# BUKTI KORESPONDENSI

# ARTIKEL INTERNASIONAL BEREPUTASI

| | | |
|---|---|---|
| Judul Artikel | : | **Exploring the Polyphenol Contents and Antioxidant Capacity of the Leaf Extracts of Selected Indonesian Syzygium Species** |
| Jurnal | : | Tropical Journal of Natural Product Research (TJNPR) |
| Penulis | : | Agustin Yumita, **Ni Putu Ermi Hikmawanti***, Endang Hanani, Cindi W. Saputri, Putri H. Hanana, Jeanne N.D. Ero, Mayang Marcelena, Tazqiyah Baytisani, Febby A. Sofiana, Amanda F. Shania, Erlina S.A. Saputri, Firda P.N. Islami |

| No. | Perihal | Tanggal |
|---|---|---|
| 1. | Bukti *submit* dan artikel yang di-*submit* | 23 Januari 2023 |
| 2. | Bukti artikel diterima | 4 Februari 2023 |
| 3. | Bukti hasil *review* dan file komentar *reviewer* | 11 Maret 2023 |
| 4. | Bukti hasil revisi dan artikel yang direvisi | 5 April 2023 |
| 5. | Bukti artikel proses *galley proof* dan artikel yang dikoreksi *galley proof* | 23 Juni 2023 |
| 6. | Bukti artikel telah terbit *online* pada website Jurnal | 2 Juli 2023 |

# 1. Bukti *submit* dan artikel yang di-*submit* (23 Januari 2023)

Uhamka
UNIVERSITAS MUHAMMADIYAH PROF. DR. HAMKA

**Ni Putu Ermi Hikmawanti <ermy0907@uhamka.ac.id>**

# [TJNPR] Submission Acknowledgement

1 message

**editor@tjnpr.org** <editor@tjnpr.org> Mon, Jan 23, 2023 at 11:22 AM
Reply-To: Prof Abiodun Falodun <editor@tjnpr.org>
To: Ni Putu Ermi Hikmawanti <ermy0907@uhamka.ac.id>

Ni Putu Ermi Hikmawanti:

Thank you for submitting the manuscript, "Exploring polyphenol and antioxidant capacity in leaf extracts of selected Indonesian Syzygium species" to Tropical Journal of Natural Product Research (TJNPR). With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

Submission URL: https://tjnpr.org/index.php/home/authorDashboard/submission/1499
Username: ermi_hikmawanti

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

Prof Abiodun Falodun

Tropical Journal of Natural Product Research (TJNPR)

Email: editor.tjnpr@gmail.com | Phone: +2348073184488

# Exploring polyphenol and antioxidant capacity in leaf extracts of selected Indonesian *Syzygium* species

Agustin Yumita[1], Ni P.E. Hikmawanti[1]*, Endang Hanani[1], Cindi W. Saputri[1], Putri H. Hanana[1], Jeanne N.D. Ero[1], Mayang Marcelena[1], Tazqiyah Baytisani[1], Febby A. Sofiana[1], Amanda F. Shania[1], Erlina S.A. Saputri[1], Firda P.N. Islami[1]

[1]Department of Pharmaceutical Biology, Faculty of Pharmacy and Sciences, University of Muhammadiyah Prof DR. HAMKA, Jakarta. Indonesia.

***Corresponding author** : ermy0907@uhamka.ac.id

## ABSTRACT

Traditionally Indonesian people use *Psidium guajava* to treat diarrhea. Over time the *P. guajava* plant was considered rare. One of the efforts is to explore the chemical content of plants still in the same family as *P. guajava*. The study aimed at exploring the chemical content and antioxidant activity of leaves of three types of guavas, *Syzygium aqueum* (Burm.f.) Alston, *Syzygium malaccense* (L.) Merr. and Perry., *Syzygium samarangense* (Blume) Merr. & L.M. Perry, which was compared to *P. guajava* leaves. The chemical similarity between the four types of guava leaves was determined based on the parameters of total tannins (vanillin-sulfuric acid reagent), flavonoids ($AlCl_3$ reagent), and phenol (Folin-Ciocalteu reagent). Antioxidant capacity was measured by the phosphomolybdate method with quercetin as a reference. For TLC analysis with $FeCl_3$ 5% for phenolics, $AlCl_3$ 5% for flavonoids, and $H_2SO_4$ for organic components. The results showed that the highest phenol, flavonoid and tannins content was found in the leaves of *P. guajava*. The chromatogram showed the similarity of the organic components of four types of guava in the three extracts based on the position and colour of the spots that appeared. The content of flavonoids, phenolics, and tannins in *P. guajava* leaves was the highest due to differences in the type and amount of chemical content between the four species. The antioxidant activity of *P. guajava* leaves is closely related to their high phenolic 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 type of plant belonging to the Myrtaceae family.[1] *Psidium guajava* (PG) leaves are widely used to treat diarrhoea and have several pharmacological activities, such as antidiabetic, antidiarrheal, antimicrobial, antihypertensive and antioxidant.[2]

Drinking herbal medicine (one of which uses PG leaves) to treat health problems is still a tradition 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 in the form of Standardized Herbal Medicines (Obat Herbal Terstandar, OHT) are registered with the Indonesian FDA (The National Agency of Drug and Food Control, NA-DFC) made from PG.[4] However, PG plants are considered rare. This causes the community or the herbal medicine industry to need help in obtaining raw materials for the leaves of this plant. One of the efforts made to meet the needs of these herbs can be realized by exploring the chemical content of the plant, which are still relatives of the same tribe as PG.

Some plants belonging to the Myrtaceae tribe 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 also easy to obtain and widely grown in Indonesia. These plants have leaves that are shadier than PG. In addition, the three types of guava leaves also have 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 is also scientifically proven as an antioxidant, antimicrobial, antifungal, antiviral, analgesic, anti-inflammatory, antihypertensive, antihyperglycemic, sedative, and spasmolytic.[5–7] Several pharmacological studies have reported that SS, SM, and SA provide their properties as antioxidants and antimicrobials. [8–11.]

The content of polyphenols found in species of the genus *Syzygium*, including phenolics, flavonoids and tannins, is closely related to their antioxidant activity. The profile of these metabolites in the extract can be studied simply using thin-layer chromatography (TLC) techniques. This antioxidant compound recovery technique is undoubtedly closely related to using extraction solvents. This research aimed to investigate the chemical content of polyphenols in terms of total phenolic, flavonoid, and tannin levels in the leaves of three selected Syzygium species and test their antioxidant activity compared to PG leaves.

## Materials and Methods

### *1. Chemicals and Solvents*

Chemicals and solvents such as n-hexane, dichloromethane, ethyl acetate, ethanol, methanol, chloroform, toluene, formic acid, sulfuric acid, hydrochloric acid, acetic acid, sodium carbonate, sodium acetate, Folin-Ciocalteu, aluminium (III) chloride, vanillin, phosphomolybdate, and silica gel plate are obtained from Merck (Darmstadt, Germany). Meanwhile, quercetin is from Sigma Aldrich Co. (St. Louis, USA), while gallic acid and catechins are from Mark Herb (Bandung, Indonesia).

### *2. Plant materials*

The four types of guava leaf were collected from the Duren Sawit sub-district, East Jakarta, in February 2022 with the determination number B-571/DI.05.07/3/2022 at the "Biosystematics and Evolutionary Research Center" BRIN, Bogor, West Java, Indonesia. Specimens of each type of guava are stored in the Pharmacognosy Laboratory of the Faculty of Pharmacy and Science, UHAMKA. After being washed with running water and cleaned of dirt, leaves were dried from water droplets and weighed. Leaves were dried in the air for 6 - 7

days, temperature 30 ± 1 ºC. After drying, the leaves were weighed again to be further powdered and stored in tightly closed, airtight, and dry bottles until the following experiment.

### *3. Extracts Preparation*

The guava leaf extraction process is carried out with different methods and solvents, as follows:

**3.1. Ethyl Acetate Extract (EAE):** ± 8 g dry leaf powder (equivalent to 25 g fresh leaves) was extracted with ethyl acetate with a solvent-to-material ratio of 1: 20 (w/v). Extraction was carried out by reflux method at 77 ºC for 30 minutes and then filtered. The extraction was repeated using the same technique until the flavonoid screening showed negative results. Each filtrate was concentrated using a vacuum rotary evaporator N-1200 BS series (EYELA, Shanghai, China) to obtain a total volume of 250 mL, after this, referred to as SEAAE, SMEAE, SSEAE, and PGEAE. Each extract was made triple.

**3.2. Ethanol 70% Extract (EE):** ± 8 g dry leaf powder (equivalent to 25 g fresh leaves) was extracted with ethanol 70% with a material-solvent ratio of 1:10 (w/v). Extraction was carried out by reflux method at 70 ºC for 30 minutes, then filtered. The extraction was repeated with the same method until the phenolic screening was negative. Each filtrate was concentrated using a vacuum rotary evaporator N-1200 BS series (EYELA, Shanghai, China) to obtain a total volume of 250 mL, starting now referred to as SAEE, SMEE, SSEE, and PGEE. Each extract was made triple.

**3.3. Water Extract (WE):** ± 3 g dry leaf powder (equivalent to 5 g fresh leaves) was extracted with hot water (90 ± 2 ºC) with a solvent-material ratio of 1: 20 (w/v) for 30 minutes, then filtered. The extraction was repeated using the same method until the tannin screening was negative. Each filtrate was concentrated using a water bath at a temperature of 65 ºC to obtain a thick extract: SAWE, SMWE, SSWE, and PGWE. The extract was made triple.

### *4. Total phenolic content (TPC) Assay*

The experiment was started by identifying the phenolic compounds in EE from the four types of guava leaves (SAEE, SMEE, SSEE and PGEE), namely the appearance of a blue-green colour with the addition of $FeCl_3$ solution. Total phenolic compound content was determined following the method of Yang et al., 2007[12] using gallic acid as a comparison (20, 33, 46, 59, and 72 ppm). Test solution 300 µL was added to Folin-Ciocalteu reagent 1.5 mL, and shaken until homogeneous. After 3 minutes, 1.2 mL of sodium carbonate 7.5% was added to the mixture. The mixture was incubated for 110 minutes at room temperature. 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 carried out in triples.

### *5. Total flavonoid content (TFC) Assay*

The flavonoid compounds in the EAE of the four types of guavas (SAEAE, SMEAE, SSEAE and PGEAE) leaves were identified by adding Mg powder and concentrated hydrochloric acid. The formation of red and pink colors indicates the presence of flavonoid compounds. Furthermore, the levels of total flavonoid compounds in the four extracts were done out utilizing the colourimetric method of Chang et al., 2002[13] with quercetin as a comparison (10, 15, 20, 25 and 30 ppm). A sample of 1 ml was added with 1.5 ml of methanol, 0.1 ml of $AlCl_3$ 10%, and 0.1 ml of sodium acetate 1 M and made up to 10 ml with methanol. The mixture was incubated for 50 minutes at room temperature. The absorbance was recorded at 438.60 nm with a UV-Vis UV-1601 Series spectrophotometer (Shimadzu,

Kyoto, Japan). Total flavonoid levels were reported as mg QE/g DW. The test was carried out in triples.

***6. Total tannins content (TTC) Assay***

First, the identification of tannin compounds in WE of the four types of guava leaves (SAWE, SMWE, SSWE, and PGWE) was carried out with the addition of a 10% gelatin solution. The appearance of a white residue indicates the existence of tannins. Determination of total tannin levels in the extracts of the four types of guava leaves was done using the colourimetric method proposed by Medini et al., (2014)[14] with catechins as a comparison (85, 148, 211, 274, and 337 ppm). Each 1 mL of the test sample was added with 2.5 mL of vanillin 4% in methanol and 2.5 mL of $H_2SO_4$ 25%. The mixture was incubated at room temperature (± 25 - 26 ºC) for 36 minutes. The absorbance was measured at 499 nm using a UV-Vis UV-1601 Series spectrophotometer (Shimadzu, Kyoto, Japan). Tannin levels are expressed in mg CE/g DW. The test was carried out in triples.

***7. Antioxidant Activity***

The antioxidant activity of EAE, EE, and WE of the four types of guava leaves was tested using the modified phosphomolybdate method following the procedure proposed by Salamah & Farahana, (2014).[15] The comparison used was quercetin. The extract and comparison samples were reacted with phosphomolybdate reagent and made up to 5 ml with distilled water. The mixture was incubated at 95 °C for 60 minutes. The absorbance was measured at 695 nm using a UV-1601 Series UV-Vis Spectrophotometer (Shimadzu, Kyoto, Japan). The test was carried out in triples.

***8. TLC analysis***

The chemical content of phenolics and flavonoids was analysed by the TLC method on the extracts prepared separately. Each guava leaf (8 g of dried leaf powder equivalent to 25 g of fresh leaves) was extracted using 80 ml of n-hexane or ethyl acetate or ethanol 70% with an ultrasonic bath (Branson) (40 kHz) for 15 minutes at room temperature. Each filtrate was concentrated with a vacuum rotary evaporator.

Chemical content was identified on silica gel plate F254 (MERCK, Germany) as the stationary phase.[16] Meanwhile, the mobile phase used was: toluene-chloroform-ethyl acetate (5:4:1) with a little formic acid added (for identification of the n-hexane and ethyl acetate extracts) and chloroform-ethyl acetate-formic acid (0.1:3.9:1) (for identification of ethanol extract). 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 also used for spot detection.[18]

***9. Statistic analysis***

The data were processed using Microsoft Office Software (Microsoft® Excel 365).

**Results and Discussion**

***Total phenolic content (TPC)***

The identification results indicated the presence of phenolic compounds in the four types of guava leaf extract. From the gallic acid calibration curve, the equation of the line is obtained, namely y = 0.0107x + 0.0112 (R2 = 0.999). Based on Figure 1A, PGEE has the highest total phenolic content compared to other guava leaves. The three guava leaves from the genus *Syzygium* had total phenolic levels that are significantly different from those of PG leaves.

Phenolic compounds are a class of secondary metabolites found throughout the plant kingdom that have aromatic groups and basic to complex structures such as tannins and lignins.[19] Many phenolic compounds are present in plants as glycosides, so their polarity generally increases. The extraction of phenolic compounds used polar ethanol 70% solvent. The Folin-Ciocalteu technique is the most popular method for quantitatively determining phenolic compounds from plant materials and extracts. It is the simplest, most reproducible method for finding total phenolic content.[20] The phosphotungstic-phosphomolybdate complex is reduced by phenolics in an alkaline medium using the Folin-Ciocalteu (F-C) technique, yielding a blue solution.[21] The blue colour formed occurs according to the total phenol content that reacts, and the intensity of the colour is calculated at a wavelength of 765.1 nm. Gallic acid is used to compare in this measurement because it is a pure and stable substance.[22] The highest phenolic compounds were in PGEE and the smallest in SMEE. The phenolic compounds contained in PG may also be of more types than other guavas, for example, guavanoic acid, guavenoic acid, guajavolida.[23]

***Total flavonoids content (TFC)***

The identification findings revealed an abundance of flavonoid compounds inside the four-leaf extracts. That quercetin calibration curve yielded the linear equation is $y = 0.0251x + 0.0002$ ($R2=0.9992$). Figure 1B shows that the three guava leaves of the genus *Syzygium* have flavonoid levels that are significantly different from those of PG leaves.

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 atoms 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-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 research measured the determination of flavonoid content in ethyl acetate extract using $AlCl_3$ using a spectrophotometer at 438.60 nm.[26] $AlCl_3$ solution forms a stable complex compound with a hydroxyl group at the position of $C_3$ and or $C_5$ with a ketone group. Complex compounds also occur when there is a hydroxyl group at the ortho position.[24] The complex that occurs causes a shift in wavelength towards the bathochromic.

***Total Tannins content (TTC)***

The identification results showed that the four types of guava leaf extract contained tannins. The catechin calibration curve's linear equation is $y = 0.002x + 0.0483$ ($R2 = 0.9997$). Based on Figure 1C, it appears that PGWE significantly has the highest total tannin content compared to other extracts.

