The Screening of Quercetin Content in Herbal Extracts Using Bioautography and Densitometry
การตรวจสอบปริมาณสารเควอซิทินในสารสกัดสมุนไพรโดยวิธี Bioautography และ Densitometry

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ABSTRACT

The spectrophotometric assay based on aluminium complex formation is one of the most commonly used procedure for determination of total flavonoid content of herbal extracts. However, this method can not characterize the herbal extract samples and unable to localize the active compounds in herbal extract. Bioautography is an effective assay for the detection of phytochemical compounds because it allows localizing the active compounds in a complex matrix. The aim of this study was to compared quercetin content of chia seeds extract, rosella calyxes extract, ginger rhizomes extract and white crane flower leaves extract using Thin-Layer chromatography (TLC) as a bioautography of each extract and densitometer method to quantitative determination of quercetin content using quercetin as a standard reference. Mobile phase consisted of toluene, ethyl acetate and formic acid with a ratio of 5 : 4 : 1, respectively. The results showed that the quercetin content of white crane flower leaves extract was 52.165 ± 6.87 mg equivalent to quercetin/g dry extract which was higher than that of ginger rhizomes extract (17.692 ± 5.08 mg equivalent to quercetin/ g dry extract) and of chia seeds extract (16.214 ± 1.43 mg equivalent to quercetin/ g dry extract) but it was not found in rosella calyxes extract. In conclusion, results from bioautography was able to localize quercetin at Rf value of 0.5. Of the herbal extracts tested, white crane flower leaves extract contained that highest quercetin. Moreover, detecting several compounds from different classes of flavonoid family such as catechins, rutin, apeginin should be further determination using bioautography.

บทคัดย่อ

วิธีสเปกโตรโฟโตเมทริก ที่อาศัยการเกิดสารประกอบเชิงซ้อนของอะลูมิเนียมเป็นวิธีการทดสอบหาปริมาณสารฟลาโวนอยด์ในสารสกัดสมุนไพรที่นิยมกันอย่างแพร่หลาย แต่อย่างไรก็ตามวิธีนี้ไม่สามารถแยกลักษณะเฉพาะและสารสำคัญที่อยู่ในสารสกัดสมุนไพรได้ วิธีไบโอออโตกราฟี เป็นวิธีที่มีประสิทธิภาพในการหาสารประกอบทางพฤกษเคมีเนื่องจากสามารถทดสอบหาสารสำคัญที่จำเพาะในตัวอย่างได้ วัตถุประสงค์เพื่อเปรียบเทียบปริมาณเควอซิทินในสารสกัดเมล็ดเจีย, ดอกกระเจี๊ยบแดง, เหง้าขิงและใบทองพันชั่งด้วยวิธีโครมาโตกราฟีแบบผิวบางซึ่งเป็นการทดสอบไบโอออโตกราฟีในสารสกัดแต่ละตัว และวิธีเดนซิโตมิเตอร์ในการคำนวณหาปริมาณสารเคอร์ซิตินโดยมีเคอร์ซิตินเป็นสารมาตรฐาน วัฏภาคเคลื่อนที่ประกอบด้วยโทลูอีน, เอธิล อะซิเตด และกรดฟอร์มิกในอัตราส่วน 5 :4 : 1 ตามลำดับ จากผลการทดลองพบว่าสารสกัดใบทองพันชั่งมีปริมาณสารเควอซิทินเท่ากับ 52.165 ± 6.87 มิลลิกรัมสมมูลเควอซิทิน/กรัมน้ำหนักสารสกัดแห้ง ซึ่งสูงกว่าสารสกัดเหง้าขิง (17.692 ± 5.08 มิลลิกรัมสมมูลเควอซิทิน/กรัมน้ำหนักสารสกัดแห้ง) สารสกัดเมล็ดเจีย (16.214 ± 1.43 มิลลิกรัมสมมูลเควอซิทิน/กรัมน้ำหนักสารสกัดแห้ง) แต่สารสกัดดอกกระเจี๊ยบแดงไม่พบปริมาณสารเคอร์ซิติน ดังนั้นจากการทดลองวิธีไบโอออโตกราฟีพบว่าเควอซิทิน มีค่า Rf เท่ากับ 0.5 และสารสกัดใบทองพันชั่งมีปริมาณเควอซิทินสูงที่สุด นอกจากนี้ยังสามารถทดสอบโดยการใช้ไบโอออโตกราฟีเพื่อหาปริมาณสารฟลาโวนอยด์ชนิดอื่นๆได้ เช่น คาเทชิน, รูติน, อะพิเจนิน เป็นต้น

