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THE PHYTOCHEMICAL SCREENING AND ANTIOXIDANT POTENTIAL OF *CINNAMOMUM JAVANICUM* BLUME LEAVES FROM CENTRAL KALIMANTAN

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Background: *Sintok lancang (Cinnamomum javanicum Blume.) is one of the typical plant of Central Kalimantan, which has not been widely studied. Local people use leaves of C. javanicum to treat various diseases, like diabetes and skin diseases. Based on this, it is necessary to do preliminary study to know phytochemical content and antioxidant potential that may be contained in this plant.* ***Methods:*** *C. javanicum leaves were extracted using percolator with 96% ethanol. The extract was tested for qualitative phytochemical with standard procedures, while the antioxidant test was carried out using 1,1-diphenyl-2-picrylhydrazyl (DPPH) method with quercetin as standard and ferric reducing antioxidant power (FRAP) with trolox as standard.* ***Results:*** *The result showed ethanolic extract of C. javanicum leaves qualitative phytochemical contained alkaloid, flavonoid, tannin and steroid. Antioxidant activity test showed leaves of C. javanicum with DPPH method IC_{50}= 26.99±0.27 ppm and antioxidant activity with FRAP method 779.73±19.66 µmoltrolox/g.* ***Conclusion:*** *It can be concluded that C. javanicum leaves have potential high antioxidant activity was tested with DPPH and FRAP method. Furthermore need further research, especially to determine specific compound of C. javanicum leaves.*

INTRODUCTION

Central Kalimantan is one of the tropical forest areas in Indonesia which has biodiversity especially natural medicine but has not been fully utilized. Traditional uses such as traditional medicine have been done, but further exploration and development have not been done much. Sintok lancang leaves (*Cinnamomum javanicum* Blume.) are one of the typical Central Kalimantan plants that have not been studied much, it's found in the Mungku Baru forest (Kawasan Hutan Dengan Tujuan Khusus) or educational forest managed by the Muhammadiyah University of Palangkaraya collaboration with the Borneo Nature Foundation (BNF). Local people use sintok lancang leaves for various diseases such as to treat abdomen pain, wounds, and diabetes.

There have not been many studies on *Cinnamomum javanicum*, especially those that grow in Indonesia, but one of the researches at the University of Jember states that different species, *Cinnamomum Sintoc* leaves has the potential as an antioxidant because it contains secondary metabolites such as flavonoids, alkaloids, steroids, terpenoids, and tannins which could prevent the free radical[1-3]. Antioxidant use is associated with reduced production of ROS (Reactive oxygen species) and free radicals[4-6]. According to previous pieces of evidence, ROS have been implicated in the pathogenesis of various diseases such as cancer, aging, and other diseases. In addition, research related to natural antioxidants are

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considered to contribute to the therapeutic approach in the treatment of COVID-19, although their mechanism against COVID-19 is still unknown and speculative[7]. Several studies provide evidence that a reduction in ROS accumulation slows the apoptosis signaling activated by a coronavirus[8].

In this study, the determination was carried out the total flavonoids, alkaloids and tannins compounds of *Cinnamomum javanicum* leaves. Antioxidant activity test was conducted by using DPPH and FRAP assay. The present data would help to explore the potential of Indonesia's natural plants, especially plants that have not been studied much so it can be further developed for the cosmetic, food and pharmaceutical industries.

METHODS

Plant collection and identification

Fresh leaves of sintok lancang (Fig. 1) were collected in the Mungku Baru forest (Kawasan Hutan Dengan Tujuan Khusus) or educational forest managed by the Muhammadiyah University of Palangkaraya collaboration with the Borneo Nature Foundation (BNF), Palangka Raya, Central Kalimantan and authenticated by Dr. R. Hendrian, M.Sc from Indonesia Institute of Sciences, Research Center for Plant Conservation and Botanic Gardens, Bogor, Indonesia.

Fig. 1: Sintok lancang (*Cinnamomum javanicum* Blume.).

Preparation of Plant Extract

The whole fresh leaves were washed, cleaned and dried in oven at 45°C[9&10]. Generally, the oven drying method is used for herb drying in the temperature range of 40-60°C[11]. The dried leaves were grindered and powdered. Extracted with ethanol 96% by percolation. Percolation is better than maceration because it is a continuous process in which the saturated solvent is constantly being replaced by fresh solvent[12]. The extract were concentrated in a rotary evaporator. The percentage yields (w/w) of the extracts were calculated using the formula[13]:

(Weight of extract ÷ Weight of starting plant material) x 100%

Phytochemical qualitative screening

The ethanolic extract of sintok lancang leaves (*C. javanicum* Blume) was screened for potential presence of alkaloid, flavonoids, tannins, saponins, and steroids by using the following standard methods[14-17].

Determination of the total alkaloid content

Ten mg extract of *C. javanicum* was weighed then dissolved in 10 ml ethanol. One ml extract was measured the absorbance with a spectrophotometer at 272 nm. The standard used for the calibration curve was caffeine[18]. The total alkaloid content was expressed as micrograms of alkaloids per milligrams of the extract.

