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Original Research Article

Exploring the Polyphenol Contents and Antioxidant Capacity of the Leaf Extracts of Selected Indonesian *Syzygium* Species

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ABSTRACT

The Myrtaceae family has about 3000 species of fruit-producing trees. The edible fruits of these trees are widely consumed by the Indonesian people. Some of the plants belonging to this family are various guavas from the genus *Syzygium*. Traditionally, the guava plant is used to treat diarrhoea. It has been shown to possess antidiabetic, antimicrobial, antihypertensive, and antioxidant activities. The present study is aimed at exploring the polyphenolic contents and antioxidant activity of the leaves of three types of guavas; *Syzygium aqueum* (Burm.f.) Alston, *Syzygium malaccense* (L.) Merr. and Perry., *Syzygium samarangense* (Blume) Merr. & L.M. Perry, and compared to *Psidium guajava* leaves.

The polyphenolic contents (total tannins, total flavonoids, and total phenols) of the four types of guava leaves were determined using standard methods. Qualitative determination of phenolics, flavonoids, and other organic components of these plants were also carried out using thin layer chromatography (TLC). The antioxidant capacity was measured by the phosphomolybdate method using quercetin as the reference standard.

The results showed that the highest phenol and tannin content was found in *Syzygium aqueum* leaves compared to two other types of guavas from the genus *Syzygium*. The TLC chromatogram showed similarity in the organic components of the three types of guavas from the genus *Syzygium*. The antioxidant activity was exhibited by *Syzygium aqueum* leaves could be related to its high phenolic and tannin content.

***Keywords*:** Antioxidant, Guava, Java apple, Malay apple, Watery rose apple.

Introduction

The Myrtaceae family has 100 genera and about 3000 species that grow in the form of shrubs or trees. *Psidium guajava* (PG) is a species of flowering plant belonging to the Myrtaceae family.[1] PG leaves are widely used to treat diarrhoea and possess other pharmacological activities, such as antidiabetic, antimicrobial, antihypertensive and antioxidant activities.[2] Consumption of herbal preparation made from PG leaves for the treatment of various health problems is still widely practiced in Indonesian society. This tradition is one of the Indonesian nation's local wisdom and cultural heritage that must be maintained.[3] Many traditional medicinal products made from PG in the form of Standardized Herbal Medicines (in Indonesia, known as *Obat Herbal Terstandar* or OHT) are registered with the Indonesian FDA (The National Agency of Drug and Food Control, NA-DFC).[4] However, PG plant is considered rare, and this has led the Indonesian communities and herbal medicine practitioners to seek alternatives to this plant. In an effort to meet the demand for the use of this herbal plant, several closely related plants belonging to the same family as PG are currently being explored.

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Some plants belonging to the Myrtaceae family are various guavas from the *Syzygium* genus, such as *Syzygium samarangense* (Blume) Merr. & L.M. Perry (SS), *Syzygium malaccense* (L.) Merr. and Perry. (SM), and *Syzygium aqueum* (Burm.f.) Alston (SA). These three guavas are easy to obtain and are widely grown in Indonesia. These three guava leaves have pharmacological properties similar to those of PG leaves. The genus *Syzygium* has traditionally been used to treat diarrheal disorders, diabetes, inflammation, bronchitis, and acid reflux/ulcers.[1] This genus has been scientifically proven to have antioxidant, antimicrobial, antifungal, antiviral, analgesic, anti-inflammatory, antihypertensive, antihyperglycemic, sedative, and spasmolytic activities.[5–7] Several pharmacological studies have reported that SS, SM, and SA have antioxidant and antimicrobial activities.[8–11]

The phenolics, flavonoids, and tannins in the polyphenolic content of *Syzygium* species contribute to their antioxidant activity. The profile of these metabolites in the plant extracts can easily be studied using thin-layer chromatography (TLC) techniques. The present study therefore, is aimed at investigating the polyphenolic contents in terms of total phenolic, flavonoids, and tannins in the leaves of three selected *Syzygium* species and evaluate their antioxidant activity in comparison to PG leaves.

Materials and Methods

Plant materials

The four types of guava leaves were collected from the Duren Sawit district (East Jakarta) in February, 2022. The plant sample was identified, authenticated, and given a voucher number B-571/DI.05.07/3/2022 by Anang Setiawan at the "Biosystematics and Evolutionary Research Center," BRIN, Bogor, West Java, Indonesia. The leaves were cleaned by flowing water, cleaned of dirt, water droplets, dried, and weighed. The leaves were dried for 6 to 7 days at

30 °C. The leaves were ground into a fine powder, weighed, and stored in tightly closed dry jars until the next experiment.

Extracts Preparation

The extraction procedures for the four samples are as follows

Extraction of Flavonoids: About 8 g each of the dried leaf powder (equivalent to 25 g fresh leaves) was extracted separately with ethyl acetate with a material to solvent ratio of 1:20 (w/v). Extraction was carried out by reflux at 77ºC for 30 min and then filtered. The extraction process was repeated using the same technique until the flavonoid test showed negative results. A vacuum rotary evaporator N-1200 BS series (EYELA, Shanghai, China) was used to concentrate the filtrate to obtain extract with a total volume of 250 mL. Furthermore, the ethyl acetate extracts of SA, SM, SS, and PG leaves were labelled as SAEAE, SMEAE, SSEAE, and PGEAE, respectively.