The solvent used to determine the tannin content is water because the solubility of tannin is quite good in the water.[27] Tannins are a phenolic group that is widely distributed in nature. The extraction method using the decoction method is simple, fast, and inexpensive. Heating in the decoction method helps increase the solubility of tannins.[26] The results of identifying tannins using $FeCl_3$ solution in PGEE produced the most concentrated colour. In PG leaves, the tannin content was the highest compared to the other three guava species, while in SM, the least. Seeing the amount of tannin in PG leaves, the type of tannin may also be the most abundant. More than 20 types of tannins have been isolated, including 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 with catechins as a standard.[28] This high tannin level supports using *P. guajava* leaves as an antidiarrheal agent.

***Antioxidant Capacity***

Based on testing with phosphomolybdate, quercetin was used as a comparison (5, 8, 11, 13, 15 g/ml), resulting in a line equation y = 0.0292x + 0.1772 (R2 = 0.9997) after reacting with phosphomolybdate. The quercetin equivalence of each sample of guava leaf extract against phosphomolybdate can be seen in Table 1.

In Figure 2, PG leaf extracts showed better antioxidant capacity than other guava leaf extracts. SM leaf extracts showed the lowest antioxidant capacity compared to the two types of guava leaves from the genus *Syzygium*. Figure 2 shows the $EC_{50}$ value of *P. guajava* water extract (PGWE), which has the highest AO among the other three types of guavas, according to the highest tannin content in the extract. The $EC_{50}$ value of *Syzygium malaccense* (SMWE) is the largest, according to the lowest tannin content 34.2303 mg CE/g) in the aqueous extract. *Syzygium aqueum* extract (SAWE) and *Syzygium samarangense* (SSWE) had no significant $EC_{50}$ values, according to the tannin content in both guavas. *Psidium guajava* leaf ethyl acetate extract (PGEAE) 35,193 ppm and *Syzygium aqueum* extract (SAEAE) 37,390 ppm had almost the same $EC_{50}$ value (Figure 2), meaning that this extract had the most potent antioxidant. One of the reasons for this activity was the flavonoid content in the extract, which was 1.843 mgQE/g extract (Figure 1B). The antioxidant strength of *S. aqueum* leaves (SAEAE), and *S. samarangense* leaves (SSEAE) ethyl acetate extracts were not statistically different ($P < 0.05$). The levels of flavonoid compounds in the ethyl acetate extract of *Syzygium malaccense* (SMEAE) 1,049 mg QE/g sample are the most minor compared to other extracts, indicating the antioxidant activity is also the smallest with an enormous $EC_{50}$ value.

The antioxidant strength of 4 types of guava was determined using the phosphomolybdate test. In this test, molybdenum (VI) decreases to molybdenum (V) in the presence of a lowering substance (antioxidant), resulting in the formation 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 known that many natural products have this reducing activity, including phenols and flavonoids.[31,32]

*Syzygium malaccense* ethanol 70% extract (SMEE) has the highest $EC_{50}$ value, which means that the antioxidant extract is in the weak category. The $EC_{50}$ value of the other three guava extracts (PGEE, SAEE, and SSEE) is relatively small, meaning that the strength of the antioxidant is quite strong. The phenolic group concentration in the ethanol 70% extract of PGEE was 79,312 mg GAE/gr extract (Figure 1A), the largest among the other three guavas. Antioxidant research using DPPH stated that ethanol 70% extract of *P. guajava* (PGEE), *S. aqueum* extract (SAEE) and *S. samarangense* (SSEE) were included in the group of solid antioxidants, each with an $IC_{50}$ value of 35.57; 38.69 and 59.16 L/mL respectively.[8,33] Based on the $IC_{50}$ value obtained from the DPPH method (138.33 g/mL), the ethanol extract of the *S. malaccense* leaf (SMEE) is included in the category of weak antioxidant.[34] This study used a comparison of quercetin with an $EC_{50}$ of 6.211 ± 0.649 g/ml, while the smallest $EC_{50}$ value, 13,142 ± 1.087 g/ml, was found in the aqueous extract of *P. guajava*.

***Correlation of $EC_{50}$ values of antioxidant activities with TPC, TFC, and TTC.***

TPC, TFC, and TTC produced from the four samples showed that PG extract had the highest compound content among other guava extract samples. This has a positive relationship with the results of antioxidant (Figure 2) and quercetin equivalence (Table 1), which means that the higher the phenolic/flavonoid/tannin levels, the tendency for the $EC_{50}$ antioxidant to be more potent and the quercetin equivalence to be higher. The levels of the compounds may affect antioxidant activity,[20] antioxidant activity increases with the rises polarity of the extraction solvent.[21] PGWE samples have an antioxidant value of 13.142 ppm, followed by PGEE 28.722 ppm and PGEAE 35.193 ppm.

***TLC analysis***

TLC is a method often used to rapidly screen organic compounds, including flavonoids as bioactive target compounds in the extracts of plants.[35] Tests using TLC were carried out to see the chromatogram pattern of the compound content of the four types of guava. The solvents used were n-hexane, ethyl acetate, and ethanol 70%, non-polar to polar, to see the number of compounds extracted in the three solvents. The experiment was conducted semi-quantitatively as the mobile phase used: 1). a solution of toluene-chloroform-ethyl acetate (5:4:1) to identify n-hexane and ethyl acetate extract; 2). ethyl acetate – chloroform - ethyl acetate - formic acid (1:39:10) for ethanol extract. Observations were carried out sequentially under conditions of 254 nm ultraviolet light, 366 nm, and using a $FeCl_3$ 5% solution (for detection of phenolic compounds), $AlCl_3$ 5% solution (for flavonoid detection) and $H_2SO_4$ 10% solution (for detection of organic compounds).[18]

In Figure 3, it is seen that in the ethyl acetate extract, more stains appeared than in the n-hexane extract. In the ethanol 70% extract, at the starting point or spot, there are still compounds that still need to be or cannot be separated from the mobile phase used. In the ethyl acetate extract, it was seen that there were similarities in the chemical content of the three types of *Syzygium*; with *P. guajava*, there were only quantitative differences. The difference is seen in *P. guajava*; there are special stains compared to other *Syzygium* species.

Figure 3. shows that the n-hexane and ethyl acetate extracts of *P. guajava* showed yellow fluorescence compounds with similar Rf, possibly flavonoids. The ethyl acetate extracts of all *Syzygium* and *P. guajava* contained purple fluorescence compounds. At the same time, in ethanol 70%, in PG, SM and SS, there were blue fluorescent stains with similar RF (altitude) values.

In Figure 4 [A] the ethyl acetate extract of the four test materials shows a blue-green stain after being sprayed with $FeCl_3$ solution. In the n-hexane extract of PG, there is a dark brown stain, this is probably a tannin compound, while in the ethyl acetate extract of PG, there is a purple-black stain, possibly a different tannin from that of PG (other Rf). In the ethanol extract, dark brown colors were found at the starting point of the spotting, meaning that the tannin compounds were not eluted by the mobile phase used.

Figure 4 [B] shows that the ethyl acetate extract of the four test materials contained a yellow fluorescence compound with a similar high Rf. These compounds may be flavonoids which are present in the four plants. In the hexane extract, yellow fluorescence compounds also appeared with a more significant Rf position, probably more non-polar flavonoid compounds than the first flavonoid.

Figure 4 [C] shows that the n-hexane and ethyl acetate extracts have much chemical content and are similar. The difference lies in the clarity/brightness of the colour. In the PG leaves in the hexane and ethyl acetate extracts, there is one compound whose colour and size are more prominent and more transparent than the leaves of the *Syzygium* type. This indicates that PG has more types of compounds than the three genera of *Syzygium*.

Blue indicates phenolic compounds, flavonoids appear yellow, and organic compounds appear by the arrival of various colors (light blue, blue, purple, purple, pink, and grey). This TLC test shows the similarity of the contents of the four test materials in the three types of extracts based on the position and colour of the stains that arise.

## Conclusion

*Psidium guajava* leaves have the highest content of tannins, flavonoids, phenols, antioxidant capacity, and other compounds than the leaves of *S. samarangense*, *S. Malaccense,* and *S. aqueum*.

**Acknowledgments**

The researchers are grateful to the Research and Development Institute of the University of Muhammadiyah Prof. Dr. Hamka, for allowing us to complete the study under contract number 383/F. 03.07/2022 Batch 2 for the year 2021.

**Conflict of interest**

The authors state that they have no competing interests.

**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. 2016;15:109–11.
2. Kafle A, Mohapatra SS, Reddy I, Chapagain M. A review on medicinal properties of Psidium guajava. J Med Plants Stud. 2018;6(4):44–7.
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):1256–81.
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 [Internet]. 2022;6(4):552–7. Available from: http://www.doi.org/10.26538/tjnpr/v1i4.5
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):707–12.
10. Choironi N, 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;102:302–8.
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):178–82.

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, Farahana L. Antioxidant activity Assay of Ethanolic Extract of Centella asiatica (L.) Urb Herb using Phosphomolybdate method. Pharmaciana [Internet]. 2014;4(1):23–30. Available from: http://dx.doi.org/10.12928/pharmaciana.v4i1.394
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. p. 26.
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):1311–6.
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).
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):2328–75.
27. Trease, Evans W. Pharmacognosy 16th Edition. In: 16th ed. Toronto: Saunders Elsevier; 2009. p. 100, 246, 269–70.
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. Available from: http://dx.doi.org/10.1016/j.phrp.2013.09.003
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):63–8.
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):1671–5.
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).

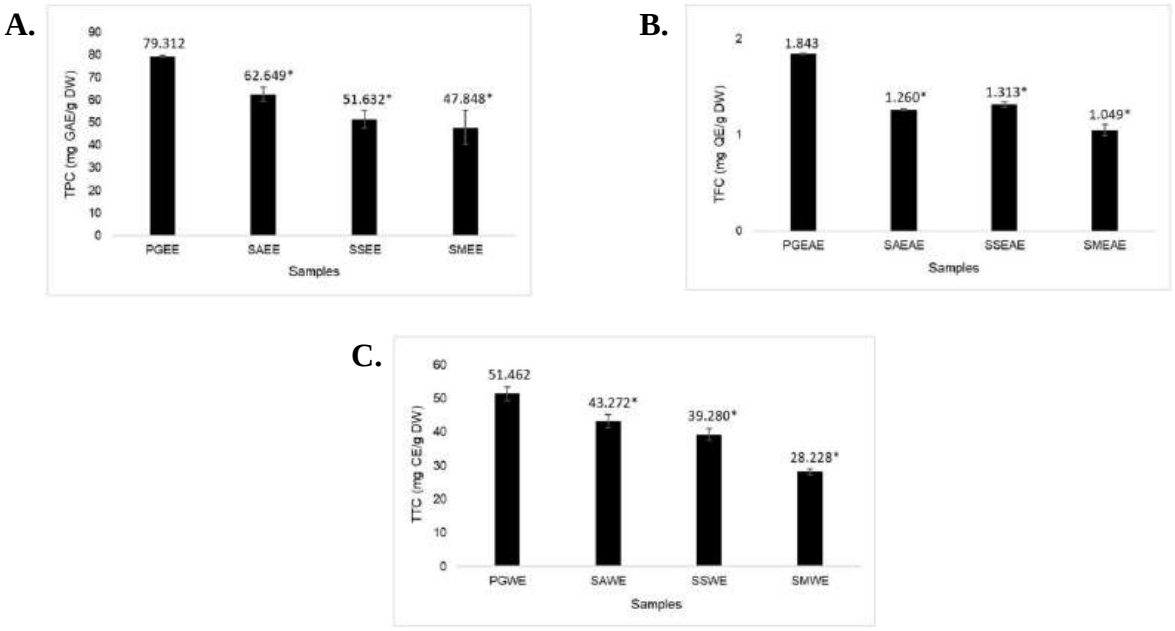



**Figure 1: [A]** Total phenolic content of guava leaf extracts. **[B]** Total flavonoid content of guava leaf extracts. **[C]** Total tannin content of guava leaf extracts. The sign (*) indicates a significant difference to PGEE, PGEAE, PGWE.

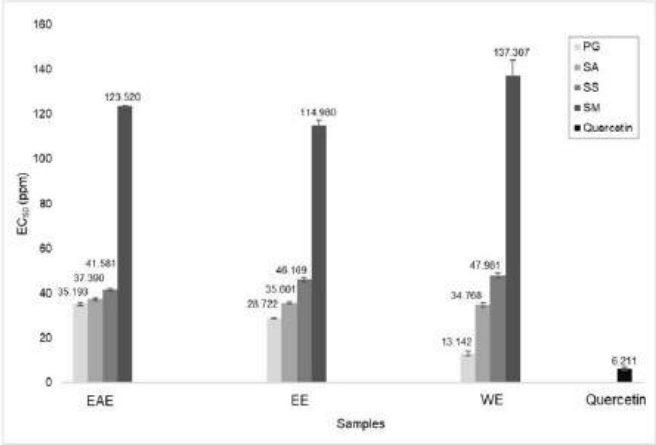



**Figure 2:** The $EC_{50}$ value of each guava leaf extract against phosphomolybdate.

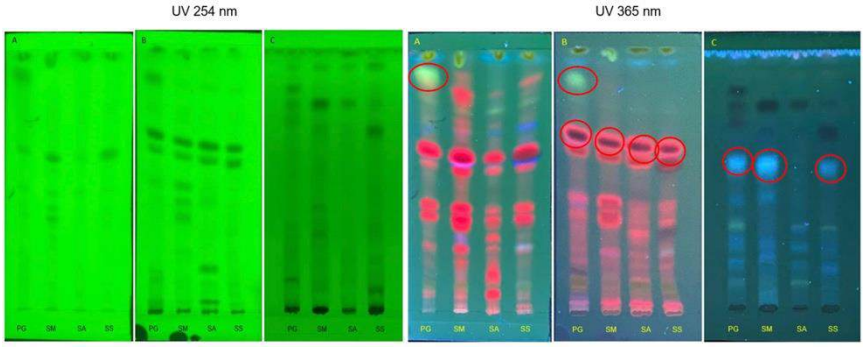



**Figure 3**: Chromatogram of n-hexane (A), ethyl acetate (B), and ethanol (C) extracts of four types of guava leaves under UV light at 254 and 365 nm.

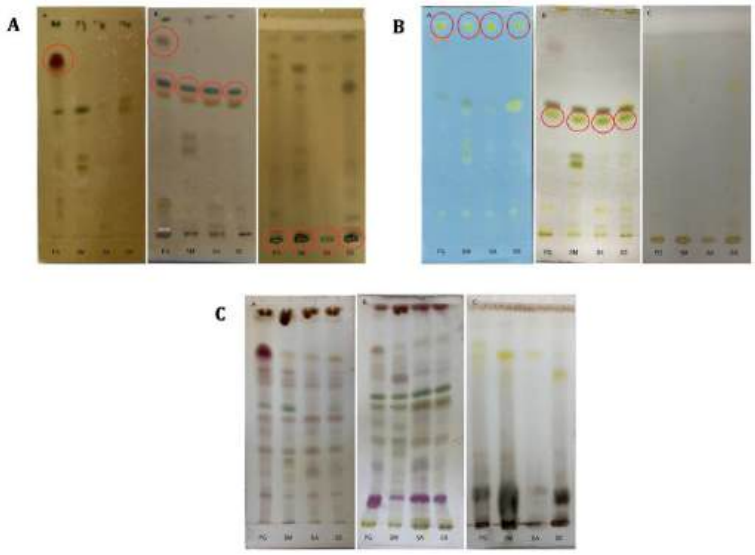


**Figure 4: [A]** Phenolic identification chromatogram of n-hexane (A), ethyl acetate (B), and ethanol (C) extracts of four types of guava leaves after spraying with 5% $FeCl_3$.
**[B]** Chromatogram identification of flavonoids from extracts of n-hexane (A), ethyl acetate (B), and ethanol (C) of four types of guava leaves after being sprayed with 5% $AlCl_3$.
**[C]** Chromatogram identification of organic compound content of extracts of n-hexane (A), ethyl acetate (B), and ethanol (C) of four types of guava leaves after spraying with 10% $H_2SO_4$.

