Original Article

Phytochemical and Free Radical Scavenging Activities of Green Arabica Coffee Beans Cultivated in Thailand

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Abstract

Introduction and Objective: In Thailand, a famous Arabica coffee is widely grown in the North. However, this species has also been grown in recent years in Phetchaburi province, but it is not yet widely known. Therefore, this research aimed to analyze phytochemicals and free radical scavenging activities, examine the identity of the extracts by means of silica gel thin-layer chromatography, and determine the chlorogenic acid and caffeine contents of six green bean samples of Arabica coffee grown in Thailand.

Methods: The samples of ground dried coffee beans were extracted with 95% ethanol. Ten groups of phytochemicals were examined, including flavonoids, alkaloids, coumarins, tannins, cardiac glycosides, steroids, terpenoids, anthraquinones, saponins, and reducing sugars. The chromatogram characteristics of the coffee samples were successively examined using thin layer chromatography technique being observed under UV wavelength of 254 nm with chlorogenic acid and caffeine as standard substances, and reacting with specific reagents of anisaldehyde in sulfuric acid and DPPH solutions on a chromatogram plate. The antioxidant activity was the determination of DPPH scavenging by using UV-spectrophotometry technique. The chlorogenic acid and caffeine contents were also determined by measuring absorbance at wavelengths of 330 and 272 nm, respectively. The statistics used in this research included percentage, mean, standard deviation and *t*-value.

Results: The same phytochemicals were found in all the coffee extract samples. The seven groups of chemical constituents were flavonoids, alkaloids, coumarins, tannins, steroids, terpenoids, and reducing sugar. All the extracts showed the same chromatogram characteristics in all gradients of organic solvent systems. In addition, most of the extracts had high 50% free radical scavenging (SC50) levels of 3.48–9.93 mcg/mL. The chlorogenic acid and caffeine contents in all green coffee bean extracts were 4.72–7.95% and 0.24–0.45%, respectively, and the differences were statistically significant at the 0.05 level.

Discussion: All samples of Arabica coffee grown at similarly high altitudes had the same key phytochemical groups by using chemical reaction test and thin layer chromatography technique. Almost all samples showed higher free radical scavenging activities than vitamin C, according to this study using a quick and convenient technique as spectrophotometry. In addition, all coffee samples had chlorogenic acid and caffeine contents in the range of the international coffee bean quality standards, and were related to high free radical scavenging activities.

Conclusion and Recommendation: The green bean samples of Arabica coffee cultivated in Phetchaburi province, western Thailand, have the same type of phytochemical groups as those grown in the North. Therefore,

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it is appropriate to conduct further studies on this coffee species in terms of nutrition, biological activities, and develop for health products

Key words: chlorogenic acid, caffeine, Arabica coffee, phytochemicals, scavenging activities

สารพฤกษเคมีและฤทธิ์ดักจับอนุมูลอิสระจากเมล็ดกาแฟดิบอาราบิกาที่ปลูกในประเทศไทย

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บทคัดย่อ

บทนำและวัตถุประสงค์: กาแฟสายพันธุ์อาราบิกาที่มีชื่อเสียงของประเทศไทยนิยมปลูกมากในภาคเหนือของประเทศ แต่ยังมีกาแฟสายพันธุ์นี้ที่เพิ่งปลูกในจังหวัดเพชรบุรี ยังไม่เป็นที่ทราบและรู้จักกันแพร่หลาย ดังนั้นงานวิจัยนี้จึงต้องการวิเคราะห์กลุ่มสารพฤกษเคมี การวิเคราะห์ฤทธิ์การดักจับอนุมูลอิสระและตรวจสอบเอกลักษณ์ของสารสกัดด้วยวิธีการทางโครมาโทกราฟีชนิดแผ่นบางและการหาปริมาณกรดคลอโรจีนิกและคาเฟอีนของเมล็ดดิบของกาแฟสายพันธุ์อาราบิกา 6 ตัวอย่างที่ปลูกในประเทศไทย