Keywords: Herbal extract, Quercetin, Thin-Layer chromatography (TLC)
คำสำคัญ: สารสกัดสมุนไพร เควอซิทิน โครมาโตกราฟีแบบผิวบาง

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Introduction

Flavonoids are an important class of secondary metabolite natural products and polyphenolic structure. They have antioxidant activity mainly by free radical scavenging (Kumar, Pandey, 2013) and inhibit UV-induced skin damage (González et al., 2011). Quercetin is an abundant flavonoid with strong antioxidant, anti-inflammatory and anti-proliferative activities (Schnekenburger, Diederich, 2015). The spectrophotometric assay based on aluminium complex formation is one of the most commonly used procedure for determination of total flavonoid content of herbal extracts. This method is unable to localize the active compounds in herbal extract even though the assay allows detecting several compounds from different classes of flavonoid family (Pekal, Pyrzynska, 2014). Bioautography is an effective assay for the detection of phytochemical compounds because it allows localizing the active compounds in a complex matrix (Rahalison et al., 1991).

Chia seed (*Salvia hispanica L.*) belongs to Lamiaceae family. It was extensively cultivated in Mexico, United States, Canada, Chile, Australia, New Zealand and Southeast Asia. Chia seed contains high in omega-3 and omega-6 which has been reported of preventing photo damage, photo aging and reducing transepidermal water loss. Thus, Chia seed appeared to reduce wrinkle and erythema from UV radiation and enhance skin moisture (Essential Fatty Acids and Skin Health, 2016). In addition, Chia seed has high antioxidant activity due to flavonoids, phenolic compound and tocopherols (Falco et al., 2017). Chia seed possessed the following polyphenolic structure compounds; Myricetin, quercetin, kaemferol, Caffeic acid, Flavonol glycosides and Chlorogenic acid (Ayerza, Coates, 2002).

Rosella (*Hibicus sabdariffa L.*) belongs to Malvaceae family. It was initially discovered in Asia (India and Malaysia) and West Africa (Widowati et al., 2017). Rosella is high antioxidant activity because its high content of polyphenols including flavonoids such as quercetin, kaempferol, myricetin, apigenin and phenolic acids such as protocatechuic, o-coumaric, p-coumaric, ferulic. In addition, Rosella contains anthocyanins, ascorbic acid, citric, hydroxycitric, malic, hibiscus and tartaric acids (Da-Costa-Rocha, et al., 2014) and it is alpha-hydroxy acids (AHAs) source to stimulate skin cell turnover and reduce acne. Moreover, it improves moisture and inhibit collagenase, elastase and hyaluronidase affect, enhance elasticity, smooth and youth of skin (Widowati et al., 2017). Thus, It has a potential prevention of free radical damage skin and tissue (Chikhoune et al., 2017).

Ginger (*Zingiber officinale L.*) belongs to Zingiberaceae family. It has been reported of ability to prevent UV-B induced wrinkle by inhibition of skin elastase and probably via inhibition of elastic tortuosity three-dimensional configuration except water content in stratum corneum (Imokawa, 2009). 6-gingerol which is antioxidant activity via inhibition of xanthine oxidase that build reactive oxygen species (ROS) such as superoxide anion etc. Furthermore, the other actives in ginger, for examples, shagol and diarylheptanoids can inhibit prostaglandin and leukotriene formation including 5-lipoxygenase or prostaglandin synthetase (anti-inflammatory) (Young et al., 2002). Ginger is a Gram positive bactericidal and inhibits *Candida albicans* (Hasan et al., 2012).

White crane flower (*Rhinacanthus nasutus L.*) belongs to Acanthaceae family which contains flavonoids (quercetin and rutin), anthraquinones, triterpenes and sterols. Its antioxidant activity appears to reduce acne scar and aging wrinkle. In addition, this plant has Gram positive bactericidal and antifungal such as *Candida albicans,*

Trichophyton mentagophyta and *Malassezia sp*. lead skin fungal infection such as ringworm or *Tinea Vesicolor* etc. (Zubaid et al., 2004).