Extraction of Phenolic: About 8 g each of the dried leaf powder (equivalent to 25 g fresh leaves) was extracted separately with ethanol (70%) with a material-solvent ratio of 1:10 (w/v). Reflux extraction at 70ºC for 30 min, followed by filtration, was performed. The extraction process was repeated until the phenolic test was negative. A vacuum rotary evaporator N-1200 BS series (EYELA, Shanghai, China) was used to concentrate the filtrate to obtain extract with a total volume of 250 mL. Furthermore, the ethyl acetate extracts of SA, SM, SS, and PG leaves were labelled as SAEE, SMEE, SSEE, and PGEE, respectively.

Extraction of Tannins: About 3 g each of dry leaf powder (equivalent to 5 g fresh leaves) was extracted with hot water (90 ± 2ºC) with a material-solvent ratio of 1:20 (w/v) for 30 min, and then filtered. The procedure was repeated until the tannin test was negative. Water bath was used to concentrate the filtrate at 65ºC until a thick extract is obtained. SAWE, SMWE, SSWE, and PGWE representing water extract of SA, SM, SS, and PG leaves, respectively.

Total phenolic content (TPC) determination

The four ethanol extracts (SAEE, SMEE, SSEE and PGEE) were qualitatively tested for phenolic compounds by the addition of $FeCl_3$ solution; formation of a blue-green colour imply the existence of phenolic compounds. The total phenolic content was determined using the method of Yang *et al*. (2007)[12] and gallic acid at concentrations of 20, 33, 46, 59, and 72 ppm as the standard. Test solution (300 µL) was added to Folin-Ciocalteu reagent (1.5 mL) and shaken until homogeneous. After 3 min, 1.2 mL of sodium carbonate (7.5%) was added to the mixture. The mixture was incubated at room temperature for 110 min. The absorbance was measured at 765.1 nm using a UV-Vis UV-1601 Series spectrophotometer (Shimadzu, Kyoto, Japan). Total phenolic content was expressed in mg GAE/g dry weight (DW). The test was done three times.

Total flavonoid content (TFC) determination

The qualitative test for flavonoids in the ethyl acetate extracts of the four types of guava (SAEAE, SMEAE, SSEAE and PGEAE) leaves was done by the addition of magnesium (Mg) powder and concentrated hydrochloric acid to aliquot quantity of the extracts. Flavonoids are present when the colour changes to red or pink. Also, the total flavonoids in the four extracts were measured using the colourimetric method suggested by Chang *et al*. (2002)[13]. Quercetin was used as a standard at 10, 15, 20, 25 and 30 ppm. Briefly, a sample of the extract (1 mL) was added to 1.5 mL of methanol, then 0.1 mL of $AlCl_3$ (10%), and 0.1 mL of sodium acetate (1 M) were added to the reaction mixture and made up to 10 mL with methanol. The mixture was left to sit for 50 min at room temperature. Using a UV-Vis spectrophotometer, the absorption was measured at 438.60 nm. The total amount of flavonoids was given as mg QE/g DW. The test was carried out in triplicates.

Total tannin content (TTC) determination

First, the qualitative test for tannins in the water extracts of the four types of guava leaves (SAWE, SMWE, SSWE, and PGWE) was done by the addition of a 10% gelatin solution to samples of the extracts. The appearance of a white residue indicates the presence of tannins.

Total tannin levels in extracts of four varieties of guava leaves were determined the colorimetric used catechin as the reference standard at concentrations of 85, 148, 211, 274, and 337 ppm.[14] The test sample (1 mL each) was added to 2.5 mL of vanillin (4% in methanol) and 2.5 mL of H_2SO_4 (25%). The mixture was kept at room temperature (25 - 26ºC) for 36 min. Using a UV-Vis spectrophotometer, the absorbance of the mixture was recorded at 499 nm. The total amount of tannins was shown as mg CE/g DW. The test was carried out in triplicates.

Antioxidant activity screening

The antioxidant activity of the four types of guava leaves was tested using the modified phosphomolybdate method following the procedure described by Salamah and Farahana, (2014).[15] Quercetin was used as the standard antioxidant compound. The extracts (50, 85, 125, 160, 200 ppm) and standard (5, 8, 11, 13, 15 ppm) samples were reacted with 1 mL phosphomolybdate reagent and made up to 5 mL with distilled water. The mixture stayed at 95°C for 60 min, using a UV-Vis Spectrophotometer, absorbance was recorded at 695 nm. The test was done three times.

TLC analysis

TLC analysis of the extract was qualitatively performed for the identification of phenolic and flavonoid content. Each guava leaf (8 g of dried leaf powder equivalent to 25 g of fresh leaves) was extracted separately using 80 mL of *n*-hexane, ethyl acetate, and ethanol (70%) using an ultrasonic bath (Branson) (40 kHz) for 15 min at 25-26ºC. Each filtrate was concentrated with a vacuum rotary evaporator. Furthermore, the *n*-hexane, ethyl acetate, and ethanol extracts of each guavas leaf are referred to as HE, EAE, and EE, respectively.