Determination of the total flavonoid content

Five mg extract of *C. javanicum* was weighed then dissolved in 10 ml ethanol. One ml extract was placed in the volumetric flask, one ml $AlCl_3$ (2% b/v) and 8 ml acetic acid (5% v/v) were added. After mixing, the solution was incubated for 20 minutes[19&20]. The solution was measured the absorbance with a spectrophotometer at 412 nm. The standard used for the calibration curve was quercetin[21]. The total flavonoid content was expressed as micrograms of flavonoids per milligrams of the extract.

Determination of the total tannin content

A total of 30 mg of sample was weighed and put into a 10 ml volumetric flask. The solution of 0.5 ml was added with 3.0 ml of vanillin 4% and 1.5 ml of concentrated HCl. The mixture was incubated for 10 minutes[21-24]. The absorbance was measured with UV Vis spectrophotometer at 498 nm. The standard used for the calibration curve was catechin[25]. The total tannin content was expressed as micrograms of tannins per milligrams of the extract.

Antioxidant activity test with DPPH method

DPPH solution with 0.4 mM concentration was prepared, the absorbance was measured at 512 nm. The absorbance result of the DPPH solution was the absorbance control. The extract of sintok lancang leaves (sample) were first dissolved in methanol with five different concentrations of 10,20,30,40, and 50 ppm. One ml of 0.4mM DPPH solution was placed into a 5 ml volumetric flask, then added with 4 ml of sample solution each of various concentrations. The mixture was stored for incubation for 30 min at room temperature, the absorbance was measured at maximum wavelength (512 nm)[26&27]. The percent inhibition or DPPH scavenging effect was calculated using the following formula:

$$\text{DPPH scavenging effect } \% = \frac{(A - B)}{A} \times 100\%$$

Where, A was the absorbance of DPPH solution and B was the absorbance of sample solution[28&29].

Antioxidant activity test with FRAP method

Sample (0.2 g) dissolved with ethanol with 10 ml volumetric flask. Two ml of sample solution was added to 3 ml of FRAP reagent in a test tube, then incubation for 16 minutes. The absorbance was measured with UV Vis spectrophotometer at 595 nm. Antioxidant activity was expressed as µmol trolox/g[28&30].

RESULTS AND DISCUSSION

Results

Yield of the extract

Four hundred grams of *C. javanicum* fresh leaves were extracted into 51.1 g of extract. Based on the rendement calculation, the extraction of *C. javanicum* yields of 12.8%. Value yield is related to the number of secondary metabolites that successfully attracted when the extraction process[13].

Phytochemical qualitative screening

Phytochemical screening of *C. javanicum* leaves by using the following standard methods[14-17]. The results of the phytochemical qualitative test of sintok lancang leaves showed the presence of alkaloids using Mayer's reagents[31], flavonoids using the Shinoda test[32], tannins and steroids (Table 1).

Table 1: The qualitative phytochemical of ethanolic extract *C. javanicum* leaves.

Phytochemical compound	Result
Alkaloids	+
Flavonoids	+
Tannins	+
Steroids	+
Saponins	-

Antioxidant activity, total alkaloid, flavonoid and tannin content

Ethanolic extract of *C. javanicum* leaves was conducted using percolation method. Antioxidant potential (DPPH & FRAP), total alkaloid content, total flavonoid content and total tannin content was calculated with standard protocols.

The results of antioxidant activity showed that the IC_{50} value of DPPH was 26.99±0.27 ppm quercetin equivalent while the FRAP method was 779.73±19.66 µmol trolox/g. Total alkaloid, flavonoid, and tannin content respectively was 20.82±1.31 µg caffeine equivalent/mg, 76.62±1.22 µg quercetin equivalent/mg, and 23.02±0.24 µg catechin equivalent/mg (Table 2).

Table 2: Antioxidant activity, total alkaloids, flavonoids and tannins content of ethanolic extract *C. javanicum leaves.*

Assay \ Sample	Ethanolic extract of *Cinnamomum javanicum*	Quercetin
Antioxidant		
-DPPH (IC_{50} ppm)	26.99 ± 0.27	6.98
-FRAP (µmol trolox/g)	779.73 ± 19.66	-
Total Alkaloid (µg caffeine equivalent/mg)	20.82 ± 1.31	-
Total Flavonoid (µg quercetin equivalent/mg)	76.62 ± 1.22	-
Total Tannin (µg catechin equivalent/mg)	23.02 ± 0.24	-

Discussion

Based on the results extract of *C. javanicum* leaves contain qualitative phytochemicals like flavonoid. Based on some literature flavonoids can prevent injury due to free radicals in various ways, one of which is the direct scavenging of free radicals. Flavonoids are oxidized by radicals, resulting in a more stable, less-reactive radical[33&34], such as the following equation:

Flavonoid (OH) + R* → Flavonoid (O*)+ RH

Besides that *C. javanicum* leaves contain alkaloids and tannins which may also potential contribute to the effectiveness of antioxidant[35&36]. Alkaloids have antioxidant properties through capturing free radicals, or binding to catalysts involved indifferent oxidation processes occurring within the human body for preventing a variety of degenerative diseases[37]. The free radical scavenging activity of tannins showed by the ability of antioxidant to donate electron to a free radical and produce a more stable and therefore less harmful radical structure. Tannins are able to bond cations of transition metals and act as protective agents against progression of some diseases, e.g. Alzheimer's or Parkinson´s disease. Antioxidant activity of tannins can also be exhibited through inhibition of prooxidative enzymes[38].

