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PRELIMINARY PHYTOCHEMICAL EVALUATION AND VITAMIN C CONTENT OF TOLIDUS (*Hornstedtia havilandii* (K. Schum) K. Schum) FROM SABAH

(Penilaian Awal Fitokimia dan Kandungan Vitamin C *Tolidus* (*Hornstedtia havilandii* (K. Schum) K. Schum) dari Sabah)

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ABSTRACT

Most of the plants in the ginger family *Zingeberaceae* are well-known for their medicinal properties. However, the genus *Hornstedtia* found in Sabah is less reported. This research aims to investigate the phytochemical constituent and vitamin C content of a fruit, locally known as the *Tolidus* fruit in Sabah. The dried fruit sample was extracted using three solvents which were water, ethanol and methanol. The phytochemical constituents were determined using standard Colour Test for the presence of alkaloid, flavonoid, saponin and tannin. Then, the content of Vitamin C was determined using the standard Colorimetric Titration and ascorbic acid as standard. The phytochemical evaluation revealed that all three targeted constituents were present in all extracts except for the alkaloid. The vitamin C content was determined in both dried and fresh sample of fruits, where 52.84 mg was quantified in the fresh fruit aqueous extract and 23.93 mg in the dried fruit aqueous extract respectively. These results are comparable to the content of vitamin C in orange and lime fruits. The phytochemical evaluation and vitamin C content of *Tolidus* suggested the potential of this underutilised fruit to be the natural and affordable source of vitamin C. Additionally, may protect the body against harmful free radicals. However, further analysis is needed to determine other constructive natural contents and evaluate the efficacy of this fruit as a natural source of antioxidant.

Keywords: *Tolidus*, phytochemical, vitamin C

1. Introduction

The Zingiberaceae family has been utilised for various purposes worldwide and has been a part of Asian culture since time immemorial. Researchers are keen to study the many species of the Zingiberaceae family due to them famed as spices, medicine and aesthetics, and their benefits in common remedy (Trimanto 2017). In Malaysia, member of the Zingiberaceae family is widely used as spice, condiment, food flavouring, vegetable, beverage, medicine, ornamental as well as in rituals associated with beliefs, customs and traditions (Ibrahim *et al*. 2007). Most Malaysian ginger is edible and are consumed fresh, cooked, pickled or boiled, whereas the plant parts that are usually consumed are the rhizomes (Jani *et al*. 2014). However, the inflorescences, fruits, seeds, young shoots and rarely leaves are also utilised (Kulip 2007). In Sabah, about 26% of all known Borneon Zingiberaceae is used as medicine, food, ornamentals and construction purposes (Kulip 2007).

The most common ginger utilisations by the Sabahan, are from the genus *Etlingera*, *Alpinia* and *Zingiber* (Sakai & Nagamasu, 2006). In this research, the genus *Hornstedtia* was selected to be studied for its phytochemical constituent and vitamin C content. In several ethnobotanical studies regarding Zingiberacea, the biochemical analysis of *Hornstedtia* is the least reported (Hlavatá 2012; Waruruaia *et al*. 2012). In this study, the species of *Hornstedtia* that are known to be consumed by the indigenous people in Sabah are also known locally as many names according to different ethnicities such as *Tolidus*, *Talirus*, *Senggang*, *Taridus* and *Tapus*. The

fruit of *Tolidus* is eaten raw or cooked by the indigenous people in Sabah. The fruit has a sweet and sour taste. The locals claimed that the fruit is able to reduce fever (Kulip 2007). *Tolidus* is known amongst rural communities only and as a traditional fruit. However, it is unknown to the other communities. The taste and colour of the fruit indicated that it has nutritional values as a source of vitamin C and antioxidant property. Therefore, it is necessary to conduct ~~a~~ scientific research about this fruit to discover its benefits as well as providing baseline study reports for other researchers in the future.

2. Materials and methods

Sample collection and preparation

The fruits of *Tolidus* were collected in Kota Marudu, Sabah, Malaysia. The fruits were rinsed with running water to remove dirt and then air-dried for three weeks until a constant weight was obtained. The dried fruits were grounded into powder and stored in an air-tight container until required for further use.

Extraction of Tolidus

The powdered form of *Tolidus* was extracted using the maceration method by soaking the sample with solvent in 1:10 ratio for 72 hours with constant shaking (Madike *et al*., 2017). The powdered sample was extracted in aqueous, ethanol and methanol (80%). Then, the mixture was filtered through Whatman No 1 filter paper and the solvent was evaporated using a rotary evaporator. The crude extracts were stored at -20 °C until further use (Hassan *et al*., 2017).

Phytochemical screening

Qualitative phytochemical screening of the targeted compounds was done using standard Colour Test according to the following methods.

Detection of alkaloid

A total of 5 mg of extracts were stirred separately with 1% HCl (2 mL) on a water bath for five minutes and filtered. Few drops of the Wagner's reagent were added to the filtrate. Formation of a brown coloured precipitate indicates the presence of alkaloids (Iqbal *et al*. 2015). Wagner's reagent: in (5 mL) of distilled water were dissolved with potassium iodide (2 g) and iodine (1.27 g) and the solution was diluted to 100 mL with distilled water (Iqbal *et al*. 2015).

Detection of flavonoids

Extracts were added with a few drops of sodium hydroxide (NaOH) solution. After a few minutes, the changes from a light-yellow colour to colourless after the addition of dilute hydrochloric acid indicates the presence of flavonoids (Fahal *et al*. 2018).

Detection of saponins

A total of 3 mL of distilled water and 3 mL plant extracts were mixed and shaken gently. The formation of a stable persistent froth was taken for the presence of saponins (Madike *et al*. 2017).