วิธีการศึกษา: ตัวอย่างกาแฟแห้งบดหยาบนำมาสกัดด้วย 95% เอทานอล ตรวจสอบกลุ่มสารพฤกษเคมี 10 กลุ่มด้วยปฏิกิริยาเคมีจำเพาะได้แก่ ฟลาโวนอยด์ แอลคาลอยด์ คูมาริน แทนนิน คาร์ดิแอคกลัยโคไซด์ สเตียรอยด์ เทอร์พีนอยด์ แอนทราควิโนน ซาโปนินและน้ำตาลรีดิวส์ การตรวจสอบลักษณะโครมาโทแกรมด้วยวิธีโครมาโทกราฟีชนิดแผ่นบาง ที่สังเกตภายใต้แสงยูวีที่ความยาวคลื่น 254 นาโนเมตร โดยมีกรดคลอโรจีนิกและคาเฟอีนเป็นสารมาตรฐาน และการทำปฏิกิริยากับสารเคมีจำเพาะชนิดแอนนิซาลดีไฮด์ในกรดซัลฟิวริกและสารละลายดีพีพีเอชบนแผ่นโครมาโทแกรม การวิเคราะห์ฤทธิ์ต้านออกซิเดชันเป็นการวิเคราะห์การดักจับดีพีพีเอชด้วยวิธียูวีวิซ-สเปคโทรโฟโทเมทรี และการวิเคราะห์หาปริมาณกรดคลอโรจีนิกและคาเฟอีนโดยวัดค่าการดูดกลืนแสงที่ความยาวคลื่น 330 และ 272 นาโนเมตร ตามลำดับ สถิติที่ใช้ในการวิจัยได้แก่ร้อยละ ค่าเฉลี่ย ส่วนเบี่ยงเบนมาตรฐานและค่าที

ผลการศึกษา: การศึกษานี้พบว่าสารสกัดของกาแฟทุกตัวอย่างประกอบด้วยสารทางพฤษเคมีเหมือนกัน 7 กลุ่ม ได้แก่ ฟลาโวนอยด์ แอลคาลอยด์ คูมาริน แทนนิน สเตียรอยด์ เทอร์พีนอยด์ และน้ำตาลรีดิวส์ และมีลักษณะโครมาโทแกรมเหมือนกัน ในระบบตัวทำละลายทุกชนิด นอกจากนี้พบว่าสารสกัดเกือบทุกชนิดแสดงค่าการดับจับอนุมูลอิสระร้อยละห้าสิบเป็น 3.48-9.93 ไมโครกรัมต่อมิลลิลิตร ร้อยละกรดคลอโรจีนิกและคาเฟอีนในสารสกัดกาแฟในเมล็ดดิบทุกชนิด 4.72-7.95 และ 0.24-0.45 ตามลำดับ และมีความแตกต่างกันมีนัยสำคัญทางสถิติที่ระดับ 0.05

การอภิปรายผล: ตัวอย่างกาแฟอาราบิกาทุกตัวอย่างปลูกในที่สูงที่ระดับความสูงใกล้เคียงกันจึงส่งผลให้ทุกสารสกัดเมล็ดกาแฟดิบทุกตัวอย่างมีสารพฤกษเคมีหลักเหมือนกัน จากการตรวจสอบด้วยปฏิกิริยาเคมีและจากวิธีโครมาโทกราฟีชนิดแผ่นบาง เกือบทุกตัวอย่างกาแฟดิบมีฤทธิ์ดักจับอนุมูลอิสระสูงกว่าวิตามินซีจากการศึกษาด้วยเทคนิคสเปโทรโฟโทเมทรีซึ่งเป็นวิธีที่สะดวกและรวดเร็ว และทุกตัวอย่างมีปริมาณกรดคลอโรจีนิกและคาเฟอีนเป็นไปตามมาตรฐานคุณภาพเมล็ดกาแฟสากลและสัมพันธ์กับฤทธิ์ต้านอนุมูลอิสระที่สูง

ข้อสรุปและข้อเสนอแนะ: ตัวอย่างเมล็ดกาแฟดิบที่ปลูกในจังหวัดเพชรบุรี ภาคตะวันตกของประเทศไทยมีคุณภาพทางเคมีเหมือนกับตัวอย่างที่เก็บจากภาคเหนือของประเทศไทย สมควรที่จะนำไปศึกษาเพิ่มเติมทางด้านสารอาหาร ฤทธิ์ทางชีวภาพและพัฒนาเป็นผลิตภัณฑ์เพื่อสุขภาพต่อไป

คำสำคัญ: กรดคลอโรจีนิก, คาเฟอีน, กาแฟอาราบิกา, สารพฤกษเคมี, การดักจับอนุมูลอิสระ

Introduction and Objectives

Currently, there were more than 85 varieties of coffee, and the most common ones were Arabica and Robusta species. The distinguished characteristics of Arabica were that it had more Chlorogenic acid (CGA, 1, Figure 1) content than other coffee varieties[1]. This secondary metabolite was an indicator of green coffee bean quality and affected decision of consumers who needed health care. This constituent was reduced in the roasted coffee beans because it was destroyed when left in high temperature[2-3].