The TLC analysis was done on silica gel F_{254} plates (MERCK, Germany).[16] The mobile phase used was toluene-chloroform-ethyl acetate (5:4:1) with a little formic acid added (for the HE and EAE) and chloroform-ethyl acetate-formic acid (0.1:3.9:1) (for the EE). The visualization was performed under visible and UV light (254 nm and 365 nm).[17] In addition, $FeCl_3$ (5%), $AlCl_3$ (5%) and H_2SO_4 (10%) spray reagents were used for spot detection.[18]

Statistical analysis

All experiments were performed in triplicate. Values were expressed as mean ± standard deviation (SD) of triplicate determinations. Statistical analysis was done using the statistical software Excel 2023 Ver. 16.73 from Microsoft Corporation (US).

Results and Discussion

Total Phenolic content

The results of the qualitative tests indicated the presence of phenolic compounds in the four types of guava leaf extracts. From the gallic acid calibration curve, the equation of the line was obtained as $y = 0.0107x + 0.0112$ ($R^2 = 0.999$). Figure 1A demonstrates that PGEE has the maximum total phenolic content compared to other guava leaves. The three guava leaves from the genus *Syzygium* had total phenolic contents that are significantly lower than that of PG leaves. Phenolic compounds are a class of secondary metabolites with aromatic groups found throughout the plant kingdom. They ranges from basic structure like phenolic acid to complex structures such as tannins and lignins.[19] Many phenolic compounds are present in plants as glycosides, so they are generally very polar. The extraction of phenolic compounds involves the use of polar solvent such as ethanol. The Folin-Ciocalteu technique is the most popular method for the quantitative determination of phenolic compounds from plant materials and extracts. It is the simplest, most reproducible method for determining total phenolic content.[20] The phosphotungstic-phosphomolybdate complex is reduced by phenolics in an alkaline medium using the Folin-Ciocalteu procedure, yielding a blue-colored solution.[21] The intensity of the blue colour formed corresponds to the total phenol content of the sample, and the intensity of the colour is measured at a wavelength of 765.1 nm. Gallic acid is used as a reference standard in this measurement because it is a pure and stable pheolic compound.[22] In this study, the highest phenolic content was observed in PGEE. Meanwhile, from the genus *Syzygium* used in this study, the highest levels of phenolic were found

in SAEE and the lowest levels in SMEE. The phenolic compounds contained in PG may be of more types than other guavas, for example, guavanoic acid, guavenoic acid, guajavolide have been found in PG.[23]

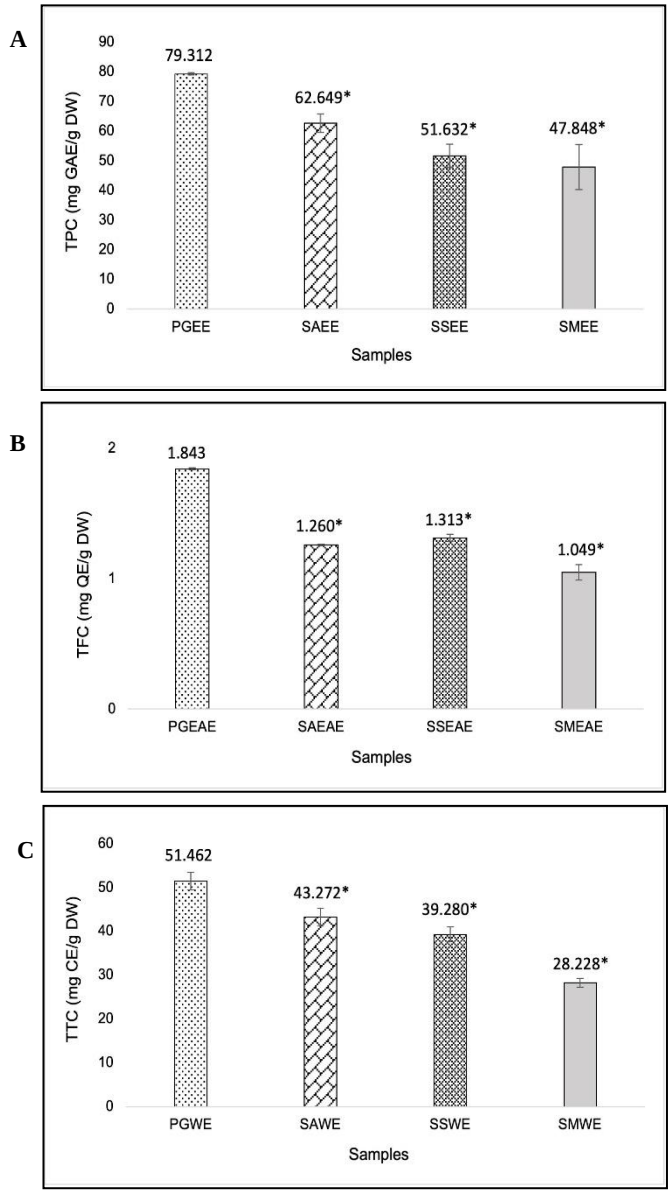

Figure 1: [A] Total phenolic content (TPC) of guava leaf extracts. [B] Total flavonoid content (TFC) of guava leaf extracts. [C] Total tannin content (TTC) of guava leaf extracts. The sign (*) indicates a significant difference.
PG – *Psidium guajava*; SA – *Syzygium aqueum*; SS – *Syzygium samarangense*; SM – *Syzygium malaccense;* EAE – Ethyl acetate extract; EE – Ethanol extract; WE – Water extract.