**Table 1.** Equivalence of quercetin of each guava leaf extract against phosphomolybdate

| **Samples** | **Quercetin equivalence (mg QE/g)** | **Samples** | **Quercetin equivalence (mg QE/g)** | **Samples** | **Quercetin equivalence (mg QE/g)** |
|---|---|---|---|---|---|
| SAEAE | 134.103 ± 0.559 | SAEE | 132.043 ± 1.53 | SAWE | 137.184 ± 2.678 |
| SMEAE | 97.256 ± 0.443 | SMEE | 101.907 ± 5.95 | SMWE | 87.893 ± 8.975 |
| SSEAE | 129.079 ± 1.711 | SSEE | 127.437 ± 2.06 | SSWE | 133.874 ± 3.156 |
| PGEAE | 168.880 ± 1.647 | PGEE | 150.990 ± 0.88 | PGWE | 168.748 ± 3.312 |

# 2. Bukti artikel diterima
# (4 Februari 2023)

Uhamka
UNIVERSITAS MUHAMMADIYAH PROF. DR. HAMKA

**Ni Putu Ermi Hikmawanti <ermy0907@uhamka.ac.id>**

# (TJNPR) Editor Decision

3 messages

**Editor-in-Chief Tjnpr** <editor.tjnpr@gmail.com> Sat, Feb 4, 2023 at 11:44 PM
To: Ni Putu Ermi Hikmawanti <ermy0907@uhamka.ac.id>

Dear Dr. Hikmawanti,

The manuscript submitted to the Tropical Journal of Natural Product Research www.tjnpr.org https://www.scopus.com/sourceid/21100933230 has been carefully reviewed by competent experts.

I am pleased to inform you that the manuscript has been accepted for publication in the Tropical Journal of Natural Product Research.

Find attached the details of the decision.

Please send your response urgently to the Editor-in-Chief, to enable us to process your manuscript for the next issue Vol 7 issue 2, 2023.
Kindly acknowledge the receipt of the mail.

**Title:** Exploring polyphenol and antioxidant capacity in leaf extracts of selected Indonesian Syzygium species
**Authors:** Agustin Yumita, Ni P.E. Hikmawanti*, Endang Hanani, Cindi W. Saputri, Putri H. Hanana, Jeanne N.D. Ero, Mayang Marcelena, Tazqiyah Baytisani, Febby A. Sofiana, Amanda F. Shania, Erlina S.A. Saputri, Firda P.N. Islami

TJNPR Editorial Decision: accepts with moderate revisions

Best regards

Abiodun

---


## Professor Abiodun Falodun, PhD

Editor-in-Chief:
Tropical Journal of Natural Product Research (TJNPR)
Head, Natural Product Research Group, University of Benin
Email:editor.tjnpr@uniben.edu; editor.tjnpr@gmail.com
www.tjnpr.org **SCOPUS, SCImago SJR Q4 0.13**
https://www.scopus.com/sources.uri

Professor of Pharmaceutical Chemistry
Fellow, Fulbright (USA)
Deputy Vice-Chancellor (Academic) 2014-2016
Faculty of Pharmacy
University of Benin
Phone: +234-807-318-4488;
email: faloabi@uniben.edu; abiodun.falodun@fulbrightmail.org
Google Scholar Citations
SCOPUS https://www.scopus.com/authid/detail.uri?

https://orcid.org/0000-0003-2929-3305authorId=12794326500#top

# Tropical Journal of Natural Product Research

**Official Journal of the Natural Product Research Group**
**Faculty of Pharmacy, University of Benin 300001, Benin City, Nigeria**
Phone: +2348073184488, Email: editor.tjnpr@gmail.com; editor.tjnpr@uniben.edu; Website: www.tjnpr.org

**ISSN**: 2616-0684 (Print); 2616-0692 (Online), **DOI**: 10.26538/tjnpr, ISI IF: 0.562 (2017)
**Abstracted/Indexed**: Index Copernicus **ICV (2017): 59.83**, Open-J-Gate, EMBASE, EBSCO Host, WorldCat, AJOL, CrossRef (USA), JIF, NCBI (PubMed), CAS

SCOPUS indexed, SCImago SjR 0.13


Ref. No. 5820901802402 DATE: 4th February 2023


Department of Pharmaceutical Biology, Faculty of Pharmacy and Sciences, Universitas Muhammadiyah Prof DR. HAMKA, Jakarta 13460. Indonesia.


Dear Dr Hikmawanti,

**Provisional Acceptance letter for Article Manuscript Number TJNPR JAN158ARN**

**Title:** Exploring polyphenol and antioxidant capacity in leaf extracts of selected Indonesian Syzygium species
**Authors:** Agustin Yumita, Ni P.E. Hikmawanti*, Endang Hanani, Cindi W. Saputri, Putri H. Hanana, Jeanne N.D. Ero, Mayang Marcelena, Tazqiyah Baytisani, Febby A. Sofiana, Amanda F. Shania, Erlina S.A. Saputri, Firda P.N. Islami

I am pleased to inform you that your manuscript sent to the Tropical Journal of Natural Product Research has been reviewed and recommended for publication as a research article.
However, before the issues raised by the Reviewers are forwarded, to enable you revise your manuscript accordingly, please pay a publication charge of **$ USD270**. The actual publication of the paper will be in the upcoming issue (**Vol 7 issue 2, 2023**).

Please, the manuscript number (TJNPR ----) should be included in the bank transfer.

Congratulations.

The money should be remitted in favour of:

| | |
|---|---|
| **Name of account**: | **Abiodun FALODUN** |
| **Bank Name**: | Access Bank Plc |
| **Account Number**: | 1456136521. |
| **Sort Code:** | 044040699 |
| **Swift code**: | ABNGNGLA |
| **Address of Bank:** | Ransome Kuti Road,University of Benin, Benin City, Edo State, Nigeria |

Sincerely,

**Professor Abiodun Falodun**
**Editor-in-Chief**

# 3. Bukti hasil review dan file komentar reviewer

## (11 Maret 2023)

Uhamka
UNIVERSITAS MUHAMMADIYAH PROF. DR. HAMKA

Ni Putu Ermi Hikmawanti <ermy0907@uhamka.ac.id>

# Editorial and Reviewer comments

6 messages

**Editor-in-Chief Tjnpr** <editor.tjnpr@gmail.com> Sat, Mar 11, 2023 at 7:38 PM
To: Ni Putu Ermi Hikmawanti <ermy0907@uhamka.ac.id>

Please see the editorial comments (below) and attached copies of the reviewer comments for manuscript title **" Exploring polyphenol and antioxidant capacity in leaf extracts of selected Indonesian *Syzygium* species."**

**Editorial comments to authors**

**Title: Names (First and Last name in full, middle name as initials) and affiliations of authors should be written correctly**

**Abstract:** begin with a brief background

Include the voucher number for the plant materials.

Materials and Methods: Include section for statistical analysis.

Move all Tables and Figures under the reference section.

**References:** Cite relevant and related references from the published articles of TJNPR www.tjnpr.org

Adhere strictly to the Journal's style for listing references. List names of all authors in place of *et al*;
Falodun A, Siraj R, Choudhary MI. GC-MS Insecticidal Leaf essential oil of *P. staudtii* Hutch and Dalz (Icacinaceae). Trop J. Pharm Res. 2009; 82:139-143.

Okolie NP, Falodun A, Oluseyi D. Evaluation of the antioxidant activity of root extract of pepper fruit (*Dennetia tripetala*), and its potential for the inhibition of Lipid peroxidation. Afr J. Trad Compl and Altern Med. 2014; 11(3):221-227. Doi: 10.4314/ajtcam. v11i3.31

**A declaration of the liability** of the authors for claims relating to the content of this article should also be included when submitting the revised manuscript. This should be stated as follows;
**Authors' Declaration**
The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

**All comments/corrections made by reviewers should be completely addressed, point by point, and make appropriate changes in the manuscript, or provide a suitable rebuttal to any specific request for change that has not been made.**
**All corrections/changes** made in the manuscript should be **highlighted in yellow** when submitting the manuscript in the revised form on or before 14th March 2023

The authors should carefully revise and correct the manuscript taking into consideration the comments of all the reviewers. 50% of the references cited should be between 2016-2020. The revised and corrected manuscript should be subjected to plagiarism checker (17% allowed in TJNPR) and English language editing. Evidence of the checks should be attached when submitting the revised/corrected manuscript.
During submission of the revised manuscript include another file labelled "**Responses to reviewers' comments**" (a matrix) clearly showing your responses to each of the issues raised by the reviewers; mention the section, page and paragraph/lines where and how the changes/corrections have been made.

Strictly adhere to the author guidelines. Make sure that all the facts and information provided in the manuscript are correct. Check grammar, spelling, spacing, other information and facts including scientific names, formulae, symbols, equations, etc.

Ensure that all the references are correctly cited in the text and list. Verify all the references from their original sources. Confirm correctness of the citation info such as authors' names (surnames, initials, spelling, arrangements, etc), year, title, journal, volume, pages, punctuation, etc. The numbers and units must be presented according to the journal style. Use clearly distinguishable patterns for the illustrations/figures (e.g., graphs and charts) such that they should be legible even for black and white printing or when reduced in size.

Proofread the whole document after effecting all the corrections. The revised version should be approved by all the co-authors before submitting it.

A manuscript not complying with these and other instructions will not be processed and may be rejected.

Please find the attached review comments for your revisions.

Best regards

Abiodun

---


## Professor Abiodun Falodun, PhD

Editor-in-Chief:
Tropical Journal of Natural Product Research (TJNPR)
Head, Natural Product Research Group, University of Benin
Email:editor.tjnpr@uniben.edu; editor.tjnpr@gmail.com
www.tjnpr.org **SCOPUS, SCImago SJR Q4 0.13**
https://www.scopus.com/sources.uri

Professor of Pharmaceutical Chemistry
Fellow, Fulbright (USA)
Deputy Vice-Chancellor (Academic) 2014-2016
Faculty of Pharmacy
University of Benin
Phone: +234-807-318-4488;
email: faloabi@uniben.edu; abiodun.falodun@fulbrightmail.org
Google Scholar Citations
SCOPUS https://www.scopus.com/authid/detail.uri?

https://orcid.org/0000-0003-2929-3305authorId=12794326500#top


University of Benin TJNPR SCOPUS Q4
www.uniben.edu www.tjnpr.org

**2 attachments**

**ca1-TJNPR-2023-M057 Editorial comments.docx**
15K

**ca2-TJNPR-2023-M057 Reviewed 1.docx**
526K

**Editorial comments to authors**

**Title: Names (First and Last name in full, middle name as initials) and affiliations of authors should be written correctly**

**Abstract:** begin with a brief background

Include the voucher number for the plant materials.

Materials and Methods: Include section for statistical analysis.

Move all Tables and Figures under the reference section.

**References:** Cite relevant and related references from the published articles of TJNPR www.tjnpr.org

Adhere strictly to the Journal's style for listing references/ list names of all authors in place of *et al*;
Falodun A, Siraj R, Choudhary MI. GC-MS Insecticidal Leaf essential oil of *P. staudtii* Hutch and Dalz (Icacinaceae). Trop J. Pharm Res. 2009; 82:139-143.

Okolie NP, Falodun A, Oluseyi D. Evaluation of the antioxidant activity of root extract of pepper fruit (*Dennetia tripetala*), and its potential for the inhibition of Lipid peroxidation. Afr J. Trad Compl and Altern Med. 2014; 11(3):221-227. Doi: 10.4314/ajtcam. v11i3.31

**A declaration of the liability** of the authors for claims relating to the content of this article should also be included when submitting the revised manuscript. This should be stated as follows;
**Authors' Declaration**
The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

**All comments/corrections made by reviewers should be completely addressed, point by point, and make appropriate changes in the manuscript, or provide a suitable rebuttal to any specific request for change that has not been made.**
**All corrections/changes** made in the manuscript should be **highlighted in yellow** when submitting the manuscript in the revised form on or before 14th March 2023

# Exploring polyphenol and antioxidant capacity in leaf extracts of selected Indonesian *Syzygium* species

## ABSTRACT

The study aimed at exploring the chemical content and antioxidant activity of leaves of three types of guavas, *Syzygium aqueum* (Burm.f.) Alston, *Syzygium malaccense* (L.) Merr. and Perry., *Syzygium samarangense* (Blume) Merr. & L.M. Perry, which was compared to *Psidium guajava* L. leaves. The chemical similarity between the four types of guava leaves was determined based on the parameters of total tannins (vanillin-sulfuric acid reagent), flavonoids ($AlCl_3$ reagent), and phenol (Folin-Ciocalteu reagent). Antioxidant capacity was measured by the phosphomolybdate method with quercetin as a reference. For TLC anaylsis with $FeCl_3$ 5% for phenolics, $AlCl_3$ 5% for flavonoids, and $H_2SO_4$ for organic components. The results showed that the highest phenol, flavonoid and tannins content was found in the leaves of *P. guajava*. The chromatogram showed the similarity of the organic components of four types of guava in the three extracts based on the position and colour of the spots that appeared. The content of flavonoids, phenolics, and tannins in *P. guajava* leaves was the highest due to differences in the type and amount of chemical content between the four species. The antioxidant activity of *P. guajava* leaves is closely related to their high phenolic content.



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


**Commented [AF1]:** What is novel in this research?

## 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 type of plant belonging to the Myrtaceae family.[1] *Psidium guajava* (PG) leaves are widely used to treat diarrhoea and have several pharmacological activities, such as antidiarrheal, antimicrobial, anti-inflammatory, antimalarial, and antioxidant.[2]

Drinking herbal medicine (one of which uses PG leaves) to treat health problems is still a tradition 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 in the form of Standardized Herbal Medicines (Obat Herbal Terstandar, OHT) are registered with the Indonesian FDA (The National Agency of Drug and Food Control, NA-DFC) made from PG.[4] However, PG plants are considered rare. This causes the community or the herbal medicine industry to need help in obtaining raw materials for the leaves of this plant. One of the efforts made to meet the needs of these herbs can be realized by exploring the chemical content of the plant, which are still relatives of the same tribe as PG.

Some plants belonging to the Myrtaceae tribe 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 also easy to obtain and widely grown in Indonesia. These plants have leaves that are shadier than PG. In addition, the three types of guava leaves also have 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 is also scientifically proven as an antioxidant, antimicrobial, antifungal, antiviral, analgesic, anti-inflammatory, antihypertensive, antihyperglycemic, sedative, and spasmolytic.[5,6] Several pharmacological studies have reported that SS, SM, and SA provide their properties as antioxidants and antimicrobials.[2,7–11.]

The content of polyphenols found in species of the genus *Syzygium*, including phenolics, flavonoids and tannins, is closely related to their antioxidant activity. The profile of these metabolites in the extract can be studied simply using thin-layer chromatography (TLC) techniques. This antioxidant compound recovery technique is undoubtedly closely related to using extraction solvents. This study aimed to explore the chemical content of polyphenols from the aspect of total phenolic, flavonoid and tannin levels in the leaves of the three selected *Syzygium* species, along with testing their antioxidant activity compared to PG leaves.

## Materials and Methods

### *1. Chemicals and Solvents*

Chemicals and solvents such as n-hexane, dichloromethane, ethyl acetate, ethanol, methanol, chloroform, toluene, formic acid, sulfuric acid, hydrochloric acid, acetic acid, sodium carbonate, sodium acetate, Folin-Ciocalteu, aluminium (III) chloride, vanillin, phosphomolybdate, and silica gel plate are obtained from Merck (Darmstadt, Germany). Meanwhile, quercetin is from Sigma Aldrich Co. (St. Louis, USA), while gallic acid and catechins are from Mark Herb (Bandung, Indonesia).