CGA and its derivatives were important bioactive compounds in green coffee bean such as anticancer, anti-diabetic, antioxidants, and anti-aging, etc[2-6].

Caffeine (2, Figure 1) was an important metabolite in coffee beans. It was found less ingredient in green coffee beans but more constituent in roasted coffee beans comparing by weight. That metabolite was a specific characteristic of coffee beans species and it was characterised roasted coffee bean standards[7] also. Especially, caffeine stimulated the nervous system, with alert and energetic reaction[7]. As a result, coffee remained a popular beverage consumed.

In addition, CGA and caffeine could indicated the type of coffee strain and the quality of the beans. The available techniques in review for determining of both key compounds were used both high-performance liquid chromatography (HPLC) and UV-Visible spectrophotometry[6,8-11].

Arabica coffee grown in Thailand was commonly grown in the northern mountains of the country[12] and in Phetchaburi province at the area on a hill more than 1,000 m above sea level, where the weather was mostly moist all year. The environmental conditions were quite similar to the climate and topography in the northern region where this coffee species was grown. Therefore, the purposes of this research were to study phytochemicals, CGA and caffeine contents, and free radical DPPH scavenging of Arabica coffee beans extracts of Thailand products, and newly grown coffee in Phetchaburi province.

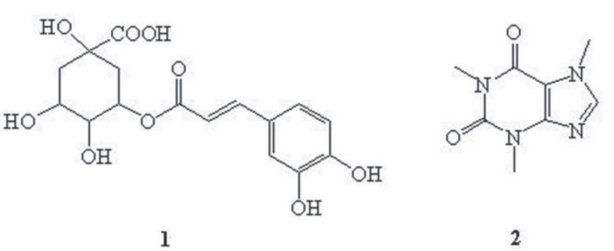


Figure 1 CGA (1) and caffeine (2)

Method

Part 1: Arabica coffee bean sampling.

All dried Arabica green coffee beans were purchased from local stores at northern of Thailand and Phetchaburi province, coding A, B, C, D, E and F in April, 2023. The all samples, except C, were coffee bean which harvest in Royal Project at the North of Thailand. Every samples were crushed and extracted with 95% EtOH in water by maceration method for 24 hours. After filtered, solutions were evaporated immediately to obtain the crude extracts of Arabica coffee green beans to determine part 2-4.

Part 2: Determination of phytochemical groups by chemical reaction[13-14], as:

Before testing with all reaction, about 100 mg of each crude extract were dissolved in 5 mL 95% ethanol.

Test for flavonoids: After adding 1 mL of Conc. HCl into 2 mL of extract, the solution was added with 1 piece of Mg, and then boiled in hot water basin for 3 minutes.

Test for alkaloids: After adding 0.5 ml of Conc. H_2SO_4 into 2 mL of extracts, the solution was shaken and then boiled for 2 minutes in a hot water basin. The residue was left, then adding 5 drops of Meyer reagent into cooled filtrate.

Test for coumarins: After soaking the chromatogram paper with few drops of 1 M NaOH, the extract was dropped on dried paper, and then the paper was heated for 3 minutes. The fluorescent observation was under wavelength 366 nm of UV light.

Test for tannins: After adding 2 mL of extracts into 2 mL of distilled water, solution was boiled for 5 minutes. Filtrate was left cold and tested with few drops of 1% $FeCl_3$. The another test, after adding 2 mL of 2 M NaOH into 1 mL extract, the change of solution colour was observed.

Test for cardiac glycosides: Five mL of extracts were added into 5 mL of 10% $Pb(OAc)_2$ and then boiled for 5 minutes. After the mixture became cold, the solution was filtrated, and then extracted with DCM 5 mL 3 times. The filtrate was concentrated by heating until solution became half of volume. Finally, solution was separated to 3 parts for testing functional groups as follows:

Test for lactone: Adding 3 drops of Kedde's reagent and 2 drops of 1 M NaOH into extract, the change of solution colour was observed.

