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ANTIOXIDANT ACTIVITIES OF DIFFERENT VARIETIES OF SPENT COFFEE GROUND (SCG) EXTRACTED USING ULTRASONIC-ETHANOL ASSISTED EXTRACTION METHOD

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Abstract: Spent coffee grounds (SCG) a by-product from coffee industries, coffee shops and domestic users contain large amounts of organic compounds which could be re-used as source antioxidants for foods or cosmetics. This project aims to study the antioxidant activity of three different spent ground coffee varieties (Robusta, Liberica and Arabica) extracted using ultrasonic-ethanol assisted extraction method utilizing 60% ethanol. The extracted samples were analysed using DPPH, FRAP, FTC and TBA, total phenolic content, total flavonoid content and also individual flavonoids to measure the quality and quantity of antioxidative activity in spent coffee. Robusta and Liberica SCGs exhibited similar activity ($p>0.05$) towards DPPH scavenging and ferric reducing reaction. Both showed 77.99±0.92 and 77.75±0.04%, respectively for DPPH, and 26.82±2.92 and 24.41±0.49 mg TE/g dry sample, respectively for FRAP. In FTC and TBA analyses, Robusta SCG was found to have the least activity with 61.07±2.43% and 2.9±0.14% respectively. Spent Arabica has similar inhibition percentage ($p>0.05$) as Liberica SCG in FTC (57.08±0.9 and 50.54±4.23% respectively), yet has different inhibition activity ($p<0.05$) in TBA (4.3±0.14 and 7.4±1.41% respectively). Total phenolic and flavonoid contents were found to be the highest in Arabica SCG with 941.04±37.25 mg GAE and 78.21 mg QE/g dry sample, respectively. Liberica and Robusta SCGs contain a total of phenolic content of 661.14±2.86 and 547.51±59.5 mg GAE/g dry sample, respectively and a total of flavonoid content of 71.64±1.85 and 20.66±7.82 mg QE/g dry sample. Individual flavonoids of luteolin and quercetin were present in all the three spent ground coffee varieties. Results from the study illustrated that the three different varieties of SCGs showed different extract yields as well as diverse traits of antioxidant activity that could serve as a good antioxidant.

Keywords: Spent coffee, antioxidant, ultrasonic-ethanol assisted technique

Introduction

Coffee is one of the commodities with a high antioxidant amount, and yet contributes to the multiplication of the solid waste (Panusa *et al*., 2013). The aforementioned species of coffee are Arabica (*Coffea arabica*), Robusta coffee (*Coffea canephora*), and Liberica coffee (*Coffea liberica*) (Schenker, 2002). Millions of cups of coffee consumed on a daily basis by people around the world contribute to tons of SCGs both in industrial and domestic sectors (Valipour, 2015). SCG is one of the most abundant by-products generated by the food industry worldwide (López-Barrera *et al*., 2016). The residues from the coffee industry are graded as high pollutants due to the presence of organic materials, specifically caffeine, tannins, and polyphenols which have a toxic nature that is dangerous to the environmental system (Chanakya & De Alwis, 2004). However, lack of information regarding these matters has resulted in the coffee and its by-products not fully utilized and fully executed.

The increasing demand for coffee is also based on the high health benefits available in the beverage, especially the antioxidants (Al-Dhabi *et al*., 2016). The antioxidants are also in high usage in many industries, mostly the food industry as they are able to maintain the quality of food for a long period of time (Moure *et al*., 2001). Basically, there are two general categories of antioxidants**:** natural and synthetic. Nevertheless, the application of synthetic antioxidants is widely spread in the manufacturing industry, especially food, for many purposes such as protecting flavour and

colour and ~~to~~ avoiding vitamin degradation (Moure *et al*., 2001), and mostly because ~~of~~ the synthetic antioxidants cost lower and easily obtained (Ranic *et al*., 2014). Despite their effectiveness, many concerns have been raised on the safety of the synthetic antioxidants to human health (Lourenço *et al*., 2019). Research have been conducted but unfortunately it is found that the synthetic antioxidants have carcinogenic effects on humans (Shebis *et al*., 2013), thus natural antioxidants are being utilized more now in order to meet public demand.