Total Flavonoid content
The qualitative analysis revealed an abundance of flavonoid compounds in the four-leaf extracts. For the quantitative determination, the quercetin calibration curve gave a linear equation as $y = 0.0251x + 0.0002$ ($R^2 = 0.9992$). Figure 1B shows that the three guava leaves of the genus *Syzygium* have flavonoid contents that are significantly lower than that of PG leaves. However, from the three guavas of the genus *Syzygium*, SSEAE had the highest levels of flavonoids followed by SAEAE and SMEAE. Flavonoids are secondary metabolites composed of a C_6-C_3-C_6 configuration with two cyclic rings connected to 3 carbon atoms, which are generally associated with O atom as heterocyclic oxygen bonds.[24] Flavonoids, including polyphenol compounds, generally have semi-polar to opposite polarity, so they can be extracted using ethyl acetate, acetone, butanol, methanol, water, or a mixture of water and alcohol. The reflux method is one of the conventional extraction methods chosen because it is simple and fast and accompanied by heating which can increase the solubility of flavonoids.[25] This present research determined the flavonoid content in ethyl acetate extract using $AlCl_3$ and measurement of the absorbance of the resulting mixture by a spectrophotometer at 438.60 nm.[26] $AlCl_3$ solution forms a stable complex with a hydroxyl group at position C_3 and/or with a ketone group at position C_5. Complex compounds also occur when there is a hydroxyl group at the ortho position.[24] The complex that occurs causes a bathochromic shift in wavelength of absorption.

Table 1: Antioxidant activity in terms of quercetin equivalence of guava leaf extracts against phosphomolybdate

Samples	Quercetin equivalence (mg QE/g)		
	EE	EAE	WE
SA	132.043 ± 1.53	134.103 ± 0.559	137.184 ± 2.678
SM	101.907 ± 5.95	97.256 ± 0.443	87.893 ± 8.975
SS	127.437 ± 2.06	129.079 ± 1.711	133.874 ± 3.156
PG	150.990 ± 0.88	168.880 ± 1.647	168.748 ± 3.312

PG – *Psidium guajava*; SA – *Syzygium aqueum*; SS – *Syzygium samarangense*; SM – *Syzygium malaccense*; EE = ethanol extract; EAE = ethyl acetate extract; WE = water extract

Total Tannin content
The results of the qualitative test for tannins showed that the four types of guava leaf extracts contained tannins. The catechin calibration curve gave a linear equation of $y = 0.002x + 0.0483$ ($R^2 = 0.9997$). From Figure 1C, it shows that PGWE has the highest total tannin content compared to other extracts. The solvent used to determine the tannin content was water because the solubility of tannin is quite good in the water.[27] Tannins are a phenolic group of compounds that are widely distributed in nature. The extraction of tannins using the decoction method is simple, fast, and inexpensive. Heating in the decoction method helps increase the solubility of tannins.[26] The results of the identification of tannins using $FeCl_3$ solution in PGEE produced the most intense colour. The tannin content was the highest in PG leaves compared to the other three guava species. While, among the guava species of the genus *Syzygium*, SA leaves had the higher tannin content than SS and SM leaves. The high amount of tannins in PG leaves may be due to the different types of tannins that have been found in high quantities in PG. More than 20 types of tannins have been isolated from PG, some of which are guavin A, B, C, and D; psidinins A, B, and C; guajavin A, B.[23] The vanillin method is often used to determine the levels of condensed tannins and proanthocyanidins using catechin as a standard.[28] This high tannin level may supports the use of PG leaves as an antidiarrheal agent.

Antioxidant activity
The total antioxidant activity of the four varieties of guava leaf extracts was evaluated using the phosphomolybdate technique with quercetin as the reference. The results were reported as quercetin equivalents determined from the line equation $y = 0.0292x + 0.1772$ ($R^2 = 0.9997$) derived from the quercetin calibration curve. In this method, molybdenum (VI) decreases to molybdenum (V) in the existence of a reducing agent (antioxidant), resulting in the forming of a green phosphomolybdate (V) complex that can be detected spectrophotometrically at 695 nm.[29,30] This test involves an electron transfer mechanism. Several studies have shown that many natural products have antioxidant activity, including phenols and flavonoids.[31,32]
Table 1 and Figure 2 illustrate the antioxidant activity of guava leaf extracts. PG leaf extract showed the highest antioxidant capacity compared to other guava leaf extracts, while SM leaf extracts showed the lowest antioxidant capacity compared to the other two types of guava leaves from the genus *Syzygium*. Figure 2 shows that PG leaf extract has the lowest EC_{50} value, indicating that PGWE has the best

antioxidant activity of the four guava leaf extracts. The significant antioxidant activity of the PG extract was also connected to its high tannin concentration. The EC_{50} value of SMWE was the highest which suggest that it has the lowest antioxidant activity which also correlated with the lowest tannin content (28.228 mg CE/g) and flavonoid content (1.049 mgQE/g) in this extract. The high antioxidant activity in the PG extract was also reflected in its high flavonoid content (1.843 mgQE/g extract) (Figure 1B). The EC_{50} values of SAWE and SSWE were not significantly different from each other, and they also have comparable tannin contents. The PGEAE and SAEAE had similar EC_{50} values of 35.193 ppm and 37.390 ppm, respectively, which means that both extracts had same potency in terms of their antioxidant activity (Figure 2). The antioxidant capacity of SAEAE and SSEAE were not statistically different ($P < 0.05$) as shown by their EC_{50} values.

