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Antioxidant activity of *jambu bol* [*Syzygium malaccense* (L.) Merr. and Perry] leaf extract

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Abstract

Jambu bol [*Syzygium malaccense* (L.) Merr. and Perry] is well known to be used in folk medicine and reports are proving the biological activity of each part of this plant. This study aimed to determine the antioxidant activity of *S. malaccense* L. leaf extract. The methods used in this study were total phenolic content (TPC) assay, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•) radical scavenging activity assay and ferric reducing antioxidant power (FRAP) assay. The results showed that the extract contained TPC of 44.10±0.06 mg GAE/g. The IC_{50} scavenging activities of DPPH and ABTS• were 0.0334 mg of Ascorbic acid E/g and 0.1352 mg of TE/g, respectively, while the antioxidant reducing power was 1013.50±0.07 mM Fe^{2+}/g. In conclusion, *S. malaccense* L. leaf extract was found to possess antioxidant activity, and this extract has potential to be developed to be used in food as a natural antioxidant agent.

1. Introduction

Antioxidants play a pivotal role in our lives nowadays because they defuse or destroy "reactive oxygen species" (ROS), also known as free radicals before they hurt living cells. Cell membrane degradation, membrane protein deterioration and DNA mutations are all results of oxidation reactions that are induced by ROS. The relationship between ROS and some common illnesses such as ageing, cancer, inflammation, skin damage, liver injuries, diabetes mellitus, inflammatory diseases, cardiovascular diseases, arteriosclerosis, coronary heart diseases, and arthritis is the oxidation reaction that ROS is responsible for its activation (Dontha, 2016). Recent reports have illustrated the benefits of phytochemicals that possess antioxidant activities in terms of eliminating the occurrence of some dangerous diseases such as cancer through frequent consumption of high-quality sources of antioxidants such as fruits. The importance of fruits is due to their richness in antioxidants such as vitamins C and E, carotenoids, flavonoids, and other phenolic compounds (Saura-Calixto and Goni, 2006; Lim and Rabeta, 2013).

Plants are known to have primary and secondary metabolites, where phenolic compounds represent a large portion of the secondary metabolites. Interestingly, phenolic compounds have been shown to have a wide range of biological activities, including antimicrobial, antioxidant, and anti-diabetic properties (Rohman *et al.*, 2010; Shahraki, 2013). According to some studies, each part of the plant (stems, fruits, roots, flowers, seeds, barks, and pulps) possesses a wide range of metabolites (Loganathan *et al.,* 2017; Jamshidi-Kia *et al.,* 2018). Plants can be classified based on their characteristics into families where each family may contain many genera that also have thousands of species, such as the Myrtaceae family, which is considered the eighth-largest family with around 140 species and 3,800–5,800 species (Ranghoo-Sanmukhiya *et al.,* 2019). *Syzygium* is the 16th largest genus of flowering plants in Myrtaceae family (Soh, 2017). This includes high diversity cultivated for many purposes such as colourful, edible and fleshy fruits (Rabeta *et al.,* 2013; Wardana *et al.,* 2018). *Syzygium malaccense* L. Merr. and Perry is one of the most popular plants in Malaysia and Indonesia and it is well known to be used in folk medicine. Different parts of *S.*

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malaccense L. extracts have been reported to have antioxidant activity (Savitha *et al.,* 2011; Arumugam *et al.,* 2014; Nunes *et al.,* 2016; Batista *et al.,* 2017). Hence, this study aimed to demonstrate the total phenolic content and the antioxidant activity of *S. malaccense* L. ethanolic leaf extract.

