

The Feasibility Study of Physicochemical Properties of Sarawak *Liberica* sp. Coffee Pulp

Elexson Nillian*, Nurhuda Syahirah Ismail, Muhamad Eddy Boli, Nick Laurence Buyong, Ngieng Nguui Sng, Dayang Salwani Awang Adeni and Awang Ahmad Sallehin Awang Hussini

Faculty of Resource Science and Technology, University Malaysia Sarawak, 94300, Kota Samarahan, Sarawak, Malaysia

ABSTRACT

Liberica coffee is a minor species that is planted all around the world. Therefore, there is little study conducted on this coffee species as only one percent is cultivated all around the world. In Malaysia, there is still no research focusing on coffee pulp from Sarawak *liberica* sp. and thus leading to this study. The wastes and by-product such as coffee pulps will become the residues as they were not needed in processing the coffee. This will create environmental pollution. Thus, this research aimed to evaluate the feasibility study on the physicochemical properties of coffee pulp from Sarawak *liberica* sp. including determination by colorimetric assays for phenolic and flavonoid content, antioxidant activity, and reducing sugar analysis. The antibacterial activities of coffee pulp were evaluated against Gram-

positive, *Staphylococcus aureus*, and Gram-negative, *Salmonella typhimurium* using a disc diffusion method. As a result, Sarawak *liberica* sp. coffee pulp extract contained total phenolic content of 24.24 mg GAE/g of coffee pulp, a total flavonoid content of 39.39 mg QE/g of coffee pulp, DPPH scavenging activity of $92.24 \pm 0.03\%$, reducing sugar analysis of 13.13 mg GE/g of coffee pulp, and there was no significant effect of antibacterial activities. Therefore, the physicochemical study determination in this study would add values toward Sarawak

ARTICLE INFO

Article history:

Received: 06 May 2020

Accepted: 20 July 2020

Published: 27 November 2020

DOI: <https://doi.org/10.47836/pjtas.43.4.05>

E-mail addresses:

nelexson@unimas.my (Elexson Nillian)

hudasyahirahismail@gmail.com (Nurhuda Syahirah Ismail)

eddy.boli@yahoo.com (Muhamad Eddy Boli)

nicklaurence30@gmail.com (Nick Laurence Buyong)

sngui@unimas.my (Ngieng Nguui Sng)

adsalwa@unimas.my (Dayang Salwani Awang Adeni)

haahmad@unimas.my (Awang Ahmad Sallehin Awang Hussini)

*Corresponding author

liberica sp. coffee pulp by-product and thus reducing the disposal of Liberica coffee wastes in the future.

Keywords: Antibacterial activity, antioxidant properties, coffee pulp, flavonoid compound, phenolic compound, reducing sugar analysis

INTRODUCTION

Coffee is a common beverage which is an important product throughout the world. Some products from coffee beans can be obtained such as essence, coffee caramel, and coffee syrup. There are three main coffee species planted throughout the world which are Arabica (80%) and Robusta (20%), while Liberica is the minimal species that is less than 1%. Unlike in Malaysia, Arabica is the minor species that were grown due to its climatic condition, while Liberica (73%) and Robusta (27%) are the major coffee species that are grown in this country (Ismail et al., 2014). Liberica and Robusta are suitable to be cultivated in Malaysia because the optimum growth temperature is 18 to 28°C, but the maximum temperature for these two species is at 34°C as stated by Ismail et al. (2014). In a developing country, over 10 million of small producers make their living growing coffee. Coffee comes from the genus *Coffea* which originated in tropical Africa (Maurin et al., 2007). It is important to know about the chemical and physical properties of coffee beans because the coffee that is freshly harvested will undergo various processes such as grinding, roasting, packing, and transporting process.

The processing of coffee berries comprises two different methods which are dry and wet (Berecha et al., 2011). The skin and pulp of the coffee beans will be removed and become the residues as they are not needed to process the coffee. As there a wide world production of coffee, it will associate with ecological damages as the residues of coffee can lead to environmental pollution if it is not handled well (Cubero-Abarca et al., 2014). Most of the coffee pulp is dumped into a landfill or river stream directly during wet processing without proper handling of waste (Geremu et al., 2016). The absence of a method that can utilize the use of coffee by-product is one of the main problems to use the coffee pulp for biomass productions due to the high moisture content (Cubero-Abarca et al., 2014). However, due to the myriad problems such as disposal problems of this by-product and cause environmental pollution, this material has received greater attention compared in the past. Many researchers have developed some ideas to reduce the environmental pollution by using the waste by-product in fermentation of edible fungi, animal feeds, extraction of active ingredients, and compost (Marcel et al., 2011; Murthy et al., 2012). The coffee pulp is rich with minerals, nutrients, amino acids, polyphenols, and caffeine (Arellano-González et al., 2011; Ploypradub et al., 2010). In addition, the coffee pulp has also been found to have antimicrobial properties against bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Escherichia coli*, and *Bacillus*

subtilis as stated by Runti et al. (2015). In Malaysia, there was a previous study that had been done on this coffee species, but it was only focused on the coffee berries and beans physical properties (Ismail et al., 2014). However, there is still no study that focuses on the physicochemical properties of coffee pulp from *liberica* sp. particularly Sarawak adapted *liberica* coffee sp. and thus leading to this study. Therefore, this research aimed to evaluate the physicochemical properties of coffee pulp from *liberica* sp., to study the antioxidant potential of coffee pulp extract using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and to investigate the antibacterial potential of coffee pulp extract against Foodborne bacteria, Gram-positive, *Staphylococcus aureus* and a Gram-negative *Salmonella typhimurium*.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