Therefore, herbal extract including chia seed, rosella calyx, ginger rhizome and white crane flower leaf appears to have the potential of antiaging properties. However, quality control of their fingerprint as well as chemical components such as total phenolic and flavonoid contents is very important to assure the quality of herbal extract raw materials. The advantages of using bioautography by Thin-Layer chromatography (TLC) and densitometry to quality control of the extracts are convenient, fast and cost effective and able to localize the active compounds in the extracts. In addition, this method can screen many samples in the same time.

Objective of the study

To compare quercetin content in herbal extracts including chia seeds, rosella calyxes, ginger rhizhomes and white crane flower leaves using Thin-Layer chromatography (TLC) and densitometer method.

Methodology

Chemicals and reagents

Quercetin was purchased from Sigma-Aldrich Co. (St.louis, MO, USA). Toluene, ethyl acetate and formic acid were purchased from QRec chemical Co., Ltd. (New Zealand). Methanol was purchased from Merck KGaA (Darmstadt, Germany).

Herbal extraction

Chia seeds were extracted by soaking in 95% Ethanol ratio 1:3 for 7 days and filtrated chia seeds extract. It was evaporated by Rotary evaporator (Buchi Co., Ltd., Thailand). Rosella calyxes, ginger rhizomes and white crane flower leaves extract were purchased from Thai-China flavours and fragrances industry Co., Ltd. (Phra Nakhon Si Ayutthaya, Thailand)

Determination of quercetin content

Quercetin content determination method used in this study was modified from Kaya et al. (2012). Quercetin 1 mg/ml was prepared as reference standard. All extract samples were prepared at a concentration of 10 mg/ml. The 200 ml of mobile phase contained toluene, ethyl acetate and formic acid in a ratio of 5 : 4 : 1. The TLC silica plate F_{254} analytical chromatography grade (Merck KGaA, Germany) was evaporated humidity at 70˚C for 10 minutes in hot air oven. Each sample and reference standard were spotted with 10, 20 and 30 µl, respectively. Reference standard and samples were spotted by Camag/LINOMAG IV (TLC spotter) on silica plate then silica plate was soaked in TLC tank contained mobile phase until mobile phase rose to solvent front level and rest until dry. Silica plate was observed band by Spectroline® Model CM-10A Fluorescence analysis cabinet (UV lamp with dark box) the UV light at λ=254 nm and then measured peak area for calculating absorbance unit (AU) by Manual winCAT scanner 3 (densitometric method). Then absorbance units (AU) were calculated for flavonoid content as mg equivalent to quercetin/g dry extract from standard curve equation.

Statistical analysis

Data were analyzed by IBM SPSS Statistics Software: Version 19.0. A one-way analysis of variance (ANOVA) followed by Tukey's range test was applied for analysis of data with the level of significance at $P < 0.05$.

Results

Table 1 Quercetin content of herbal extracts

Sample	Quercetin content (mg equivalent to quercetin/ g dry extract)
Chia (*Salvia hispanica L.*)	16.214 ± 1.43 [a]
Rosella (*Hibiscus sabdariffa L.*)	-
Ginger (*Zingiber officinale Ros.*)	17.692 ± 5.08 [a]
White crane flower (*Rhinacanthus nasutus L.*)	52.165 ± 6.87 [b]

Note: a, b were described significance level of quercetin content analyzed by ANOVA and Tukey's test. If the result was significant difference ($p < 0.05$), it showed different letter.

