^{Aa}	659.96 ± 23.28^{Bb}
LB	826.67 ± 19.92^{Ab}	942.11 ± 25.18^{Aa}	657.54 ± 21.19^{Ca}	327.15 ± 16.43^{Db}
FB	825.83 ± 35.22^{Aa}	909.52 ± 24.77^{Aa}	529.73 ± 18.16^{Da}	433.93 ± 17.00^{Cb}
Pyrogallol	56.97 ± 2.79^{D}	86.13 ± 9.00^{D}	59.43 ± 3.15^{C}	27.91 ± 1.87^{D}
Protocatechuic acid	250.04 ± 12.25^{C}	589.80 ± 36.40^{B}	325.22 ± 23.27^{A}	313.73 ± 19.86^{A}
Caffeic acid	212.61 ± 10.42^{B}	754.14 ± 45.77^{A}	251.37 ± 31.13^{B}	115.80 ± 10.26^{C}
Syringic acid	354.66 ± 17.38^{A}	457.82 ± 24.13^{C}	272.94 ± 15.73^{B}	186.74 ± 14.91^{B}
LNA*	347.18±17.01	ND	ND	ND
Cisplatin**	ND	38.88±3.91	22.75±1.97	35.11±2.67

ND means not determined. * L-NA (L-Nω-nitroarginine): positive control for NO inhibition. ** Cisplatin (cis-diamminedichloroplatinum (II): anti-cancer drug. Values are presented as mean ± SD (n=3). Different uppercase letters in the same column within the same standards indicate significant difference ($p<0.05$). The RC, CC, LC and FC samples and the RB, CB, LB and FB samples denote *C. porrectum* leaves with root beer, cajuput, lemongrass with orange and flower with spice odor produced as herbal tea by un-treated (control) and blanched processes, respectively.

6.7.**Conclusion**

In the cell culture studies, all herbal teas from both blanched and without blanched at concentration lower than 50 µg/ml had no toxicity on RAW264.7 cells (percentage of cell viability more than 80%). The flower with spice odor (both blanched and un-blanched) and lemongrass with orange (blanched) provided a higher ability on NO inhibition more than positive control: L-NA (IC_{50}=30.21±1.48 µg/ml). Un-blanched lemongrass with orange odor showed the lowest toxicity (CC_{50}= 922.76±50.11 µg/ml) on HEK293 cells. The cytotoxicity on HEK293 can be reduced by blanching process. Before blanching process, the cajuput odor provided the highest anti-colon cancer on HT-29 (CC_{50} = 438±30.06 µg/ml) while the flower with spice odor and lemongrass with orange odor showed a higher cytotoxicity on Caco-2 cells. Therefore, the root beer odor should be blanched before making herbal tea. The cajuput odor provided the highest of anti-colon cancer on HT-29 cells. The lemongrass with orange odor and flower with spice odor provided a high ability on NO inhibition, higher toxicity on both colon cancer cells.

CHAPTER 6

SUMMARY AND SUGGESTION

6.1. Summary

1. The *C. porrectum* leaves (root beer odor) produced by drying at 60 ºC and steeping time for 10 min exhibited the highest of TPC and TFC. Blanching for 60 s was suitable for pre-treatment due to the highest of TPC, TFC and ABTS activities. Blanching can significantly decrease safrole and methyleugenol more than 89 and 68%, respectively, in addition, it can help open structure porosity in powder and preserve chlorophyll contents leading to a higher green color preservation.

2. Different odor types of *C. porrectum* leaves had different nutritional composition and physiochemical properties. In blanched group, the TPC, TFC and FRAP activity were increased in root beer odor leaves and lemongrass with orange odor leaves. Based on TPC, all samples were classified as moderated antioxidant category. The CC sample provided the highest of DPPH, ABTS and FRAP activities.

3. The phenolics composition of *C. porrectum* herbal tea hydrolyzed extracts consist of pyrogallol, gallic acid, protocatechuic acid, catechin, caffeic acid, syringic acid, *p*-coumaric acid and rutin.