The Sarawak *liberica* coffee sp. pulp was used as the main substrate in this study. The Sarawak *liberica* sp., the coffee sample was supplied by RekaJaya Plantation Sdn. Bhd. in Kuching, Sarawak. The pulp of the coffee beans was separated from the beans and crushed to obtain a greater surface area. Consequently, the crushed coffee pulp sample was oven-dried at 105°C for 5 days. The dried coffee pulp sample was analyzed for its moisture content as well as dry matter.

Dry Matter and Moisture Content

A drying dish was oven-dried at 60°C for 10 min and subsequently was cooled to room temperature. The dried drying dish was

weighed using an analytical balance. Then, the coffee pulp sample was dried at 105°C in an oven for 5 days, cooled to ambient temperature, and was weighed again. The drying, cooling, and weighing processes were repeated until a persistent weight was obtained. The percentage of moisture content of coffee pulp was computed using the formula:

Moisture content (%)

$$= \frac{(w_1 - w_2)}{w_1} \times 100\%$$

According to this formula, the weight of an amount of coffee pulp was determined as w_1 and the weight of dried coffee pulp sample was determined as w_2 . The percentage of dry matter was determined using the formula below:

$$\text{Dry matter (\%)} = 100\% - \text{moisture content (\%)}$$

Extraction of Sarawak *Liberica* sp. Coffee Pulp Sample

The extraction of coffee pulp using methanol as a solvent was done by referring to the previous procedure by Redfern et al. (2014). The coffee pulp sample was dried and ground using mortar and pestle to fine powder form. The powdered coffee pulp was collected and placed inside a thimble of the Soxhlet extractor. An amount of 200 mL of solvent which was methanol was added into the Soxhlet apparatus. Next, the solvent was heated using the isomantle and evaporated.

This process was run for 8 hours. After the process was finished, the methanol was removed, leaving about 2 to 3 mL yield of the essential oil.

Total Phenolic Content Determination

The total phenolic content (TPC) was investigated using the Folin-Ciocalteu method as exhibited by Rahayu et al. (2013). Gallic acid (GA) was used as a standard thus the TPC was indicated as mg/g gallic acid equivalence (GAE) from the calibration curve ($R^2=0.9995$). To form the stock solution, 10 mg of GA was dissolved in 100 mL of distilled water as the solvent (0.1 mg/mL). For the standard curve, different concentration of 0, 0.02, 0.04, 0.06, 0.08, and 0.10 mg/mL of (GA) were made from the stock solution. Then, 100 μ L of the extract was added with 0.75 mL of the stock Folin-Ciocalteu's reagent. The mixture was let to settle down at room temperature for 5 min before adding 0.75 mL of 6% sodium carbonate solution. The mixture was then shaken thoroughly before allowing to mix at room temperature for 90 min. UV-Vis spectrometer was then used to measure the absorbance at 725 nm. Each sample then was repeated thrice for this procedure.

Total Flavonoid Content Determination

The total flavonoid content (TFC) of the pulp extracts was determined referring to the procedure of colorimetric assay done by Ahlem et al. (2014). First, 75 μ L of 5% sodium nitrite (NaNO_2) solution was added to 125 μ L of the extract. Then, the mixture left for 6 min, before inserting

150 μ L of 10% aluminum trichloride (AlCl_3). After leaving for another 5 min, 750 μ L of NaOH (1M) was added into the combination. The mixture then was added with distilled water to reach 2500 μ L mark. After 15 min of incubation, the mixture was observed to turn pink in coloration. The absorbance of the blend then was measured at 510 nm. Next, the calibration curve was made from multiple concentrations of quercetin. In this study, quercetin was used as a standard flavonoid and conveyed as Quercetin equivalence (mg QE/mL extract). Quercetin stock solution was made by mixing 20 mg quercetin with 20 mL of methanol thus obtaining a concentration of 1.0 mg/mL. Next, it was diluted to 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL of quercetin. Absorbance was measured by using the spectrophotometer at 510 nm.