### *2. Plant materials*

The four types of guava leaf were collected from the Duren Sawit sub-district, East Jakarta, in February 2022 and determined at the BRIN "Biosystematics and Evolutionary Research Center", Bogor, West Java, Indonesia. Specimens of each type of guava are stored in the Pharmacognosy Laboratory of the Faculty of Pharmacy and Science, UHAMKA. After

**Commented [AF2]:** should

**Commented [AF3]:** why?

**Commented [AF4]:** State the herbarium number of the plant samples

being washed with running water and cleaned of dirt, leaves were dried from water droplets and weighed. Leaves were dried in the air for 6 - 7 days, temperature 30 ± 1 ºC. After drying, the leaves were weighed again to be further powdered and stored in tightly closed, airtight, and dry bottles until the following experiment.

***3. Extracts Preparation***

The guava leaf extraction process is carried out with different methods and solvents, as follows:

**3.1. Ethyl Acetate Extract (EAE):** ± 8 g dry leaf powder (equivalent to 25 g fresh leaves) was extracted with ethyl acetate with a solvent-to-material ratio of 1: 20 (w/v). Extraction was carried out by reflux method at 77 ºC for 30 minutes and then filtered. The extraction was repeated using the same technique until the flavonoid screening showed negative results. Each filtrate was concentrated using a vacuum rotary evaporator N-1200 BS series (EYELA, Shanghai, China) to obtain a total volume of 250 mL, after this, referred to as SEAAE, SMEAE, SSEAE, and PGEAE. Each extract was made triple.

**3.2. Ethanol 70% Extract (EE):** ± 8 g dry leaf powder (equivalent to 25 g fresh leaves) was extracted with ethanol 70% with a material-solvent ratio of 1:10 (w/v). Extraction was carried out by reflux method at 70 ºC for 30 minutes, then filtered. The extraction was repeated with the same method until the phenolic screening was negative. Each filtrate was concentrated using a vacuum rotary evaporator N-1200 BS series (EYELA, Shanghai, China) to obtain a total volume of 250 mL, starting now referred to as SAEE, SMEE, SSEE, and PGEE. Each extract was made triple.

**3.3. Water Extract (WE):** ± 3 g dry leaf powder (equivalent to 5 g fresh leaves) was extracted with hot water (90 ± 2 ºC) with a solvent-material ratio of 1: 20 (w/v) for 30 minutes, then filtered. The extraction was repeated using the same method until the tannin screening was negative. Each filtrate was concentrated using a water bath at a temperature of 65 ºC to obtain a thick extract: SAWE, SMWE, SSWE, and PGWE. The extract was made triple.

***4. Total phenolic content (TPC) Assay***

The experiment was started by identifying the phenolic compounds in EE from the four types of guava leaves (SAEE, SMEE, SSEE and PGEE), namely the appearance of a blue-green colour with the addition of $FeCl_3$ solution. Total phenolic compound content was determined following the method of Yang et al., 2007[12] using gallic acid as a comparison (20, 33, 46, 59, and 72 ppm). Test solution 300 µL was added to Folin-Ciocalteu reagent 1.5 mL, and shaken until homogeneous. After 3 minutes, 1.2 mL of sodium carbonate 7.5% was added to the mixture. The mixture was incubated for 110 minutes at room temperature. 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 carried out in triples.

***5. Total flavonoid content (TFC) Assay***

The flavonoid compounds in the EAE of the four types of guavas (SAEAE, SMEAE, SSEAE and PGEAE) leaves were identified by adding Mg powder and concentrated hydrochloric acid. The formation of red and pink colors indicates the presence of flavonoid compounds. Furthermore, the levels of total flavonoid compounds in the four extracts were carried out using the colourimetric method of Chang et al., 2002[13] with quercetin as a comparison (10, 15, 20, 25 and 30 ppm). A sample of 1 ml was added with 1.5 ml of methanol, 0.1 ml of $AlCl_3$ 10%, and 0.1 ml of sodium acetate 1 M and made up to 10 ml with methanol. The mixture was incubated for 50 minutes at room temperature. The absorbance was measured

**Commented [AF5]:** Delete
Replace with container

What temperature were they stored?

**Commented [AF6]:** How did you dry the 250ml extract?

**Commented [AF7]:** Why hot water?

**Commented [AF8]:** What informed the choice of solvent for the extraction?

at 438.60 nm using a UV-Vis UV-1601 Series spectrophotometer (Shimadzu, Kyoto, Japan). Total flavonoid levels were expressed in mg QE/g DW. The test was carried out in triples.

***6. Total tannins content (TTC) Assay***

First, the identification of tannin compounds in WE of the four types of guava leaves (SAWE, SMWE, SSWE, and PGWE) was carried out with the addition of a 10% gelatin solution. The appearance of a white precipitate indicates the presence of tannins. Determination of total tannin levels in the extracts of the four types of guava leaves was done using the colourimetric method proposed by Medini et al., (2014)[14] with catechins as a comparison (85, 148, 211, 274, and 337 ppm). Each 1 mL of the test sample was added with 2.5 mL of vanillin 4% in methanol and 2.5 mL of $H_2SO_4$ 25%. The mixture was incubated at room temperature (± 25 - 26 ºC) for 36 minutes. The absorbance was measured at 499 nm using a UV-Vis UV-1601 Series spectrophotometer (Shimadzu, Kyoto, Japan). Tannin levels are expressed in mg CE/g DW. The test was carried out in triples.

***7. Antioxidant Activity***

The antioxidant activity of EAE, EE, and WE of the four types of guava leaves was tested using the modified phosphomolybdate method following the procedure proposed by Salamah & Farahana, (2014).[15] The comparison used was quercetin. The extract and comparison samples were reacted with phosphomolybdate reagent and made up to 5 ml with distilled water. The mixture was incubated at 95 °C for 60 minutes. The absorbance was measured at 695 nm using a UV-1601 Series UV-Vis Spectrophotometer (Shimadzu, Kyoto, Japan). The test was carried out in triples.

***8. TLC analysis***

The chemical content of phenolics and flavonoids was analysed by the TLC method on the extracts prepared separately. Each guava leaf (8 g of dried leaf powder equivalent to 25 g of fresh leaves) was extracted using 80 ml of n-hexane or ethyl acetate or ethanol 70% with an ultrasonic bath (Branson) (40 kHz) for 15 minutes at room temperature. Each filtrate was concentrated with a vacuum rotary evaporator.

Chemical content was identified on silica gel plate F254 (MERCK, Germany) as the stationary phase.[16] Meanwhile, the mobile phase used was: toluene-chloroform-ethyl acetate (5:4:1) with a little formic acid added (for identification of the n-hexane and ethyl acetate extracts) and chloroform-ethyl acetate-formic acid (0.1:3.9:1) (for identification of ethanol extract). 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 also used for spot detection.[18]

## Results and Discussion

***Total phenolic content (TPC)***

The identification results indicated the presence of phenolic compounds in the four types of guava leaf extract. From the gallic acid calibration curve, the equation of the line is obtained, namely $y = 0.0107x + 0.0112$ ($R2 = 0.999$). Based on Figure 1A, PGEE has the highest total phenolic content compared to other guava leaves. The three guava leaves from the genus *Syzygium* had total phenolic levels that are significantly different from those of PG leaves.

Phenolic compounds are a group of secondary metabolites widely distributed in the plant kingdom, with aromatic groups and simple to complex structures such as tannins and lignins.[19] In plants, many phenolic compounds are present in the form of glycosides, so their polarity generally increases. The extraction of phenolic compounds used polar ethanol 70% solvent. Of

the many methods for determining total phenolic content, The Folin-ciocalteu method is the first choice because of its simple, reproducible, and widely used method for quantitatively determining phenolic compounds from both plant materials and extracts.[20] The Folin-Ciocalteu (F-C) method reduces the phosphotungstic-phosphomolybdate complex by phenolics in an alkaline medium resulting in a blue solution.[21] The blue colour formed occurs according to the total phenol content that reacts, and the intensity of the colour is calculated at a wavelength of 765.1 nm. In this test, gallic acid is used as a comparison, which is a pure substance and is stable.[22] The highest phenolic compounds were in PGEE and the smallest in SMEE. The phenolic compounds contained in PG may also be of more types than other guavas, for example, guavanoic acid, guavenoic acid, guajavolida.[23]

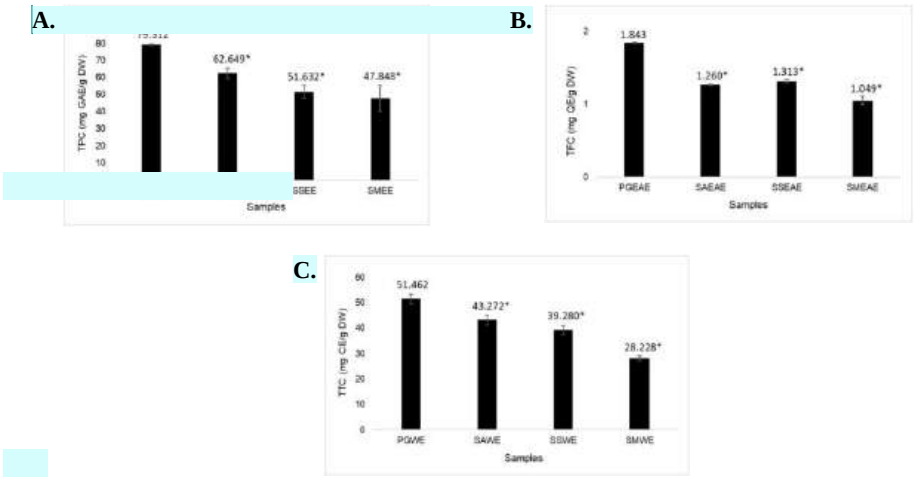



**Figure 1: [A]** Total phenolic content of guava leaf extracts. **[B]** Total flavonoid content of guava leaf extracts. **[C]** Total tannin content of guava leaf extracts. The sign (*) indicates a significant difference to PGEE, PGEAE, PGWE.

**Commented [AF9]:** Tables and figures should be moved to the last page after the list of references

***Total flavonoids content (TFC)***

**Commented [AF10]:** All subtitles should be removed

The identification results indicated the presence of flavonoid compounds in the four-leaf extracts. The linear equation obtained from the quercetin calibration curve is y = 0.0251x + 0.0002 (R2=0.9992). Figure 1B shows that the three guava leaves of the genus *Syzygium* have flavonoid levels that are significantly different from those of PG leaves.

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 atoms 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-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] In this study, the determination of flavonoid levels in ethyl acetate extract using $AlCl_3$ was measured using a spectrophotometer at 438.60 nm.[26] $AlCl_3$ solution forms a stable complex compound with a hydroxyl group at the position of $C_3$ and or $C_5$ with a ketone group. Complex compounds also occur when there is a hydroxyl group

at the ortho position.[24] The complex that occurs causes a shift in wavelength towards the bathochromic.

***Total Tannins content (TTC)***

The identification results showed that the four types of guava leaf extract contained tannins. The linear equation of the catechin calibration curve is y = 0.002x + 0.0483 (R2 = 0.9997). Based on Figure 1C, it appears that PGWE significantly has the highest total tannin content compared to other extracts.

The solvent used to determine the tannin content is water because the solubility of tannin is quite good in the water.[27] Tannins are a phenolic group that is widely distributed in nature. The extraction method using the decoction method is simple, fast, and inexpensive. Heating in the decoction method helps increase the solubility of tannins.[26] The results of identifying tannins using $FeCl_3$ solution in PGEE produced the most concentrated colour. In PG leaves, the tannin content was the highest compared to the other three guava species, while in SM, the least. Seeing the amount of tannin in PG leaves, the type of tannin may also be the most abundant. More than 20 types of tannins have been isolated, including 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 with catechins as a standard.[28] This high tannin level supports using *P. guajava* leaves as an antidiarrheal agent.

***Antioxidant Capacity***

Based on testing with phosphomolybdate, quercetin was used as a comparison (5, 8, 11, 13, 15 g/ml), resulting in a line equation y = 0.0292x + 0.1772 (R2 = 0.9997) after reacting with phosphomolybdate. The quercetin equivalence of each sample of guava leaf extract against phosphomolybdate can be seen in Table 1.

**Table 1.** Equivalence of quercetin of each guava leaf extract against phosphomolybdate

| Samples | Quercetin equivalence (mg QE/g) | Samples | Quercetin equivalence (mg QE/g) | Samples | Quercetin equivalence (mg QE/g) |
|---|---|---|---|---|---|
| SAEAE | 134.103 ± 0.559 | SAEE | 132.043 ± 1.53 | SAWE | 137.184 ± 2.678 |
| SMEAE | 97.256 ± 0.443 | SMEE | 101.907 ± 5.95 | SMWE | 87.893 ± 8.975 |
| SSEAE | 129.079 ± 1.711 | SSEE | 127.437 ± 2.06 | SSWE | 133.874 ± 3.156 |
| PGEAE | 168.880 ± 1.647 | PGEE | 150.990 ± 0.88 | PGWE | 168.748 ± 3.312 |

In Figure 2, PG leaf extracts showed better antioxidant capacity than other guava leaf extracts. SM leaf extracts showed the lowest antioxidant capacity compared to the two types of guava leaves from the genus *Syzygium*. Figure 2 shows the $EC_{50}$ value of *P. guajava* water extract (PGWE), which has the highest AO among the other three types of guavas, according to the highest tannin content in the extract. The $EC_{50}$ value of *Syzygium malaccense* (SMWE) is the largest, according to the lowest tannin content 34.2303 mg CE/g) in the aqueous extract. *Syzygium aqueum* extract (SAWE) and *Syzygium samarangense* (SSWE) had no significant $EC_{50}$ values, according to the tannin content in both guavas. *Psidium guajava* leaf ethyl acetate extract (PGEAE) 35,193 ppm and *Syzygium aqueum* extract (SAEAE) 37,390 ppm had almost the same $EC_{50}$ value (Figure 2), meaning that this extract had the most potent antioxidant. One of the reasons for this activity was the flavonoid content in the extract, which was 1.843 mgQE/g extract (Figure 1B). The antioxidant strength of the ethyl acetate extract of *Syzygium aqueum* leaves (SAEAE) and *Syzygium samarangense* (SSEAE) was not significantly different ($P < 0.05$). The levels of flavonoid compounds in the ethyl acetate extract of *Syzygium*

**Commented [AF11]:** Move to the last page after the list of references

**Commented [AF12]:** Use the journal table format visit www.tjnpr.org

*malaccense* (SMEAE) 1,049 mg QE/g sample are the most minor compared to other extracts, indicating the antioxidant activity is also the smallest with an enormous $EC_{50}$ value.

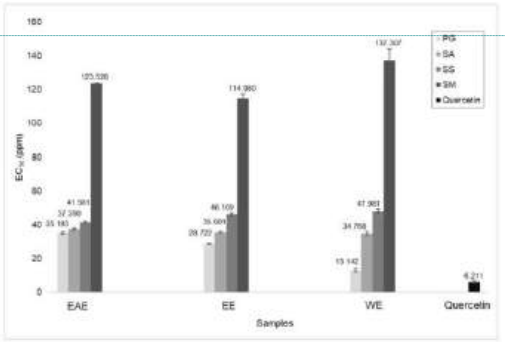



**Figure 2:** The $EC_{50}$ value of each guava leaf extract against phosphomolybdate.

The antioxidant strength of 4 types of guava was determined using the phosphomolybdate test. In this test it is based on molybdenum (VI) which is reduced to molybdenum V) in the presence of a reducing agent (antioxidant), thus forming a green phosphomolybdate (V) complex, which can be evaluated spectrophotometrically at 695 nm.[29,30] This test involves an electron transfer mechanism. Several studies have known that many natural products have this reducing activity, including phenols and flavonoids.[31]

*Syzygium malaccense* ethanol 70% extract (SMEE) has the highest $EC_{50}$ value, which means that the antioxidant extract is in the weak category. The $EC_{50}$ value of the other three guava extracts (PGEE, SAEE, and SSEE) is relatively small, meaning that the strength of the antioxidant is quite strong. The content of the phenolic group in the ethanol 70% extract of *P. guajava* leaves (PGEE) was 79,312 mg GAE/gr extract (Figure 1A), the largest among the other three guavas. Antioxidant research using DPPH stated that ethanol 70% extract of *P. guajava* (PGEE), *S. aqueum* extract (SAEE) and *S. samarangense* (SSEE) were included in the group of solid antioxidants, each with an $IC_{50}$ value of 35.57; 38.69 and 59.16 L/mL respectively.[7,32,33] Based on the $IC_{50}$ value obtained from the DPPH method (138.33 g/mL), the ethanol extract of the *S. malaccense* leaf (SMEE) is included in the category of weak antioxidant.[34] This study used a comparison of quercetin with an $EC_{50}$ of 6.211 ± 0.649 g/ml, while the smallest $EC_{50}$ value, 13,142 ± 1.087 g/ml, was found in the aqueous extract of *P. guajava*.

