Original research article

Preliminary Phytochemical Screening and Antioxidant Activity of Robusta Coffee Blossom

Vanida Chairgulprasert* and Kittiya Kongsuwankeeree
Department of Science, Faculty of Science and Technology, Prince of Songkla University, Pattani Campus, Mueang, Pattani 94000, Thailand

Received 5 August 2016; Received in revised form 19 September 2016
Accepted 27 September 2016; Available online 24 March 2017

ABSTRACT

The Robusta coffee flowers were analyzed for phytochemical composition and antioxidant activity. Flower powders at three different stages were extracted successively with hexane, dichloromethane and methanol, resulting in the extracts of varying yields (1.61-16.01%). All crude extracts were constituted of flavonoids, terpenoids and alkaloids. The methanol crude extracts exhibited the best antioxidant activity on both DPPH (EC_{50} 0.03-0.13 mg/mL) and reducing power (EC_{50} 0.19-0.46 mg/mL). It also contained the highest amount of total phenols (28.32-139.88 mg GAE/g extract).

Keywords: Phytochemical screening; Antioxidant; Total phenols; Robusta blossom

Introduction

Robusta coffee, *Coffee robusta* Pierre ex A Frochner, belongs to the family of Rubiaceae [1]. Its origin is in central and western sub-Sahara Africa, but it is now cultivated in more than 70 countries such as Indonesia, India, Uganda, Vietnam, Brazil and Thailand [2,3]. The planted area of Robusta coffee is in the southern part of Thailand. According to the agricultural statistics for 2015, the harvested area was 185,167 rais and the total production was 17,028 tons [4]. The coffee plants thrive best at altitudes of 200-900 meters and produce white flowers with strong aroma after the first shower of the rainy season [3]. An adult trees can produce around 30000-40000 flowers. The Robusta coffee is about 30% of the world market production [5]. Coffee is rich in antioxidants [6-8]. The coffee beverage has been consumed as a stimulant, diuretic, antioxidant, and antipyretic, and also to relieve spasmodic asthma. Tea from coffee flowers has also been consumed in some countries. In Thailand, it is widespread in the northern and southern parts. It is believed that coffee can reduce blood cholesterol, lower blood sugar and prevent obesity, liver and heart diseases.

Currently, people have to face and tolerate numerous pollutants all day long, especially free radicals which could deteriorate health and cause chronic problems. Free radicals, such as superoxide anion and hydroxyl radical are the main cause of oxidative stress leading to early aging, and many ailments including rheumatoid arthritis, cancer, diabetes and cardiovascular disease [9]. Antioxidants are required for everybody to prevent early aging and promote health. Many natural foods are good sources of dietary antioxidants which

***Corresponding author:** vanida.c@psu.ac.th

are of much interest to be consumed for their health promoting or pharmaceutical properties.

The aim of this work was to evaluate the antioxidant property and total phenolic content of Robusta coffee blossoms. The phytochemical screenings of flavonoids, terpenoids and alkaloids were also investigated.

Methodology

Robusta flowers

Robusta blossoms were obtained from a coffee farm in Surat Thani province, Thailand. The blossoms were collected at three different times (January, February and March, 2016) and they have three different ages: 5, 7 and 9 days. They were dried naturally in the shade, and ground into fine powders using a blender.

Preparation of crude extracts

Each Robusta flower powder at three different ages was macerated successively with hexane, dichloromethane (DCM) and methanol at flower powders: solvent ratio of 1:1.5-2.0 for 24 h. extracting three times with each solvent. Each combined extract was evaporated under reduced pressure to produce hexane, dichloromethane and methanol crude extracts which were used for determination of phytochemical screening, total phenols and antioxidant activity.

Phytochemical screening

The crude extracts of Robusta flowers were screened for the flavonoids, terpenoids and alkaloids. Flavonoids were determined by DPPH and Sofowara reagents. Mayer and Wagner reagents were employed to test alkaloids. Salkowski and Liebermann-Burchard reagents were used to verify the terpenoids [10-11].

DPPH's test

To each extract solution 0.2 mM DPPH was added. A yellow coloured solution will indicate the presence of flavonoids.

Sofowara's test

Each extract solution was treated with ammonia and sulfuric acid**.** A yellow coloured solution will indicate the presence of flavonoids.