4. The LB, FB and FC samples provided the NO inhibition more than L-NA. The CC sample had the highest on HT-29 cells cytotoxicity. Only LC, LB, FB and FC samples showed a lower toxicity in both normal cells and showed a higher ability on both colon cancer.

6.2. Suggestion

Based on the cytotoxicity of both normal cells (RAW264.7 and HEK293) and colon cancer cells (HT-29 and Caco-2), consuming of this herbal tea should be postpone until animal and/ or clinical trial can be proved.

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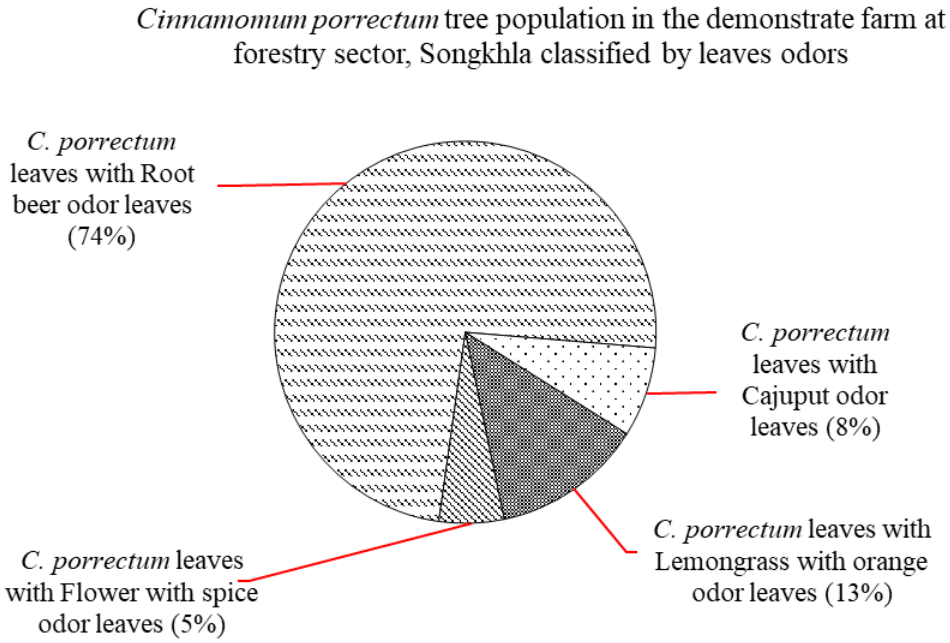
APPENDIXES

Appendix 1

Determination of sampling size

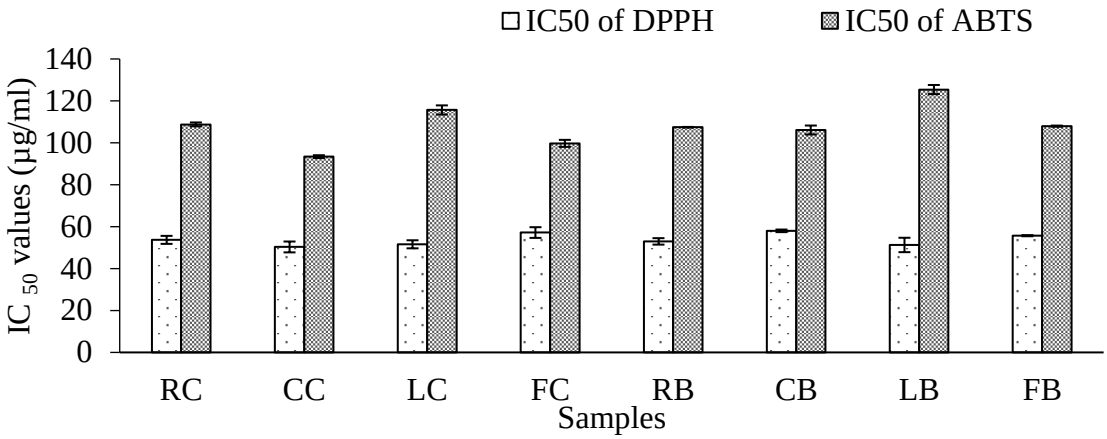
The sample of this research was calculated by using modified Taro Yamane (Yamane, 1973) formula with 95% confidence level. At least 20% of total plant odors was marked and selected. The four odor types of *C. porrectum* leaves consisted of root beer odor (R), cajuput odor (C), lemongrass with orange odor (L) and flower with spice odor (F) from Technology Research Centre of Forestry sector, Songkhla were marked with expert person. At least 10 kgs of the *C. porrectum* leaves with root beer odor and 5 kgs of others odor types were selected. From each odor type were pooled together before taken to remove the branch. All leaves were washed and drained before taken to further step.



