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Phytochemical Screening, Total Phenolic Content and Antioxidant Activity of *Syzygium zeylanicum* Leaves

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Abstract

Syzygium zeylanicum is a species that is rarely known especially among the present community. Although the plant is palatable, lack of research has been carried out on it benefits to human health. The leaves of Syzygium zeylanicum were extracted using hexane, ethanol and water; and evaluated for its phytochemicals, total phenolic content and antioxidant activities. Specific reagents were used in the phytochemical screening to check for the presence of secondary metabolites. Total phenolic content was determined using the Folin-Ciocalteu's method and antioxidant activities using the DPPH free radical scavenging assay and ferric reducing antioxidant power (FRAP). The highest yield of extract was from the ethanol (64.52 ± 0.05%), followed by water (17.65 ± 0.84%) and hexane (4.61 ± 0.56%). Phytochemical screening on the leaves extracts showed the presence of flavonoids, saponins, tannins, alkaloids, terpenoids and steroids. Total phenolic content was highest in the extract of water (0.24 ± 0.02 μg/mL GAE), followed by ethanol (0.19 ± 0.01 μg/mL GAE) and hexane (0.02 ± 0.001 μg/mL GAE). Both free radical scavenging assays of ethanol and water extracts demonstrated stronger antioxidant activities in which the IC_{50} values were 79.48 μg/mL and 75.98 μg/mL respectively, than the commercial butylated hydroxyl toluene which was 197.60 μg/mL. A strong antioxidant capacity in both water (751.75 ± 12.36 mg/mL) and ethanol 741.64 ± 6.96 mg/mL) extracts were also shown in the FRAP method. The highest total phenolic content and antioxidant activities in the leaves extract of Syzygium zeylanicum indicated that the plant has the potential to be used as a functional food.

Key Words: *Syzygium Zeylanicum, Phytochemicals, Total Phenolics, Antioxidant*.

Introduction

Syzygium zeylanicum belongs to the family Myrtaceae, is also known in Malaysia by its local name as Gelam Tikus or Kelat Nenasi. It is an evergreen large shrub which can grow up to 8 m height with widespread branches. When the tree blooms, it produces white flowers and fruits, giving the tree a snowy effect. *Syzygium zeylanicum* is considered as an edible wild plant and in some regions the young leaves

have been used as salad to be eaten with rice or laksa (a type of traditional noodle in Malaysia). *Syzygium zeylanicum* was reported to have stimulant, antimicrobial, vermifuge and anti-rheumatic effects (Pullaiah, 2006). The extracts of water and methanol from the plant were identified to have positive relationship among alpha-glucosidase inhibitory activities, antioxidant activities and polyphenol content (Mai et al., 2007).

Synthetic antioxidants for example butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA) have known to cause toxicity at fairly high doses, thus limit their usage (Peteros & Uy, 2010). Effords have been made to identify new resources from natural antioxidants compounds which recognized as being safer and more effective in the context of their efficiency and non-toxicity (Dudonne et al., 2009). The presence of particular phytochemicals constituents and its beneficial biological effects of natural sources have attracted nutritional scienties to extensively study on its ability in prevention and treatment of various diseases. Polyhenolic is one of which widely known for its ability to react as antioxidant and its activities are important in maintaining health. Many studies revealed positive coorelation between polyphenolic compounds and antioxidant properties (Katalinić et al., 2010; Kilicgun & Altiner, 2010; Šeruga et al., 2011). Crude extracts of edible plant materials rich in phenolics are increasingly of interest in the food industries because of their potential to retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food.

To date, there have been very few studies on phytochemical constituents in *Syzygium zeylanicum* and its relationship as a potential antioxidant agent. Therefore, the objective of the present study are to investigate the presence of secondary metabolites, the amount of total phenolic content and antioxidant activities in the leaves of the plant spesies.