Test for deoxy sugar: The extract was added with 5 drops of 1% $FeCl_3$, and 3 drops of Conc. HOAc. After shaking, the test tube was tilted about 45°C then 1 mL of Conc. H_2SO_4 was dropped along the test tube wall. Testing was complete by observing the colour of the ring on the seam of mixture.

Test for steroid: After adding 3 drops of Conc. HOAc into 2 mL of extract, then the solution was shaken and dropped slowly with Conc. H_2SO_4. The colour change was observed using the positive test.

Test for terpenoids: After 2 mL of extract was separated with DCM, the soluble DCM was tested with 1 mL of Conc. H_2SO_4 by tilting the test tube for about 45 o and then dropping Conc. H_2SO_4 gently in the test tube wall. The change of colour of the ring at the seam of solution was carefully observed.

Test for anthraquinones: Five drops of 10% H_2SO_4 were added into 2 mL extract, and then the solution was shaken and boiled for 2 minutes. After leaving it cold, the solution was added with 1 mL of 10% NH_3.

Test for saponins: After adding 5 mL of distilled water into 2 mL of extract, solution was mixed vigorously. The frothing was mixed with few drops of olive oil and mixed vigorously and then the foam appearance was observed.

Test for reducing sugar: After 1 mL of Benedict's reagent was added to 2 mL of extract, solution was shaken and boiled for 5 minutes. The copper colour of the precipitate was observed.

Part 3: Plant identity testing was determined by using Thin Layer Chromatography (TLC) technique. Silica gel GF_{254} was stationary phase and isocratic solvent as organic solvent, and acetone and mixed organic solvent system were mobile phase including DCM:MeOH (9:1), MeOH: $CHCl_3$ (1:9), EtOH:DCM (1:9), EtOH:$CHCl_3$ (1:9), and CHCl3: acetone: Conc. HCOOH (6.5:1.5:2.0). The identity of plant extracts was observed under UV light at a wavelength of 254 nm. Although chromatogram characteristics of secondary metabolites were appeared on TLC plates[15] by comparing with standards, two chemical reaction with reagents as anisaldehyde in Conc. H_2SO_4 and DPPH solution on plate were tested.

Part 4: DPPH free radical scavenging activity was investigated by using UV-Visible spectrophotometry technique at wavelengths of 517 nm in ethanol solvents. For vitamin C as a positive standard, its concentration of 15, 20, 25, 30 and 35 μg/mL was prepared. After 100 μL of standard or extract solutions were added into solution of 0.002 mM, DPPH 200 μL and ethanol volume 3.8 mL, the testing solution was incubated in a dark place for 30 minutes[13]. The analysis of absorption was determined with spectrophotometry technique at wavelength 517 nm by using UV-Vis spectrophotometer (model SP8001 of Metertech), with ethanol as blank. The percentage of free radical scavenging activity, was calculated with the formula (1-[As/Ab]) X 100, when As was the absorption value of the sample or standard and Ab was the absorption value of the solvent. The scavenging DPPH concentration at 50% (SC_{50}) was analysed by using formula of curve

comparing with vitamin C as a positive standard. The experiment was repeated 3 times.

Part 5: Determination of CGA and caffeine in green coffee was successively measured using method from Navarra *et al*.[11] The absorbance was read at wavelength 330 and 272 nm, respectively. Analysis was done in triplicate for each extract. Standard solutions of CGA and caffeine in range of concentration 7.5-22.5 and 2.5-20.0 μg/ml were used to obtain a calibration curve. The CGA and caffeine contents were exposed as a percentage of CGA and caffeine in green coffee beans. Both chemical standards were dissolved in 70% EtOH. The samples were uniformly dissolved in EtOH. UV/V is absorbance spectra were recorded to determine the molar extinction coefficient values for the caffeine and CGA, as well as expected when ethanol was mainly used as solvent. The determination of the molar extinction coefficients of caffeine and CGA was realized by applying the well-known Lambert-Beer law, by fitting linearly the dependence of the absorbance versus the sample concentration.

Statistical analysis

Each sample analysis was performed in triplicate. All of the presented results are means (± standard deviation) of at least three independent experiments. One way analysis of variance (ANOVA) was used for test of significance at $p = 0.05$.

