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Potential of Malaysian Cherry Leaves (*Muntingia calabura*) as an Antioxidant Agent

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ABSTRACT

Muntingia calabura has a high phytochemical content, especially the phenolic group that can act as antioxidant. In Malaysia country, this *M. calabura* also known as 'kerukup siam' or 'Ceri Kampung' and it belongs to *Muntingiaceae* family. This research was conducted to determine the potential of antioxidant activity application of cherry leaves (*M. calabura*) from various solvent extracts (methanol, ethyl acetate, and n-hexane). The phytochemical contents were screening by using the established standard procedure. Total phenolic content (TPC) was determined according to the Folin-Ciocalteau colorimetric method, while the antioxidant activity was carried out using 2,2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging assay. Phytochemical screening on the leaves part methanolic extracts revealed that the presence of various biochemicals like flavonoids, phenols, steroids, triterpenes, tannins, reducing sugars, and saponins except the alkaloids. Among the three extracts, the methanol leaf extract gave the highest content of phenolics (8.20 mg GAE/g extract). Analyses of antioxidant activity with DPPH method showed that cherry leaf methanolic extracts produced high antioxidant activity with IC_{50} value of 167.70 µg/mL. The present study confirms that the presence of various phytochemicals which shows good antioxidant activity of *M. calabura* leaves. Therefore, it has the potential as a therapeutic antioxidant agent and can be used in cosmeceutical and food products.

Keywords: *Muntingia calabura; Ceri kampung; Total phenolic content; Antioxidant*

INTRODUCTION

Muntingia calabura is one of the many species from the family *Muntingiaceae* used in folk medicine throughout the world. It is commonly known as the Jamaican cherry in Brazil and 'Kerukup Siam' in Asian region, including Malaysia. Various parts of *M. calabura* such as leaves, barks, flowers, and roots have been employed as a treatment for traditional medical, prevention, rehabilitation and health promotion [1].

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Mostly, plants are producing a variety of secondary metabolites or bioactive compounds such as flavonoids [2][3], phenolics [4], tannins [5], quinones [6], glycosides [7] and terpenes [8] that are used as herbal medicine. The scientific evaluations on *M. calabura* have been revealed several pharmacological activities possessed by the plant. *M. calabura* leaves have been reported to exhibit significant anti-inflammatory, antipyretic [9], antinociception [10], antitumor [11], antiproliferative, antioxidant [9], and antibacterial [11] activities. Flavonoids, saponins, tannins, triterpenes, and steroids have been detected also in the leaves of *M. calabura* [3]. Several types of flavonoids have been isolated and identified from the leaves, roots, and stem barks of *M. calabura* [12].

Natural antioxidants have been used also in the cosmetic industries including a great number of substances and extracts obtained from a variety of plants, grains and fruits, either by reducing the skin oxidative stress or protecting the skin from oxidative degradation [13]. Plants that are efficacious as antioxidants are plants that contain carotenoids and polyphenols, especially flavonoids which can be formulated as natural antioxidants in oral dosage forms such as vitamins and topicals for skin care products. Therefore, the aim of this study was to screen the phytochemical present in leaf extracts with three types of solvent polarities using maceration method, to determine the antioxidant activity and total phenolic content of *M. calabura*.

EXPERIMENTAL

Materials and Instruments

The list of solvents used are acetone, methanol, ethyl acetate, and n-hexane were purchased from (HmBG chemicals) while the list of chemicals used are gallic acid, Folin-Ciocalteu reagent were purchased from (R&M Chemical Supplier), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from (QReC). Instrument used is T80/T80+ UV-Visible spectrometer in the range 500-700 nm.

Plant material and preparation of extracts

M. calabura leaves were collected in August 2018 from the Alor Gajah, Melaka. The fresh leaves were air-drying for about two weeks at room temperature and grinded into a fine powder. The extracts were prepared by maceration of 100 grams of the powdered sample in methanol, ethyl acetate, and n-hexane (800 mL of each), by using orbital shaker for 72 hrs at room temperature. Then, the extracts were filtered, and concentrated via rotary evaporation (IKA HB 10) at 40 °C. Crude extracts were stored in sealed containers until further analysis [14].

Phytochemical Screening

The leave extracts from of *M. calabura* were subjected to phytochemical screening to test the presence of tannins [15], flavonoids [16], alkaloids, phenols [17], steroids, triterpenes, saponins, and reducing sugar [18] following the standardized methods [19].

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Determination of Total Phenolic Content (TPC)

Total phenolic content (TPC) of methanol, ethyl acetate and n-hexane extracts of *M. calabura* leave was carried out by using modified Folin-Ciocalteau colorimetric method [20]. Stock solution of gallic acid was prepared by dissolving 0.01 g of gallic acid and marked up in 100 mL of volumetric flask with distilled water. The mixture was left in the dark for 1 hour. The absorbance of the samples was measured at 760 nm using a UV-Vis Spectrometry. Results were expressed as mg gallic acid equivalent (GAE)/g plant extract. All the experiments were carried out in triplicate. The average absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve.

