

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF *Psidium guajava*

(Saringan Fitokimia dan Aktiviti Antioksidan terhadap *Psidium guajava*)

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Abstract

Psidium guajava or commonly known as guava is useful to treat gastroenteritis, dysentery, stomach pain and indigestion. In this study, the leaves of this species were screened for its phytochemical and antioxidant activity. The phytochemicals were extracted by sequential maceration by using *n*-hexane, chloroform and methanol, while phytochemical screening was performed using various chemical tests. Meanwhile, its antioxidant activity was assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Steroids and terpenoids were found to be present in the *n*-hexane extract, while phenols and terpenoids were detected in the chloroform extract. The methanol extract contained flavonoids, steroids, saponins, phenols and terpenoids. Among the tested extracts, the methanolic extract demonstrated strong DPPH radical scavenging activity with an IC₅₀ value of 45.52 µg/mL.

Keywords: *Psidium guajava*, phytochemical screening, antioxidant

Abstrak

Psidium guajava atau dikenali sebagai jambu batu berguna bagi mengubati gastroenteritis, disenteri, sakit perut dan masalah pencernaan. Dalam kajian ini, daun spesies ini telah disaring untuk fitokimia dan aktiviti antioksidan. Fitokimia telah diekstrak menggunakan rendaman berturutan menggunakan *n*-heksana, kloroform dan metanol, manakala saringan fitokimia telah dijalankan menggunakan pelbagai ujian kimia. Sementara itu, aktiviti antioksidan telah dinilai menggunakan kaedah 2,2-difenil-1-pikrilhidrazil (DPPH). Steroid and terpenoid telah didapati hadir di dalam ekstrak *n*-heksana, manakala fenol dan terpenoid telah dikesan di dalam ekstrak kloroform. Ekstrak metanol telah mengandungi flavonoid, steroid, saponin, fenol dan terpenoid. Di kalangan ekstrak yang diuji, ekstrak metanol menunjukkan aktiviti perencatan radikal DPPH yang kuat dengan nilai IC₅₀ 45.52 µg/mL.

Kata kunci: *Psidium guajava*, saringan fitokimia, antioksidan

Introduction

Reactive oxygen species (ROS) such as superoxide (O₂⁻), hydroxyl (HO[•]) and peroxide radicals (ROO[•]) are chemically reactive molecules that are produced from oxygen metabolism [1]. Their excessive production can cause severe damage to cells and tissues, loss of cellular function, oxidative stress, and ultimately, apoptosis or necrosis. Collectively, these damages are linked to many health issues, such as heart disease and carcinogenesis [2, 3]. In order to counteract the biomolecules against a ROS attack, antioxidants are needed to neutralise the excessive free radicals, protect the cells against their toxic effects and prevent diseases such as arthritis, stroke and chronic bronchitis [4]. In recent years, an intensive interest to search and identify natural and safe antioxidants, especially

those from a plant source has grown rapidly. These natural antioxidants may be either phenolic compounds (i.e. flavonoids, phenolic acids and tannins), nitrogen-containing compounds (i.e. alkaloids, amino acids, peptides and amines), carotenoids, tocopherols, or ascorbic acid and its derivatives [5].

Psidium guajava is commonly known as guave, goyave, or goyavier in France; guave, Guavenbaum, or Guayave in Germany; banjiro in Japan; goiaba or goiabeiro in Portugal; araçá-goiaba, araçá-guaçu, or guaiaba in Brazil; guayaba, or guayabo in Spain and guava in English [6]. This small tree is a native plant of tropical American regions, which is distributed throughout Southern Mexico, South America, European, Africa and Asia [6, 7]. Its leaves, barks, fruits, shoots and flower buds are applied for ethnomedicinal purposes, specifically in the treatment of several ailments such as gastroenteritis, dysentery, bacterial infection, diabetes, hypertension, caries and wounds, as well as a pain reliever and fever reducer. Meanwhile, this plant is also utilised commercially as food and in carpentry, house construction and toy manufacturing [6]. Biological investigations on *P. guajava* have revealed that its different extracts and isolated compounds exhibited antimicrobial, antihyperglycaemic, anti-inflammatory, analgesic, antipyretic, spasmolytic, central nervous system (CNS)-depressant, antidiarrhoeal, antimalarial, hepatoprotective, antigenotoxic, antimutagenic, antiallergic, antioxidant, anticancer, cardiovascular, hypotensive, antinociceptive and wound healing activities [6-8]. Furthermore, phytochemical studies undertaken by previous researchers have disclosed that this species can produce flavonoids, triterpenoids, tannins, phenolics, carotenoids and essential oils [6, 7, 9-14]. To date, there is only one study found detailing the phytochemical screening and antibacterial activity of *P. guajava* leaves extract, which have been collected from Selangor, Malaysia [14]. Accordingly, geographical distribution affects the chemical compositions of plants, while their biological activities are impacted conversely. In this study, the leaves of *P. guajava* were collected from Johor, Malaysia. The investigation was carried out to identify phytochemicals in the *P. guajava* leaves extracts and their relationship with the resulting antioxidant activity.