Results

All extracts of green coffee beans extracted with 95% ethanol in water consisted of similar 10 phytochemical groups, including flavonoids, alkaloids, coumarins, tannins, cardiac glycosides, steroids, terpenoids, anthraquinones, saponins and reducing sugars. (Table 1)

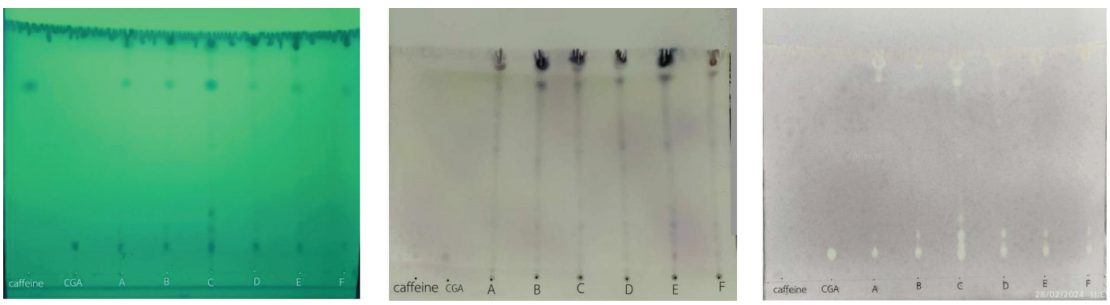
Table 1 Qualitative analysis of phytochemicals in all coffee beans extracts

Phytochemicals	Results					
	A	B	C	D	E	F
Flavonoids	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Cardiac glycosides	–	–	–	–	–	–
Steroids	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Anthraquinones	–	–	–	–	–	–
Saponins	–	–	–	–	–	–
Reducing sugar	+	+	+	+	+	+

Note: + means positive test
– means negative test

Plant extract identity was tested by using thin layer chromatography, silica gel GF_{254} as absorbance in a solvent system of $CHCl_3$ to acetone to Conc. HCOOH (6.5:1.5:2.0). Additionally, the identity was observed under UV light at 254 nm wavelength and the reaction on other sheet plates with anisaldehyde in Conc. H_2SO_4 and 0.02 mM DPPH. All green coffee extracts displayed that all chromatogram were very similar. (Figure 2)

The major chemical constituents, caffeine and CGA, were found with R_f values at 0.69 and 0.12 (Figure 2) in order of low to high polar compounds. They exhibited absorption properties under UV light at a wavelength of 254 nm. The identity secondary metabolites were found when reacting with anisaldehyde in Conc. H_2SO_4, with R_f = 0.81 (brown), and 0.12 (pink), the later was a CGA. In addition, bleaching of DPPH reagent was occurred on TLC plates, which was CGA, with $R_f = 0.12$[16]. (Figure 2 and Table 2)



UV 254 nm anisaldehyde-Conc. H_2SO_4 DPPH

Figure 2 Finger prints of green coffee beans extracts under UV 254 nm and reacted with chemical reagent

Table 2 The R_f of active secondary metabolites of green coffee extracts

Mobile phase systems	CGA	caffeine	Mobile phase systems	CGA	caffeine
Acetone	0.00	0.55	MeOH:$CHCl_3$ (1:9)	0.00	0.67
$CHCl_3$:acetone:Conc. HCOOH (6.5:1.5:2.0)	0.12	0.69	EtOH:DCM (1:9)	0.00	0.58
EtOH:$CHCl_3$ (1:9)	0.00	0.51	MeOH:DCM (1:9)	0.00	0.65

The percentages of extract weight per weight of dried coffee beans were in the range of 1.26-3.18% as shown in Table 3. All sample extracts showed free radical scavenging activities of DPPH with SC_{50} value between 3.48-55.70 μg/mL, the C sample (Phetchaburi coffee bean) was the highest activity of 3.48 μg/mL . Whereas, SC_{50} of vitamin C, positive

control, was 27.06 ± 0.02 μg/mL as shown in Table 2. All extracts showed different DPPH free radical scavenging activities with statistical significance at the 0.05 level. (Figure 3)

Table 3 Percentages of green coffee beans extracts and DPPH free radical scavenging activities

Samples	Beans (g)	Extracts (g)	%w/w	SC_{50} (μg/ml)	S.D.
A	31.8380	0.4018	1.26	9.93[a]	0.08
B	32.0282	1.0189	3.18	55.70[b]	2.20
C	32.2756	0.7298	2.26	3.48[c]	0.13
D	31.9683	0.738	2.31	7.15[d]	0.45
E	32.2010	0.8124	2.52	4.46[e]	0.04
F	31.9725	0.5424	1.70	5.71[f]	0.06
Vitamin C	-	-	-	27.06[g]	0.02

Note: a different letter in a column meant there was a statistically significant difference at a confidence level of 0.05.