There are many methods for the extraction of antioxidants from natural sources and ultrasonic assisted methods which are extensively utilized these recent days due to lower cost and simple instruments required (Nadar *et al*., 2018). Research has been carried out on antioxidant activity and potential contributory compounds in green and roasted coffee beans. However, studies examining the antioxidant activity of the residues in the coffee industry are still limited (Shukri *et al*., 2020). Although recent research has been conducted focusing on the effort to fully utilize all the products of the coffee industry by investigating the chemical composition**,** especially the antioxidant compound, inadequate information on specified coffee varieties still arises. The use of ultrasonic methods as an extraction technique for coffee compounds has also not been fully discovered (Zainol *et al*., 2018). This study was therefore conducted to measure the antioxidant properties of the different SCG coffee varieties extracted using an ultrasonic-ethanol assisted technique.

Materials and Methods

Raw material

The different varieties of roasted coffee beans are: (a) Arabica beans (*C. arabica*) from PacificBru, Pulau Pinang, (b) Robusta beans (*C. canephora*) from Tekka Shop, Selangor and (c) Liberica beans (*C. liberica*) from My Liberica, Johor.

Sample preparation

The spent coffee ground (SCG) was separated using plunger coffee maker, referring to a method from Bravo *et al*. (2012) with a slight modification. Roasted coffee beans were freshly ground using a mortar grinder (RETSCH, type RM100) for 90 sec to a course-size ground before being subjected to the brewing process through the plunger method for 4 min. The resulting SCG was dried in the oven at 100°C until it reached a moisture value of 4%. The dried spent ground coffees obtained were kept in airtight containers at ambient and dark rooms until the extraction process.

Ultrasonic-ethanol assisted extraction of spent ground coffee

The ultrasonic-assisted extraction of spent ground coffees was performed according to the method described by Zainol *et al*. (2018) with some modifications. The extraction was carried out in 720 ml 60% ethanol using an ultrasonic cleaner bath UC-10 (JEIO Tech, Korea) at 28°C for 4 h and 30 min to prevent possible denaturation of antioxidant compounds and the duration. The extracted SCG sample was then filtered and centrifuged prior to the reduction in a rotary evaporator supplied with cooling and vacuum control (BUCHI Rotavapor R-215, Switzerland). The resulting extract was kept in a covered container at 4°C until further use.

Determination of antioxidant activity

2, 2 –Diphenyl–1–Picrylhydrazyl (DPPH) radical scavenging activity method

The free radical scavenging of spent ground coffee was evaluated using the DPPH method as previously described by Malik *et al* (2017) with a slight modification. The sample extract was first adjusted to 6 mg/ml by dissolving it in methanol prior to the addition of DPPH reagent. Butylated hydroxytoluene (BHT) and α-tocopherol were used as positive control while pure methanol was used as negative control. The absorbance was measured at 517 nm and converted into percentage inhibition of the DPPH radical determined using the equation below:

$$\% \text{ DPPH} = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \times 100$$

Ferric reducing antioxidant power (FRAP) method

The ferric reducing antioxidant power (FRAP) assay was performed using an established method by Ng *et al*. (2019). The FRAP reagent was prepared from 2.5 ml of a TPTZ (10 mM) in hydrochloric acid (40 mM) and 2.5 ml of $FeCl_3$ solution (20 mM) mixed with 25 ml of an acetate buffer (300 mM, pH 3.6). Trolox, a water-soluble analogue of vitamin E, was used for the analysis calibration. Absorbance was taken at 593 nm and the resulting values were expressed as mol of Trolox equivalent (μmoles/ml).

Ferric thiocyanate (FTC) method

The dried SCG sample was first mixed with absolute ethanol before being added with linoleic acid in absolute ethanol, phosphate buffer of pH 7.0 and distilled water. The mixture was placed in an amber glass bottle and incubated in a dark oven at 40°C for 1 h. After adding ferrous chloride to hydrochloric acid, ethanol and ammonium thiocyanate were then added and absorbance measured at 500 nm precisely 3 min. Butylated hydroxytoluene (BHT) and α-tocopherol were used as positive control, whereas a mixture without the sample was used as the negative control (Kikuzaki and Nakatani 1993; Ng *et al*. (2019).