Methodology

Collection and Preparation of Plant Materials

The study was carried out on the leaves of *Syzygium zeylanicum* obtained from tropical bushes in Beserah, Kuantan, Pahang, Malaysia. The leaves were separated from the brunch, washed, tossed and dried in the drying cabinet for three days at 40^{o}C. The dried samples were ground into powder and stored in air-tight container. Extraction was carried out using three different extractants polarities; hexane, ethanol and water. Ethanol and hexane extractions were carried out using Soxhlet at 60^{o}C for 4 hours or until clear, followed by evaporation using rotary evaporator. For water extraction, the samples were agitated in a water bath at 60^{o}C for 12 hours, centrifuged (Buchi) and filtered the extract through Whatman filter paper No. 42. The filtrates were freeze dried, weighed and stored in plastic screw-cap bottle. The extraction was carried out in triplicates. The extract leaves of samples were weighed for their percentage of mass residue.

Phytochemicals Screening Test

Phytochemicals screening for major secondary metabolites of the leaves were carried out using standard qualitative methods as described by Raaman (2006). The samples extracts were screened for the presence of bioactive compounds such as flavanoids, saponins, tannins, terpenoids and/or steroids and alkaloids.

Total Phenolic Content

The phenolic content was determined using the Folin-Ciocalteu (FC) method (Blainski et al., 2013) with slightly modifications. Ethanol crude extract of 0.1 g was weighed and dissolved in 5 ml methanol, then made up to 100 ml with distilled water to get a final concentration of 1 mg/ml. 0.4 ml of sample was taken from the stock solution and added with 2 ml of 50% Folin-Ciocalteu's reagent and mixed well using vortex mixer for 5 minutes. The solution mixture was allowed to react for 5 minutes before further reacted with 4 ml of 5% Na_2CO_3 and placed in a dark for 1 hour. The absorbance was measured at 765 nm using UV

visible spectrophotometer with distilled water as blank. Stock solution of gallic acid (GA) was prepared with the concentration of 500 µg/ml standards in the range of 50 – 500 µg/ml. The absorbance values were compared with the calibration curves of standard and determined using a simple regression test. All the experiments were done in triplicate and the result obtained were expressed in µg/ml.

DPPH Free Radical Scavenging Assay

The antioxidant activity of the leaves extracts was determined using 1, 1 – diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay described by Brand-Williams, et al., (1995) and Kulisic, et al., (2004) with slightly modification. The crude was mixed with 95% methanol to prepare the stock solution of 1,250 – 50,000 µg/ml. A series of test samples are prepared from the stock solution by diluting with methanol to attain a concentration within range 20 - 50,000 µg/ml. DPPH solution of 24µg/ml was feshly prepared in methanol to measure percentage of DPPH free radical scavenging activities. The selected crude extract solution of 1 ml was added to each of the test tube containing 6 ml of DPPH solution. The absorbance was read at 517 nm after 10 minutes reaction using uv-vis spectrophotometer. Butylated Hydroxyl Toulene (BHT) was used as a positive control with 100 µg/ml concentration. The DPPH solution without sample was used as negative control while a solution of 95% methanol was used as a blanck in this test. Percent scavenging of the DPPH free radical was measured using the following equation:

$$\% \text{ DPPH radical scavenging} = [1 - (A_s/A_c)] \times 100$$

$$A_c = \text{Absorbance}$$

$$As = \text{Absorbance of sample solution}$$

Ferric Reducing Antioxidant Power (FRAP) Assay

Frap assay was performed as in the method described by Benzie and Strain (1999) with slightly modification. 0.4 mL extracted samples were mixed with 6 mL FRAP reagent in test tubes and undergoes vortex. Blank samples and leaves extracted samples were prepared and incubated in water bath for 30 minutes at 37°C. The absorbance of samples was determined against blank at 593 nm. An aqeous solution of $FeSO_4.7H_2O$ was used to prepare as a standard curve by using a series of stock solution at the range of 100 – 600 mg/L.

Result and Discussion

Extraction of Raw Material

Ethanol extract of *Syzygium zeylanicum* leave contains the most material in term of yield of mass with the average of 64.52 ± 0.05%, followed by water and hexane with the average of 17.65 ± 0.84% and 4.61 ± 0.56% (Table 1). The characteristic of the ethanol extract was slightly sticky and dark green colour mass. This character observed for ethanol leaves extract was similar with the result obtained by Anoop and Bindu (2014) on the same species. Meanwhile, the hexane and freeze dried water extracts have character of yellowish sticky mass and brown powder, relatively.