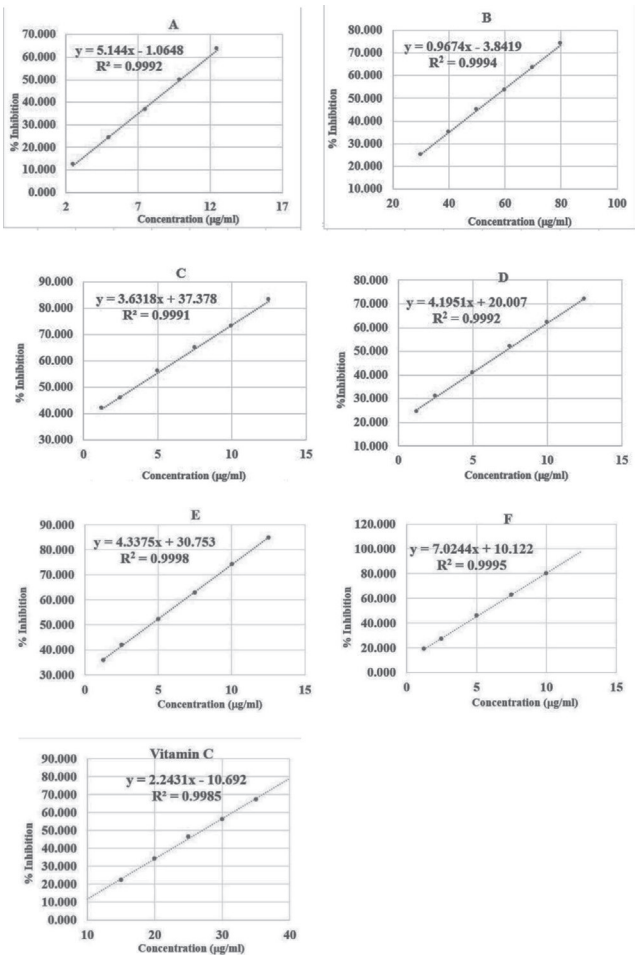


Figure 3 DPPH scavenging activities of all extracts and vitamin C

There is a growing interest in the monitoring and quantification of caffeine and CGA in coffee beans. Therefore, UV-Vis spectrophotometry was used in this study, which caffeine and CGA were measured at wave lengths of 330 and 272 nm, respectively. From this method, 70% EtOH in water as solvent[11], it was found that all 6 extracts contained CGA contents of 4.72-7.95% (Table 4). The CGA standard curves were of R^2 = 0.9992 (Figure 4). The caffeine contents were analysed from the standard curve of R^2 = 0.9989 (Figure 4) and caffeine amounts were in the range of 0.24-0.45 % (Table 4). It was also found that each type of Arabica green coffee bean extract was significantly difference at the 0.05 level.

Table 4 The percentage of CGA and caffeine contents of all samples

Samples	pH	%CGA	S.D.	%caffeine	S.D.
A	5.02	5.45[a]	0.02	0.28[a]	0.01
B	4.95	6.48[b]	0.01	0.35[b]	0.01
C	4.98	7.95[c]	0.02	0.45[c]	0.01
D	5.10	4.72[d]	0.01	0.26[d]	0.02
E	5.12	5.64[e]	0.03	0.29[e]	0.02
F	5.01	5.06[f]	0.02	0.24[f]	0.01

Note: a different letter in a column meant there was a statistically significant difference at a confidence level of 0.05.