The SMEE has the highest EC_{50} value, which means that the extract has the least antioxidant activity. The EC_{50} values of the other three guava extracts (PGEE, SAEE, and SSEE) were relatively low, meaning that their antioxidant activity is quite strong. The phenolic content in the PGEE was 79.312 mg GAE/g extract (Figure 1A), the highest among all the guava extracts. Studies on the antioxidant activity of PGEE, SAEE and SSEE using DPPH radical scavenging activity revealed that these extracts have good antioxidant activity with IC_{50} values of 35.57 g/mL, 38.69 g/mL, and 59.16 g/mL, respectively, while the SMEE showed the low antioxidant activity with IC_{50} value of 138.33 g/mL.[8,33,34] These observations agrees with the findings from the present study which shows PG extract as the highest antioxidant activity with EC_{50} value of 13.142 ± 1.087 g/mL.

The polyphenolic contents of extracts have been found to affect their antioxidant activity.[20] In this study, TPC, TFC, and TTC test results showed that the PG extract had more phenolic, flavonoid, and tannin contents than the other guava extracts. This correlates positively with antioxidant activity, implying that the higher the phenolic, flavonoid, or tannin content, the higher the antioxidant activity. Extraction solvent polarity has also been found to have profound effect on the antioxidant activity of the resulting extract; the higher the polarity of the extraction solvent, the higher the antioxidant activity of the extract.[21] This assumption is corroborated by the findings of our study, which reveal that as the polarity of the solvent increases, so does its antioxidant activity. Hence, the antioxidant activity was in the following order; PGWE > PGEE > PGEAE with corresponding EC_{50} values of 13.142 ppm, 28.722 ppm, and 35.193 ppm, respectively. Furthermore, SAEE, SAEE, and SAWE have the potential to be good sources of antioxidants compared to other extracts from the genus *Syzygium* in this study.

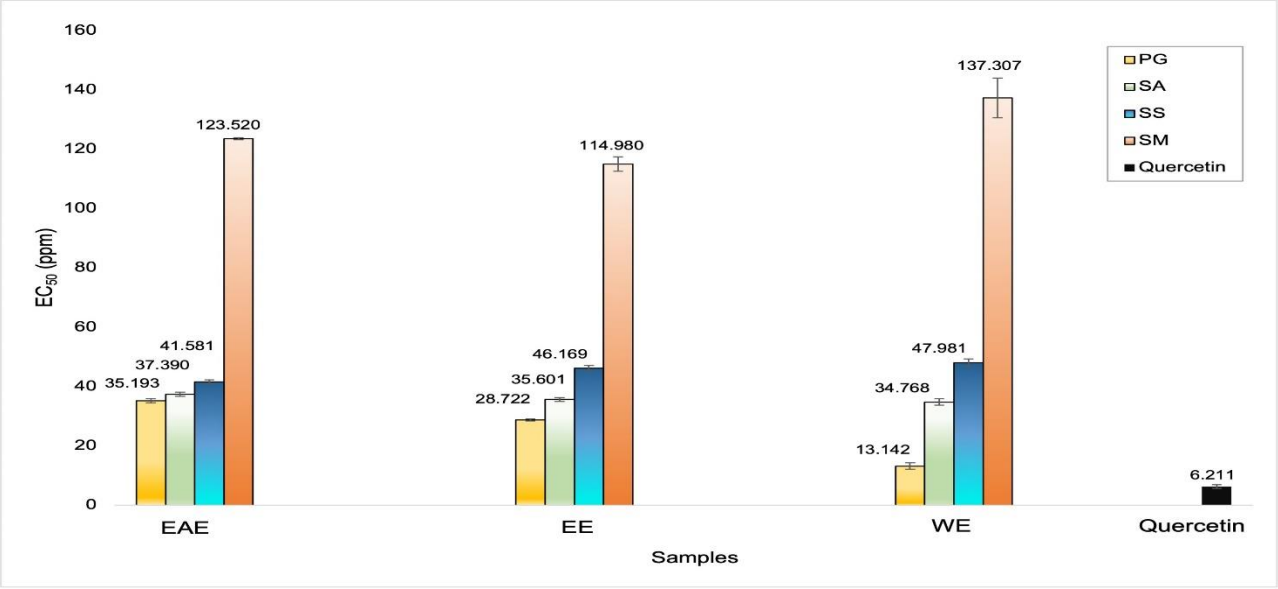

Figure 2: Antioxidant activity (EC_{50} values) of guava leaf extracts against phosphomolybdate.
PG – *Psidium guajava*; SA – *Syzygium aqueum*; SS – *Syzygium samarangense*; SM – *Syzygium malaccense*; EAE – Ethyl acetate extract; EE – Ethanol extract; WE – Water extract.