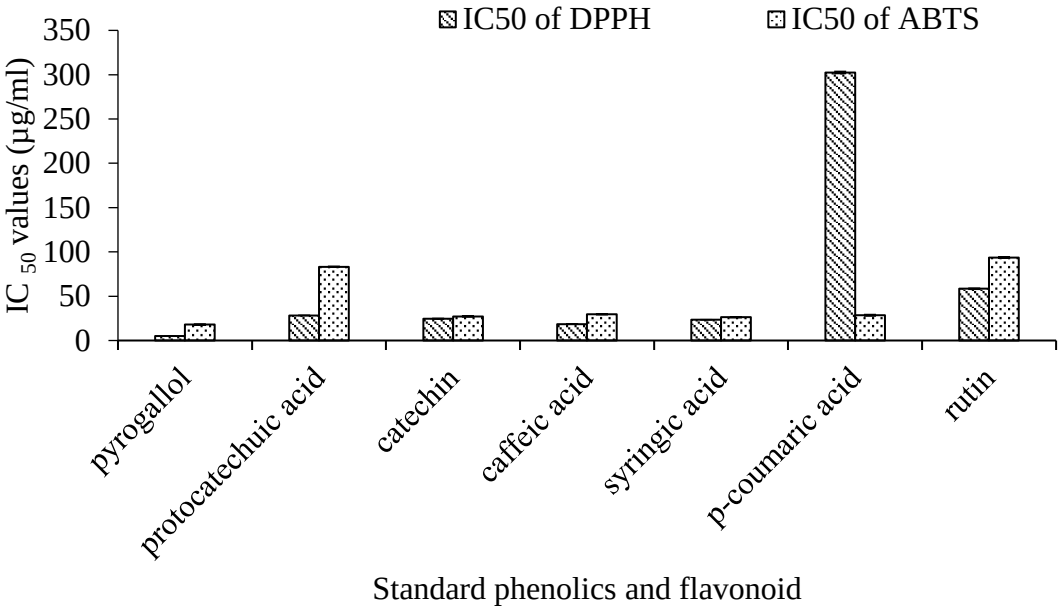
Appendix-1.1 The *Cinnamumum porrectum* tree population in the demonstrate farm of Technology research center of forestry sector, Rattaphoom, Songkhla

Appendix 2

IC_{50} value of *C. porrectum* herbal tea and phenolics standards on DPPH and ABTS



Appendix-2.1 IC_{50} of *C. porrectum* herbal tea analyzed by DPPH and ABTS assays



Appendix-2.2 IC_{50} of standard phenolics and flavonoids on DPPH and ABTS assay.

Appendix 3

Cell culture analysis

Appendix-3.1 Effect of *C. porrectum* herbal tea on percentage of cell viability, CC_{20} and CC_{50} values on RAW264.7 cells