2. Materials and methods

2.1 Plant sampling and extraction

Syzygium malaccense L. Leaves were collected in February 2020 from Taman Pertanian, Universiti Putra Malaysia (UPM). The chosen leaves were uniform in shape, fresh, healthy, and mature. Samples were directly cleaned under running tap water to clean them up from dust and dirt and then washed again with distilled water. The extraction method was performed using the maceration technique as described by Rukayadi *et al.* (2013), with slight modifications. Cleaned leaves were dried in the shade for 2 days and then complete drying was done in a hot-air oven (Euroasaia, Malaysia) at 43±2°C temperature for 3 hrs. Dried leaves were crushed to a fine powder using stainless steel grinder (sieving was done after each grinding process to avoid the existence of big particles). A hundred grammes of powdered leaves were soaked in 400 mL of absolute ethanol (RandM Chemicals, UK) in a universal bottle and incubated for 24 hrs in a water bath incubator shaker (Heidolph, Germany) at 40±3°C with 110 revolutions per minute (rpm). The ethanolic solution was filtered using Whatman filter paper size No. 2 (Whatman International Ltd., England) by using a continuous vacuum filtration machine, and then it was concentrated using a rotary evaporator (Heidolph, Germany) at 40±3° C and a speed of 110 rpm. The crude extract was diluted in DMSO to obtain 100 mg/mL and then further diluted in 1:10 (v/v) distilled water to get 10 mg/mL stock solutions.

2.2 Total phenolic content and antioxidant activity determination

2.2.1 Total phenolic content

The total phenolic content (TPC) in *S. malaccense* L. leaf extract was determined by the Folin-Ciocalteu spectrophotometric method as described by Oki *et al.* (2002), with slight modifications. Briefly, different concentrations of the extract were prepared in a range from 5 – 0.001 mg/mL. Folin- Ciocalteu (RandM Chemicals, Essex, UK) reagent (FCR) was prepared by dissolving 1 mL of the reagent in 9 mL of distilled water to get a 10% concentration of FCR. A 7.5% sodium carbonate (Na_2CO_3) solution (Firma Chempur, Piekary lskie, Poland) was prepared by dissolving 0.75 g of Na_2CO_3 in 10 mL of distilled water. 20 µL of the extract was transferred into a sterile 96-well microtiter flat bottom plate. Then, 100 µL of FCR was added to the extract and homogenised properly, and then left for 5 mins. Then, 80 µL of Na_2CO_3 was added to the solution, bringing the total volume to 200 µL. The microtiter plate was wrapped in aluminium foil and left in the dark for 30 mins. The absorbance was read at a 750 nm wavelength using the BIO-RAD 170-6930 Benchmark Plus Microplate Spectrophotometer (BIO-RAD, California, United States) and the results was obtained using Microplate Manager Software. The same protocol was applied to gallic acid (Acros Organics, New Jersey, United States) at a range of 0.100-0.006 mL to create a calibration standard curve. Results were presented as gallic acid in milligram equivalents per mL (mg GAE/ mL). The following equation was obtained from the calibration standard curve ($y = 0.011x + 0.0308$, $R^2 = 1$)

2.2.2 Antioxidant activity

2.2.2.1 DPPH radical scavenging activity assay

The antioxidant activity of *S. malaccense* L. leaf extract was performed *in vitro* by a method described by Gerhäuser *et al.* (2003) with some modification, and it was measured by the free radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. Briefly, a range of 5–0.001 mg/mL of *S. malaccense* L. leaf extract concentrations was prepared by dissolving the extract in methanol. Ascorbic acid (RandM Chemicals, Essex, UK) as a positive control was prepared in different concentrations from 0.1-0.001 mg/mL. The DPPH radical (Alfa Aesar, Massachusetts, UK) was prepared by dissolving 3.9 g of DPPH in 100 mL of methanol where the last adjusted optical density (OD) using BIO-RAD was 0.93±0.02 at 517 nm wavelength. An amount of 100 µL of the extract concentrations was transferred into a sterile 96-well microtiter and then mixed with 100 µL of prepared DPPH radicals. The same steps were carried out for the ascorbic acid, and then the microtiter plate was kept in the dark for 30 mins. The plate was then applied to the 170-6930 Benchmark Plus Microplate Spectrophotometer to get the OD values of absorbance at 517 nm wavelength while the results were obtained using Microplate Manager Software. The results were measured as the percentage of scavenging activity by using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 represents the absorbance of the negative control and A_1 represents the absorbance of the sample. The results were plotted as IC_{50} values and ascorbic acid equivalents (AAE, mg/mL) of *S. malaccense* L. leaves extract.