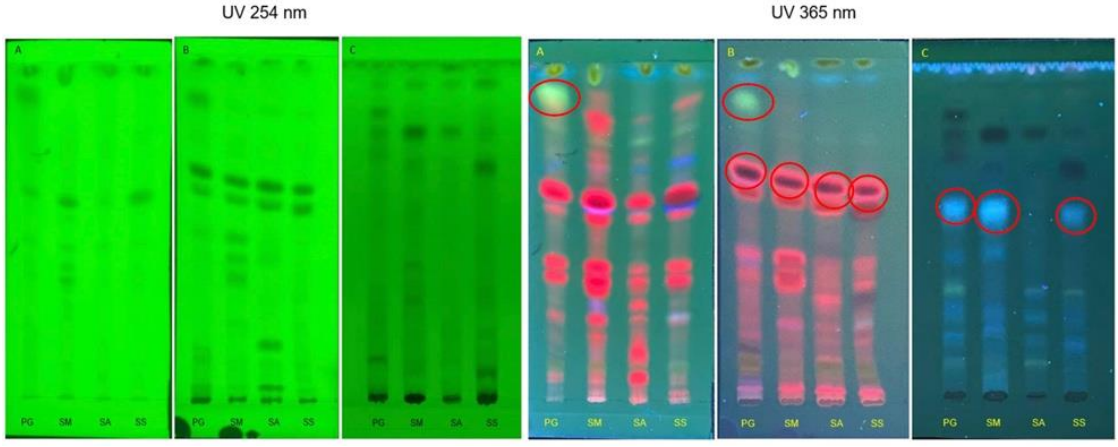

Figure 3: TLC Chromatogram of HE (A), EAE (B), and EE (C) of four types of guava leaves under UV light at 254 and 365 nm.
PG – *Psidium guajava*; SA – *Syzygium aqueum*; SS – *Syzygium samarangense*; SM – *Syzygium malaccense*; HE – *n*-Hexane extract; EAE – Ethyl acetate extract; EE – Ethanol extract.

TLC Profile

TLC is often used to rapidly identify organic compounds, including flavonoids as bioactive target compounds in the plant extracts.[35] TLC analysis was used to identify the nature of phytoconstituents concerning the polyphenolic chemicals found in the four varieties of guava leaves. The extracting solvents used ranges from non-polar to polar (*n*-hexane, ethyl acetate, and ethanol), to determine the number of compounds extracted by the three solvents. The mobile phase used include; (i) a solution of toluene–chloroform–ethyl acetate (5:4:1) to identify compounds in the HE and EAE, (ii) chloroform– ethyl acetate–formic acid (0.1:3.9:1) to identify compounds in the EE. Visualization of the TLC plates was done under UV light (254 nm and 366 nm) and by spray reagents using a 5% $FeCl_3$ solution (for detection of phenolic compounds), 5% $AlCl_3$ solution (for flavonoid detection) and 10% solution of H_2SO_4 (for detection of other organic compounds).[18]

As shown in Figure 3, the EAE had more spots in all leaves extracts than the HE. In contrast, the EE had more unresolved spots at the origin. In the EAE, it was observed that there were similarities in the chemical constituents of the three types of *Syzygium*. Whereas, in PG extracts, there were quantitative differences, as there appeared some unique spots which were not seen in the extracts of the three *Syzygium* species.

The HE and EAE of PG showed yellow fluorescence compounds at 365 nm with similar spot location (Rf is around 0.80). The EAE of all three *Syzygium* species and PG leaves showed purple fluorescence compounds (Rf is around 0.56-0.58). Similarly, in EE of PG, SM and SS, there were blue fluorescent spots with similar Rf values (Rf is around 0.56) (Figure 3).

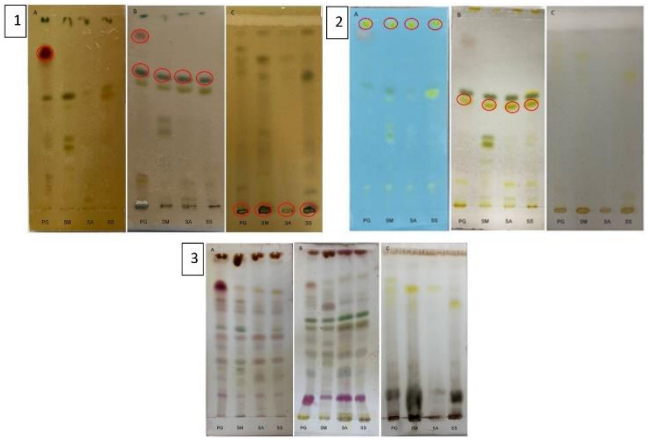

Figure 4: TLC chromatogram for identification of **[1]** phenolic compounds in HE (A), EAE (B), and EE (C) of four types of guava leaves after spraying with 5% $FeCl_3$. **[2]** flavonoids in HE (A), EAE (B), and EE (C) of four types of guava leaves after spraying with 5% $AlCl_3$. **[3]** other organic compounds in HE (A), EAE (B), and EE (C) extracts of four types of guava leaves after spraying with 10% H_2SO_4.
PG – *Psidium guajava*; SA - *Syzygium aqueum*; SS - *Syzygium samarangense*; SM - *Syzygium malaccense*; HE – *n*-Hexane extract; EAE – Ethyl acetate extract; EE – Ethanol extract.