Liebermann-Burchard's test

Each extract solution was treated with glacial acetic acid and sulfuric acid**.** A red coloured solution will indicate the presence of terpenoids.

Salkowski's test

Each extract solution was treated with chloroform and sulfuric acid**.** A brownish coloured solution will indicate the presence of terpenoids.

Mayer's test

Each extract solution was treated with 2 drops of hydrochloric acid and potassium mercuric iodide. A yellow precipitate will showed the present of alkaloids.

Wagner's test

Each extract solution was treated with 2 drops of hydrochloric acid and iodine in potassium iodide. A reddish precipitate will indicate the presence of alkaloids.

Determination of total phenols

Each extract was analysed for total phenol content by using Folin-Ciocalteu reagent [12]. Folin-Ciocalteu reagent 0.75 mL was diluted 10 times with distilled water and added to 0.1 mL of 5 mg/mL extract solution. The mixture was shaken and left to stand for 5 min. The 0.75 mL of 10% sodium carbonate in 10 mL of distilled water was then added. It was mixed thoroughly and allowed to stand at room temperature. After 90 min, the absorbance was recorded at the wavelength 725 nm.

Antioxidant assays

Each crude extract was assessed for antioxidant activity by 2 methods including DPPH and reducing power as follows.

Scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals

The antioxidant activity was evaluated on the basis of its activity in scavenging the DPPH radical [13]. Each extract was diluted in methanol to give at least 5 different concentrations. A volume of 2.5 mL of each concentration was mixed with 2.5 mL of 0.2 mM DPPH and left to stand for 30 min in the dark. The absorbance (Abs) was then measured at the wavelength 517 nm against a blank using a spectrophotometer. The scavenging effect was calculated as followed.

$$\text{Scavenging effect (\%)} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}})}{\text{Abs}_{\text{control}}} \times 100$$

The corresponding EC_{50} values, obtained from the graph, are the concentration of extracts that scavenged DPPH radicals in 50%.

Reducing power

The reducing power of the extracts was determined using the reported procedure [14]. The crude extract in methanol 1 mL was mixed with 2.5 mL of sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The sample was incubated at 50°C for 30 min and 2.5 mL of 10% trichloroacetic acid was added. The mixture was centrifuged for 10 min. The 5 mL of supernatant was mixed with 5 mL of deionised water and 1 mL of 0.1% ferric chloride. After 10 min, the absorbance of the sample was measured at 700 nm against a blank. The EC_{50} value represents the concentration of the extract at which the absorbance was 0.5.

Results and Discussion

Solvent extractions

The 50.35, 150.24 and 170.75 g of Robusta flower powders at three different ages (Stage 1, 2 and 3) were extracted continuously with hexane, dichloromethane and methanol in different yields as depicted in Fig. 1. It was found that Robusta blossom provided the methanol crude extracts in the highest percentage yield (5.52-16.06%), followed by dichloromethane (1.67-4.12%) and hexane extracts (1.61-2.23%). Significantly, the highest yield was obtained from the first stage of Robusta coffee.

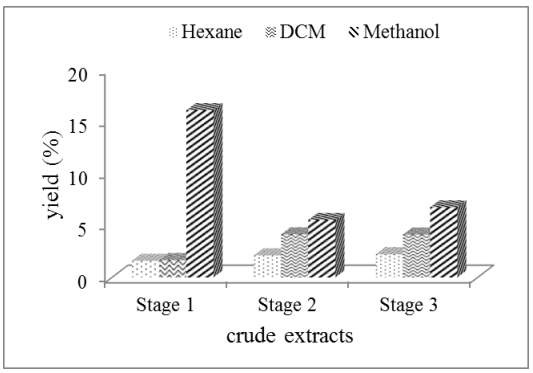


Fig. 1. The weight of crude extracts from Robusta coffee blossoms.

Chemical constituents

The results of preliminary phytochemical tests of hexane, dichloromethane and methanol crude extracts are shown in Table 1. It was revealed that terpenoids, flavonoids and alkaloids were present in all crude extracts. Flavonoids were found a little more in the methanol crude extracts than dichloromethane and hexane crude extracts, respectively. Alkaloids were found equally in dichloromethane and methanol crude extracts and at higher concentrations than in hexane crude extracts. Additionally, terpenoids were found in comparable amounts in all crude extracts.