Table 1: Percentage of yield extracted by each of the three extractant of dried leaves

Extractant	Percentage of extract[a]
Water	17.65 ± 0.84%
Ethanol	64.52 ± 0.05%
Hexane	4.61 ± 0.56%

Note. [a] Value as mean ± SD and analyzed individually in triplicate

Phytochemical Screening of Leaves Extracts

Preliminary phytochemical screening of *Syzygium zeylanicum* leaves extract revealed the presence of all the investigated compounds including flavanoids, saponins, tannins, alkaloids and terpenoids and steroids (Table 2). Ethanol was considered as the best extractant due to its ability to extract all types of the compounds. The effectiveness of ethanol in extracting compounds could be associated to its properties as a universal solvent that able to extract both polar and non-polar compounds. Alkaloid was solely absent in the aqueous extract of the plant. Meanwhile, the hexane extract indicated the presence of terpenoids, steroids and alkaloids. Two difference types of tannins detected in the leaves, in which gallic acid in aqueous extract and catecholic acid in ethanol extract. Previous study on *Syzygium zeylanicum* also showed the presence of various chemical constituents such as alkaloids, glycosides, phenolics, flavanoids, tannins, saponins, carbohydrates and steroids (Anoop & Bindu, 2014).

Table 2: Phytochemicals constituent present in *Syzygium zeylanicum* leaves extracts

Phytochemicals	Agents of Extraction		
	Hexane	Ethanol	Water
Flavanoids	-	+++	++
Saponins	-	+	++
Tannins	-	++ (gallic, dark blue)	+++ Catecholic, dark green)
Terpenoids & steroids	+	+++	++
Alkaloids (Dragendroff's reagent)	ND	ND	ND
Alkaloids (Mayer's reagent)	++	+++	-

Note: Note : - = absent + = Slightly clear ++ = Medium clear
+++ = Very clear ND = Not detected

Total Phenolic Content (TPC)

Gallic acid was used as representative of phenolic compounds present in the three different extractants; hexane, ethanol and water. The highest phenolic content in *Syzygium zeylanicum* leaves extract was identified in water crude extract with the concentration of 0.24 ± 0.02 µg/mL GAE, followed by ethanol and hexane, 0.19 ± 0.01 µg/mL GAE and 0.02 ± 0.001 µg/mL GAE), respectively (Table 3). Although ethanol extraction gave the highest yield than water, but the amount of total phenolic content was lower than water.

Table 3: Total phenolic content, radical scavenging activity (IC_{50}) and FRAP value of the extracts of *Syzygium zeylanicum* leaves

Extractant	TPC Mean of Concentration[a] µg/mL GAE	IC_{50} value µg/mL	FRAP mg/mL samples[a]
Water	0.24 ± 0.02	75.98	751.75 ± 12.36
Ethanol	0.19 ± 0.01	79.48	741.64 ± 6.96
Hexane	0.02 ± 0.001	3591.77	ND
BHT	NR	197.60	NR

Note. [a] Value as mean ± SD and analyzed individually in triplicate
IC_{50} = the concentration of substrate that cause 50% loss of the DPPH color.
ND = Not Detected
NR = Not Relevant

Antioxidant Activity

The aqueous and ethanol extracts of *Syzygium zeylanicum* considered as almost equal antioxidant activities in which IC_{50} value for water and ethanol extracts were 75.98 µg/mL and 79.48 µg/mL, respectively (Table 3). Interestingly, it was found that both the water and ethanol extracts indicated higher antioxidant activities than the BHT standard (commercial antioxidant). This means that the standard required higher concentration up to 197.60 µg/mL than the water and ethanol extract in order to achieve a 50% radical scavenging. Antioxidant activity exhibited by hexane extract was extremely low and in fact in the FRAP method the activity could not even detected. The result for phenolic content was consistent with the value of antioxidant activities as indicated both in DPPH radical scavenging and FRAP assay. Water extract containing highest phenolic compound showed the highest antioxidant capacity than the ethanol, which was slightly lower. Higher FRAP value indicated higher antioxidant capacity because it is based on reducing ion, where antioxidants are the reducing agent. Meanwhile,

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