***Correlation of $EC_{50}$ values of antioxidant activities with TPC, TFC, and TTC.***

TPC, TFC, and TTC produced from the four samples showed that PG extract had the highest compound content among other guava extract samples. This has a positive relationship with the results of antioxidant (Figure 2) and quercetin equivalence (Table 1), which means that the higher the phenolic/flavonoid/tannin levels, the tendency for the $EC_{50}$ antioxidant to be more potent and the quercetin equivalence to be higher. The levels of the compounds may affect antioxidant activity,[20] antioxidant activity increases with increasing polarity of the extraction solvent.[21] PGWE samples have an antioxidant value of 13.142 ppm, followed by PGEE 28.722 ppm and PGEAE 35.193 ppm.

***TLC analysis***

**Commented [AF13]:** Move to the last page after the list of references

TLC is a method often used to rapidly screen organic compounds, one of which is flavonoids as pharmacological target compounds in plant extracts.[35] Tests using TLC were carried out to see the chromatogram pattern of the compound content of the four types of guava. The solvents used were n-hexane, ethyl acetate, and ethanol 70%, non-polar to polar, to see the number of compounds extracted in the three solvents. The experiment was conducted semi-quantitatively as the mobile phase used: 1). a mixture of toluene-chloroform-ethyl acetate (5:4:1) to identify n-hexane and ethyl acetate extract; 2). ethyl acetate – chloroform - ethyl acetate - formic acid (1:39:10) for ethanol extract. Observations were carried out sequentially under conditions of 254 nm ultraviolet light, 366 nm, and using a $FeCl_3$ 5% solution (for detection of phenolic compounds), $AlCl_3$ 5% solution (for flavonoid detection) and $H_2SO_4$ 10% solution (for detection of organic compounds).[18]

In Figure 3, it is seen that in the ethyl acetate extract, more stains appeared than in the n-hexane extract. In the ethanol 70% extract, it can be seen that at the starting point or spot, there are still compounds that still need to be or cannot be separated from the mobile phase used. In the ethyl acetate extract, it was seen that there were similarities in the chemical content of the three types of *Syzygium*; with *P. guajava*, there were only quantitative differences. The difference is seen in *P. guajava*; there are pretty special stains compared to other *Syzygium* species.

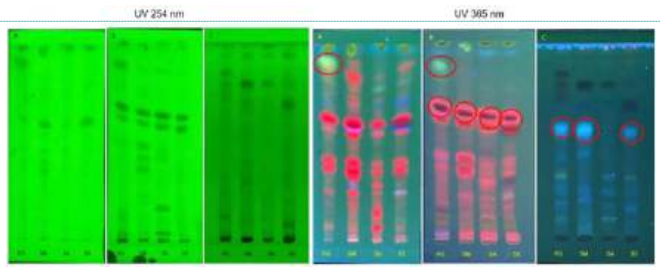
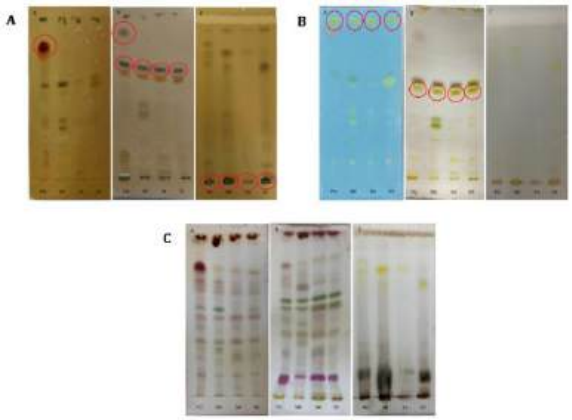



**Figure 3**: Chromatogram of n-hexane (A), ethyl acetate (B), and ethanol (C) extracts of four types of guava leaves under UV light at 254 and 365 nm.

Figure 3. shows that the n-hexane and ethyl acetate extracts of *P. guajava* showed yellow fluorescence compounds with similar Rf, possibly flavonoids. The ethyl acetate extracts of all *Syzygium* and *P. guajava* contained purple fluorescence compounds. At the same time, in ethanol 70%, in PG, SM and SS, there were blue fluorescent stains with similar RF (altitude) values.

In Figure 4 [A] the ethyl acetate extract of the four test materials shows a blue-green stain after being sprayed with $FeCl_3$ solution. In the n-hexane extract of PG, there is a dark brown stain, this is probably a tannin compound, while in the ethyl acetate extract of PG, there is a purple-black stain, possibly a different tannin from that of PG (other Rf). In the ethanol extract, dark brown colors were found at the starting point of the spotting, meaning that the tannin compounds were not eluted by the mobile phase used.




**Commented [AF14]:** Move to the last page after the list of references

**Figure 4: [A]** Phenolic identification chromatogram of n-hexane (A), ethyl acetate (B), and ethanol (C) extracts of four types of guava leaves after spraying with 5% $FeCl_3$.
**[B]** Chromatogram identification of flavonoids from extracts of n-hexane (A), ethyl acetate (B), and ethanol (C) of four types of guava leaves after being sprayed with 5% $AlCl_3$.
**[C]** Chromatogram identification of organic compound content of extracts of n-hexane (A), ethyl acetate (B), and ethanol (C) of four types of guava leaves after spraying with 10% $H_2SO_4$.

Figure 4 [B] shows that the ethyl acetate extract of the four test materials contained a yellow fluorescence compound with a similar high Rf. These compounds may be flavonoids which are present in the four plants. In the hexane extract, yellow fluorescence compounds also appeared with a more significant Rf position, probably more non-polar flavonoid compounds than the first flavonoid.

Figure 4 [C] shows that the n-hexane and ethyl acetate extracts have much chemical content and are similar. The difference lies in the clarity/brightness of the colour. In the PG leaves in the hexane and ethyl acetate extracts, there is one compound whose colour and size are more prominent and more transparent than the leaves of the *Syzygium* type. This indicates that PG has more types of compounds than the three genera of *Syzygium*.

Blue indicates phenolic compounds, flavonoids appear yellow, and organic compounds appear by the arrival of various colors (light blue, blue, purple, purple, pink, and grey). This TLC test shows the similarity of the contents of the four test materials in the three types of extracts based on the position and colour of the stains that arise.

## Conclusion

*Psidium guajava* leaves have the highest content of tannins, flavonoids, phenols, antioxidant capacity, and other compounds than the leaves of *S. samarangense, S. Malaccense,* and *S. aqueum*.


## Acknowledgments

The authors would like to thank the Research and Development Institute of the University of Muhammadiyah Prof. Dr. Hamka, who has allowed us to complete the research with contract number 383/F. 03.07/2022 Batch 2 the Year 2021.


## Conflict of interest

The authors declare no conflict of interest.

**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. 2016;15:109–11.
2. Guti'erez R, Mitchell S, Solis R. Psidium guajava: A review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol. 2008;117:1–27.
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, Aung EE, et al. Plant description, phytochemical constituents and bioactivities of Syzygium genus: A review. Open Chem. 2020;18(1):1256–81.
6. Ahmad B, Baider C, Bernardini B, Biffin E, Brambach F, Burslem D, et al. Syzygium (Myrtaceae): Monographing a Taxonomic Giant via 22 Coordinated Regional Revisions. Peer J Prepr. 2016;
7. Itam A, Mutia Siska Wati, Vina Agustin, Nursal Sabri, Rafika Aris Jumanah, Mai Efdi. 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.
8. Hariyati T, Jekti D, Andayani Y. Pengaruh Ekstrak Etanol Daun Jambu Air (Syzygium aqueum) terhadap Bakteri Isolat Klinis. J Penelit Pendidik IPA. 2015;1(2).
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):707–12.
10. Choironi N, 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, R Paulino, 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;102:302–8.
13. Chang C, MH Yang, Wen HM, Chern JC. Estimation of Total Flavonoid Cintent in Propolis by Two Complementary Colorimetric Methods. J Food Drug Anal. 2002;10(3):178–82.
14. Medini F, H F, 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, Farahana L. Antioxidant activity Assay of Ethanolic Extract of Centella asiatica (L.) Urb Herb using Phosphomolybdate method. Pharmaciana [Internet]. 2014;4(1):23–30. Available from: http://dx.doi.org/10.12928/pharmaciana.v4i1.394
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. Shah B, Seth AK. Textbook of Pharmacognosy and Phytochemistry. first edit. Chauhan SK, editor. Haryana: Elsevier; 2010. 587 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.
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):1311–6.
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).
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.
25. Farmakope Herbal Indonesia II. Farmakope Herbal Indonesia Edisi II. Jakarta: Kementerian Kesehatan RI, Direktorat Jenderal Bina Kefarmasian dan Alat Kesehatan; 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):2328–75.
27. Trease, Evans W. Pharmacognosy 15th Edition. In: 15th ed. Toronto: Elsevier; 2009.
28. Ajila CM, Brar SK, Verma M, Tyagi RD, Godbout S, Valéro JR. Extraction and Analysis of Polyphenols : Recent trends. Crit Rev Biotechnol. 2011;31(3):227–249.
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. Available from: http://dx.doi.org/10.1016/j.phrp.2013.09.003
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. Seo J, Lee S, Elam M, Johnson S, Kang J, Arjmandi B. Study to find the best extraction solvent for use with guava leaves (Psidium guajava L.) for high antioxidant efficacy. Food Sci Nutr. 2013;2((2)):174–80.
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):1671–5.
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).

**Commented [AF15]:** Journal titles should be abbreviated in line with the international standard

**Commented [AF16]:** All botanical names should be italicized

# 4. Bukti hasil revisi dan artikel yang direvisi
# (5 April 2023)

**Ni Putu Ermi Hikmawanti** <ermy0907@uhamka.ac.id> Wed, Apr 5, 2023 at 5:47 AM
To: Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

Dear Editor,

Sorry we just got back to you. Based on the results of comments from all reviewers, we appreciate everything well. We have followed the revisions we sent based on all these comments to make the manuscript perfect. Here's our response:

1. The writing of the author's name is correct, where the first and last names are not abbreviated, only in the middle name.
2. Abstract: We add a brief background according to the reviewer's comments.
3. Materials and Methods: "Plant materials" we have included the plant collection number.
4. We have moved the pictures and tables after the references.
5. We have replaced several references with the latest ones so that the 2016-2023 references exceed 50%. We have also included two references from the TJNPR journal.
6. References by writing et al., we have improved. We make sure all the references we quote are correct.
7. The revised draft has been checked for plagiarism; the result is <17%. We have included the results of the plagiarism check.
8. Statistical analysis has been included.
9. All authors agree to publish the results of this study in the TJNPR journal. The agreement is stated in the approval letter (*letter attached).

We thank you for providing comments on improving our manuscript. Once again, we apologize for being late in submitting the revision of the manuscript. We are waiting for the next Editor's direction. Thank You.

Best Regards,
Ermi
[Quoted text hidden]
--
Ni Putu Ermi Hikmawanti, M.Farm.

Office:
Department of Pharmaceutical Biology
Faculty of Pharmacy and Sciences
Universitas Muhammadiyah Prof. DR. HAMKA
Jl. Delima II/IV Klender, East Jakarta, Indonesia
Post code 13460
Phone: +62 852 5087 4147
Email: ermy0907@uhamka.ac.id

**4 attachments**

**Responses to reviewers' comments'.pdf**
57K

**AGREEMENTFORM.pdf**
346K

**rev1_Exploring polyphenol and antioxidant capacity in leaf extracts of selected Indonesian Syzygium species.doc**
1427K

**Check plagiarism results.pdf**
3157K

**Editor-in-Chief Tjnpr** <editor.tjnpr@gmail.com> Wed, Apr 5, 2023 at 10:06 PM
To: Ni Putu Ermi Hikmawanti <ermy0907@uhamka.ac.id>

Dear author, Thanks for the efforts made so far. Unfortunately, the manuscript still needs more revision. I will suggest you contact a science English editor to assist you. Dr Erahruyi ( osayemwenre.erharuyi@uniben.edu) can assist you

Best regards

Abiodun

---


## Professor Abiodun Falodun, PhD

Editor-in-Chief:
Tropical Journal of Natural Product Research (TJNPR)
Head, Natural Product Research Group, University of Benin
Email:editor.tjnpr@uniben.edu; editor.tjnpr@gmail.com
www.tjnpr.org **SCOPUS, SCImago SJR Q4 0.13**
https://www.scopus.com/sources.uri

Professor of Pharmaceutical Chemistry
Fellow, Fulbright (USA)
Deputy Vice-Chancellor (Academic) 2014-2016
Faculty of Pharmacy
University of Benin
Phone: +234-807-318-4488;
email: faloabi@uniben.edu; abiodun.falodun@fulbrightmail.org
Google Scholar Citations
SCOPUS https://www.scopus.com/authid/detail.uri?

https://orcid.org/0000-0003-2929-3305authorId=12794326500#top




University of Benin TJNPR SCOPUS Q4
www.uniben.edu www.tjnpr.org

[Quoted text hidden]

**needs correctionmanuscript.doc**
1434K

---

**Ni Putu Ermi Hikmawanti** <ermy0907@uhamka.ac.id> Thu, Apr 6, 2023 at 3:21 AM
To: Agustin Yumita <agustinyumita@yahoo.com>

[Quoted text hidden]

**needs correctionmanuscript.doc**
1434K

---

**Ni Putu Ermi Hikmawanti** <ermy0907@uhamka.ac.id> Wed, Jun 14, 2023 at 3:20 PM
To: Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

Dear Editor TJNPR,

Based on your recommendation, we contacted the scientific language editor Dr Erahruyi. The following manuscripts have been edited in Grammar, Spelling, Punctuation, Sentence Structure, and Formatting Style. We hope that the edits can improve the scientific quality of our manuscript. Hopefully well received.

Regards,
Authors

[Quoted text hidden]

**2 attachments**

**Certificate of English Editing - Yumita et al..pdf**
525K

**corrected manuscript-3 Edited.doc**
1151K

---

**Editor-in-Chief Tjnpr** <editor.tjnpr@gmail.com> Wed, Jun 14, 2023 at 7:02 PM
To: Ni Putu Ermi Hikmawanti <ermy0907@uhamka.ac.id>

Received. The editorial team will get back to you as soon as possible.

Best regards

Abiodun

---


## Professor Abiodun Falodun, PhD; FAAS, FISPON

Editor-in-Chief:
Tropical Journal of Natural Product Research (TJNPR)
Head, Natural Product Research Group, University of Benin
Email: editor.tjnpr@gmail.com
www.tjnpr.org SCOPUS, SCImago SJR Q4 0.13
https://www.scopus.com/sources.uri

Professor of Pharmaceutical Chemistry, FAAS
Fellow, Fulbright (USA)
Deputy Vice-Chancellor (Academic) 2014-2016
Faculty of Pharmacy
University of Benin
Phone: +234-807-318-4488;
email: faloabi@uniben.edu; abiodun.falodun@fulbrightmail.org
Google Scholar Citations
SCOPUS https://www.scopus.com/authid/detail.uri?

https://orcid.org/0000-0003-2929-3305authorId=12794326500#top

University of Benin TJNPR SCOPUS Q4
www.uniben.edu www.tjnpr.org


[Quoted text hidden]

**Dear Editor in-Chief**
**Tropical Journal of Natural Product Research (TJNPR)**

Based on the results of comments from all reviewers, we appreciate everything well. We have followed the revisions we sent based on all these comments to make the manuscript perfect. Here's our response:

1. The writing of the author's name is correct, where the first and last names are not abbreviated, only in the middle name.
2. Abstract: We add a brief background according to the reviewer's comments.
3. Materials and Methods: "Plant materials" we have included the plant collection number.
4. We have moved the pictures and tables after the references.
5. We have replaced several references with the latest ones so that the 2016-2023 references exceed 50%. We have also included two references from the TJNPR journal.
6. References by writing et al., we have improved. We make sure all the references we quote are correct.
7. The revised draft has been checked for plagiarism; the result is <17%. We have included the results of the plagiarism check.
8. Statistical analysis has been included.
9. All authors agree to publish the results of this study in the TJNPR journal. The agreement is stated in the approval letter (*letter attached).