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2.2.2.2 *ABTS• radical scavenging activity assay*

The ABTS• assay, also known as the 2,2′-azinobis (3 -ethylbenzthiazoline-6-sulphonic acid) assay, was used to determine the scavenging activity of *S. malaccense* L. leaf extract, with some modifications to the protocol described by Kanagasabapathy *et al.* (2011). In brief, the ABTS• radical solution was prepared by reacting 1 mL of ABTS (Sigma-Aldrich, Missouri, United States) (7 mM) with 1 mL of potassium persulfate (Emsure, Darmstadt, Germany) ($K_2S_2O_8$ - 2.45 mM). The solution was kept in a universal bottle wrapped with aluminium foil for 12 hrs to produce a dark solution of the ABTS• radical. In the preliminary experiment, the ABTS• radical solution was diluted with 50 mL of distilled water and then adjusted to an absorbance of 0.70±0.02 at 734 nm using the BIO-RAD 170-6930 Benchmark Plus Microplate Spectrophotometer. In the experiment, 20 µL of different prepared concentrations of the extract were transferred into a 96-well microtiter plate with a flat bottom. Following that, 200 L of the adjusted ABTS• radical was added to the extract, and the 96-microtiter plate was placed in the dark for 10 mins. The absorbance was recorded at a wavelength of 734 nm. The same procedures for the ABTS• radical were performed with Trolox (Sigma-Aldrich, Missouri, United States), and the scavenging activity was determined as the following equation:

$$ABTS \bullet \text{ scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 represents the absorbance of the negative control and A_1 represents the absorbance of the sample. The results were plotted as IC_{50} value which is the concentration of the extract that is necessary for 50% reduction of ABTS•.

2.2.2.3 Ferric reducing antioxidant power

The antioxidant activity of *S. malaccense* L. leaf extract was determined by a ferric reducing antioxidant power (FRAP) assay according to the method described by Benzie and Strain (1996) with some modifications. Briefly, the FRAP reagent was freshly prepared by mixing the following solutions together and they were 300 mM acetate buffer (pH 3.6), 10 mM 4,6-tripryridyls-triazine (TPTZ) (Sigma-Aldrich, Missouri, United States) solution, 40 mM HCl (RandM Chemicals, Essex, UK) and 20 mM ferric chloride (Sigma-Aldrich, Missouri, United States) in the ratio of 10:1:1 (v: v: v). Before the beginning of the experiment, the temperature of FRAP reagent was increased up to 37°C using the calibrated oven. 20 µL of *S. malaccense* L. leaves extract (different concentrations) were transferred into a 96-microtiter plate followed by 180 µL of FRAP reagent. A 180 µL of FRAP reagent was added to 20 µL of distilled water to represent the reagent blank. After incubating the microtiter plate at 37°C for 5 mins, BIO-RAD 170-6930 Benchmark Plus Microplate Spectrophotometer was used to measure the absorbance at 593 nm wavelength. To generate a standard curve, the same procedures were applied to different concentrations of ferrous sulphate heptahydrate (Sigma-Aldrich, Missouri, United States) ($FeSO_4 \bullet 7H_2O$) aqueous solution. The absorbance value of the extract tested after subtraction of reagent blank and blank sample was translated into FRAP value (mmol of $FeSO_4 \bullet 7H_2O$ equivalents) using the $FeSO_4 \bullet 7H_2O$ calibration plot with the following equation formula: $y = 0.0002x + 0.1853$, $R^2 = 0.9804$.

2.3 Statistical analysis

The results of total phenolic content, ferric reducing power, DPPH, and ABTS• scavenging activities were calculated using Microsoft Excel version 16.0 and presented as mean ± standard deviation (SD) of triplicate experiments.