However, the methanol crude extracts contained flavonoids in amounts comparable to alkaloids and terpenoids. The dichloromethane extracts found alkaloids and terpenoids at higher concentrations than flavonoids, whereas the hexane crude extracts exposed higher concentrations of terpenoids than alkaloids and flavonoids.

Total phenols

Each extract was evaluated for total phenol concentration as gallic acid equivalents (GAE) per gram of extract and the results were detailed in Table 2. The concentration of total phenols was varied from 6.43 to 139.88 mg GAE/g of extract. The oldest blossoms contained a greater total of phenols than the second and the first stages, respectively. Additionally, the methanol crude extracts showed the highest concentration of phenols (28.32-139.88 mg GAE/g of extract) whereas the hexane crude extracts demonstrated the lowest concentration (6.43-87.74 mg GAE/g of extract). The total phenols found in Robusta blossoms were considerably more than those found in the Robusta green bean and instant coffee (56.73 and 140.78 mg/g) [7].

Antioxidant properties

Three crude extracts including hexane, dichloromethane and methanol showed different efficiency on DPPH radical scavenging as indicated in Fig. 2. The results showed that the radical scavenging effect on DPPH increased with increasing of extract concentrations. The methanol crude extracts showed the highest scavenging effect. At 0.2 mg/mL, the methanol extracts of Robusta blossom stage 3, 2 and 1 showed the efficiency of 71.0, 65.6 and 56.9 % respectively. Obviously, the antioxidant activities of all extracts in stage 3 were better than those of stage 2 and 1. The corresponding EC_{50} values of each extract are shown in Table 2. The extracts with the lower values of EC_{50} conformed to the higher antioxidant activity. All crude extracts revealed some different antioxidant efficiency. Amongst them, all crude extracts of coffee flowers in stage 3 had the highest activity with the lowest EC_{50} on scavenging DPPH radicals (0.01-0.10 mg/mL), followed by coffee flowers in stage 2 and 3, respectively. Moreover, the methanol crude extracts exhibited the highest efficiency on DPPH radical scavenging. The dichloromethane crude extracts were slightly less effective and the hexane crude extracts are the significantly least active. However, they were still less effective than the synthetic antioxidants of BHA (EC_{50} = 0.002 mg/mL) and ascorbic acid (EC_{50} = 0.004 mg/mL).

Table 1. Phytochemical constituents of extracts from Robusta blossom powders.

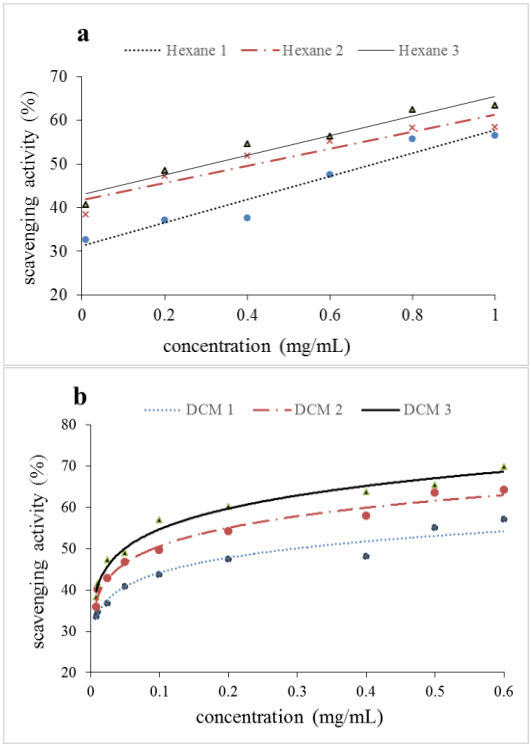
Phytochemical Constituents	Methods	Crude extracts		
		Hexane	Dichloromethane	Methanol
Terpenoids	Liebermann-Burchard	+++	+++	++
	Salkowski	+++	+++	+++
Flavonoids	DPPH	+	++	+++
	Sofowora	+	+	+++
Alkaloids	Mayer	+	+++	+++
	Marquis	+	++	++

+++ : Present in high concentration, ++ : Present in moderate concentration,
+ : Present in low concentration

Table 2. Total phenols, IC_{50} values of DPPH and reducing power activity of extracts from Robusta coffee blossoms.