Sample	Cell viability (%) at various concentration (µg/ml)						CC_{20} µg/ml	CC_{50} µg/ml
	50	100	250	500	1000	2000		
RC	80.42±1.42	73.85±0.46	63.21±4.38	53.44±1.81	31.26±1.81	10.55±1.07	$50.29±5.72^{Cb}$	$654.94±33.78^{Db}$
CC	87.35±3.05	81.62±1.70	72.37±4.11	61.43±2.26	38.09±1.03	16.50±2.55	$223.73±9.77^{Bb}$	$754.06±37.14^{Cb}$
LC	92.54±1.79	87.66±2.67	82.78±1.14	72.04±0.85	51.07±1.80	32.72±0.82	$349.53±13.89^{Aa}$	$1008.92±68.39^{Aa}$
FC	90.98±0.93	88.05±0.56	82.45±0.64	72.39±1.48	37.09±2.28	13.37±1.08	$315.67±14.70^{Aa}$	$831.88±27.09^{Ba}$
RB	82.27±1.24	76.16±2.07	70.13±2.53	60.20±1.45	42.47±1.17	23.98±0.77	$90.92±3.64^{Ba}$	$702.43±25.29^{Ba}$
CB	95.55±4.40	86.46±2.63	71.28±2.44	64.59±4.18	44.00±0.58	29.47±4.45	$326.62±37.00^{Aa}$	$832.70±37.20^{Aa}$
LB	95.65±1.01	87.86±1.53	80.21±0.40	59.33±1.04	44.63±0.77	28.66±1.15	$333.01±15.77^{Aa}$	$826.67±19.92^{Ab}$
FB	93.88±1.72	86.47±1.87	76.76±1.11	69.95±1.42	40.28±1.54	27.19±1.77	$322.70±18.56^{Aa}$	$825.83±35.22^{Aa}$

Values are presented as mean ± SD (n=3). Different uppercase letters in the same column within the same leaves odor indicate significant difference ($p< 0.05$). Different lowercase letters in the same column within the same process indicate significant difference ($p<0.05$). The RC, CC, LC and FC samples and the RB, CB, LB and FB samples denote *C. porrectum* leaves with root beer, cajuput, lemongrass with orange and flower with spice odor produced as herbal tea by un-treated (control) and blanched processes, respectively.

Appendix-3.2 NO inhibition of *C. porrectum* herbal tea, phenolics and positive control on LPS-stimulated RAW264.7 cells