Thiobarbituric acid (TBA) method

The sample used in the TBA analysis was taken from the previous method FTC. Aqueous trichloroacetic acid (TCA) and aqueous thiobarbituric acid (TBA) were added to the same sample before it was kept for 10 min in boiling water. The sample was then centrifuged at 3000 rpm for 20 min measured at 531 nm. The antioxidant activity was recorded based on the absorbance of the final day of the FTC assay (Kikuzaki and Nakatani, 1993).

Determination of antioxidant quantity

Total phenolic contents

Total phenolic contents were determined using the Folin Ciocalteau reagent by Looi *et al*. (2020) with some modifications. Briefly, the sample, distilled water, sodium carbonate, and Folin-Ciocalteau reagent mixture were held for 2 h before the absorption was measured at 765 nm. The TPC was determined according to a standard curve prepared with gallic acid as standard prepared in five different concentrations (50, 100, 150, 200, 250 and 300 mg/L). The results were then expressed as milligrams of gallic acid equivalent (GAE) per gram of the dried sample (mg GAE/g).

Total flavonoid content

Total flavonoid content was determined using aluminium chloride colourimetric method adapted from Ebrahimzadeh *et al*., (2008) with slight modifications. In short, the ethanolic sample was mixed with 50 mg/10 ml of 99% ethanol, aluminum chloride, potassium acetate and distilled water, then the absorbance was taken at 415 nm. The result was expressed as milligrams of quercetin equivalents (QE) per gram of dried sample (mg QE/g), using a calibration curve obtained with standard solutions of quercetin in five different concentrations (12.5, 25, 50, 75 and 100 mg/L).

Statistical analysis

All data were analysed statistically using a one-way analysis of variance (ANOVA) and p values < 0.05 were regarded as significant using Fisher's Least Significant Difference (LSD) test (MINITAB 14 statistical software package).

Results and Discussion

Yield of extractions

Table 1: Yield and antioxidant activities of SCG extracted using ultrasonic-ethanol method

	Yield (%)	DPPH (% inhibition)	FRAP (% inhibition)	FTC (% inhibition)	TBA (% inhibition)
Arabica SCG	9.87±1.07^{a}	75.04±1.46^{c}	21.54±5.87^{b}	57.08±5.53^{b}	4.33±0.46^{d}
Robusta SCG	10.00±2.01^{a}	77.99±4.58^{b}	26.82±8.84^{a}	50.54±9.76^{c}	2.94±0.25^{d}
Liberica SCG	8.05±1.34^{a}	77.75±3.21^{b}	24.41±6.48^{ab}	61.07±5.89^{b}	7.41±1.44^{c}
BHT		89.78±2.87^{a}		69.51±4.31^{a}	17.45±3.24^{a}
α-tocopherol		89.56±.4.87^{a}		40.47±3.17^{d}	11.93±2.27^{b}

Values represent the mean ± standard deviation. Values with the same superscript letters in the same row are not significantly different (P>0.05)

The yields from spent ground coffee samples, namely Arabica, Robusta and Liberica SCGs extracted using ultrasonic extraction method, are shown in Table 1. Robusta SCG demonstrated a higher extraction yield (10.00±1%), followed by Liberica SCG (8.01±1%). Likewise, Bermejo *et al*. (2013) obtained an extract yield of 0.4-8.7 % which also used ethanol as an extraction solvent. On the other hand, Kiattisin *et al*. (2016) and Vignoli *et al*. (2011) found that Robusta produced higher amount of extract than that of Arabica SCG, reasoning the larger CGA content of Robusta SCG than Arabica SCG that contributed to the results. Yield of extraction could also be controlled by ~~the~~ ethanol with varying polarity, pH, temperature, extraction time, and composition of the sample. Under the same extraction time and temperature, ethanol and composition of sample are labelled as the most important parameters. Zuorro and Lavecchia (2012) stated that the use of ethanol as extraction ethanol may be one of the contributing points to the high yield of extraction, since the phenolic compound has a higher affinity to a more polar ethanol. Do *et al*. (2014) cited that several factors influence the extraction efficiency of various methods applied, including the extraction process itself, the chemical composition of phytochemicals, the sample particle size, the ethanol used and the presence of interfering substances.