Stages of Blossom	Crude extracts/ Synthetic antioxidants	Total phenol content (mg GAE/g crude extract)	EC_{50} (mg/mL)	
			DPPH	Reducing power
1	Hexane	6.43	0.71	5.56
	Dichloromethane	25.96	0.30	1.75
	Methanol	28.32	0.13	0.46
	(overall)	(60.71)		
2	Hexane	50.41	0.42	1.05
	Dichloromethane	52.34	0.10	1.69
	Methanol	112.63	0.08	0.22
	(overall)	(215.38)		
3	Hexane	87.74	0.31	0.46
	Dichloromethane	95.04	0.05	0.31
	Methanol	139.88	0.03	0.19
	(overall)	(322.66)		
	BHA	Not tested	0.002	0.026
	Ascorbic acid	Not tested	0.004	0.016

It was noted that the DPPH radical scavenging activity showed an association with the total phenols and flavonoid contents. The methanol crude extracts having the highest total phenols and flavonoids also showed the best DPPH scavenging activity. This result agreed with the previous reports [15]. The radical scavenging effect depended on the structural of constituents and the number of available hydroxyl groups of phenols [16]. At 200 ppm concentrations conserves of Robusta coffee extracted exhibit 78-87% [17] which was a little higher than this work. These results indicate that Robusta blossoms perform a noticeable effect on scavenging free radicals.



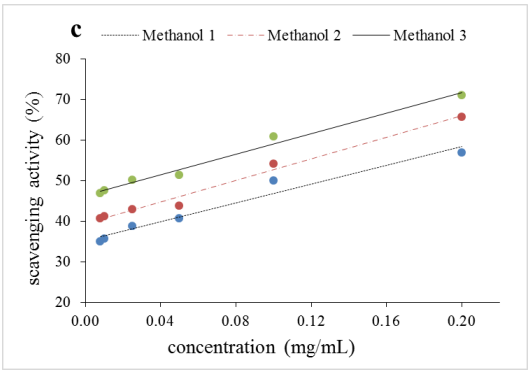


Fig. 2. Scavenging activity on DPPH radical of crude extracts from Robusta coffee blossom (a) Hexane (b) Dichloromethane (c) Methanol.

Similarly, the reducing power of the extracts increased with concentration. Each state of blossoms showed different activities. However, all extracts from the 3rd stage were the most effective (Fig. 3). As can be seen from Table 2, the methanol crude extracts displayed the highest reducing power with EC_{50} of 0.19-0.46 mg/mL). In contrast, the hexane crude extract at stage 1 exhibited the least in reducing power activity. Again, their efficiencies were less than BHA (EC_{50} = 0.026 mg/mL) and ascorbic acid (EC_{50} = 0.016 mg/mL There was also a correlation between the DPPH radical scavenging and the reducing power. The methanol extracts giving the highest DPPH scavenging, exhibited the greatest reducing power as well. Hence, in addition to phenolic compounds, the methanol extracts also contain other substances that promote reducing activity.