Antioxidant assay

The DPPH radical scavenging activity of *M. calabura* has been tested by following the reported method with modifications [21]. The sample in methanol (0.2 mL) with concentrations ranging from 7.81 until 1000 µg/mL and were mixed with the DPPH solution (3.8 mL). The reaction mixture was then left to stand in the dark for 30 min at room temperature. The absorbance of the reaction mixture was record at 517 nm. Ascorbic acid was applied as a standard antioxidant, while DPPH solution was used as DPPH blank. The percentage inhibition DPPH was calculated using the following Equation 1:

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

All tests were performed in triplicates and were expressed as mean ± standard deviation. The graph of percentage inhibition was plotted against the concentration (in µg/mL) to determine the concentration of *M. calabura* extract required to scavenge 50% of DPPH free radicals. The results were reported as IC_{50} values (in µg/mL).

RESULTS AND DISCUSSION

Phytochemical analysis

Freshly prepared extracts were subjected to a preliminary phytochemical screening for various constituents. The results of the phytochemical analysis of the different extracts (n-hexane, ethyl acetate, and methanol) of cherry leaf have shown a remarkable variation, are presented in Table 1. The methanolic extracts showed presence of secondary metabolite classes due to high quantity of flavonoids, phenols, steroids, triterpenes, tannins, reducing sugars, and saponins with absence of alkaloids.

On the other hand, ethyl acetate and n-hexane extract revealed the flavonoids, phenols and steroids. These constituents have ability to be hydrogen donor makes it suitable to acts as antioxidants [22]. Meanwhile, the three of solvent extraction was absent in the alkaloids test for *M. calabura*. The present study regarding the qualitative analysis of the selected medicinal plants is in agreement with the previous findings by the various researchers [23-27].

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Table 1: Phytochemical profiles of Cherry leaf (*M.calabura*) extracts

Leaf Extracts	Constituents							
	Alkaloids	**Flavonoids**	**Phenols**	**Steroids**	**Tannins**	**Saponins**	**Triterpenes**	**Reducing sugars**
n-hexane	-	+	+	+	-	-	+	-
E. Acetate	-	++	++	+	-	-	-	-
Methanol	-	+++	+++	++	++	++	++	+

- = absent; + = less present; ++ = moderate; +++ = high [28]

Based on the above discussion, the phytochemicals present in the crude extracts of *M. calabura* leaf can serve as a valuable source of information and provide appropriate standards to establish a base for identification and elucidation of the different types of bioactive chemicals.

Quantitative Total Phenolic Content and DPPH Radical Scavenging Activity

Total phenolic content and DPPH radical scavenging activity of n-hexane, ethyl acetate, and methanol extracts of the leaves of *M. calabura* are presented in Table 2. Among the extracts, the methanol extract was displayed the highest total phenolic content (8.20 mg GAE/g) and the lowest IC_{50} value (167.70 µg/mL) as compared to the ethyl acetate and n-hexane extract. In this study the DPPH method was used to obtain IC_{50} values from a plant extract.

IC_{50} is the concentration of the sample to inhibit 50% of free radicals. Experimentally, the purple color of DPPH solution decolorized into yellow color as hydrogen from the antioxidant source was accepted. The lower the IC_{50} value, the higher the antioxidant properties of the extracts sample and vice versa [29]. Based on the results of the study of IC_{50}, leaves methanol extract of *M. calabura* is very strong antioxidant due to the lower value of DPPH, while for IC_{50} of leaves n-hexane extract is slightly higher due to the lower of total phenolic content.

Table 2: Total phenolic compounds (TPC) and antioxidant activity (DPPH) of different phenolics fractions of Cherry leaf (*M. calabura*) extracts

Assay	Solvents		
	n-hexane	**Ethyl Acetate**	**Methanol**
TPC (mg GAE/g extract)[a]	2.80±0.0	4.42±0.0	8.20±0.0
DPPH (IC_{50}, µg/mL)[a]	408.80±0.5	404.03±0.7	167.70±0.6

[a]Data represent mean ± standard deviation of three replicate experiments;
Positive control (ascorbic acid = 10.68±0.9 µg/mL)

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It was reported that the IC_{50} values obtained from *M. calabura* leaves extracts were: 496.18±4.56 µg/mL for petroleum ether extract, 107.99±6.24 µg/mL for chloroform extract, 79.96±0.91µg/mL for ethanol extract, and 97.638±2.06 µg/mL for aqueous extract in comparison to ascorbic acid 40.43±3.95 µg/mL [30]. However, their IC50 values were higher than that of ascorbic acid (10.68 µg/mL). It can be seen that the methanol extract of *M. calabura* has the strongest antioxidant activity because it has a very high content of phenolic compounds. The radical scavenging activity of the extracts were in the order of polarity of solvent methanol > ethyl acetate > n-hexane.

Also, the result are agreeing with the previous study who are reported that the methanol extract of *M. calabura* was shown the significant antioxidant activities, due to high containing of phenolic compounds [31]. From the phytochemical screening, the flavonoids and phenolics compound had the strongest presence in methanol extracts of *M. calabura* as shown in Table 1. These compounds were give the inhibition against the free radical for antioxidants assay. Therefore, these results indicated that the antioxidant activity of extracts was well correlated with TPC [32].

CONCLUSIONS

The phytochemical analysis showed that the leaves extracts of *Muntingia calabura* contain flavonoids, phenols, steroids, triterpenes, tannins, reducing sugars, and saponins. The significance TPC value and the potent antioxidant activity (DPPH) of the leaf methanol extract indicated that this plant could be beneficial as a source of natural antioxidant. From these findings, it is suggested that the methanol extract can be further subjected for the isolation and characterization of phytochemicals that have antioxidant effect.

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