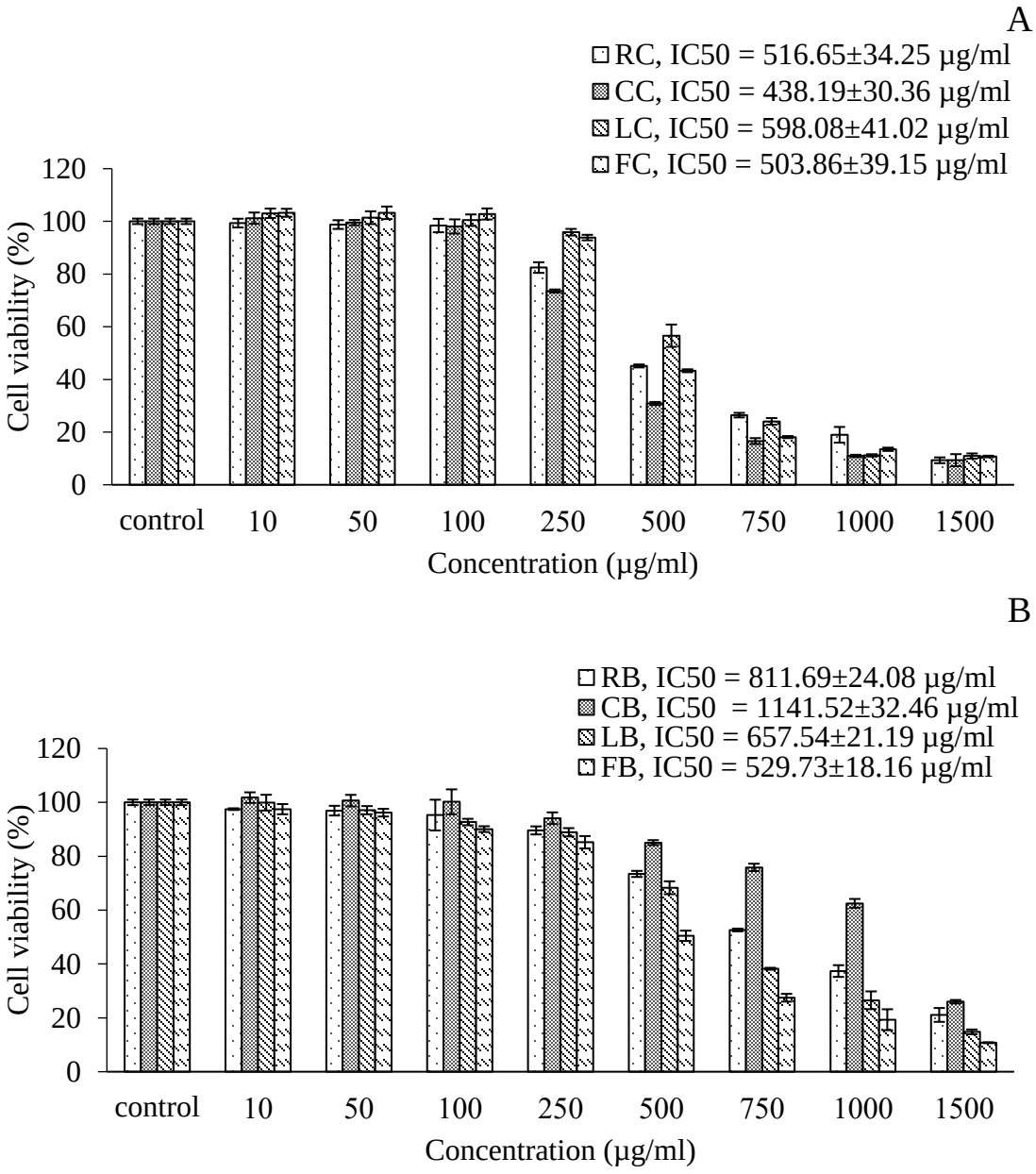
Sample/ phenolics/ positive control	**Nitric oxide inhibition (%) at various concentrations (µg/ml)**						**IC_{50} value (µg/ml)**
	1	**5**	**10**	**25**	**50**	**100**	
RC	20.69±3.45	25.86±1.72	28.74±2.63	ND	39.66±5.17	Not observed	Not observed
CC	17.24±3.45	28.16±1.00	32.18±1.00	ND	50.00±2.99	Not observed	$49.79±3.04^{Aa}$
LC	30.46±2.63	44.83±2.99	50.00±2.72	ND	54.02±1.00	Not observed	$35.63±2.17^{Ba}$
FC	28.74±5.27	32.76±1.72	44.83±1.72	ND	62.64±1.99	Not observed	$27.28±1.66^{Ca}$
RB	27.01±7.18	34.48±2.99	41.38±3.45	ND	52.30±2.63	Not observed	$42.29±2.58^{A}$
CB	33.33±5.54	36.78±1.00	43.10±2.99	ND	54.02±1.00	Not observed	$34.05±2.08^{Ba}$
LB	35.06±1.00	43.10±4.56	45.98±1.00	ND	61.49±2.63	Not observed	$24.15±1.47^{Cb}$
FB	32.76±4.56	39.08±4.34	48.85±1.99	ND	60.92±1.99	Not observed	$22.59±1.38^{Cb}$
Pyrogallol	ND	ND	32.31±3.08	55.38±1.54	Not observed	Not observed	$21.50±1.05^{D}$
Protocatechuic acid	ND	ND	11.79±3.87	21.03±2.35	36.92±0.89	60.00±1.78	$79.20±3.88^{A}$
Caffeic acid	ND	ND	29.74±3.87	45.64±2.35	81.03±0.89	89.74±1.78	$28.76±1.41^{C}$
Syringic acid	ND	ND	28.21±6.41	40.00±1.54	58.46±4.07	90.77±1.54	$39.94±1.94^{B}$
L-NA*	ND	ND	38.25±2.65	46.67±1.22	65.61±2.19	77.19±2.19	30.21±1.48

*L-NA: positive control for NO inhibition. Values are presented as mean ± SD (n=3). Different uppercase letters in the same column within the same leaves odor indicate significant difference ($p< 0.05$). Different lowercase letters in the same column within the same process indicate significant difference ($p<0.05$). The RC, CC, LC and FC samples and the RB, CB, LB and FB samples denote *C. porrectum* leaves with root beer, cajuput, lemongrass with orange and flower with spice odor produced as herbal tea by un-treated (control) and blanched processes, respectively.