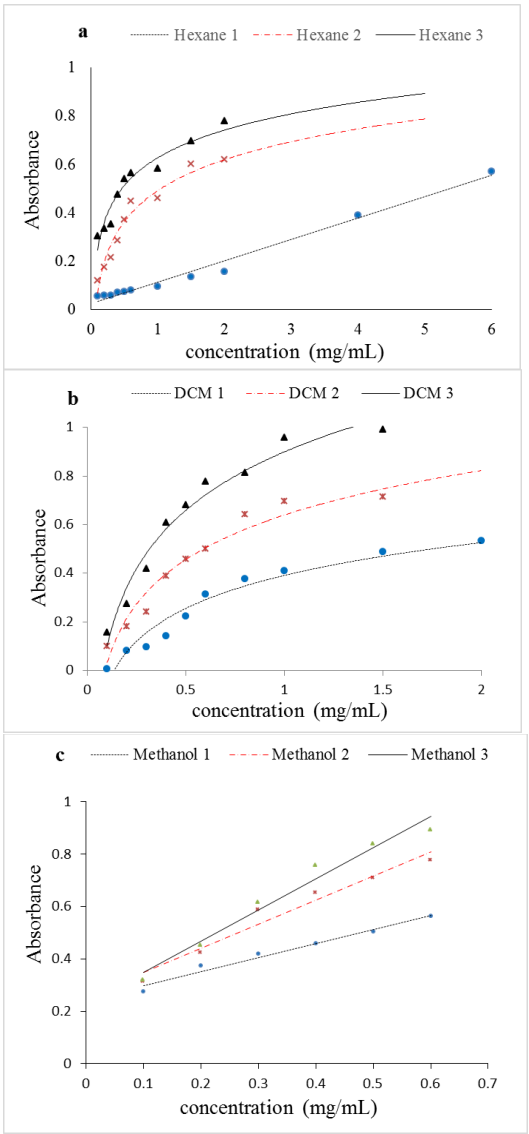


Fig. 3. Absorbance on reducing power of crude extracts from Robusta coffee blossom (a) Hexane (b) Dichloromethane (c) Methanol.

Conclusion

This study provided a brief investigation of phytochemical constituents of Robusta coffee flowers. The hexane, dichloromethane and methanol crude extracts contained terpenoids, flavonoids and alkaloids in different amounts. Their antioxidant properties were also proved to be effective. The antioxidant activity and total phenol capacity can be adjusted by varying the extract solvent. These findings would be of much interest for consumers and researchers. The data of the current report is useful as a baseline for further studies to identify bioactive compounds that are responsible for the antioxidant property.

Acknowledgement

We would like to thank Division of Chemistry, Department of Science, Faculty of Science and Technology, Prince of Songkla University, Pattani Campus, Thailand, for support with chemicals and equipment.

References

[1] Dagoon J. Agriculture & Fishery Technology IV. Manila: Rex Bookstore, 2005.

[2] Damatta FM, Ronchi CP, Maestri M, Barros RS. Ecophysiology of coffee growth and production. Braz J Plant Physiol 2007;19(4):485-510.

[3] Coffee: World Markets and Trade 2016. Foreign Agricultural Service, [cited 2016 July 20]. Available from: http://www.fas.usda.gov/data/coffee-world-markets-and-trade

[4] Office of Agricultural Economics: Agricultural statistics of Thailand 2015 [Internet]. [cited 2015 September 17]. Available from: http://www.oae.go.th/download/download_journal/2559/yearbook58.pdf

[5] Pohlan HAJ, Janssen MJJ. Growth and production of coffee in soils, plant growth and crop production. Oxford: EOLSS Publishers; 2010.

[6] Yashin A, Yashin Y, Wang JY, Nemzer B. Antioxidant and antiradical activity of coffee. Antioxidants 2013; 2:230-45.

[7] Vignoli JA, Viegas MC, Bassoli DG, Benassi MT. Roasting process affects differently the bioactive compounds and the antioxidant activity of Arabica

and Robusta coffee. Food Res Int 2014;61:279-85.

[8] Hernandez LMP, Quiroz KC, Juarez LAM, Meza NG. Phenolic characterization, melanoidins, and antioxidant activity of some comm ercial coffees from *Coffea Arabica* and *Coffea canephora*. J Mex Chem Soc 2012; 56(4):430-5.

[9] Tsao R, Deng Z. Separation procedures for naturally occurring anti oxidant phytochemicals. J Chromatogr B 2004;812:85-99.

[10] Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review. IPS 2011;1(1): 98-106.

[11] Ashfaq M, Shah KW, Ahmad S, Singh D. Preliminary phytochemical screen ing of alcoholic & aqueous extracts of *Mentha Longifolia* Linn. Leaves. Int J Pharm Biol Sci 2012;3(3) 384-6.

[12] Singleton VL, Rossi J. Calorimetry of total phenolic with phosphomolybdic-phosphotungstic acid agents. Am J Enol Vitic 1965;16:144-158.

[13] Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autooxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem 1992;40:945-8.

[14] Oyaizu M. Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr 1986;44:307-315.

[15] Chairgulprasert V, Prasertsongskun S, Junpra-ob S, Sanjun M. Chemical constituents of the essential oil, antioxidant and antibacterial activities from *Elettariopsis curtisii* Baker. Songklanakarin J Sci Technol 2008;30(5):591-6.

[16] Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebensm Wiss Technol 1995;28:25-30.

[17] Naidu MM, Sulochanamma G, Sampathu SR, Srinivas P. Studies on extraction and antioxidant potential of green coffee. Food Chem 2008; 107(1):377-384.