Antioxidative activities analysis

2, 2 – diphenyl – 1 – picrylhydrazyl (DPPH) radical activity

Table 1 also shows the antioxidant activity measured using the DPPH radical scavenging effect. Robusta SCG displayed the highest antioxidant activity, which could be considered that the compounds in the spent coffee samples were strong electron donors and could put an end to the oxidation chain reactions by turning free radicals into a stable form (Moon & Shibamoto, 2009). Both Robusta and Liberica coffee extracts spent showed some significant differences ($p<0.05$) compared to coffee extract spent in Arabica (75.04±1%). All three SCG extracts displayed an antioxidant activity marginally lower than that of the standards used (BHT and α-tocopherol). This research is in accordance with the analysis by Murthy *et al*. (2012) which, although in different quantities, obtained higher DPPH radical scavenging activity on standards than the coffee samples. In addition, Oliveira *et al*. (2014) stated that the different extraction method and the ethanol selected for use may influence the antioxidant potential of the extracts obtained.

Ferric reducing antioxidant power (FRAP)

The findings showed that Robusta SCG (26.82±3 mg TE / g) has a higher reaction to the Fe (III) complex reduction compared to

Liberica (24.41±0.49 mg TE/g) and Arabica SCG (21.5±2 mg TE/g) (Table 1). The finding is in accordance with Yashin *et al*. (2013) who found the antioxidant activity of coffee against Fe (III) reduction complex to be within the range of 26 to 38 mg TE / g sample. In addition, Saw *et al*. (2015) found that Liberica and Robusta coffee had higher antioxidant activity compared to Arabica coffee when compared to Fe (III), whereas Moreira *et al*. (2005) and Vignoli *et al*. (2011) reported that Robusta coffee had higher reduction capacity than Arabica coffee.

Ferric thiocyanate (FTC) analysis

The spent coffee Arabica, Robusta and spent Liberica coffee extracts exhibited stronger antioxidant activities ($p < 0.05$) as all surpassed α-tocopherol (40.47±0.25% inhibition) in the FTC analysis on the 5th day of incubation (Table 1). However, the SCG extracts samples were found to be significantly lower ($p < 0.05$) than that of standard BHT (69.51±0.25% inhibition). This ~~finding~~ is found to be in agreement with the finding of Emami *et al*. (2013) and Shafekh *et al*. (2012) who obtained the percentage of lipid peroxidation inhibition to be increasing from α-tocopherol to the samples and with standard BHT showing the highest among the three. The data exhibit that both spent Arabica (57.08±0.90%) and Liberica coffee (61.07±2.43%) extracts showed significant difference ($p < 0.05$) when compared with Robusta SCG extract (50.54±4.23%). The result also showed that Arabica, Robusta, and Liberica SCGs are more efficient than α-tocopherol in donating electrons to end the oxidation chain reactions by reducing the oxidized intermediates to a stable form.

Thiobarbituric acid (TBA) analysis

Table 1 also reveals that the Liberica SCG spent has substantially higher TBA inhibition values ($p<0.05$) relative to Arabica spent and that Robusta SCG spent has 7.4±1.41 % inhibition. Nevertheless, no substantial difference between spent Arabica (4.3±0.14 % inhibition) and Robusta SCG (2.9±0.14 %) was observed ($p > 0.05$). TBA analysis is performed to assess the antioxidant activity of SCGs against lipid peroxidation, indicating precisely the amount of peroxide in the secondary stage of lipid peroxidation (Zuki *et al*., 2011). This analysis is performed after FTC analysis using the same samples. Table 1 also shows that BHT and α-tocopherol as standards had better and significantly higher antioxidant capabilities ($p<0.05$) towards the secondary stage of lipid peroxidation than the SCG samples, with 17.45±1.48% and 11.9±0.71% respectively. It is shown to be consistent with earlier work by Emami *et al*. (2013) and Shafekh *et al*. (2012), which considered a higher percentage of lipid peroxidation inhibition in the secondary stage of standard BHT compared to samples and a higher percentage of lipid peroxidation inhibition in samples compared to standard α-tocopherol. Comparing the FTC and TBA results, both determined the antioxidant activity towards lipid peroxidation, which showed that Liberica SCG exhibited a higher antioxidant activity than that of Arabica and Robusta SCGs. The antioxidant activity of the FTC method for Liberica SCG is also higher than that of the TBA method. Heemann *et al*. (2019) reported that the higher antioxidant activity found from the FTC method indicated that the amount of peroxide in the initial stage of lipid peroxidation was greater than the amount of peroxide in the secondary stage. Thus**,** it can be assumed from these analyses that spent Liberica SCG has a better beneficial effect against lipid peroxidation compared to spent Arabica and Robusta SCG.