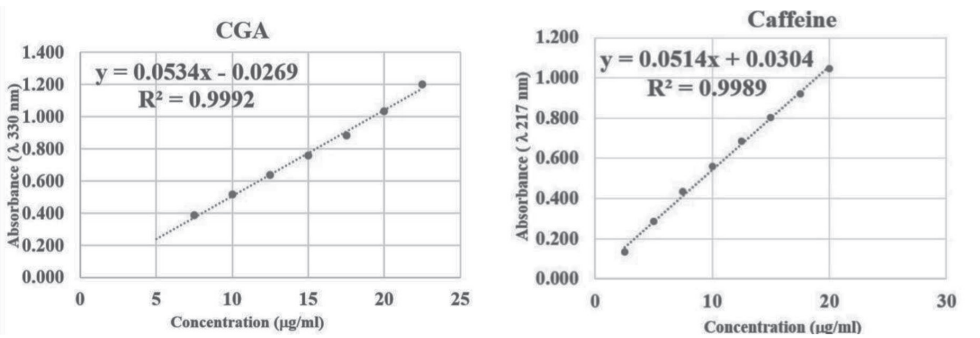


Figure 4 Standard curve of CGA and caffeine

Discussions

All 6 samples of coffee beans with different sources in Thailand producing coffee beans with similar quality contained the same major secondary phytochemicals. There were 6 secondary metabolite groups including flavonoids. alkaloids, coumarins, steroids, terpenoids and reducing sugars. In addition, there were also reports that Arabica coffee had constituents as flavonoids, alkaloids, ter-

penoids, and phenols[17].

This research results agreed with the literature report for the main chemical composition of green coffee beans, which each group of substances had different pharmacological effects[17], for example, many low-polar substances such as alkaloid group including caffeine, threobromine and theophylline, and terpenoid group including cafestol and kahweol and α-cocopherol. Triacylglycerols and steroids had also been found in coffee beans. The high-polar substances were also found, including phenolic groups such as CGA and its derivatives, etc. Therefore, this research was additional information for investigating the preliminary quality of the basic phytochemical groups of Arabica coffee beans, especially cultivated in Phetchaburi province.

There were many substance group analysis reports, however, the chromatogram characteristics of the Phetchaburi bean extracts had not been reported. This technique used the movement of substances on silica gel GF_{254} sheets to compare the key chemical components of green coffee beans. This herb had biologically important because of its health effects, so chromatogram was used for quantity analysis by comparing with CGA and caffeine standards.

In the gradient solvent system, MeOH to DCM (1:9), the substance with an R_f value of 0.65 was caffeine. Its identity showed an R_f value of 0.55 in a single solvent, acetone, and R_f values of 0.69, 0.67, 0.58 and 0.51 for mixed solvent system of $CHCl_3$ to acetone to Conc. HCOOH (6.5:1.5:2.0), MeOH to $CHCl_3$ (1:9), EtOH to DCM (1:9), and EtOH to $CHCl_3$ (1:9), which this information was also found[15]. In contrast, CGA, a high-polar component, showed an R_f value of 0.12 in a solvent mixture of $CHCl_3$ to acetone to Conc. HCOOH (6.5:1.5:2.0). However, the R_f value of other main substances were 0.00 in all systems of solvents, including acetone and MeOH to $CHCl_3$ (1:9), EtOH to DCM (1:9), and EtOH to $CHCl_3$ (1:9), which referred that CGA was a strong polar compound[16].

It was also found that all sample coffee beans had the same main composition characteristics not only seen in visible light, but also under wave length of 254 nm UV light, and reaction with anisaldehyde in Conc. H_2SO_4 and 0.02 mM DPPH. This study also revealed that the secondary metabolite spot of CGA and caffeine were at R_f of 0.12 and 0.69, respectively, in EtOH to DCM (1:9) system. Due to observation after spraying DPPH onto the TLC sheet, the purple bleaching of DPPH occurred the most quickly and clearly than other components. It was found that the main constituents of green coffee beans had strong identity and specificity on CGA spot.

Moreover, when all 6 green coffee samples were tested for antioxidant activity of 0.02 mM DPPH comparing with vitamin C standard

as shown in Figure 3, it was found that the SC_{50} values were in range of 3.48-55.70 μg/ml, agreeing with the reported research[18-19]. The reported research displayed that high-polar extracts had higher antioxidant activity than low-polar extracts. So this research showed that the strong active anti-oxidative agents included caffeine and CGA[18], which was observed in DPPH's bleached TLC sheets as shown in reaction (Figure 5). This work used DPPH scavenging method for evaluate anti-oxidant activity, because the DPPH assay is an easy and rapid way to determine.