Materials and Methods

Plant material and preparation of extracts

The leaves of *P. guajava* were collected in February 2018 from Muar, Johor, Malaysia. The leaves were subjected to air-dried and ground into fine powder. The powdered leaves (200 g) were soaked sequentially with *n*-hexane (2 L), chloroform (2 L) and methanol (2 L) for three days each. The extracts were filtered using Whatman paper No. 1 and concentrated using a rotatory evaporator to afford *n*-hexane, chloroform and methanol extracts.

Phytochemical screening

The extracts were tested for the presence of alkaloids, flavonoids, phenolic compounds, terpenoids, steroids and saponins.

Test for alkaloids (Wagner's test)

Six drops of Wagner's reagent were added to 2 mL of crude extract. The Wagner's reagent was prepared by mixing iodine in potassium iodide solution. The formation of brown or reddish precipitate indicates the presence of alkaloids [15].

Test for flavonoids (Ferric chloride test)

Crude extract (2 mL) was treated with a few drops of ferric chloride solution. The intense green colour of the solution shows the presence of flavonoids [16].

Test for phenolics (Ferric chloride test)

A few drops of ferric chloride solution were added into each crude extract (2 mL). The appearance of a bluish black colour shows the presence of phenolic compounds [17].

Test for terpenoids (Salkowski test)

Each crude extract (1 mg) was mixed with chloroform (2 mL) and concentrated sulphuric acid (1 mL). The formation of reddish-brown colour at the interface indicates the presence of terpenoids [2].

Test for steroids (Salkowski test)

About 100 mg of dried crude extract was dissolved in 2 mL of chloroform. About 2-3 drops of sulphuric acid was carefully added to the extract to form a lower layer. A reddish-brown colour at the interface was indicative of the presence of steroidal ring [2, 18].

Test for saponins (Foam test)

Distilled water (2 mL) was added to 2 mL of crude extract. The mixture was shaken vigorously for 15 minutes period. The persistence of foam produced after 15 minutes indicates the presence of saponins [19].

Antioxidant assay

Antioxidant activity was performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The method was performed as described by the previous study with slight modifications [20]. Each sample (1.0 mg) was dissolved in methanol (1 mL) to obtain a stock concentration of 1000 µg/mL. The stock solution was further diluted to final concentrations of 500, 250, 125, 62.5, 31.3, 15.63 and 7.81 µg/mL in methanol. The DPPH solution was freshly prepared in MeOH to acquire final concentration of 50 µM. Then, 3.8 mL of DPPH solution was added to 0.2 mL of sample solution of different concentrations. The mixture was allowed to react at room temperature in the dark. After 30 minutes, the absorbance of the reaction mixture was recorded at 517 nm. Ascorbic acid was used as positive control, while the DPPH solution (3.8 mL DPPH and 0.2 mL methanol) was used as DPPH blank. The percentage inhibition (%) was calculated using the following formula [20]:

$$\text{Percentage Inhibition (\%)} = [A_{\text{DPPH blank}} - (A_{\text{Sample}} - A_{\text{blank sample}})] / A_{\text{DPPH blank}} \times 100 \quad (1)$$

where $A_{\text{DPPH blank}}$ is the absorbance of DPPH reagent and methanol, A_{sample} is the absorbance of sample solution with DPPH reagent and $A_{\text{blank sample}}$ is the absorbance of sample solution with methanol. The IC_{50} value was defined as the concentration of each sample required to scavenge 50% of the DPPH radical and was determined using GraphPad Prism 5. The assay was carried out in triplicate and the results were reported as means $\pm$ standard deviation. The Independent t -test was performed by using SPSS for Windows (version 22) software for comparison between samples and positive control. A value of $p < 0.05$ was considered significantly different.