Thus, in our response regarding comments from all reviewers, we thank you for providing comments on improving our manuscript.

Thank You.

Best Regards,
Authors.

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

Agustin Yumita[1], Ni P.E. Hikmawanti[1]*, Endang Hanani[1], Cindi W. Saputri[1], Putri H. Hanana[1], Jeanne N.D. Ero[1], Mayang Marcelena[1], Tazqiyah Baytisani[1], Febby A. Sofiana[1], Amanda F. Shania[1], Erlina S.A. Saputri[1], Firda P.N. Islami[1]

[1]Department of Pharmaceutical Biology, Faculty of Pharmacy and Sciences, University of Muhammadiyah Prof DR. HAMKA, Jakarta. Indonesia.

***Corresponding author** : ermy0907@uhamka.ac.id

## 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 phenols, flavonoids, and tannins contents were found in the leaves of *P. guajava*. The TLC chromatogram showed similarity in the organic components of the four types of guavas. The highest antioxidant activity was exhibited by *P. guajava* leaves, and this could be related to their high phenolic 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 specie of flowering plant belonging to the Myrtaceae family.[1] *Psidium guajava* (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 (Obat Herbal Terstandar, 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. 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 types of guavas leaves have 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 minutes and then filtered. The extraction process was repeated using the same technique until the flavonoid test showed negative results. Vacuum rotary evaporator N-1200 BS series (EYELA, Shanghai, China) was used to concentrate the filtrate to a total volume of 250 mL which was labelled as

SAEAE, SMEAE, SSEAE, and PGEAE for *Syzygium aqueum* ethyl acetate extract, *Syzygium malaccense* ethyl acetate extract, *Syzygium samarangense* ethyl acetate extract, and *Psidium guajava* ethyl acetate extract, 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 minutes, followed by filtration, was performed. The extraction process was repeated until the phenolic test was negative. Vacuum rotary evaporator was used to concentrate the filtrate to a total volume of 250 mL for each sample which were coded as SAEE, SMEE, SSEE, and PGEE for *Syzygium aqueum* ethanol extract, *Syzygium malaccense* ethanol extract, *Syzygium samarangense* ethanol extract, and *Psidium guajava* ethanol extract, 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 minutes, and then filtered. The procedure was repeated until the tannin test was negative. Water bath was used to concentrate the filtrate at 65ºC until the extract was viscous: SAWE, SMWE, SSWE, and PGWE representing *Syzygium aqueum* water extract, *Syzygium malaccense* water extract, *Syzygium samarangense* water extract, and *Psidium guajava* water extract, 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 minutes, 1.2 mL of sodium carbonate (7.5%) was added to the mixture. The mixture was incubated at room temperature for 110 minutes. 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 minutes 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 minutes. 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 minutes, using a UV-Vis Spectrophotometer, absorbance was recorded at 695 nm. The test was done three times.

***TLC analysis***

TLC analysis was also performed on the phenolic and flavonoid content of the extracts. Each guava leaf (8 g of dried leaf powder equivalent to 25 g of fresh leaves) was extracted using 80 mL of n-hexane or ethyl acetate or ethanol (70%) with an ultrasonic bath (Branson) (40 kHz) for 15 minutes at 25 - 26ºC. Each filtrate was concentrated with a vacuum rotary evaporator.

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 n-hexane and ethyl acetate extracts) and chloroform-ethyl acetate-formic acid (0.1:3.9:1) (for the ethanol extract). 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 involve 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 (F-C) 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] The highest phenolic content was observed in PGEE and the lowest was found 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]

*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. Of the three *Syzygium* leaves extracts, the ethyl acetate extract of *Syzygium malaccense* (SMEAE) had the lowest flavonoid content of 1.049 mg QE/g. 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.

*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 SM leaves had the least tannin content. 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 *P. guajava* 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 *P. guajava* water extract (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 *Syzygium malaccense* water extract (SMWE) was the highest which suggest that it has the lowest antioxidant activity which also correlated with the lowest tannin content (34.2303 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 *Syzygium aqueum* extract (SAWE) and *Syzygium samarangense* extract (SSWE) were not significantly different from each other, and they also have comparable tannin contents. *Psidium guajava* leaf ethyl acetate extract (PGEAE) and *Syzygium aqueum* extract (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 *S. aqueum* leaves (SAEAE), and *S. samarangense* leaves (SSEAE) ethyl acetate extracts were not statistically different ($P < 0.05$) as shown by their $EC_{50}$ values. *Syzygium malaccense* ethanol extract (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 70% ethanol extract of PG (PGEE) was 79.312 mg GAE/g extract (Figure 1A), the highest among all the guava extracts. Studies on the antioxidant activity of 70% ethanol extract of *P. guajava* (PGEE), *S. aqueum* extract (SAEE) and *S. samarangense* (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 ethanol extract of the *S. malaccense* leaf (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.

*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 n-hexane and ethyl acetate extracts, (ii) chloroform - ethyl acetate - formic acid (0.1:3.9:1) to identify compounds in the ethanol extract. 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 ethyl acetate extract had more spots than the n-hexane extract. In contrast, the 70% ethanol extract had more unresolved spots at the origin. In the ethyl acetate extract, it was observed that there were similarities in the chemical constituents of the three types of *Syzygium*, whereas in *P. guajava* extracts, there were quantitative differences, as there appeared some unique spots which were not seen in the extracts of the three *Syzygium* species.

*P. guajava* extracts in n-hexane, and ethyl acetate showed yellow fluorescence compounds with similar Rf, possibly flavonoids. The ethyl acetate extracts of all three *Syzygium* species and *P. guajava* leaves showed purple fluorescence compounds. Similarly, in ethanol extracts of PG, SM and SS, there were blue fluorescent spots with similar Rf values (Figure 3).

After being sprayed with $FeCl_3$ solution, the ethyl acetate extracts of the four test samples displayed a blue-green spot, as shown in Figure 4(A). In the n-hexane extract of PG, there was a dark brown spot, which probably indicated a tannin compound, while in the ethyl acetate extract of PG, there was a purple-black spot, suggesting a possibly different type of tannin from that of PG n-hexane extract. In the ethanol extract, dark brown colours were found at the origin, meaning that the tannin compounds present in this extract were not eluted by the mobile phase used.

The ethyl acetate extract of the four test samples contained yellow fluorescence compounds with similar Rf, as shown in Figure 4(B). These compounds may be flavonoids which are present in the four plants. In the n-hexane extract, yellow fluorescence compounds also appeared with different Rf, this may suggest the presence of more non-polar flavonoids in the n-hexane extract than in the ethyl acetate extract.

Figure 4(C) showed that the phytochemical contents of the n-hexane and ethyl acetate extracts are more similar, with the main difference being the intensity of the colors. A spot with a different color and size appeared in the n-hexane and ethyl acetate extracts 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 coloured (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*.

**Acknowledgments**
The authors are grateful to the Research and Development Institute, University of Muhammadiyah Prof. Dr. Hamka, for support for the study under contract number 383/F. 03.07/2022 Batch 2 for the year 2021.

**Conflict of interest**
The authors report having no competing interests.

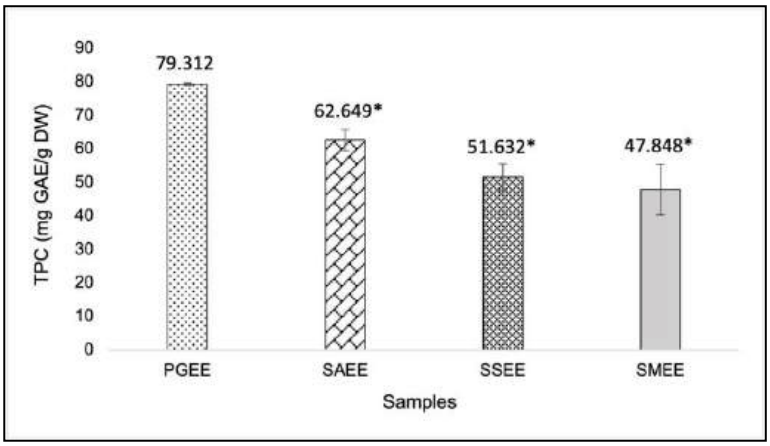
**References**

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

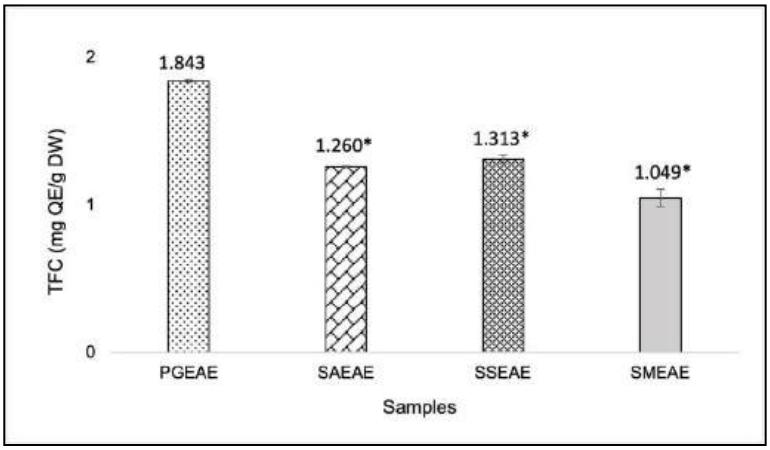
the plant *Limonium delicatulum*. J Taibah Univ Sci. 2014; 8(3):216–224.
15. Salamah N and Farahana L. Antioxidant activity Assay of Ethanolic Extract of *Centella asiatica* (L.) Urb Herb using Phosphomolybdate method. Pharmaciana [Internet]. 2014; 4(1):23–30.
16. Waksmundzka-hajnos M, Sherma J, Kowalska 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).
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).

**A.**




**B.**




**C.**

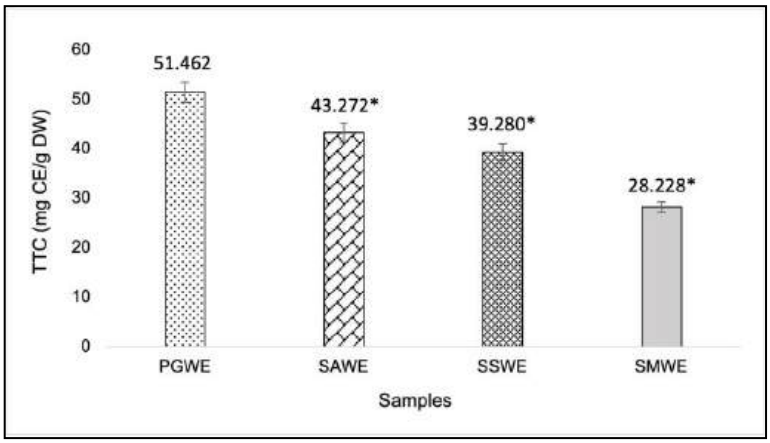



**Figure 1: [A]** Total phenolic content of guava leaf extracts. **[B]** Total flavonoid content of guava leaf extracts. **[C]** Total tannin content of guava leaf extracts. The sign (*) indicates a significant difference.

PG – Psidium guajava; SA - *Syzygium aqueum*; SS - *Syzygium samarangense*; SM - *Syzygium malaccense*.

EAE – Ethyl acetate extract; EE – Ethanol extract; WE – Water extract.

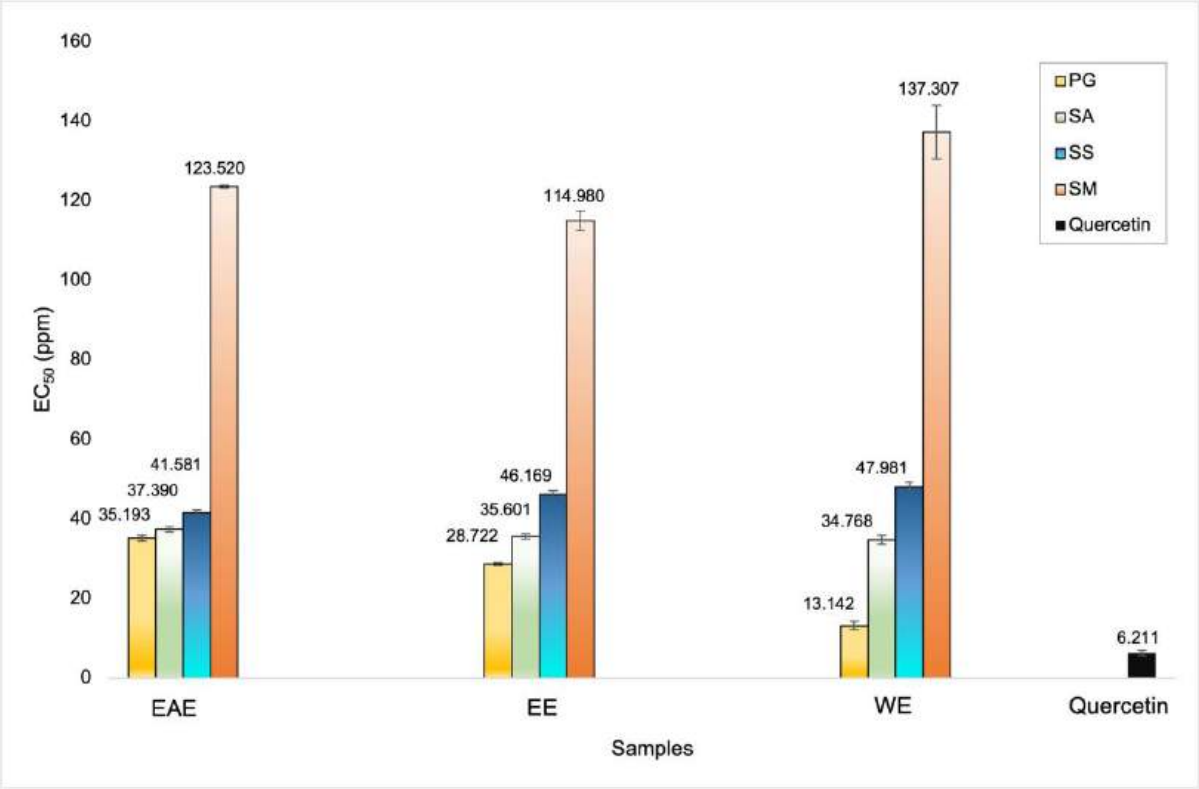



**Figure 2:** Antioxidant activity ($EC_{50}$ values) of guava leaf extracts against phosphomolybdate.
PG – Psidium guajava; SA - *Syzygium aqueum*; SS - *Syzygium samarangense*; SM - *Syzygium malaccense*.
EAE – Ethyl acetate extract; EE – Ethanol extract; WE – Water extract.