In order to check the coffee cultivating places affecting the main secondary metabolites for all substances characterising chromatogram, the extract polarity was not separated. All samples in this study were harvested from the similar height of cultivation, so the caffeine and CGA were in range of standard contents[19]. Consequently, for the analysis of caffeine content using UV-Vis spectrophotometry technique, Arabica coffee species grown in Ethiopia at altitudes above sea level from 1,400-2,508 m showed the caffeine content of water extract in range of 0.62-1.16%. However, this research showed its amount less than previously data, because the sample were extracted in hot water[20]. Sample C harvested from Phetchaburi province was different CGA and caffeine contents from each other with statistical significance at the 0.05 level, but they were in range of standard caffeine level[21]. The pH of those were in range of weak acid about 5. Not only the difference in altitude of growing places, but also all Arabica green coffee grown in Thailand displayed the higher level of free radicals scavenging than vitamin C about 3-9 times by compared both in ethanolic extracts. The previous report found that the SC_{50} was higher than that found in this study, because in this research the samples were extracted by maceration with ethanol at the room temperature, but in that report the samples were extracted with hot water 90°C for 15 mins[22]. Because in this research the bean crude was extracted at low temperature, CGA, a potential active substances were not thermally destroyed[5,21-23]. So, there were a lower SC_{50}, but higher antioxidant activity.

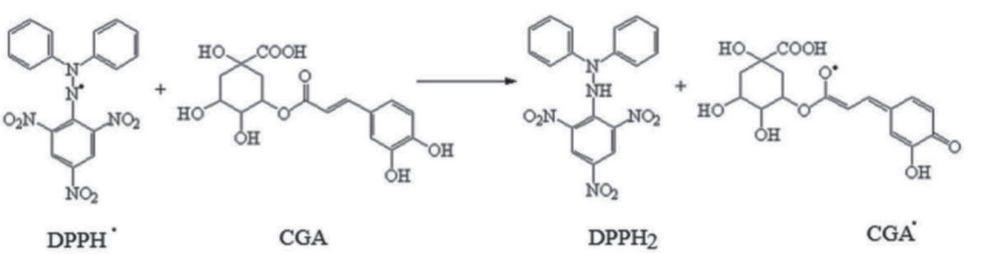


Figure 5 Reaction of CGA with DPPH free radical

In the previous report of CGA and caffeine amounts in Arabica coffee were the 3.4-14.4 % and less than 10%, respectively[3,23]. All 6 Arabica green coffee beans cultivated in northern of Thailand had key secondary metabolites, including CGA and caffeine. Their amounts were in range of 4.72-7.95 % and 0.24-0.45%, respectively. which were in range of a standard quantity[3,23]. In this study, all ethanolic coffee extracts had significant scavenging effects on the DPPH radical which increased with decreasing activity in the 3.48-55.70 μg/ml range; C sample had high DPPH radical scavenging, 3.48 ± 0.13 μg/ml, compare to all 5 northern coffee beans and vitamin C in the DPPH radical scavenging assay was 27.06 ± 0.02 μg/ml, all samples including A-F and standard had a statistically significant result ($p < 0.05$). On TLC sheet, the CGA position was the most rapid active with DPPH reaction. The positive DPPH scavenging test, CGA standard, suggested that all 6 bean samples cultivated different places in Thailand had CGA, as strong free radical DPPH scavenging activity. From all 6 samples, C sample had the highest CGA content, 7.95 ± 0.02%, so this sample showed potential strong DPPH scavenging activity too. According to the percentage of CGA in green from Brazil, Columbia and Kenya (11 samples), which were acquired from several stores in Jordan, was 5.43 ± 0.89%, espectively. However, all those coffee samples extractions were extracted using hot water at 75-85°C at a ratio of 1:100, that extracted condition was high polarity solvent and hot condition to separated metabolite. The CGA was more destroyed easily in hot water than cold maceration. On the other hand caffeine amount in our work was less than that report, because caffeine content was less separated with alcoholic solvent than water[25].

Conclusion

The Phetchaburi green coffee bean, C sample, showed the highest amount of CGA, 7.95%, according to the highest DPPH scavenging activity of 3.48 μg/ml. In terms of the highest antioxidant activity of bean in this research comparing with vitamin C, it was found that 3 g of green coffee bean ethanolic extract had the same activity as 54 tablets of 500 mg vitamin C. In summary, this research presented that samples of green coffee beans grown in Phetchaburi province contained the highest levels of two important substances than those grown in other areas, because it was grown in the abundant mineral and nutrient foothill and specially, this beans were the first harvested. Although it had quite similar weather and geography to northern Thailand, thus, Arabica coffee plants synthesized secondary metabolites similar chemical

groups to those in the same species grown in other regions[26], but they had not in the same quantity and quality. Therefore, this research supported Green coffee bean consumers were more confident that the coffee beans grown in Phetchaburi province had high quality level. This research data led to the promotion of this Arabica coffee bean was a unique agricultural product of Phetchaburi province.

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