3. Results and discussion

The total phenolic content and the antioxidant activity of *S. malaccense* leaf extract were determined using TPC, FRAP, DPPH, and ABTS• assays. In this study, it was found that the increases in the antioxidant activity of the ethanolic extract were relatively related to the increases in the concentrations of the extract.

3.1 Total phenolic content and antioxidant determination

3.1.1 Total phenolic content

Plants contain antioxidant compounds that are important with redox properties that are responsible for antioxidant activity. In the present study, the determined TPC amount in *S. malaccense* L. leaf extract was 44.10±5.26 mg GAE/g (Table 1), which is less than what has been reported in previous studies. Arumugam *et al.* (2014), reported that the TPC of *S. malaccense* L. leaf extract was 125.81 mg GAE/g which is higher results from this current study. This difference may be contributed to the pre-extraction and the extraction method where they used an air circulation oven at 40°C for the drying process, ethanol as a solvent with ratio 1:20 (w/v), and then kept the solution in an incubator shaker at 37°C for 24 hrs which were all factors influenced the TPC content in the extract. According to other studies, the TPC of methanolic *S. malaccense* L. leaf extract was 53.77 and 108.08 mg GAE/g, while the aquatic extract was 82.07 mg GAE/g (Savitha *et al.,* 2011; Batista *et al.,* 2017). Although the variation among the results from different studies is small, leaves of *S. malaccense* L. seem to contain more TPC compared

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Table 1. TPC value and antioxidant activity as determined using FRAP assay in *S. malaccense* L. leaves extract.

Sample	Concentration (mg/mL)	TPC (mg GAE/g)	FRAP (mM Fe^{2+}/g)
S. malaccense L.	0.312 mg/mL	44.10±5.26	1013.54±0.37

Values are presented as mean±SD, n = 3.

with the other portions of the plant, such as peels and fruits, which contain 12.58 and 8.03 mg GAE/g of TPC, respectively (Nunes *et al.,* 2016). The values of phenolic content in the current study varied compared to those in the other studies. This variation may be due to the presence of different amounts of phytochemicals or the duration, geographical variation, or methods of extraction, which may alter the amounts of phenolics (Aryal *et al.,* 2019). However, phenolic compounds play a major role in plant self-defence mechanisms against incursive microorganisms or against any other threats as well as environmental stress, such as wounding and excessive light or ultraviolet radiation (Harborne *et al.,* 1994; Wallace and Fry, 1994). These compounds can act as antioxidants by chelating metal ions, preventing radical formation and improving the antioxidant endogenous system (Al-Azzawie and Alhamdani, 2006).