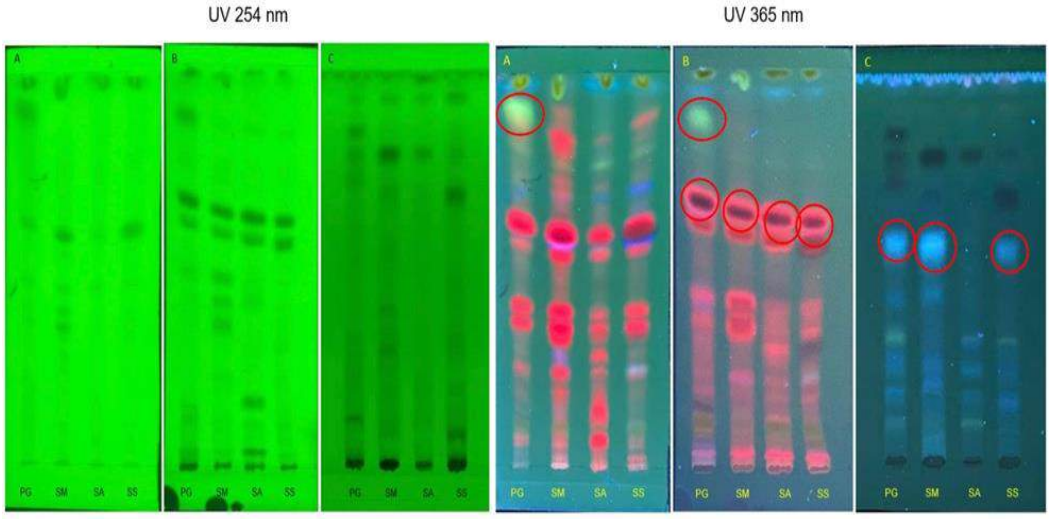



**Figure 3**: **TLC** Chromatogram of n-hexane (A), ethyl acetate (B), and ethanol (C) extracts of four types of guava leaves under UV light at 254 and 365 nm.
PG – Psidium guajava; SA - *Syzygium aqueum*; SS - *Syzygium samarangense*; SM - *Syzygium malaccense*.

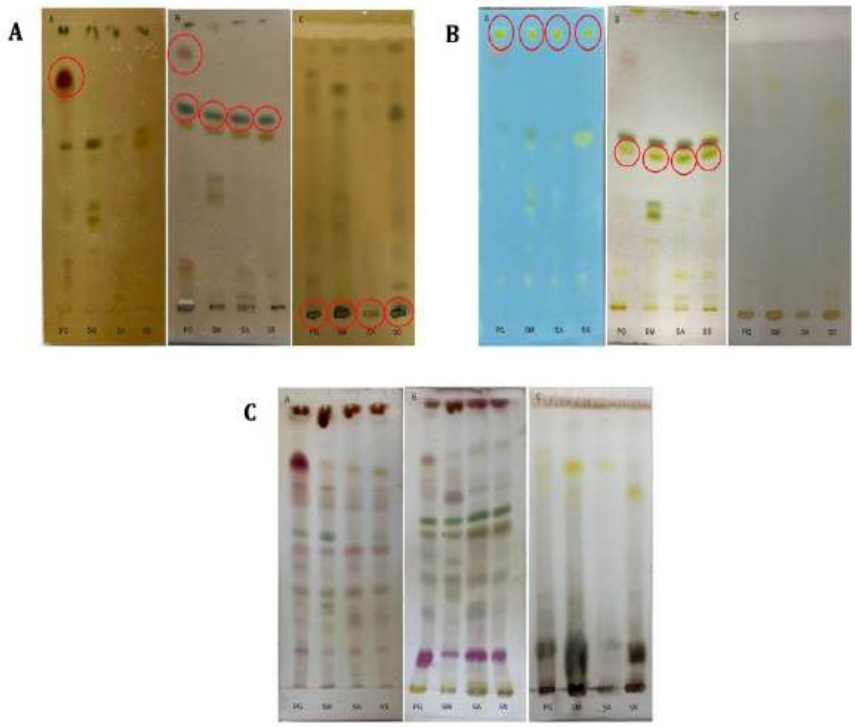



**Figure 4:** TLC chromatogram for identification of **[A]** phenolic compounds in n-hexane (A), ethyl acetate (B), and ethanol (C) extracts of four types of guava leaves after spraying with 5% $FeCl_3$. **[B]** flavonoids in n-hexane (A), ethyl acetate (B), and ethanol (C) extracts of four types of guava leaves after spraying with 5% $AlCl_3$. **[C]** other organic compounds in n-hexane (A), ethyl acetate (B), and ethanol (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*.

**Table 1:** Antioxidant Activity in Terms of Quercetin Equivalence of guava leaf extracts against phosphomolybdate

| Samples | Quercetin equivalence (mg QE/g) | | |
|---|---|---|---|
| | **EtOH (EE)** | **EtoAc (EAE)** | **Water (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*.

# 5. Bukti artikel proses *galley proof* dan artikel yang dikoreksi *galley proof* (23 Juni 2023)

Uhamka
UNIVERSITAS MUHAMMADIYAH PROF. DR. HAMKA

**Ni Putu Ermi Hikmawanti <ermy0907@uhamka.ac.id>**

# Check galley proof

2 messages

**Editorial Team** <et.tjnpr@gmail.com> Fri, Jun 23, 2023 at 6:26 PM
To: Ni Putu Ermi Hikmawanti <ermy0907@uhamka.ac.id>
Cc: Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

Dear Author,

See the attached **galley proof** manuscript with title **" Exploring the Polyphenol Contents and Antioxidant Capacity of the Leaf Extracts of Selected Indonesian *Syzygium* species"** for authors perusal

Please, as soon as possible (not later than 48 hours) kindly respond with receipt along with any comment or observations. Also respond to the comment(s) where indicated.

**All corrections/changes made in the manuscript should be highlighted in yellow ink when submitting the manuscript in the revised form.**

Regards

**TJNPR-2023-M057 Galley Proof.docx**
899K

**Ni Putu Ermi Hikmawanti** <ermy0907@uhamka.ac.id> Sat, Jun 24, 2023 at 1:44 PM
To: Editorial Team <et.tjnpr@gmail.com>

Dear Editor,
We submit our Galley proof review. Some parts we deliberately add sentences to make it more detailed. In other parts, we change the sentence pattern. However, this effort does not change the essence of the previous article. In the image, we edited the label name to make it easier to read. Some of the words that have previously been abbreviated we uniformly use. Hope you are OK. We are waiting for news of the publication of our article soon. Thank You.

Regards,
Ermi

[Quoted text hidden]

--
Ni Putu Ermi Hikmawanti, M.Farm.

Office:
Department of Pharmaceutical Biology
Faculty of Pharmacy and Sciences
Universitas Muhammadiyah Prof. DR. HAMKA
Jl. Delima II/IV Klender, East Jakarta, Indonesia
Post code 13460
Phone: +62 852 5087 4147
Email: ermy0907@uhamka.ac.id

**TJNPR-2023-M057 Galley Proof-check.docx**
870K

Trop J Nat Prod Res, June 2023; 7(6): **ISSN 2616-0684 (Print)**
**ISSN 2616-0692 (Electronic)**

# Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

*Original Research Article*

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

Agustin Yumita[1], Ni P.E. Hikmawanti[1]*, Endang Hanani[1], Cindi W. Saputri[1], Putri H. Hanana[1], Jeanne N.D. Ero[1], Mayang Marcelena[1], Tazqiyah Baytisani[1], Febby A. Sofiana[1], Amanda F. Shania[1], Erlina S.A. Saputri[1], Firda P.N. Islami[1]

[1]*Department of Pharmaceutical Biology, Faculty of Pharmacy and Sciences, University of Muhammadiyah Prof DR. HAMKA, Jakarta. Indonesia*

ARTICLE INFO

*Article history:*
Received 27 January 2023
Revised 14 June 2023
Accepted 23 June 2023
Published online ****



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.

**Commented [NPEH1]:** We adjusted the conclusions with the objectives of this study.

## 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.

*Corresponding author. E mail: ermy0907@uhamka.ac.id
Tel: +62852 50874147

**Citation:** Yumita A, Hikmawanti NPE, Hanani E, Saputri CW, Hanana PH, Ero JND, Marcelena M, Baytisani T, Sofiana FA, Shania AF, Saputri ESA, Islami FPN. Exploring the Polyphenol Contents and Antioxidant Capacity of the Leaf Extracts of Selected Indonesian *Syzygium* Species. Trop J Nat Prod Res. 2023; 7(6):

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

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 ºC. 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

**Commented [NPEH2]:** We simplify sentences so that they are not too long and easy to understand.

**Commented [NPEH3]:** We use abbreviations so that all extract label names are uniform

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]

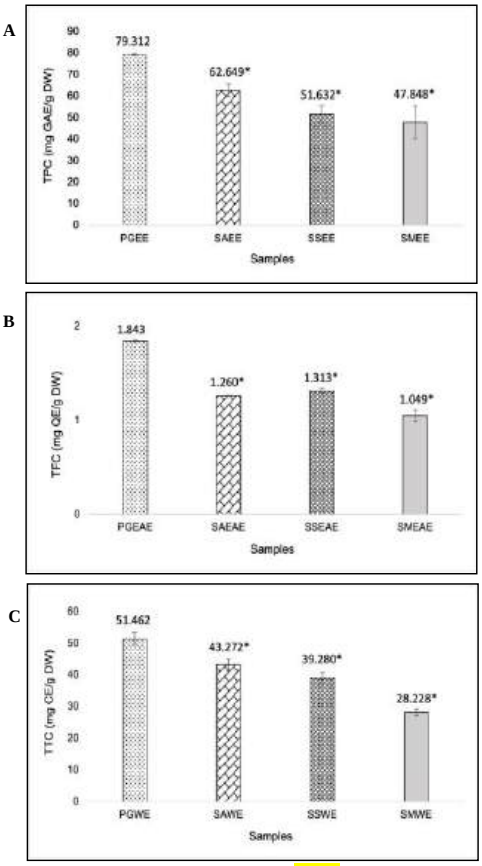



**Figure 1:** [A] Total phenolic content (TPC) of guava leaf extracts. [B] Total flavonoid content (TFC) of guava leaf extracts. [C] Total tannin content (TTC) of guava leaf extracts. The sign (*) indicates a significant difference.
PG – *Psidium guajava*; SA – *Syzygium aqueum*; SS – *Syzygium samarangense*;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

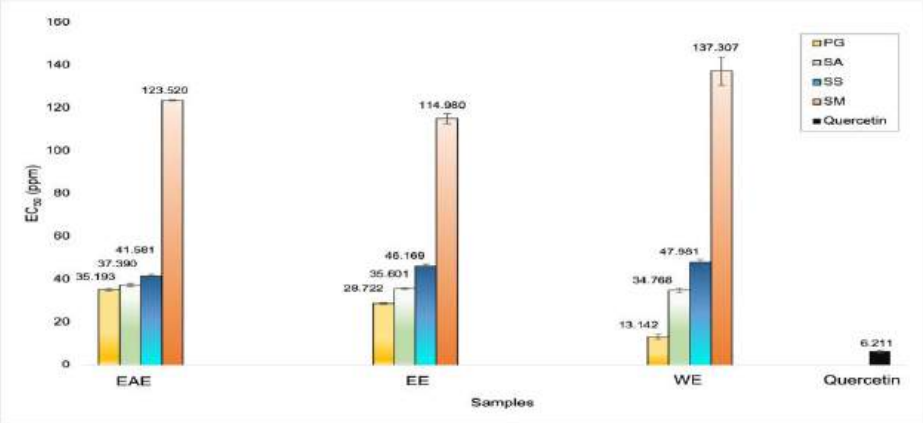



**Figure 2:** Antioxidant activity ($EC_{50}$ values) of guava leaf extracts against phosphomolybdate.
PG – *Psidium guajava*; SA – *Syzygium aqueum*; SS – *Syzygium samarangense*; SM – *Syzygium malaccense*;
EAE – Ethyl acetate extract; EE – Ethanol extract; WE – Water extract.

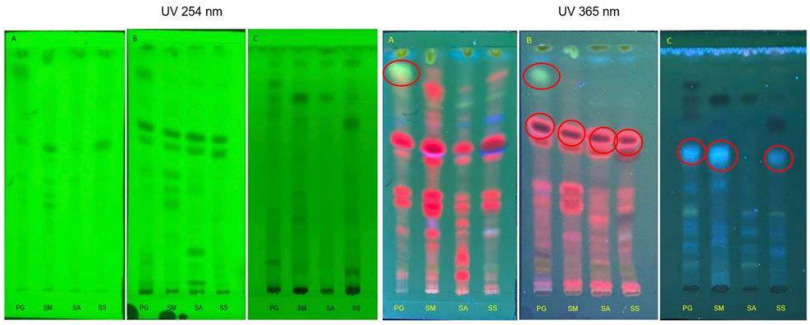



**Figure 3**: TLC Chromatogram of HE (A), EAE (B), and EE (C) of four types of guava leaves under UV light at 254 and 365 nm.
PG – *Psidium guajava*; SA – *Syzygium aqueum*; SS – *Syzygium samarangense*; SM – *Syzygium malaccense*;
HE – *n*-Hexane extract; EAE – Ethyl acetate extract; EE – Ethanol extract.

*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).

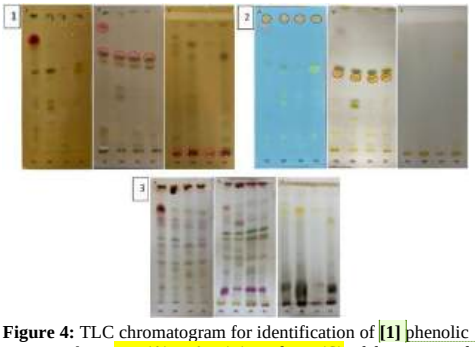


**Figure 4:** TLC chromatogram for identification of **[1]** phenolic compounds in HE (A), EAE (B), and EE (C) of four types of guava leaves after spraying with 5% $FeCl_3$. **[2]** flavonoids in HE (A), EAE (B), and EE (C) of four types of guava leaves after spraying with 5% $AlCl_3$. **[3]** other 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 (Rf 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 Rf (Rf 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.

## Acknowledgements

The authors are grateful to the Research and Development Institute, University of Muhammadiyah Prof. Dr. HAMKA, for support for the study under contract number 383/F. 03.07/2022 Batch 2 for the year 2021.

## 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

**Commented [NPEH5]:** We add Rf values to make the information clearer.

**Commented [NPEH6]:** We add two sentences to make the conclusion sharper.

**Commented [NPEH4]:** We changed the image coding order from A, B, and C to 1, 2, and 3 so that there are no double letter codes. We hope you are okay.

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.