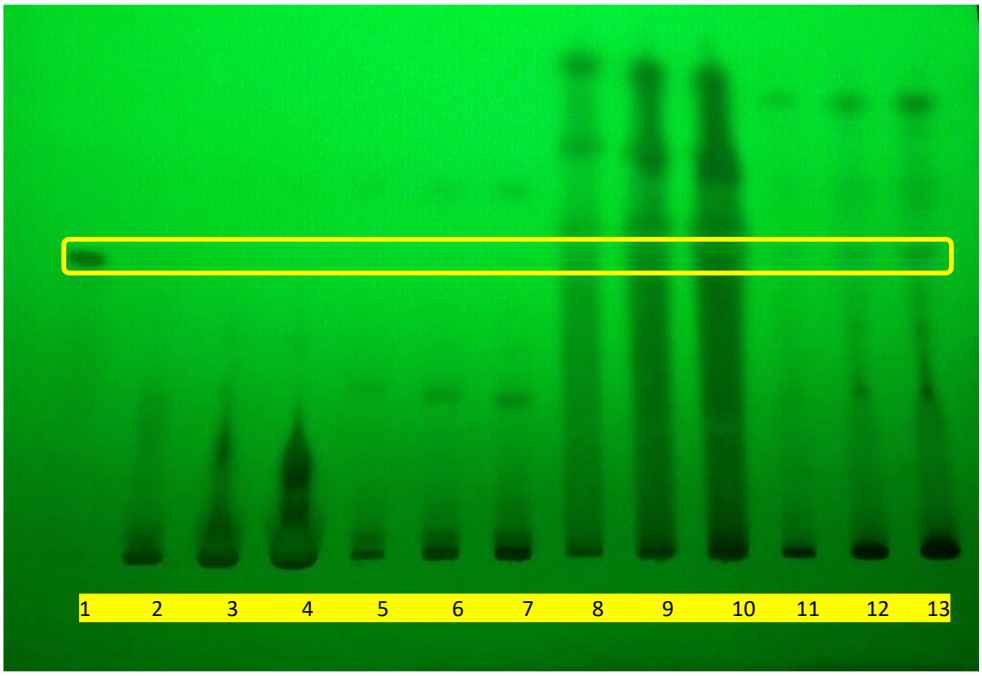

Figure 1 Showed the band of quercetin (No. 1), rosella calyxes extract (No. 2-4), chia seeds extract (No. 5-7), ginger rhizomes extract (No. 8-10) and white crane flower leaves extract (No. 11-13) by Thin-layer chromatography (TLC) and observed bands by Spectroline® Model CM-10A Fluorescence analysis cabinet (UV lamp dark box) at 254 nm.

Determination of quercetin content in herbal extracts by Thin-layer chromatography (TLC) and under curve area calculation by using Absorbance unit (AU). Figure 1 show TLC fingerprints of all tested extracts and quercetin reference standard in lane. Rf value of quercetin which is a ratio of distance of solute and distance of solvent. was 5 cm/10 cm = 0.5. As a result, quercetin in each extract was determine at Rf value of 0.5. However, the same Rf did not mean that it is only quercetin, it could be any other groups of compounds. Moreover, ginger extract bands were quite unclear when compare to the other extracts. As shown in figure 2-4, the quercetin content in white crane flower leaves extract was 52.165 ± 6.87 mg equivalent to quercetin/ g dry extract, which had significantly higher than that of ginger rhizomes extract (17.692 ± 5.08 mg equivalent to quercetin/ g dry extract) and chia seeds extract (16.214 ± 1.43 mg equivalent to quercetin/ g dry extract) ($p < 0.05$) whereas the quercetin content in ginger rhizomes extract was not significantly different from that of chia seeds extract . Interestingly, it was not found in rosella calyxes extract.