3.1.2 Antioxidant activity

3.1.2.1 DPPH radical scavenging activity assay

Antioxidants upon interaction with DPPH either transfer an electron or hydrogen atom to DPPH, thus neutralizing its free radical character. The colour of the reaction mixture changes from purple to yellow, and the degree of discoloration indicates the scavenging potential of the antioxidants (Foti *et al.,* 2004; Villaño *et al.,* 2007). Table 2 shows the half-maximal inhibitory concentration (IC_{50}) of *S. malaccense* L. leave extract that inhibited 50% of the DPPH radical and was compared with ascorbic acid. The IC_{50} values of *S. malaccense* L. leaf extract and ascorbic acid were 0.0334 and 0.0037 mg/mL, respectively. The scavenging activity was increased to 92.29±4.12% at a higher concentration of the extract (0.078 mg/mL). Ascorbic acid as a positive control was more effective as a pure antioxidant than the extract, where its highest scavenging activity was 90.37±6.11% at 0.0125 mg/mL. According to the study by Arumugam *et al.* (2014), the ethanolic *S. malaccense* L. leaf extract had a lower IC_{50} value to scavenge DPPH radicals (0.016 mg/mL). Savitha *et al.* (2011) determined the IC_{50} of the methanolic extract of *S. malaccense* L. leaves against DPPH radicals at 0.025 mg/mL. Another study mentioned that the IC_{50} of *S. malaccense* L. leaf extract using hexane, methanol, and ethyl acetate as solvents was 0.510, 0.010, and 0.031 mg/mL, respectively (Ramadhania *et al.,* 2017). From the literature, it was found that methanolic leaf extract of *S. malaccense* L. has higher activity in terms of DPPH radical scavenging. *Syzygium aqueum* fruits, also known as water apple, were reported to have powerful scavenging activity in terms of the DPPH radical scavenging activity assay where the recorder IC_{50} was 0.004 mg/mL (Saptarini and Herawati, 2017). Furthermore, *S. aqueum* methanolic leaf extract was found to have high DPPH radical scavenging activity, as *S. aqueum* fruits have an IC_{50} of 0.006 mg/mL (Sobeh *et al.*, 2018). *Syzygium samarangense,* which is known as wax apple, had reordered antioxidant activity where the IC_{50} of methanolic pulp and seed power in scavenging DPPH radicals were 0.072 mg/mL and 0.078 mg/mL, respectively (Simirgiotis *et al.,* 2008). Similarly, the DPPH radical scavenging activity of *S. samarangense* leaf extracts was reported to have an IC_{50} of 0.094 mg/mL, 0.074 mg/mL, and 0.929 mg/mL using n-Hexane, athel acetate, and methanol as solvents, respectively (Budiono *et al.,* 2019). From the given data, the ethanolic leaf extract of *S. malaccense* L. was found to have less powerful DPPH scavenging activity than *S. aqueum* fruits and leaf extracts but better than that reported for *S. samarangense* pulp, seeds, and leaf extracts. However, *S. malaccense* L. leaves extract had powerful antioxidant activity because the IC_{50} value was less than 50 μg/mL (Saptarini and Herawati, 2017).

3.1.2.2 ABTS• radical scavenging activity assay

The determination of the scavenged ABTS• radicals was established using *S. malaccense* L. leaf extract in order to evaluate the extract's antioxidant activity by delocalization of the ABTS• through measuring the reduction of the radical cation as the percentage inhibition of absorbance at 734 nm (Abdullah and Jamil, 2019). Trolox was used as a positive standard control. As shown in Table 2, the highest scavenging activities of the extract and Trolox were observed at 0.625 mg/mL and 3.8 mg/mL, with scavenging powers of 98.60% and 99.90%, respectively. The IC_{50} of the extract and the control were 0.135 mg/mL and 0.547 mg/mL, respectively. The extract exhibits more antioxidant activity when compared to Trolox. Arumugam *et al.* (2014) reported that *S. malaccense* L. leaf extract has powerful antioxidant activity and estimated the IC_{50} of ethanolic leaf extract of *S. malaccense* L. at 0.047 mg/mL, which is less than the results obtained from the present study. Leaves methanolic extract of *S. aqueum* was investigated by Osman *et al.* (2009) to determine its scavenging activity against ABTS• radicals and it was observed that 0.035 mg/mL had almost 65% of the scavenging power, which is higher scavenging

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Table 2. Antioxidant activity of *S. malaccense* L. leaves extract as determined using DPPH and ABTS• assays.

Sample	DPPH		
	Concentration (mg/mL)	Scavenging activity (%)	IC_{50}
S. malaccense L.	0.078	92.29±0.07	0.0334
	0.001	4.80±0.12	
Ascorbic acid	0.0125	90.37±0.13	0.0037
	0.001	9.87±0.03	
Sample	ABTS•		
	Concentration (mg/mL)	Scavenging activity (%)	IC_{50}
S. malaccense L.	0.625	98.60±0.18	0.13
	0.001	17.56±0.33	
Ascorbic acid	1.90	81.78±0. 27	0.54
	0.01	17.75±0.03	

Values are presented as mean±SD, n = 3.