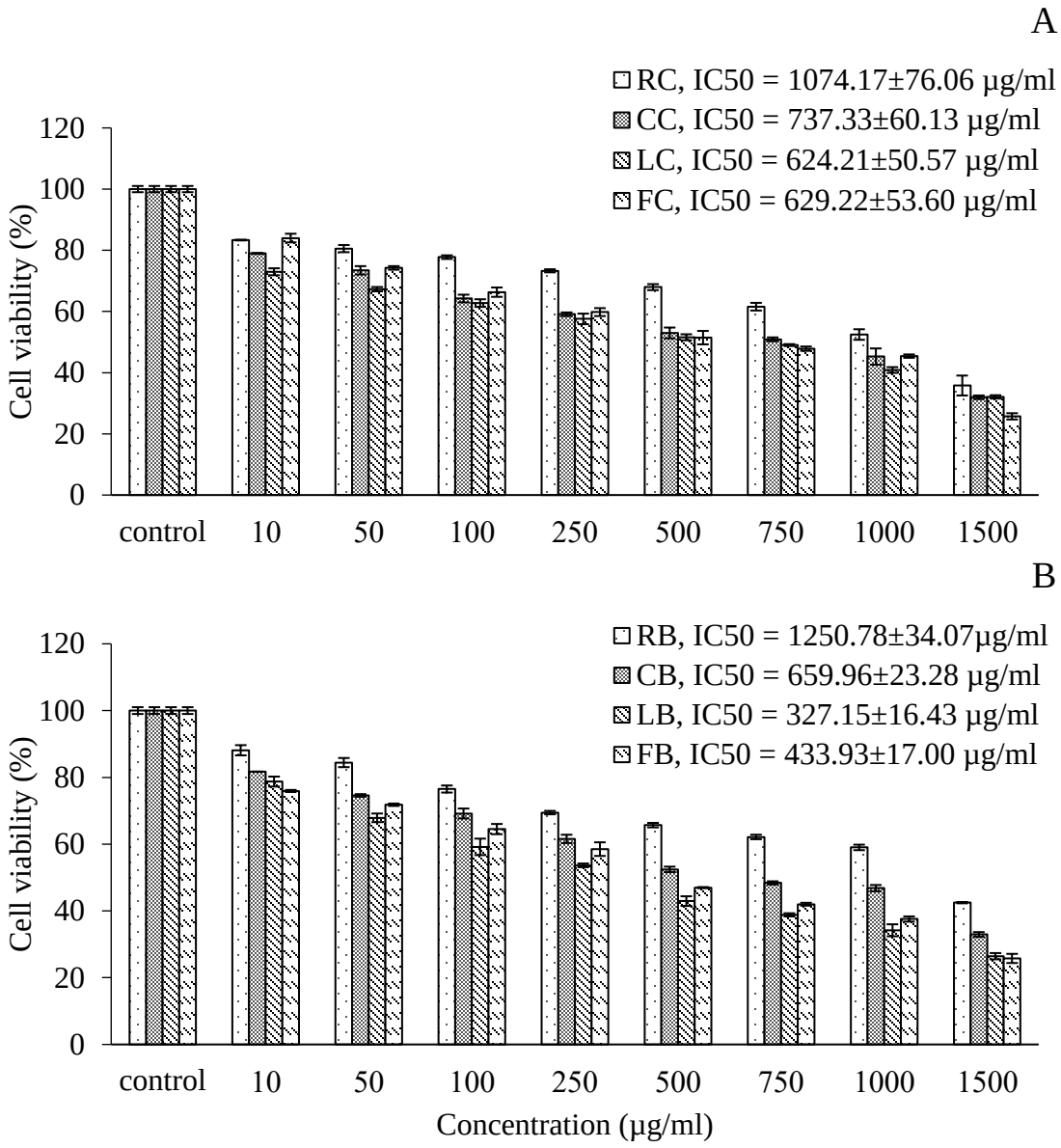
Appendix-3.3 The effect of *C. porrectum* on percentage of cell viability and CC_{50} values on HEK293 cells