After being sprayed with $FeCl_3$ solution, the EAE of the four test samples displayed a blue-green spot, as shown in Figure 4(1). In the HE of PG, there was a dark brown spot (R_f is around 0.80), which probably indicated a tannin compound. While, in the EAE of PG, there was a purple-black spot, suggesting a possibly different type of tannin from the HE of PG (Rf is around 0.80). In the EE of the four test samples, dark brown spots were found at the origin, meaning that the tannin compounds present in this extract were not eluted by the mobile phase used.

The EAE of the four test samples contained yellow fluorescence compounds with similar R_f (R_f is around 0.53-0.55), as shown in Figure 4(2). These compounds may be flavonoids which are present in the four plants. In the HE of all leaves, yellow fluorescence compounds also appeared with different Rf (Rf is around 0.85), this may suggest the presence of more non-polar flavonoids in the HE than in the EAE.

Figure 4(3) showed that the phytochemical contents of the HE and EE are more similar, with the main difference being the intensity of the colors. A spot with a different color and size appeared in the HE and EAE of PG leaves than in the extracts of *Syzygium* species. This indicates that PG has more compounds than the three species of *Syzygium*.

Summarily, appearance of blue spot indicates phenolic compounds; yellow spot indicates flavonoids, while varieties of other organic compounds were indicated by various colour (light blue, blue, purple, purple, pink, and grey) spots. The TLC profile has shown the similarity in the type of phytochemical constituents in the extracts of the four test guava leaves.

Conclusion

The findings from the present study shows that *Psidium guajava* leaves have the highest contents of tannins, flavonoids, phenols, as well as the highest antioxidant capacity compared to the other three guava leaves from the genus *Syzygium* which are *S. samarangense*, *S. malaccense*, and *S. aqueum*. The chemical components of the four guava have similarities which may be related to their membership in the Myrtaceae. Besides, this study concluded that *S. aqueum* is a species of *Syzygium* that has the potential to be developed as a source of polyphenols and antioxidants compared to the other two species in this study.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Gayen P, Ama H, Saifuzzaman M, Faroque A. Anthelmintic activity of ethanolic extract of *Syzygium samarangense* (Blume) Merril & Perry. Dhaka Univ J Pharm Sci. 2016; 15:109–111.
2. Kafle A, Mohapatra SS, Reddy I, Chapagain M. A review on medicinal properties of *Psidium guajava*. J Med Plants Stud. 2018; 6(4):44–47.
3. Tilaar M, Widjaja B, Ranti A. The Green Science of Jamu. PT Dian Rakyat, editor. Jakarta: PT Dian Rakyat; 2010.
4. The National Agency of Drug and Food Control (NA-DFC). Statistik Produk Yang Mendapat Persetujuan Izin Edar [Internet]. https://cekbpom.pom.go.id. 2021. Available from: https://cekbpom.pom.go.id//home/produk/p5i4kt4kfpe75r9po7ng9gqap7/all/row/10/page/2/order/4/DESC/search/5/psidii
5. Aung E, Kristanti A, Aminah N, Takaya Y, Ramadhan R. Plant description, phytochemical constituents and bioactivities of *Syzygium* genus: A review. Open Chem. 2020; 18(1):1256–1281.
6. Group S working. Syzygium (Myrtaceae): Monographing a taxonomic giant via 22 coordinated regional revisions. PeerJ Prepr [Internet]. 2016;(4:e1930v1). Available from: https://doi.org/10.7287/peerj.preprints.1930v1