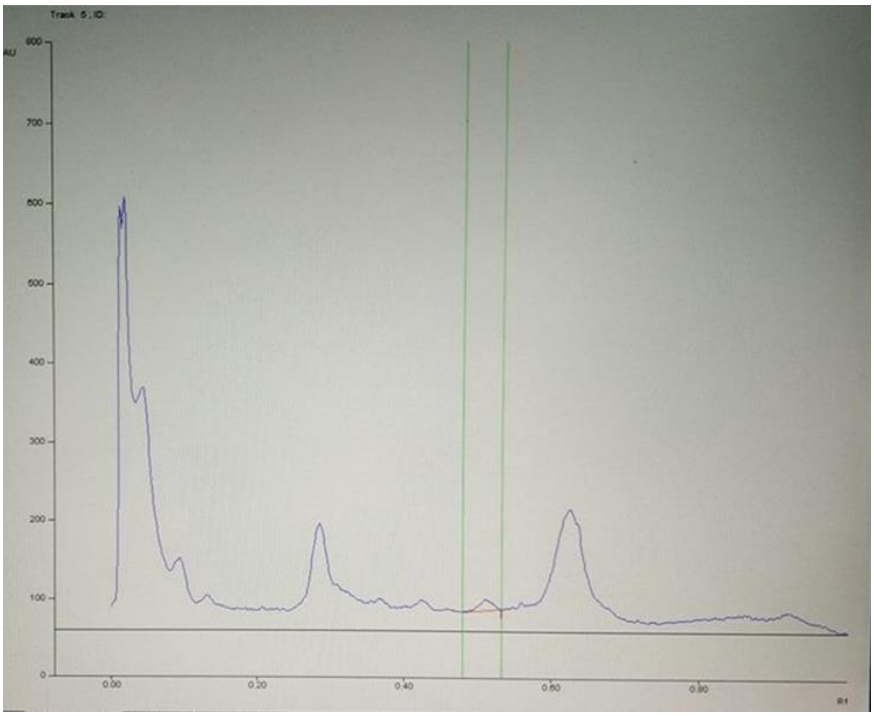

Figure 2 Showed under curve area was Absorbance unit (AU) of chia seeds extract by Manual winCAT scanner 3 (densitometer) and calculated quercetin content.

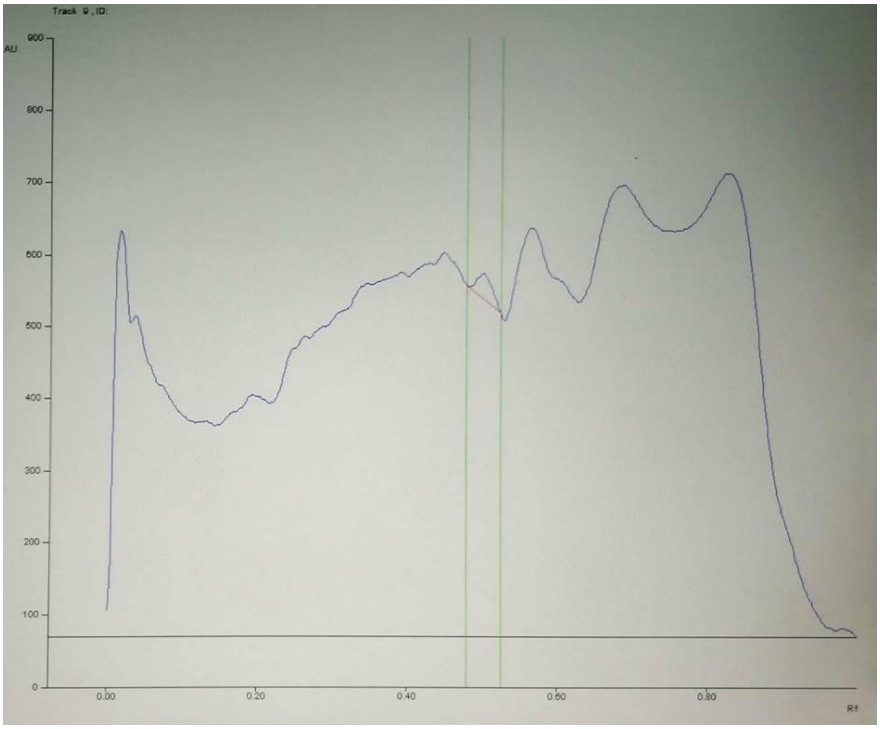

Figure 3 Showed under curve area was Absorbance unit (AU) of ginger rhizomes extract by Manual winCAT scanner 3 (densitometer) and calculated quercetin content.