Antioxidant Activity of Sarawak *Liberica* sp. Coffee Pulp

The antioxidant properties of the extract were determined by the procedure previously done by Daniel and Workneh (2017) with slight modifications. By using 2,2-diphenyl-1-picrylhydrazyl (DPPH), the free radical scavenging activity of the extract was formulated below:

% RSA

$$= \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100 \%$$

Firstly, a concentration of 1 mg/mL of coffee pulp extracts was used as a working standard. It was diluted to obtain

concentration 1000, 500, 250, 125, 62.5, 31.25, 15.63, and 7.8 $\mu\text{g}/\text{mL}^{-1}$. An amount of 0.5 mL of diluted solvent extracts was inserted into a respective test tube and 1 mL of (0.006% in methanol) of DPPH was added. The solutions were left for half an hour in darkroom temperature. After 30 min, the absorbance was determined using a UV-Visible spectrophotometer at 520 nm wavelength. Ascorbic acid was set as a positive control. The mixture without any extracts, but with the DPPH and methanol would be used as control. The DPPH free radical-scavenging activity of the coffee pulp extract would be calculated as the following equation and the IC_{50} value was determined from the graph of the percentage DPPH free radical scavenging activity against concentrations of the samples.

Reducing Sugar Analysis Determination

The DNS (3,5-dinitrosalicylic acid) assay was the way used to analyze reducing sugars such as glucose referred to the previous research done by Marisa et al. (2017) with some modifications. Solution A and solution B were prepared as DNS reagent. For solution A, 1.0 g of DNS was diffused in 20 mL of 2M NaOH. For solution B, 30.0 g of potassium sodium tartrate tetrahydrate (Rochelle salt) was dissolved in 50 mL of distilled water. Both solutions were stirred until complete dissolution. Then, solutions A and B were mixed and heated to homogenize. By using distilled water, the volume was fixed to 100 mL and stored in an amber bottle at 4°C. After that, 1 mL of coffee pulp extracts and 1 mL of DNS reagent

were placed in tubes of 10 mL. Then, the tubes were taken to a bath thermostated at 100°C for 5 min. Then, it was cooled down to room temperature. After that, the volume was completed with 8 mL of distilled water and homogenized. Then, the absorbance was read at 540 nm in a spectrophotometer and subsequently was analyzed to determine glucose concentration. The concentration of 1.0 mg/mL of glucose solution was prepared as a working standard stock solution. It was diluted to obtain 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL glucose working standard. A standard was plotted by using glucose to estimate glucose equivalent values. The reading was compared against a reagent blank solution.

Antibacterial Activity of Coffee Pulp Extract

Bacteria Inoculum Preparation. In this study, a Gram-positive, *Staphylococcus aureus*, and a Gram-negative *Salmonella typhimurium* were selected for testing. These bacteria were acquired from the Microbiology Laboratory of Faculty of Resource Science and Technology (FRST), UNIMAS culture collection. These bacteria were pre-cultured in Mueller-Hinton Broth and incubated at 37°C for 24 hours. The inoculum of each bacteria was standardized to 0.5 McFarland standard and measured at 620 nm via UV-vis spectrophotometer.

Antibacterial Susceptibility Test. The antimicrobial properties of the extract were tested using the disc diffusion method referring to the procedure done by Mohamed

et al. (2018) with few adjustments. First, the coffee pulp extract was redissolved in 1% dimethyl sulphoxide (DMSO) to obtain the final concentration of 100 mg/mL. The solution was then diluted further with 1% DMSO to the concentration of 10, 50 and 100 mg/mL. An amount of 100 μ L of bacterial inoculums prepared was spread onto Mueller-Hinton Agar. Then the disc (6 mm in diameter) was dripped with 10 μ L of different concentrations of coffee pulp extracts were placed on the agar. Gentamicin (10 μ g) was used as positive control while 1% of DMSO as a negative control. The agar plate was incubated at 37°C for 24 hours and the experiment was done thrice. The diameters of inhibition zones were measured.

RESULT AND DISCUSSION

Total Phenolic Content

Polyphenols play a crucial role in human health to protect from diseases caused by oxidative stress and free radical (Geremu et al., 2016). In this assay, GA was used as standard because it can inhibit the oxidation and hydrolysis of oils and fats because of their free radical scavenging and antioxidant nature (Kahkeshani et al., 2019). This colorimetric assay is easy to perform, low cost, and rapid making it extensively used in the UV/Vis spectrophotometric method. In this colorimetric reaction, a blue complex was formed because polyphenol in the coffee extract was reacted with redox reagents which are a Folin-Ciocalteu

reagent that can be quantified with UV/Vis spectrophotometry (Blainski et al., 2013).

As a result, an amount of 24.24 mg GAE/g was found in the *Coffea liberica*. In this test, the TPC in the coffee pulp extract was estimated in terms of GA equivalent (mg GAE/g of coffee pulp). Based on a previous study by Geremu et al. (2016), coffee pulp from *Coffea arabica* exhibited the highest amount of phenolic content when it was extracted with methanol compared to ethanol and acetone. In this study, methanol was used as a solvent in the extraction of coffee pulp samples. Methanol is also known to be a competent solvent and is usually used to extract natural oxidative components from plants or natural materials. This is because methanol mixture has greater efficiency and high polarity towards the extraction of polar phytochemicals such as phenolic and flavonoids. Nevertheless, methanol is the recommended solvent when extracting polyphenols due to its capability to obstruct the activity of polyphenol (Geremu et al., 2016). Other previous studies also indicate that the capacity of phenolic content in the coffee pulp depends on a few reasons including the variety of extraction methods. Samar et al. (2018) had stated that the total polyphenols were notably differed in the roasted, raw, and spent coffee that could be influenced due to the heat treatment. Based on the studied, roasted coffee bean showed a very low amount of total polyphenol content in the coffee bean. This factor might also influence the amount of phenolic content in the coffee pulp extract from *Coffea liberica*

as the coffee pulp sample was oven-dried until the sample was completely dried.