activity than that which was obtained from *S. malaccense* L. leaf extract in the present study. In terms of ABTS• radical scavenging activity, Hidayati *et al.* (2017) reported that among *S. polyanthum* leaf extracts (methanol, ethyl acetate, dichloromethane, and n-hexane), the methanolic extract showed the highest scavenging activity with an IC_{50} value of 0.017 mg/mL. According to Stalin (2018), the methanolic leaf extract of *Syzygium caryophyllatum* had high antioxidant activity and the IC_{50} of scavenging ABTS• radicals was 0.013 mg/mL. Generally, methanol is known to be evaporated at 64.7 °C which is far less than the boiling point of ethanol, this difference makes the plant extraction process more convenient with methanol as less temperature is needed to complete the extraction process and less damage to the phytochemical compounds occurred. Furthermore, methanol has been generally found to be more efficient in the extraction of lower molecular weight polyphenols than other solvents which is suggested to be the reason methanolic plant extracts have higher antioxidant activity than other extracts produced by other solvents (Do *et al*., 2014). Ethanolic leaves extract of *Syzygium jambos* had its antioxidant activity evaluated and it was found that the concentration of 0.057 mg/mL was the IC_{50} that scavenged 50% of ABTS• radicals (Hossain *et al.,* 2018). These studies revealed that the ethanolic leaf extract of *S. malaccense* L. has less scavenging activity for ABTS• radicals than the other extracts from *S. aqueum, S. polyanthum, S. caryophyllatum,* and *S. jambos.*

3.1.2.3 Ferric reducing antioxidant power

The reducing power assay measures the electron-donating ability of antioxidants using the potassium ferricyanide reduction method. Antioxidants cause the reduction of the ferricyanide complex to its ferrous form. The reducing power of *S. malaccense* L. leaf extract at a 0.312 mg/mL concentration was 1013.54±61.37 mM Fe^{2+}/g (Table 1). According to Arumugam *et al.* (2019), the ethanolic extract of *S. malaccense* L. had ferric reducing power equal to 847.24 mM Fe^{+2} /g sample. This revealed the current excrement presented higher activity in ferric reducing power than in the previous report. However, the presence results from reducing Fe^{3+} to Fe^{2+} by using the leaf extract of *S. malaccense* L. had better activity than the peels and the edible part of the same plant (Nunes *et al.,* 2016). The present FRAP results were found to be comparable with a study by Abdullah and Jamil (2019) in which they investigated the antioxidant activities of eight different species of the *Syzygium* genus. According to Lim and Rabeta (2013), solvents that were used in the extraction process had a high impact factor on the antioxidant activity of the produced extracts. However, using acetone as a solvent in the extraction process to extract bioactive compounds from *S. malaccense* L. leaves and *S. aqueum* leaves had significantly higher ferric reducing power than the extracts extracted by using water (Lim and Rabeta, 2013). The ferric reducing power of acetonic leaves of *S. malaccense* L. and *S. aqueum* extracts were 2062.78 and 3545.56 M Fe (II) per g of freeze-dried sample, respectively, while water had 378.33 and 847.22 M Fe (II) per g of freeze-dried sample, respectively. From this comparison, it was remarkable that the ethanolic extract that was used in the present study had higher ferric reducing power than both acetonic extract and water-based extracts. Generally, in the presence of 2,4,6-tripyridyl-s-triazine, phenolic compounds are responsible for electron transfer, leading to a reduction of Fe^{3+} to Fe^{2+}. This reduction reaction appears and can be observed by the formation of a coloured complex with Fe^{2+} absorption at 593 nm (Romes *et al.,* 2019).

4. Conclusion

The present study showed that the ethanolic leaf extract of *S. malaccense* L. had less total phenolic content than the other reported results. The reason has been suggested to be the steps of the pre-extraction

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process, such as the drying process or the size of powdered particles during the grinding step, the extraction process itself, and the solvent used in the extraction process. Antioxidant activity of *S. malaccense* L. leaf extract showed better activity than some of the other species of *Syzygium* plants, and that was in terms of DPPH, ABTS•, and FRAP assays. This study concluded that *S. malaccense* L. leaf extract had noteworthy antioxidant activity and that it may be developed to be used alone or in combination with other extracts as food additives for antioxidant purposes.

Conflict of interest

The authors declare no conflict of interest.

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