Total phenolic content

Table 2: Total phenolic content and total flavonoid content of SCG extracted using ultrasonic-ethanol

	Total phenolic content (mg GAE/g dry sample)	Total flavonoid content (mg QE/g dry sample)
Arabica SCG	941.04±31.65[a]	78.24±7.76[a]
Robusta SCG	547.51±21.76[b]	20.66±5.15[b]
Liberica SCG	661.14±11.96[b]	71.64±2.57[a]

Values represent the mean ± standard deviation. Values with the same superscript letters in the same row are not significantly different (P>0.05)

Table 2 shows that Arabica SCG contains the highest ($p < 0.05$) phenolic content (941.04±37 mg GAE/g), followed by Liberica SCG (661.14±2 mg GAE/g) and Robusta SCG (547.51±59 mg GAE/g). The results are in line with studies by Bravo *et al*. (2012) and Panusa *et al*. (2013), who reported that Arabica SCG contains higher total phenolic content than that of Robusta SCG. The variability of polyphenols content in SCG can be contributed by several factors, for example, the variety of the SCGs, roasting process it has undergone through, the maturity, storage condition and brewing method (Rothwell *et al*., 2013). The non-specific nature of the assay could be the major reason for different results obtained in between the present and previous studies. Saw *et al*. (2015) stated that the transfer of electrons from the phenolic compounds in alkaline medium to molybdenum to form blue complexes that happen in the assay can be monitored spectroscopically. This reaction however, is not only limited to phenolic compounds but other reducing species such as ascorbic acid, Cu (I), aromatic amines and even sugars are also reported to give a positive response. SCG, as reported by Mussatto *et al*. (2011), contains many other chemical compounds besides polyphenol and carbohydrates was found to constitute the most portion of the dry weight which is the SCG is rich in sugars polymerized into cellulose and hemicelluloses, showing similarity with the coffee beans. The differing concentration of phenolic content observed may be due to the presence of sugar in the SCG sample resulting from the different extraction methods and ethanol used may also be one of the reasons for the difference of values affecting the efficacy of the reagent (Patay *et al*., 2016).

Total flavonoid content (TFC)

Table 2 also presents the findings of the total flavonoid content (TFC) analysis which shows a similar trend to the total phenolic content analysis (TPC). Arabica SCG exhibited the highest TFC value (78.21±24 mg QE/g) followed by Liberica SCG (71.64±2 mg QE/g) and these two were not significantly different ($p > 0.05$) with each other. Robusta SCG contains the least total flavonoid content among the three SCG samples with the amount of 20.66±8 mg QE/g and is significantly different ($p < 0.05$) when compared with the other two SCG samples. In contrast, Panusa *et al*. (2013) reported that the TPC in SCG to be in the range of 2.11 to 8.03 mg QE/g dry sample. This phenomenon could be due to the different extraction methods applied or different extraction ethanol used which can become a very influential factor on the amount of compound able to be withdrawn from a sample (Andrade *et al*., 2012).

Conclusion

Robusta SCG extract exhibited the highest antioxidant activity towards the radical scavenging activity. Liberica SCG extract showed that the uppermost activity in both FTC and TBA analysis while Robusta SCG extract showed the lowest reaction on peroxide inhibition in FTC and TBA, respectively. Studies on the total phenolic content found that the Arabica SCG extract ~~to~~ contain the highest concentration followed by spent Liberica and then Robusta SCG extracts. Considering this finding, SCG could serve as a good source of antioxidant and enhance nutritionally and antioxidant properties for human consumption using SCG extract in food products.

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