Total Flavonoid Content

Flavonoid is the most familiar, major, and extensively dispersed sole group of phenols. It is available in plants with tremendous efficacious antioxidants and by producing combination with metal ions, it is also obstructed metal-initiated lipid oxidation (Samar et al., 2018). In this study, quercetin was used as standard because it is a plant pigment that is a vigorous antioxidant flavonoid and more specifically a flavonol that is found in most plants (David et al., 2016). The TFC was determined using a colorimetric assay. The yellow coloration produced is due to the pattern of a compound between the aluminum ion, the carbonyl groups, and the hydroxyl groups of flavonoids (Al-Farsi et al., 2018).

The color changes indicate that the coffee pulp sample from *Coffea liberica* has an amount of flavonoid content. The TFC in coffee pulp extract was estimated as quercetin equivalent (mg QE/g of coffee pulp). A total of 39.39 mg QE/g of coffee pulp was found in the coffee pulp sample extract that determined *Coffea liberica* has a slight amount of flavonoid. In the previous study that was conducted by Geremu et al. (2016), roasted coffee beans had the lowest amount of flavonoid content compared to raw and spent coffee bean which was 30.65 mg catechin/g while raw and spent coffee beans were 52.07 and 34.32 mg catechin/g respectively. In general, the number of total flavonoid contents in the coffee pulp varied

because it was influenced by the extraction method. It was described that the flavonoid content was influenced by the temperature in the extraction method which was above 40°C (Geremu et al., 2016). The solvent polarity in the extraction method plays an important part in increasing phenolic solubility and it is proven that the regaining of flavonoid is influenced by the solvent and the polarity (Nadiah & Uthumporn, 2015).

Antioxidant Activity of Sarawak *Liberica* sp. Coffee Pulp

The antioxidant is a molecule that is ready to give an electron to a charged free radical and counteracts it, hence decreasing its magnitude for cell corruption. The radical scavenging activity is one of the various mechanisms to measure the antioxidant activity of plant extract. 2,2-diphenyl-1-picrylhydrazyl (DPPH) is one of the compounds that have a proton free radical and shows maximum absorption at 520 nm. This study used the DPPH method because it is low cost and easy to evaluate the radical scavenging activity. In agreement with Sagar and Singh (2011), this method was able to react with the varieties of DPPH concentration regarding the sample type and allowed DPPH to react gradually even with weak antioxidants and with sufficient time given. Besides, the colorimetric assay was conducted to measure the antioxidant activity of coffee pulp sample extract. The purple color dims rapidly when DPPH confronts proton radical scavengers (Daniel & Workneh, 2017).

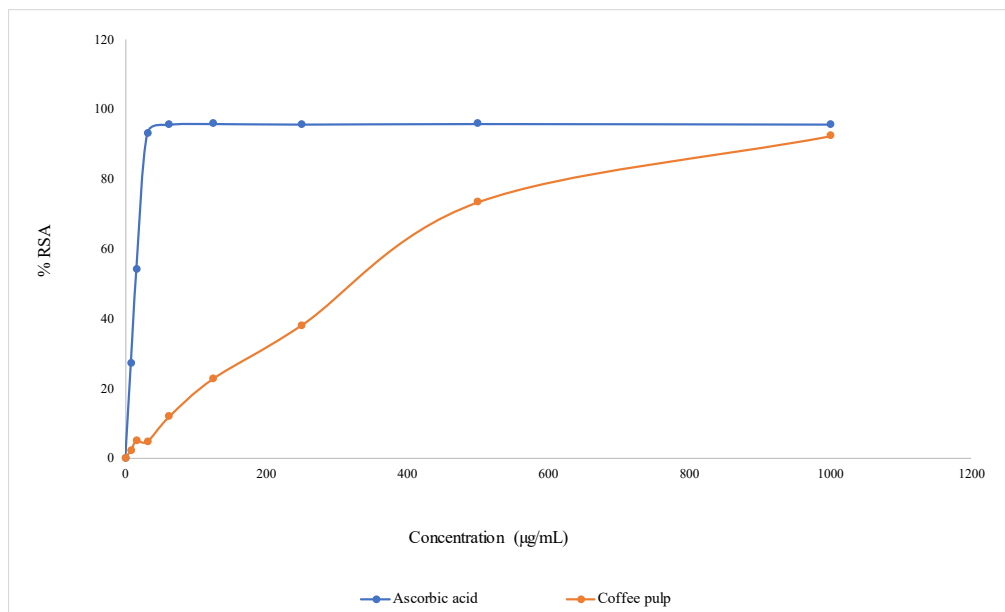


Figure 1. Radical scavenging activity of ascorbic acid and coffee pulp extract

The antioxidant activity of coffee pulp sample extract was calculated as a percent of radical scavenging activity. Based on the result (Figure 1), Liberica coffee pulp sample extract had a high level of radical scavenging activity which was $92.24 \pm 0.003\%$ RSA. This is in agreement with Geremu et al. (2016) stated that the interaction of solvents in the extraction played a crucial part in the effects of DPPH scavenging activity. Based on the study, methanolic extract gives a higher value of radical scavenging activity compared to ethanol and acetone. The study indicates that polyphenols play an important role due to their rummage capability on free radicals which they have hydroxyl groups and the extracts performed the highest antioxidant