Detection of tannins

A total of 1 mL of 3 % of ferric chloride and 1 mL of the extract were mixed. The formation of brownish-green colour development indicated the presence of tannins (Fahal *et al*. 2018).

Determination of Vitamin C content

Preparation of the titration method of vitamin C

Preparation quantification of vitamin C both standard and sample from the fresh and dried fruits of *Tolidus* was analysed using the titration method (Chowdhury *et al*. 2016).

Preparation of reagents

Preparation of 0.1 M iodine solution

0.1 M iodine solution 10 g of potassium iodide (KI) and 35 mL of distilled water was mixed and heated. Then, the solution was cooled to room temperature and added with 3.15 g of iodine powder. After that, preparation 0.005 M of iodine solution where 2 g of KI were dissolved in 100 mL of distilled water and 1.3 g of iodine powder was stirred with a small quantity of water to 1 litter (Chowdhury *et al*. 2016).

Preparation of 0.5 % starch solution

In 50 mL warm distilled water, 0.25 g of starch powder was dissolved as the starch was not soluble in cold water and needed to be boiled to stay in solution (Chowdhury *et al*. 2016).

Preparation of standard solution ascorbic acid

10 mg ascorbic acid were dissolved in 100 mL of methanol (Chowdhury *et al*. 2016).

Preparation of sample solution for vitamin C quantification

100 g of fresh fruit was blended with 50 mL of distilled water. Then, the pulp was strained through a cheesecloth. The extract was make-up to 100 mL in a volumetric flask (Chowdhury *et al*. 2016).

Standard Colorimetric Titration

Standard solution and sample solution with iodine solution 20 mL of the standard solution or sample balance with 150 mL distilled water, separately. The titrant, either sample solution or standard solution was run against analyse iodine solution. About five to six drops of starch solution were added to the analyte and the evaluation of titration began. The outcome will be noted when analyse present blue in colour (Chowdhury *et al*. 2016).

Quantification of vitamin C content

The quantification of vitamin C content was determined using the method used by Ciancaglini *et al*. (2001). Relative molecular mass (MW) of ascorbic acid which is 176 and amount of vitamin C can be calculated in g/L with the equation: Mass Vitamin C (g) = M juice vitamin C × V Juice (1) × MW vitamin C.

3. Results and Discussion

Phytochemical screening

The phytochemical compounds of alkaloid, flavonoid, saponin and tannin from the *Tolidus* fruit were extracted using water, ethanol and methanol respectively are shown in Table 1. A polyamine alkaloid known as squalamine leads to bacterial membrane disruption by exerting detergent-like mechanisms against Gram-negative bacteria that cause disturbance of the outer membrane as well as depolarizing Gram-positive bacterial membranes and quinoline alkaloid also act as antimalarial agent that by killing the malaria parasites during development stage in the liver and red blood cells (Othman *et al*. 2019; Widelski 2017).

Saponin acts as a natural antioxidant that reacts by avoiding the oxidation of cholesterol in the colon through attaching to the cholesterol, changing its biological process (Dorota *et al*.

2017). As for tannins, the biological activity as antimicrobial agent happens by binding with proteins through covalent and non-covalent interfaces that are ultimately incapacitating microorganisms by changing the morphology, inhibiting growth and protease activity of microorganisms (Othman *et al*. 2019).

Polyphenols in flavonoids pass through gastrointestinal system without being absorbed, which then affect intestinal microbiota by inhibiting pathogenic bacteria and enriching beneficial bacteria, reducing the risk of gastrointestinal cancer (Othman *et al*. 2019).

Table 1 Phytochemical constituents of dried fruit extracts using different solvents

Compound	Water	Ethanol	Methanol
Alkaloid	-	-	-
Flavonoid	+	+	+
Saponin	+	+	+
Tannin	+	+	+

Key: Present (+), Absent (-)

Quantification of vitamin C of Tolidus fruit aqueous extract and other selected citrus fruits

Table 2 shows the quantity of vitamin C in the fruit using titrimetric analysis including vitamin C from other citrus fruits. The results showed that the high content of vitamin C was present in fresh fruit extract, which is 52.84 mg compared to dried fruit extract, which is 23.93 mg. Fresh fruit extract contains higher vitamin C because dried extract became dehydroascorbic acid which causes vitamin C content being oxidized. Factors that cause the oxidation of vitamin C include pH, light, temperature and oxygen (Ishaq & Obirinakem 2015).

Table 2 The quantity of vitamin C in the fruit using titrimetric analysis including vitamin C from other citrus fruits

Fruit	**Amount of Vitamin C**
Orange	56.30 mg*
***Tolidus* (fresh)**	**52.84 mg**
Grapefruit	49.15 mg*
Lemon	43.96 mg*
Lime	27.78 mg*
***Tolidus* (Dried)**	**23.93 mg**
Musk Lime	18.62 mg*

*source**:** Najwa and Azrina (2017)

The present study reveals that *Tolidus* has essential vitamin C and could act as an antioxidant. Antioxidant activity of vitamin C can prevent the development of certain diseases such as neurodegenerative diseases, cancer, heart diseases, common cold and diabetes. Vitamins C also could protect our body against the damage effects of free radical (Ciancaglini *et al*. 2001).

4. Conclusions

The experimental work concludes that *Tolidus* fruit aqueous, methanol and ethanol extracts possess phytochemical compounds with the potential antioxidant property which are flavonoid, tannin and saponin. However, the compound alkaloid is absent in all extracts. The fruits also contained high vitamin C, especially in the fresh fruit. Therefore, it is suggested that *Tolidus* can be the natural and affordable source of vitamin C and antioxidant agent. However, further analysis is needed to determine other constructive natural contents and evaluate the efficacy of this fruit as a natural source of antioxidant. Further investigation is also prominent to determine more potential bioactivity and nutritional composition of *Tolidus*.

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