Sample	Cell viability (%) at various concentration of sample µg/ml					CC_{50} µg/ml
	50	100	250	500	1000	
RC	86.56±2.27	73.80±4.09	61.92±0.77	52.09±1.52	35.02±0.28	$585.18±42.41^{Cb}$
CC	85.68±0.80	80.22±0.80	64.89±1.82	53.06±1.14	35.43±0.98	$595.16±40.15^{Cb}$
LC	84.13±1.36	82.03±2.99	75.68±1.76	64.84±1.83	48.93±1.48	$922.76±50.11^{Aa}$
FC	85.70±2.43	81.43±1.40	71.32±1.46	55.16±0.93	37.99±1.78	$752.39±41.25^{Bb}$
RB	88.71±1.22	84.06±1.29	75.98±0.17	63.52±0.56	36.78±0.80	$752.39±21.21^{Ca}$
CB	91.27±1.24	85.25±1.26	72.61±1.15	66.55±1.00	39.14±1.78	$912.47±23.84^{Ba}$
LB	89.83±0.80	81.53±0.94	72.09±4.54	65.00±0.80	49.84±0.73	$942.11±25.18^{Aa}$
FB	93.88±1.72	86.47±1.87	76.76±1.11	69.95±1.42	40.28±1.54	$909.52±24.77^{Aa}$

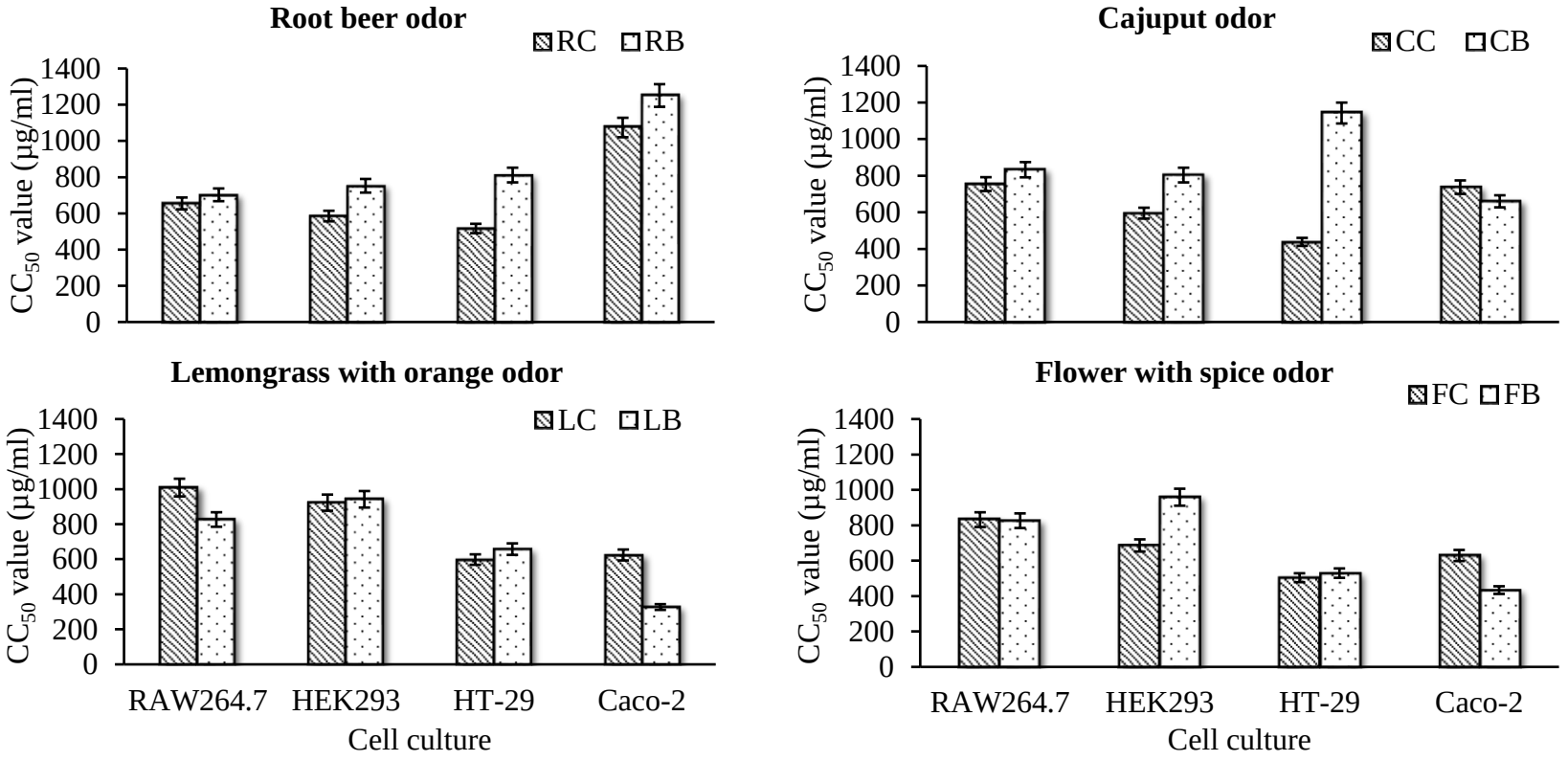
Values are presented as mean ± SD (n=3). Different uppercase letters in the same column within the same leaves odor indicate significant difference ($p< 0.05$). Different lowercase letters in the same column within the same process indicate significant difference ($p<0.05$). The RC, CC, LC and FC samples and the RB, CB, LB and FB samples denote *C. porrectum* leaves with root beer, cajuput, lemongrass with orange and flower with spice odor produced as herbal tea by un-treated (control) and blanched processes, respectively.