activity have the highest concentration of polyphenols. Sultana and Anwar (2008) also described that the increase in the magnitude of DPPH free radical scavenging capacity and the increased antioxidant activity were due to the increase in the accumulation of polyphenol compounds also. Therefore, polyphenol also plays an important role that may contribute to the antioxidant action.

The inhibition concentration (IC_{50}) value is defined as the concentration of antioxidant required for 50 % scavenging of DPPH radicals. The IC_{50} was calculated for the extraction of coffee pulp sample extract. It is a parameter that is extensively used to compute the antioxidant activity of organic and non-organic compounds. In this step, the standard used was ascorbic acid and it had a

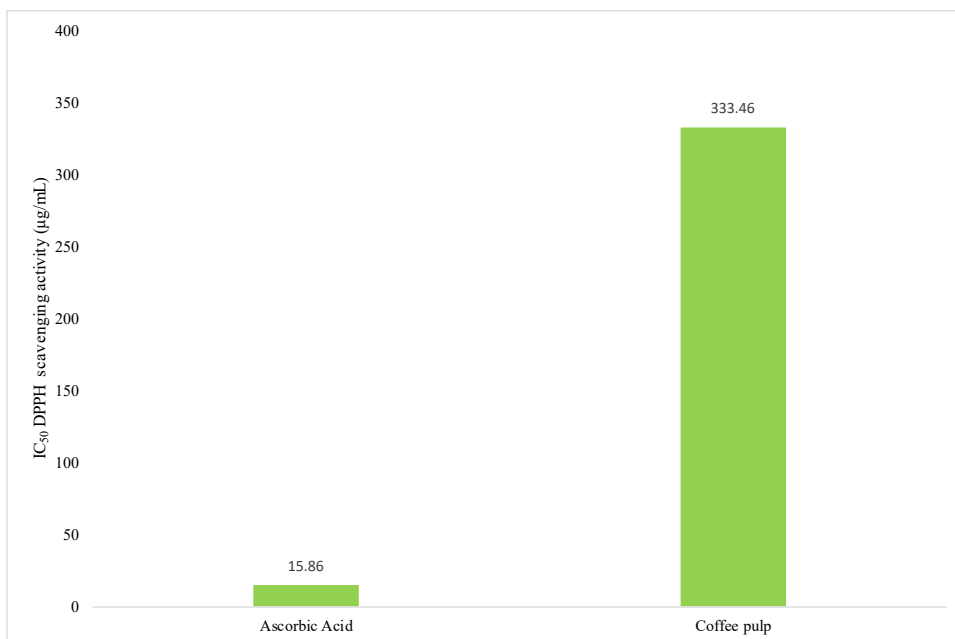


Figure 2. IC₅₀ of DPPH scavenging activities in ascorbic acid and coffee pulp extract of *Liberica* coffee

lower value of IC₅₀ which was 15.86 µg/mL. Based on Figure 2, the coffee pulp sample extract showed a high-value IC₅₀ which was 333.46 µg/mL and it might correspond to a low antioxidant activity compared to ascorbic acid. This was supported by Maisuthisakul et al. (2007) that a smaller value of IC₅₀ correlated to higher antioxidant activity and ascorbic acid was an example of a substance that had high antioxidant activity. Referring to the study conducted by Geremu et al. (2016), the solvent also influenced the IC₅₀ value because ethanol extract in the studied gave the highest value of IC₅₀ which indicates that it had least potent antioxidant activity. The lowest IC₅₀ value was also estimated when extracting using methanolic solvent due to the higher

antioxidant activity of methanolic extracts and the total amount of polyphenols. The differences IC₅₀ values between solvent properties might be because of their reaction with the solvent used and internal properties of plant compounds

Reducing Sugar Analysis of Sarawak *Liberica* sp. Coffee Pulp

Reducing sugar is any sugar that is capable of becoming as a reducing agent because of its free ketone group or aldehyde group. It is most commonly determined by the reaction with a stabilized alkaline solution of a copper salt. In this study, the DNS assay was conducted to determine the reducing sugar activity in the coffee pulp sample extract. The DNS method is a colorimetric method

that comprises a redox reaction between the reducing sugar and 3,5-dinitro salicylic acid available in the sample. The carbonyl group of sugars has a reducing power which can be oxidized to the carboxyl group by mild oxidizing agents. The DNS reagent is yellow colored and is reduced to 3-amino-5-nitro salicylic acid which is red-brown color. Marisa et al. (2017) had stated that the brightness of the color was in correlation to the sugar concentration.