# 6. Bukti artikel telah terbit *online* pada website Jurnal

# (2 Juli 2023)

Tropical Journal of Natural Product Research
Official Journal of Natural Product Research Group
ISSN: 2616-0684 (Print)
ISSN: 2616-0692 (Online)



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

http://www.doi.org/10.26538/tjnpr/v7i6.8

pdf (https://tjnpr.org/index.php/home/article/view/2072/2878)
doi (https://tjnpr.org/index.php/home/article/view/2072/2887)

**Published:** Jul 2, 2023

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

**Agustin Yumita**
Department of Pharmaceutical Biology, Faculty of Pharmacy and Sciences, University of Muhammadiyah Prof DR. HAMKA, Jakarta. Indonesia

**Ni P.E. Hikmawanti**
Department of Pharmaceutical Biology, Faculty of Pharmacy and Sciences, University of Muhammadiyah Prof DR. HAMKA, Jakarta. Indonesia

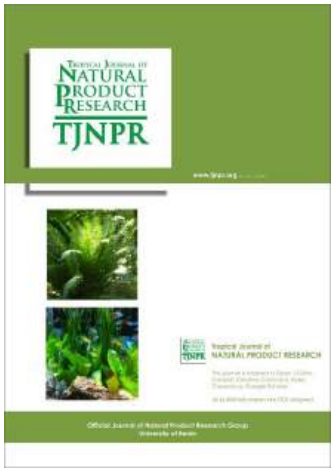
**Endang Hanani**
Department of Pharmaceutical Biology, Faculty of Pharmacy and Sciences, University of Muhammadiyah Prof DR. HAMKA, Jakarta. Indonesia

**Cindi W. Saputri**
Department of Pharmaceutical Biology, Faculty of Pharmacy and Sciences, University of Muhammadiyah Prof DR. HAMKA, Jakarta. Indonesia

**Putri H. Hanana**
Department of Pharmaceutical Biology, Faculty of Pharmacy and Sciences, University of Muhammadiyah Prof DR. HAMKA, Jakarta. Indonesia

**Jeanne N.D. Ero**





Department of Pharmaceutical Biology, Faculty of Pharmacy and Sciences, University of Muhammadiyah Prof DR. HAMKA, Jakarta. Indonesia

**Mayang Marcelena**
Department of Pharmaceutical Biology, Faculty of Pharmacy and Sciences, University of Muhammadiyah Prof DR. HAMKA, Jakarta. Indonesia

**Tazqiyah Baytisani**
Department of Pharmaceutical Biology, Faculty of Pharmacy and Sciences, University of Muhammadiyah Prof DR. HAMKA, Jakarta. Indonesia

**Febby A. Sofiana**
Department of Pharmaceutical Biology, Faculty of Pharmacy and Sciences, University of Muhammadiyah Prof DR. HAMKA, Jakarta. Indonesia

**Amanda F. Shania**
Department of Pharmaceutical Biology, Faculty of Pharmacy and Sciences, University of Muhammadiyah Prof DR. HAMKA, Jakarta. Indonesia

**Erlina S.A. Saputri**
Department of Pharmaceutical Biology, Faculty of Pharmacy and Sciences, University of Muhammadiyah Prof DR. HAMKA, Jakarta. Indonesia

**Firda P.N. Islami**
Department of Pharmaceutical Biology, Faculty of Pharmacy and Sciences, University of Muhammadiyah Prof DR. HAMKA, Jakarta. Indonesia

# 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.



How to Cite

Yumita, A., Hikmawanti, N. P., Hanani, E., Saputri, C. W., Hanana, P. H., Ero, J. N., Marcelena, M., Baytisani, T., Sofiana, F. A., Shania, A. F., Saputri, E. S., & Islami, F. P. (2023). Exploring the Polyphenol Contents and Antioxidant Capacity of the Leaf Extracts of Selected Indonesian Syzygium Species: http://www.doi.org/10.26538/tjnpr/v7i6.8. *Tropical Journal of Natural Product Research (TJNPR)*, *7*(6), 3119-3124. https://tjnpr.org/index.php/home/article/view/2072 (https://tjnpr.org/index.php/home/article/view/2072)



Issue

Vol. 7 No. 6 (2023): Tropical Journal of Natural Product Research (TJNPR) (https://tjnpr.org/index.php/home/issue/view/70)

Section

Articles



## Keywords


## Publisher

Dean's Office,
Name of Publisher: Dr. Vincent O. Imieje,
Room 1, Second Floor,
Faculty of Pharmacy,
University of Benin,
PMB 1154
Benin City
Edo State, Nigeria.
Email: vincent.imieje@uniben.edu (mailto:vincent.imieje@uniben.edu); vincent.imieje@gmail.com (mailto:vincent.imieje@gmail.com)
Tel: +2348037890763

**How to Cite**

Yumita, A., Hikmawanti, N. P., Hanani, E., Saputri, C. W., Hanana, P. H., Ero, J. N., Marcelena, M., Baytisani, T., Sofiana, F. A., Shania, A. F., Saputri, E. S., & Islami, F. P. (2023). Exploring the Polyphenol Contents and Antioxidant Capacity of the Leaf Extracts of Selected Indonesian Syzygium Species: http://www.doi.org/10.26538/tjnpr/v7i6.8. *Tropical Journal of Natural Product Research (TJNPR)*, *7*(6), 3119-3124. https://tjnpr.org/index.php/home/article/view/2072 (https://tjnpr.org/index.php/home/article/view/2072)

**More Citation Formats**

# References

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.

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.

Tilaar M, Widjaja B, Ranti A. The Green Science of Jamu. PT Dian Rakyat, editor. Jakarta: PT Dian Rakyat; 2010.

The National Agency of Drug and Food Control (NA-DFC). Statistik Produk Yang Mendapat Persetujuan Izin Edar [Internet]. https://cekbpom.pom.go.id (https://cekbpom.pom.go.id). 2021. Available from: https://cekbpom.pom.go.id//home/produk/p5i4kt4kfpe75r9p (https://cekbpom.pom.go.id//home/produk/p5i4kt4kfpe75r9p)

o7ng9gqap7/all/row/10/page/2/order/4/DESC/search/5/psidii

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.

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 (https://doi.org/10.7287/peerj.preprints.1930v1)

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.

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.

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.

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.

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.

Yang J, Paulino R, Janke-Stedronsky S, Abawi F. Freeradical-scavenging Activity and Total Phenols of Noni (Morinda citrifolia L.) Juice and Powder in Processing and Storage. Food Chem. 2007; 102:302–308.

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.

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.

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.

Waksmundzka-hajnos M, Sherma J, Kowalska T. Thin Layer Chromatography in Phytochemistry. Cazes J, editor. CRC Press, Taylor & Francis Group; 2008. 888 p.

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.

Sarker S, Latif Z, Gray A. Natural Products Isolation. Second. New Jersey: Humana Press; 2006.

Hanani E. Pharmacognosy. Jakarta: Penerbit Uhamka Press; 2021. 177–178 p.

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.

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.

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.

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.

Hanani E. Analisis Fitokimia (In Bahasa) “Phytochemistry Analysis.” Jakarta: EGC Medical Publisher; 2015; 69(83)110, 148–149, 202, 232–233 p.

Ministry of Health Republic of Indonesia. Farmakope Herbal Indonesia Edisi II. Jakarta: Ministry of Health Republic of Indonesia; 2017. 5–6 p.

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.

Trease, Evans W. Pharmacognosy 16th Edition. In: 16th ed. Toronto: Saunders Elsevier; 2009. p. 100, 246, 269–270.

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).

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.

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.

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.

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.

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.

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.

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.

Trop J Nat Prod Res, June 2023; 7(6):3119-3124 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

*Original Research Article*

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

Agustin Yumita[1], Ni P.E. Hikmawanti[1]*, Endang Hanani[1], Cindi W. Saputri[1], Putri H. Hanana[1], Jeanne N.D. Ero[1], Mayang Marcelena[1], Tazqiyah Baytisani[1], Febby A. Sofiana[1], Amanda F. Shania[1], Erlina S.A. Saputri[1], Firda P.N. Islami[1]

[1]*Department of Pharmaceutical Biology, Faculty of Pharmacy and Sciences, University of Muhammadiyah Prof DR. HAMKA, Jakarta. Indonesia*

ARTICLE INFO

*Article history:*
Received 27 January 2023
Revised 14 June 2023
Accepted 23 June 2023
Published online 01 July 2023



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.

*Corresponding author. E mail: ermy0907@uhamka.ac.id
Tel: +62852 50874147

**Citation:** Yumita A, Hikmawanti NPE, Hanani E, Saputri CW, Hanana PH, Ero JND, Marcelena M, Baytisani T, Sofiana FA, Shania AF, Saputri ESA, Islami FPN. Exploring the Polyphenol Contents and Antioxidant Capacity of the Leaf Extracts of Selected Indonesian *Syzygium* Species. Trop J Nat Prod Res. 2023; 7(6): 3119-3124 http://www.doi.org/10.26538/tjnpr/v7i6.8

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

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]

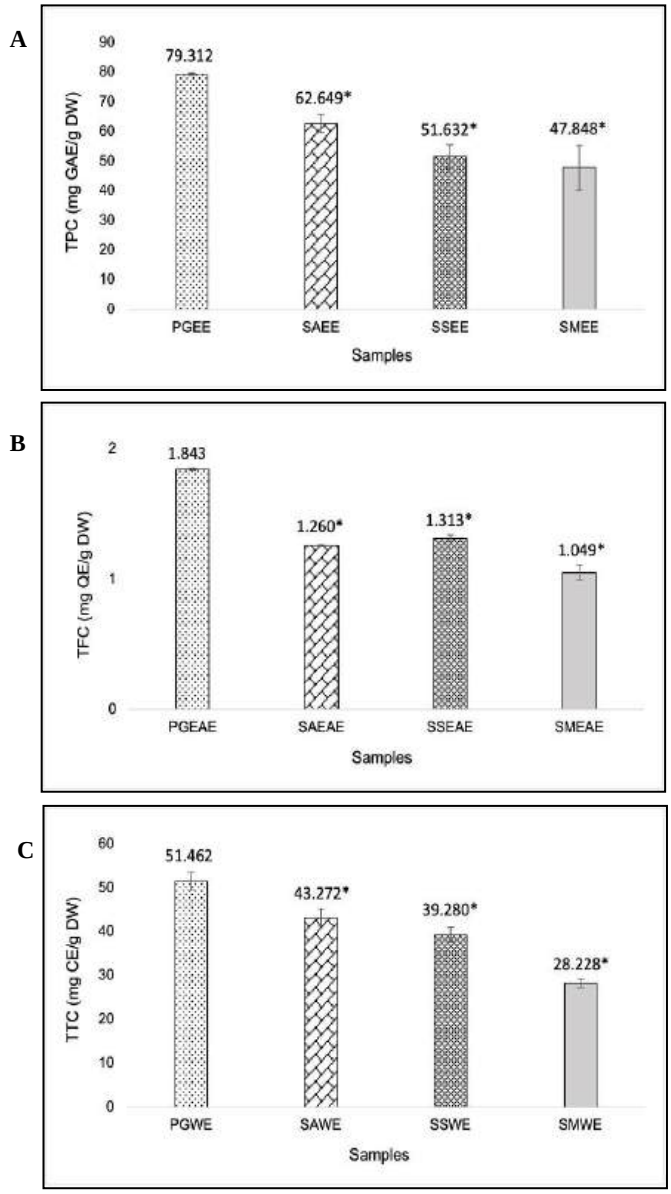



**Figure 1:** [A] Total phenolic content (TPC) of guava leaf extracts. [B] Total flavonoid content (TFC) of guava leaf extracts. [C] Total tannin content (TTC) of guava leaf extracts. The sign (*) indicates a significant difference.
PG – *Psidium guajava*; SA – *Syzygium aqueum*; SS – *Syzygium samarangense*;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

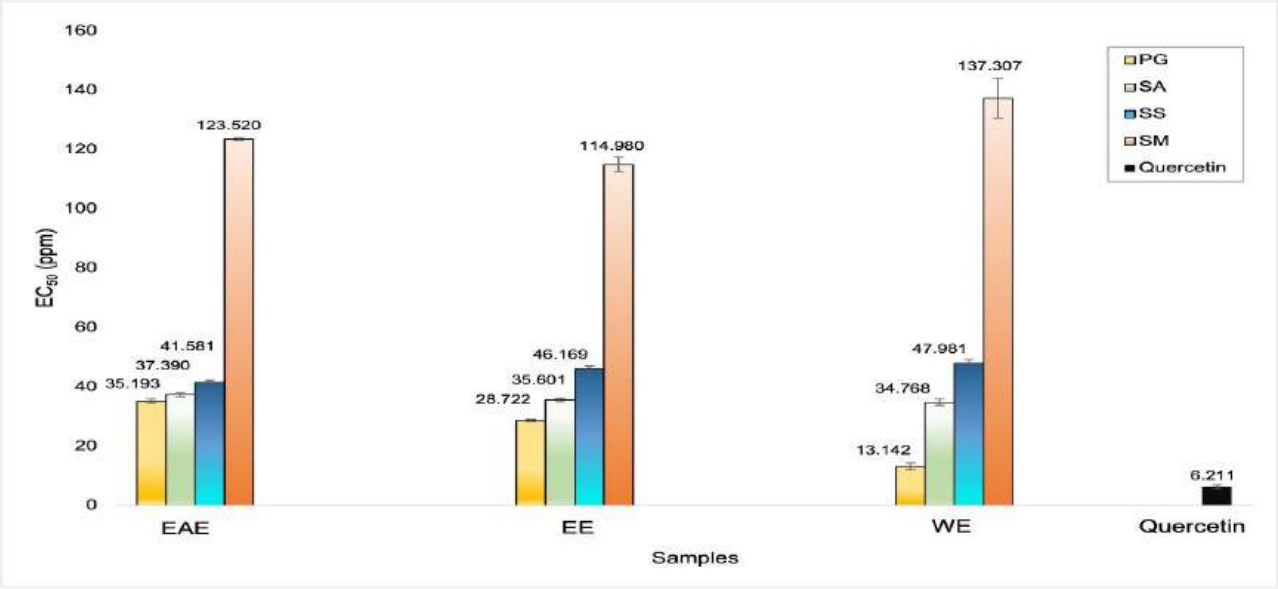



**Figure 2:** Antioxidant activity ($EC_{50}$ values) of guava leaf extracts against phosphomolybdate.
PG – *Psidium guajava*; SA – *Syzygium aqueum*; SS – *Syzygium samarangense*; SM – *Syzygium malaccense*; EAE – Ethyl acetate extract; EE – Ethanol extract; WE – Water extract.

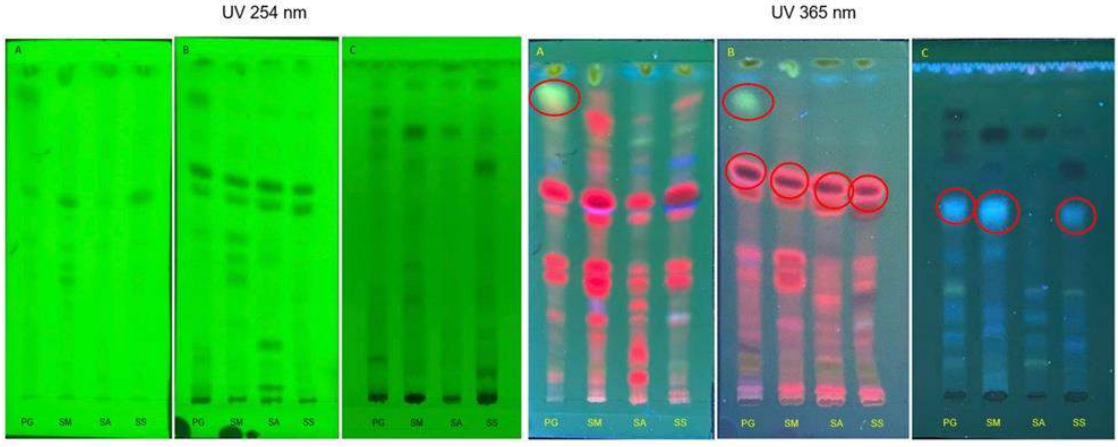



**Figure 3**: TLC Chromatogram of HE (A), EAE (B), and EE (C) of four types of guava leaves under UV light at 254 and 365 nm.
PG – *Psidium guajava*; SA – *Syzygium aqueum*; SS – *Syzygium samarangense*; SM – *Syzygium malaccense*; HE – *n*-Hexane extract; EAE – Ethyl acetate extract; EE – Ethanol extract.

*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).

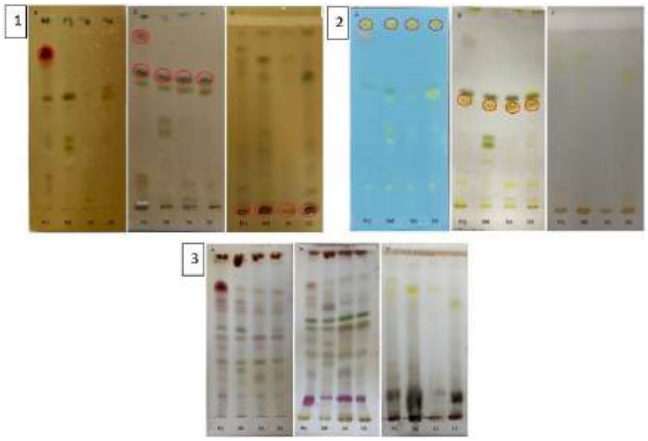



**Figure 4:** TLC chromatogram for identification of **[1]** phenolic compounds in HE (A), EAE (B), and EE (C) of four types of guava leaves after spraying with 5% $FeCl_3$. **[2]** flavonoids in HE (A), EAE (B), and EE (C) of four types of guava leaves after spraying with 5% $AlCl_3$. **[3]** other 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.

## Acknowledgements

The authors are grateful to the Research and Development Institute, University of Muhammadiyah Prof. Dr. HAMKA, for support for the study under contract number 383/F. 03.07/2022 Batch 2 for the year 2021.

## 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.