Appendix-3.4 Effect of *C. porrectum* herbal tea with various odor types of leaves and blanching process on colon cancer cell cytotoxicity on HT-29 cells of control (A), blanched groups (B). The RC, CC, LC and FC samples and the RB, CB, LB and FB samples denote *C. porrectum* leaves with root beer, cajuput, lemongrass with orange and flower with spice odor produced as herbal tea by un-treated (control) and blanched processes, respectively.



Appendix-3.5 Effect of *C. porrectum* herbal tea with various odor types of leaves and blanching process on colon cancer cell cytotoxicity on Caco-2 cells of control (A) and blanched groups (B). The RC, CC, LC and FC samples and the RB, CB, LB and FB samples denote *C. porrectum* leaves with root beer, cajuput, lemongrass with orange and flower with spice odor produced as herbal tea by un-treated (control) and blanched processes, respectively.



Appendix-3.6 Effect of different odor types of leaves and blanching process of *C. porrectum* herbal tea) on CC_{50} values of RAW264.7, HEK293, HT-29 and Caco-2 cells (Means ± standard derivative) were calculated along with a line graph. The RC, CC, LC and FC samples and the RB, CB, LB and FB samples denote *C. porrectum* leaves with root beer, cajuput, lemongrass with orange and flower with spice odor produced as herbal tea by un-treated (control) and blanched processes, respectively.

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- Grant-in-aid for dissertation from Graduate School, Prince of Songkla University.

List of Publication and Proceedings

1. Saetan, P., Usawakesmanee, W. and Siripongvitikorn, S. 2016. Influence of hot water blanching process on nutritional content, microstructure, antioxidant activity and phenolic profile of *Cinnamomum porrectum* herbal tea. Funct Food Health Dis. 6: 836-854.
2. Saetan, P., Usawakesmanee, W, Siripongvitikorn, S. Yupanqui, C. T. 2017. Reduction of safrole content of *Cinnamomum porrectum* leaves by blanching and the effect on the antioxidant and anti-inflammatory activities of its herbal tea. Funct Food Health Dis. 7: 936-957.
3. Saetan, P., Usawakesmanee, W. and Siripongvitikorn, S. 2013. Effect of enzyme inactivation on total phenolic, flavonoid content and their antioxidant activities of *Cinnamomum porrectum.* International conference on Food and Applied Bioscience 2016. February 4-5, 2016. pp. 240-252.