Based on this study, an amount of 13.13 mg GE/g of the coffee pulp of reducing sugar analysis was identified in the coffee pulp sample extract from *Coffea liberica* that indicates that coffee pulp sample has a slight amount of sugars. The reducing sugar analysis was estimated in the terms of glucose equivalent (mg GE/g of coffee pulp). The color changes during the DNS assay of coffee pulp sample extract showed a change from yellow to red-brown color that indicates that the coffee pulp sample has an amount of sugar. Glucose was used as a standard in this study and the color changes were correlational to the glucose concentration. The changes from yellow coloration to red-brown coloration was increasing with the increasing of the concentration of glucose. In this assay, the 3,5-dinitrosalicylic acid acted as an antioxidant; Rochelles salt that avoids the dissolution of oxygen in the reagent, and lastly, sodium hydroxide were to give the medium needed for the redox reaction to happen (Marisa et al., 2017).

Antibacterial Activity of Sarawak *Liberica* sp. Coffee Pulp

The antibacterial properties of coffee pulp were conducted using the disc diffusion method and evaluating the susceptibility of *Coffea liberica* against foodborne bacteria. The results revealed that there was no antibacterial activity of the coffee pulp extract against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Salmonella typhimurium*) bacteria as no inhibition zone was observed against these bacteria (Figures 3 and 4). Duangjai et al. (2016) stated that coffee pulp extracts showed powerful nutrients and antioxidant activities. Different concentration of 10, 50, and 100 mg/mL of coffee pulp was used in this test. The coffee pulp sample extract was redissolved in Dimethyl Sulfoxide (DMSO) because Awoufack et al. (2013) stated that DMSO was suitable to dissolve the compounds because in the previous studied, acetone was used as the solvents but it did not dissolve all the compounds well.

The coffee pulp sample extract showed no inhibition zone and it might due to the concentration variation. Some reports suggested the antibacterial activity of plant extracts was influenced by phytochemicals such as phenolic acids, malic acids, tannin, caffeine and hydroxycinnamic acid that are the reason for the antibacterial activity (Kabir et al., 2014). The coffee pulp sample extract might have low phytochemical properties that cause no formation of an inhibition zone. It is difficult to predict

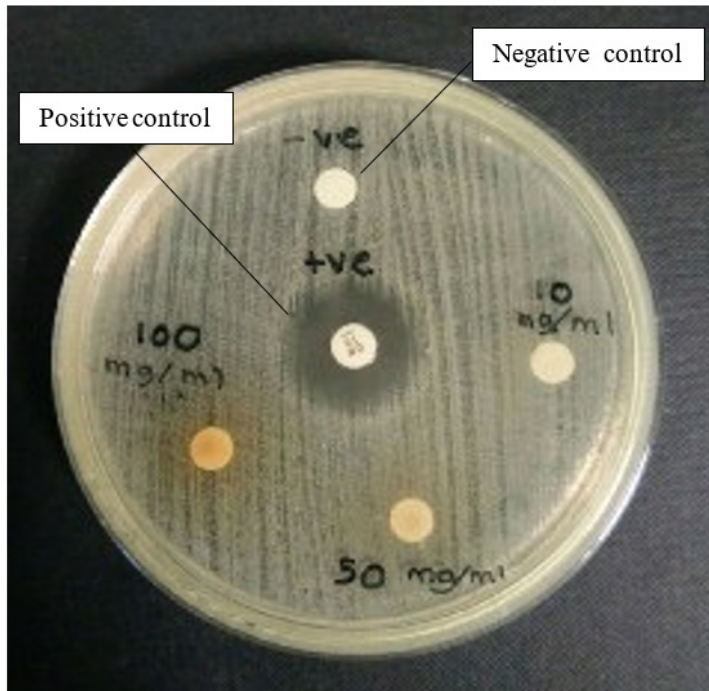


Figure 3. Disk diffusion test of Sarawak *liberica* sp. coffee with different concentration against *Staphylococcus aureus*