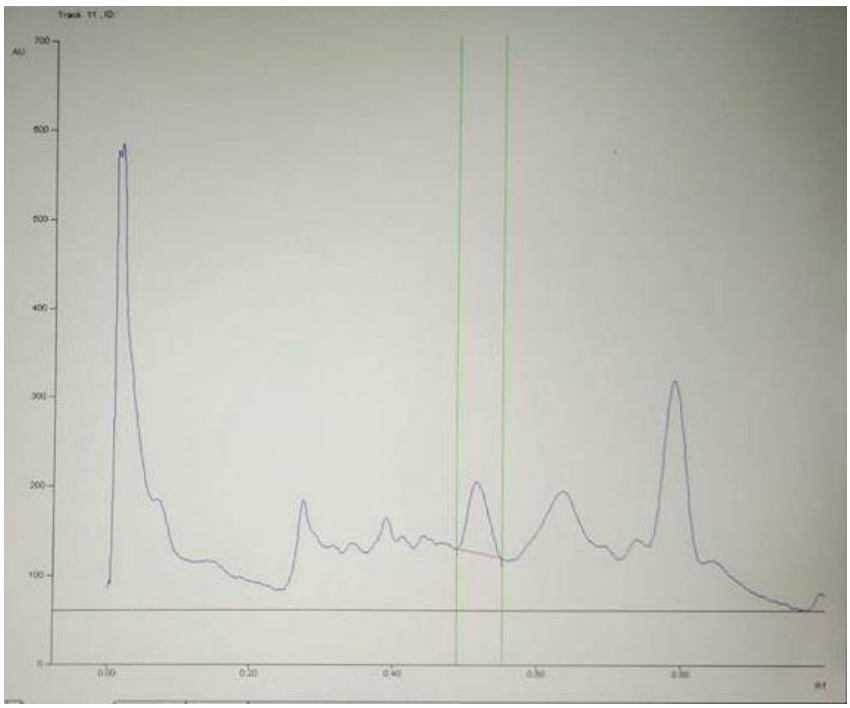

Figure 4 Showed under curve area was Absorbance unit (AU) of white crane flower leaves extract by Manual winCAT scanner 3 (densitometer) and calculated quercetin content.

Discussion and conclusion

Flavonoids are an important class of natural products. Particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure, widely found in fruits, vegetables and certain beverages. They have miscellaneous favourable biochemical and antioxidant effects associated with various diseases such as cancer, Alzheimer's disease (AD), atherosclerosis, etc. (de Falco et al., 2017). Scapin et al. (2016) studied flavonoids content of chia seeds extract in different condition showed that the highest flavonoids condition was chia seeds extract in 80% ethanol at 60 ˚C had 0.162 ± 0.003 g/kg EQ while the present study was 1783.498 ± 157.752 mg equivalent to quercetin g extract. There was different results due to different sources , chia seeds extraction and flavonoids calculation method (Scapin et al., 2016). In addition, Chia seed contains other flavonoids including Myrecetin, Kaempferol, Glycitin, Diadzin, Genistein, Genistin and Glycetein (Falco et al., 2017). Formagio et al. (2015) found that flavonoid content of rosella leaves was higher than calyxes (140.29 ± 3.14 and 97.43 ± 2.51 mg/g, respectively) (Formagio et al., 2015). In the present study used rosella calyxes extract purchased from Thai-China flavours and fragrances industry Ltd. Lot no. 60121100-1 and Thin-layer chromatography (TLC) did not contain quercetin content. Qadir et al. (2017) determined flavonoids content of herbal extract in 4 solvents including 80% ethanol, 80% methanol, 80% acetone and distilled water. Herbal extract including garlic, onion, ginger, Thyme leaves, mint leaves, aloe vera and oak to compare with catechin as standard found that thyme leaves in 80% ethanol had highest flavonoids content 17.64 mg CE/100 g, ginger in 80% ethanol about 11 mg CE/100 g, oak in distilled water was 0.41 mg CE/100 g. Because this study used different standard and herbal sources, flavonoids content in herbal extract were different (Abdul Qadir, Shahzadi, Bashir, Munir, & Shahzad, 2017). Ginger rhizome contains flavonoids content including quercetin, rutin, catechin, epicatechin, kaempferol and naringenin (Ghasemzadeh et al., 2010).

In conclusion, bioautography using TLC chromatogram could be used as a fingerprint characteristic of herbal extract as well as localize the active compound in tested herbal extract. This study could not use only quercetin to represent as total flavonoids. Ginger extract should be determined with more suitable mobile phase to show clear band and rosella extract should be determined by using more suitable method such as HPTLC. Moreover, at the same fingerprint TLC chromatogram, the other flavonoids content could be determined by densitometer. Once the mobile phase system has been established, this method will be rapid and cost effective for quality control of herb materials as well as localize the flavonoids in any herbal extracts.

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