Results and Discussion**Percentage yield of extracts**

The removal of solvents under reduced pressure yielded *n*-hexane, chloroform and methanol extracts as shown in Table 1. The methanol extract gave the highest extraction yield of 9.65%, followed by chloroform (2.22%) and *n*-hexane (1.49%) extracts. These results indicate that the extraction yield increases alongside the increasing polarity of solvent utilized in the extraction process [21]. Therefore, the higher percentage yield shown by the methanol extract may be due to the high polarity of the methanolic solvent, which can extract abundant types of compounds [22].

Table 1. Yield of *P. guajava* leaves extracts

Extracts	Yield (g, %)
<i>n</i> -Hexane	2.98, 1.49
Chloroform	4.44, 2.22
Methanol	19.29, 9.65

Preliminary phytochemical screening

The results of qualitative phytochemical analysis for the *n*-hexane, chloroform and methanol extracts are presented in Table 2. From the findings, it was found that *n*-hexane extract contained steroids and terpenoids, while chloroform extract showed the presence of phenols and terpenoids. Flavonoids, steroids, saponins, phenols and terpenoids were detected in methanol extract. Furthermore, the current study revealed the existence of terpenoids in all extracts in which a reddish-brown colour was observed. However, these three extracts did not give brown or

reddish precipitate when treated with Wagner's reagent, thereby indicating the absence of alkaloids. The qualitative phytochemical analysis undertaken in this study showed identical findings for the existence of flavonoids and phenols compared to the reports by earlier researchers [11, 12, 14].

Table 2. Phytochemical screening of *P. guajava* leaves extracts

Phytochemicals	Extracts		
	<i>n</i> -Hexane	Chloroform	Methanol
Alkaloids	-	-	-
Flavonoids	-	-	+
Steroids	+	-	+
Saponins	-	-	+
Phenols	-	+	+
Terpenoids	+	+	+

+ = present; - = absent

The phytochemicals detected in the leaves of *P. guajava* have been reported to offer countless benefits of medicinal importance. For examples, phenolics and flavonoids demonstrate many biological activities, such as antioxidant [23], antimicrobial [24], anticancer, anti-inflammatory and wound healing properties [25]. On the other hand, steroids are known to have antibacterial, insecticidal and cardiogenic properties [2], while saponins can treat diabetes [26]. Similarly, terpenoids have been used to alleviate human diseases such as cancer, malaria, inflammation and various infectious diseases [27].

DPPH radical scavenging activity

Data on the DPPH radical scavenging activity of *n*-hexane, chloroform and methanol extracts of *P. guajava* leaves along with the standard antioxidant, ascorbic acid is presented in Table 3. The samples that showed $p < 0.05$ were considered to have a statistically significant difference as compared to ascorbic acid as the positive control. Among the extracts, only methanol extract demonstrated radical scavenging activity with a percentage inhibition of 85.70% and an IC_{50} value of 45.52 μ g/mL. However, the IC_{50} value was higher than that of ascorbic acid, which has an IC_{50} value of 25.32 μ g/mL. The other extracts did not show radical scavenging activity since their respective percentage inhibitions were less than 50% at the concentration of 1000 μ g/mL. The potent antioxidant activity of the methanol extract may be due to the existence of flavonoids and phenolics, which are known to be capable of donating their hydrogen atoms [23].

Table 3. DPPH radical scavenging activity of *P. guajava* leaves extracts

Samples	Inhibition at 1000 μ g/mL (%)	IC_{50} (μ g/mL)
<i>n</i> -Hexane extract	15.95 $\pm$ 0.92 ^b	ND
Chloroform extract	40.51 $\pm$ 1.25 ^b	ND
Methanol extract	85.70 $\pm$ 0.80 ^b	45.52 $\pm$ 3.71 ^a
Ascorbic acid	95.53 $\pm$ 0.10	25.32 $\pm$ 0.44

Data represent mean $\pm$ standard deviation of three replicate experiments; ND = not determined

a = $p < 0.01$, b = $p < 0.001$

Conclusion

The phytochemical screening revealed that the leaves extracts of *P. guajava* from Johor, Malaysia comprise of various beneficial phytochemicals such as flavonoids, steroids, saponins, phenols and terpenoids. This study also

disclosed that the methanol extract yielded the highest extraction yield and good DPPH radical scavenging activity. The present data may be useful as an initial step to determine the potential natural antioxidants that can be applied in pharmaceutical and therapeutic industries. In order to identify these antioxidants, further purification and characterization steps are suggested to be done in future study.

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