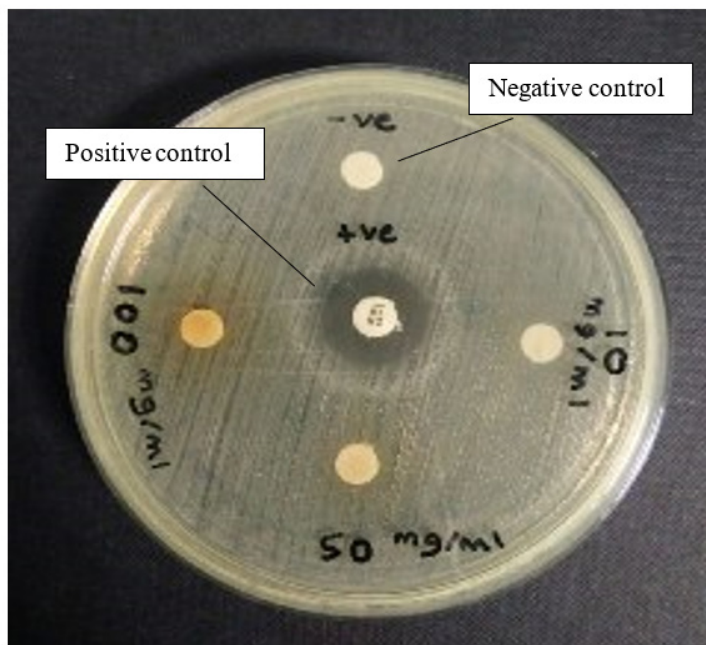


Figure 4. Disk diffusion test of Sarawak *liberica* sp. coffee with different concentration against *Salmonella typhimurium*

the susceptibility to antibacterial agents because results were reported in the previous studies using different types and parts of the plants. It is necessary to isolate the bioactive compounds associated with the antibacterial activity to properly identify the mode of action of antibacterial agents and test against numerous different strains and species of bacteria.

CONCLUSION

This research has successfully determined the physicochemical properties, antioxidant and antibacterial activities of Sarawak *liberica* sp. coffee pulp. The Sarawak *liberica* sp. coffee pulp showed the potential of values added product in conjunction with the amount of phenolic, flavonoid contents, reducing sugar analysis, and a higher level of radical scavenging activity that could have great importance as therapeutic for beneficial use. Comprehensive improvement can be made by estimating the physicochemical properties of coffee pulp using different processing methods and solvents for extraction.

ACKNOWLEDGEMENTS

Research fund was sponsored by F07/MIRG/1906/2019, Mobility Initiative Research Grant, Universiti Malaysia Sarawak (UNIMAS), Kota Samarahan, Sarawak, Malaysia in collaboration with Rekajaya Plantation Sdn. Bhd.

REFERENCES

- Ahlem, R., Souad, I. B., Béatrice, B., Valérie, M. L., Fathi, M., Evelyne, O., ... Malika, T. A. (2014). Total phenolic, total flavonoid, tannin content, and antioxidant capacity of *Halimium halimifolium* (Cistaceae). *Journal of Applied Pharmaceutical Science*, 5(1), 52-57. doi: 10.7324/japs.2015.50110
- Al-Farsi, M., Al-Amri, A., Al-Hadhrami, A., & Al-Belushi, S. (2018). *Color, flavonoids, phenolics and antioxidants of Omani honey*. Retrieved April 28, 2020, from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6236019/pdf/main.pdf>
- Arellano-González, M. A., Amírez-Coronel, A. R., Torres-Mancera, T., Pérez-Morales, G. G., & Saucedo-Castañeda, G. (2011). Antioxidant activity of fermented and nonfermented coffee (*Coffea arabica*) pulp extracts. *Food Technology and Biotechnology*, 49(3), 374-378.
- Awoufack, M. D., McGaw, L. J., Gottfried, S., Mbouangoure, R., Tane, P., Spiteller, M., & Eloff, J. N. (2013). Antimicrobial activity and cytotoxicity of the ethanol extract, fractions and eight compounds isolated from *Eriosema robustum* (Fabaceae). *BMC Complementary Alternative Medicine*, 13(1), 289. doi: 10.1186/1472-6882-13-289
- Berecha, G., Lemessa, F., & Wakjira, M. (2011). Exploring the suitability of coffee pulp compost as growth media substitute in greenhouse production. *International Journal of Agricultural Research*, 6(3), 255-267. doi: 10.3923/ijar.2011.255.267
- Blainski, A., Lopes, G. C., & Palazzo de Mello, J. C. (2013). Application and analysis of the Folin-Ciocalteu method for the determination of the total phenolic content from *Limonium brasiliense* L. *Molecules*, 18(6), 6852-6865. doi: 10.3390/molecules18066852