7. Kokilananthan S, Bulugahapitiya VP, Manawadu H, Gangabadage CS. Comparative Evaluation of Different Extraction Techniques on Phytochemicals and Antioxidant Activity of *Psidium Guajava* L. Leaves. Trop J Nat Prod Res. 2022; 6(4):552–557.
8. Itam A, Wati MS, Agustin V, Sabri N, Jumanah RA, Efdi M. Comparative Study of Phytochemical, Antioxidant, and Cytotoxic Activities and Phenolic Content of *Syzygium aqueum* (Burm. f. Alston f.) Extracts Growing in West Sumatera Indonesia. Hindawi. Sci World J Vol. 2021;1–9.
9. Arumugam B, Manaharan T, Heng C, Kuppusamy U. Antioxidant and Antiglycemic Potentials of a Standardized Extract of *Syzygium malaccense*. Food Sci Technol. 2014; 59(2):707–712.
10. Choironi N and Fareza M. Phytochemical screening and antibacterial activity of ethanolic extract of *Syzygium samarangense* leaves. J Kartika Kim. 2018; 1(1):1–4.
11. Majumder R, Nur-E-Hasnat, Ashraf-Uz-Zaman M, Alam M. *In vivo* evaluation of the pharmacological activities of *Syzygium samarangense* (Blume) Merr. & L.M. Perry. Adv Biol Res (Rennes). 2014; 8(3):107–115.
12. Yang J, Paulino R, Janke-Stedronsky S, Abawi F. Free-radical-scavenging Activity and Total Phenols of Noni (*Morinda citrifolia* L.) Juice and Powder in Processing and Storage. Food Chem. 2007; 102:302–308.
13. Chang C, Yang M, Wen H, Chern J. Estimation of Total Flavonoid Cintent in Propolis by Two Complementary Colorimetric Methods. J Food Drug Anal. 2002; 10(3):178–182.
14. Medini F, Fellah H, Ksouri R, Abdelly C. Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant *Limonium delicatulum*. J Taibah Univ Sci. 2014; 8(3):216–224.
15. Salamah N and Farahana L. Antioxidant activity Assay of Ethanolic Extract of *Centella asiatica* (L.) Urb Herb using Phosphomolybdate method. Pharmaciana [Internet]. 2014; 4(1):23–30.
16. Waksmundzka-hajnos M, Sherma J, Kowalska T. Thin Layer Chromatography in Phytochemistry. Cazes J, editor. CRC Press, Taylor & Francis Group; 2008. 888 p.
17. Rafi M, Heryanto R, Septaningsih DA. "ATLAS" Kromatografi Lapis Tipis Tumbuhan Obat Indonesia (In Bahasa), Thin Layer Chromatography of Indonesian Medicinal Plants. In: Volume 1. Bogor, Indonesia: Pusat Studi Biofarmaka Tropika, LPPM IPB; 2017. 26 p.
18. Sarker S, Latif Z, Gray A. Natural Products Isolation. Second. New Jersey: Humana Press; 2006.
19. Hanani E. Pharmacognosy. Jakarta: Penerbit Uhamka Press; 2021. 177–178 p.
20. Hikmawanti NPE, Fatmawati S, Arifin Z, Vindianita. Pengaruh Variasi Metode Ekstraksi Terhadap Perolehan Senyawa Antioksidan Pada Daun Katuk (*Sauropus androgynus* (L.) Merr). J Farm Udayana. 2021; 10(1):1.
21. Hikmawanti NPE, Hanani E, Sapitri Y, Ningrum W. Total phenolic content and antioxidant activity of different extracts of *Cordia sebestena* L. leaves. Pharmacogn J. 2020; 12(6):1311–1316.
22. Senet M, Raharja I, Darma I, Prastakarini K, Dewi N, Parwata I. Determination of total flavonoid and total phenol content of kersen root (*Mutingia calabura*) and the antioxidant activity. J Chem. 2018; 12(1), 13-18.
23. Achmad S, Hakim E, Makmur L, Syah Y, Juliawaty L, Mujahidin D. Tumbuh-tumbuhan Obat Indonesia Jilid 1. Penerbit ITB, editor. Bandung: Penerbit ITB; 2008.
24. Hanani E. Analisis Fitokimia (In Bahasa) "Phytochemistry Analysis." Jakarta: EGC Medical Publisher; 2015; 69(83) 110, 148–149, 202, 232–233 p.
25. Ministry of Health Republic of Indonesia. Farmakope Herbal Indonesia Edisi II. Jakarta: Ministry of Health Republic of Indonesia; 2017. 5–6 p.
26. Khoddami A, Wilkes M, Roberts T, Khoddami A, Wilkes MA, Roberts TH. Techniques for analysis of plant phenolic compounds. Molecules. 2013; 18(2):2328–2375.
27. Trease, Evans W. Pharmacognosy 16th Edition. In: 16th ed. Toronto: Saunders Elsevier; 2009. p. 100, 246, 269–270.
28. Hayat J, Akodad M, Moumen A, Baghour M, Skalli A, Ezrari S, Belmalha S. Phytochemical screening, polyphenols, flavonoids and tannin content, antioxidant activities and FTIR characterization of *Marrubium vulgare* L. from 2 different localities of Northeast of Morocco. Heliyon. 2020; 6(e05609).
29. Prieto P, Pineda M, Aguilar M. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. Anal Biochem. 1999; 269:337–341.
30. Jan S, Khan MR, Rashid U, Bokhari J. Assessment of Antioxidant Potential, Total Phenolics and Flavonoids of Different Solvent Fractions of *Monotheca Buxifolia* Fruit. Osong Public Heal Res Perspect [Internet]. 2013;4((5)):246–54.
31. Ahmed D, Khan MM, Saeed R. Comparative Analysis of Phenolics, Flavonoids, and Antioxidant and Antibacterial Potential of Methanolic, Hexanic and Aqueous Extracts from *Adiantum caudatum* Leaves. Antioxidants. 2015; 4((2)):394–409.
32. Brindha P, Ragamanvitha A, Narendran R, Sriram S, Vadive V. Antioxidant Activity and Phytochemical Composition of Aqueous Extract of *Markhamia lutea*(Benth) K. Schum. Leaves. Trop J Nat Prod Res. 2017; 1(2):63–68.
33. Habisukan U, Elfita, Widjajanti H, Setiawan A, Kurniawati A. Antioxidant and Antimicrobial Activity of Endophytyc Fungi Isolated from *Syzygium aqueum* Leaves. J Phys Conf Ser ICASMI Sumatera. 2020; 1–8.
34. Primadiastri IZ, Wulansari ED, Suharsanti R. Perbandingan Kandungan Fenolik Total, Flavonoid Total Aktivitas Antioksidan Ekstrak Etanol Daun Jambu Bol (*Syzigium malaccense* L .) Dan Daun Jambu Air Kancing (*Syzigium aqueum*). J Media Farm Indones. 2021; 16(2):1671–1675.
35. Hikmawanti NPE, Wiyati T, Abdul Muis M, Nurfaizah FA, Septiani W. Total Flavonoids Content of Polar Extracts of *Cayratia trifolia* Leaves. IOP Conf Ser Earth Environ Sci. 2021; 819(1), 1-4.