- Cubero-Abarca, R., Moya, R., & Valaret, J. (2014). Use of coffee (*Coffea arabica*) pulp for the production of briquettes and pellets for heat generation. *Ciência e Agrotecnologia*, 38(5), 461-470. doi: 10.1590/s1413-70542014000500005
- Daniel, A., & Workneh, M. (2017). *Determination of total phenolic content and antioxidant activities of five different brands of Ethiopian coffee*. Retrieved April 29, 2020, from <https://escipub.com/Articles/IJFNR/IJFNR-2017-03-1301>
- David, A. V. A., Arulmoli, R., & Parasuraman, S. (2016). Overviews of biological importance of quercetin: A bioactive flavonoid. *Pharmacognosy Review*, 10(20), 84-89. doi: 10.4103/0973-7847.194044
- Duangjai, A., Suphrom, N., Wungrath, J., Ontawong, A., Nuengchamnon, N., & Yosboonruang, A. (2016). Comparison of antioxidant, antimicrobial activities and chemical profiles of three coffee (*Coffea arabica* L.) pulp aqueous extracts. *Integrative Medicine Research*, 5(4), 324-331. doi: 10.1016/j.imr.2016.09.001
- Geremu, M., Bekele, T. Y., & Sualeh, A. (2016). Extraction and determination of total polyphenols and antioxidant capacity of red coffee (*Coffea arabica* L.) pulp of wet processing plants. *Chemical and Biological Technologies in Agriculture*, 3(1), 25-30. doi: 10.1186/s40538-016-0077-1
- Ismail, L., Anuar, M. S., & Shamsudin, R. (2014). Physical properties of Liberica coffee (*Coffea liberica*) berries and beans. *Pertanika Journal of Science and Technology*, 22(1), 65-79.
- Kabir, F., Katayana, S., Tanji, N., & Nakamura, S. (2014). Antimicrobial effects of chlorogenic acid and related compounds. *Journal of the Korean Society for Applied Biology Chemistry*, 57(3), 359-365. doi: 10.1007/s13765-014-4056-6
- Kahkeshani, N., Farzaei, F., Fotouhi, M., Alavi, S. S., Bahramsoltani, R., Naseri, R., ... Bishayee, A. (2019). Pharmacological effects of gallic acid in health and diseases: A mechanistic review. *Iranian Journal of Basic Medical Sciences*, 22(3), 225-237. doi: 10.22038/ijbms.2019.32806.7897
- Maisuthisakul, P., Suttajit, M., & Pongsawatmanit, R. (2007). Assessment of polyphenols content and free radical scavenging capacity of some Thai indigenous plants. *Food Chemistry*, 100(4), 1409-1418. doi: 10.1016/j.foodchem.2005.11.032
- Marcel, B. K. G., André, K. B., Viviane, Z. T., & Séraphin, K. C. (2011). Potential food waste and by-products of coffee in animal feed. *Electronic Journal of Biology*, 7, 74-80.
- Marisa, G., Melisa, A., & Alicia, M. (2017). Determination of reducing sugars in extracts of *Undaria pinnatifida* (harvey) algae by UV-visible spectrophotometry (DNS method). *Actas de Ingeniería*, 3, 173-179.
- Maurin, O., Davis, A. P., Chester, M., Myungi, E. F., Jauferally-Fakim, Y., & Fay, M. (2007). Towards a phylogeny for *Coffea* (Rubiaceae): Identifying well-supported lineages based on nuclear and plastid DNA sequences. *Annals of Botany*, 100(7), 1565-1583. doi: 10.1093/aob/mcm257
- Mohamed, S. B., Hafida, H., Chahrazed, B., & Djamel, E. A. (2018). Evaluation of antibacterial activity of some medicinal plants extracts commonly used in Algerian traditional medicine against some pathogenic bacteria. *Pharmacognosy Journal*, 10(3), 507-512. doi: 10.5530/pj.2018.3.83
- Murthy, P. S., Manjunatha, M. R., Sulochannama, G., & Naidu, M. M. (2012). Extraction, characterization and bioactivity of coffee anthocyanins. *European Journal of Biological Sciences*, 4(1), 13-19.
- Nadiah, N. I., & Uthumporn, U. (2015). Determination of phenolic and antioxidant properties in tea and spent tea under various extraction method and determination of catechins, caffeine and gallic acid by HPLC. *International Journal of*

- Advanced Science Engineering Information Technology*, 5(3), 158-164. doi: 10.18517/ijaseit.5.3.520
- Ploypradub, C., Cheamsuphakit, B., & Punbusayakul, N. (2010). Antioxidant properties of different parts of Arabica coffee berry and spent coffee ground. *The Journal of Agricultural Science*, 41, 577-580.
- Rahayu, U., Nurhasniza, K., Mohd, A. S., Mawardi, R., Ahmad, B., & Dachriyanus. (2013). Phenolic contents, antioxidant and cytotoxic activities of *Elaeocarpus floribundus* Blume. *Pakistan Journal of Pharmaceutical Sciences*, 26(2), 245-250.
- Redfern, J., Kinninmonth, M., & Burdass, D. (2014). Using Soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. *Journal of Microbiology and Biology Education*, 15(1), 45-46. doi: 10.1128/jmbe.v15i1.656
- Runti, G., Pacor, S., Colomban, S., Gennaro, R., Navarini, L., & Scocchi, M. (2015). Arabica coffee extract shows antibacterial activity against *Staphylococcus epidermidis* and *Enterococcus faecalis* and low toxicity towards a human cell line. *LWT-Food Science and Technology*, 62(1), 108-114. doi: 10.1016/j.lwt.2014.12.039
- Sagar, B. K., & Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, 48(4), 412-422. doi: 10.1007/s13197-011-0251-1
- Samar, A. A., Khitma, A. E., & Amro, B. H. (2018). Estimation of phenolic and flavonoid compounds and antioxidant activity of spent coffee and black tea (processing) waste for potential recovery and reuse in Sudan. *Recycling*, 3(2), 27. doi: 10.3390/recycling3020027
- Sultana, B., & Anwar, F. (2008). Flavonols (kaempferol, quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants. *Food Chemistry*, 108(3), 879-884. doi: 10.1016/j.foodchem.2007.11.053