There hasn't been much study on *C. javanicum* leaves. However, some studies have stated that *C.javanicum* found in Sumatra can reduce fever, others claim that it has antioxidant potential but there are no studies of *C.javanicum* that grows in Kalimantan[39&40].

Yuan et al stated that *C.javanicum* both leaf and stem extract showed antimicrobial activity against *Listeria monocytogenes*[41]. The essential oil of *C.javanicum* also showed antibacterial activities against four strains of food pathogenic bacteria: *Staphylococcus aureus* (ATCC 29213), *Listeria monocytogenes* (ATCC 7644), *Salmonella typhimurium* (ATCC 25922) and *Salmonella enteritidis* (ATCC 29213)[42]. The various study results can be the basis for further research.

According to some literature, antioxidant activity with DPPH method classified by IC_{50} into: very strong (< 50 ppm), strong (50-100 ppm), moderate (101-150 ppm) and low (> 150 ppm)[28&43], while antioxidant activity with FRAP method classified into very low FRAP (< 10 µmol/g), low FRAP (10-50 µmol/g), good FRAP (50-100 µmol/g), high FRAP (100-400 µmol/g), and very high FRAP (> 400 µmol/g)[44]. The ethanolic extract of *C. javanicum* leaves included in the very strong antioxidant activity (26.99±0.27 ppm) and very high FRAP (779.73±19.66 µmol trolox/g). When compared with *C. javanicum* leaves study conducted in Malaysia with DPPH method (223.5 ppm)[45], *C. javanicum* leaves in Indonesia, especially Central Kalimantan give better antioxidant activity.

DPPH is a stable free radical with an unpaired electron that is delocalized over the entire molecule. The DPPH assay is based on both electron transfer and hydrogen atom transfer reactions. The benefits of the DPPH assay is easy to do, rapid method and economic. Even though the DPPH assay is simple, its sensitivity may be affected by several factors, such as the type of solvent, reaction time, temperature and freshness of DPPH reagent[46&47], while the FRAP assay is a non-specific, redox-linked, colorimetric assay that is related to the molar concentration of the antioxidant present. The FRAP assay is a typical electron transfer based method that measures the reduction of ferric ion (Fe^{3+})-ligand complex to the ferrous (Fe^{2+}) complex by antioxidants in acidic media. One limitation of FRAP assay is the tendency to precipitate, forming a suspension and staining the measuring cuvette. Therefore, time to add $FeCl_3$ is essential to prevent error interpretation. However, the FRAP or DPPH assay is simple, economic, rapid and not require specialized equipment[47].

Antioxidants are widely used for protection of various diseases such as coronary heart, cancer. Besides that, it is also often applied at the industrial level such as cosmetic for anti-aging and acne[48]. The role of the food industry and mass media were explored to focus on health promotion of the older person in the present[49].

Based on the results *C. javanicum* leaves has the potential to be cultivated to keep sustainability and developed into antioxidant material.

Conclusion

It can be concluded ethanolic extract of *C. javanicum* leaves has the potential high antioxidant activity tested by DPPH and FRAP methods. Furthermore, it is necessary to further identify the specific compounds that have antioxidant activity in *C. javanicum* leaves.

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نشرة العلوم الصيدلية
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المسح الكيميائي النباتي والفاعلية كمضادات الأكسدة لأوراق سيناموم جافانيكم بلوم من كاليمانتان الوسطى

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[١]قسم الصيدلة ، كلية العلوم الصحية ، جامعة المحمدية في بالانغكارايا ، بالانغكا رايا ، كاليمانتان الوسطى ، إندونيسيا

[٢]قسم الغابات ، كلية الزراعة والغابات ، جامعة المحمدية في بالانكارايا ، بالانجكا رايا ، كاليمانتان الوسطى ، إندونيسيا

الخلفية: نبات سيناموم جافانيكم بلوم هو أحد النباتات النموذجية في وسط كاليمانتان ، والتي لم تتم دراستها على نطاق واسع. يستخدم السكان المحليون أوراق سيناموم جافانيكم بلوم لعلاج أمراض مختلفة ، مثل مرض السكري والأمراض الجلدية. بناءً على ذلك، من الضروري إجراء دراسة أولية لمعرفة المحتوى الكيميائي النباتي والفاعلية كمضادات الأكسدة لهذا النبات.

الطريقة: تم استخلاص أوراق نبات سيناموم جافانيكم باستخدام ٩٦٪ إيثانول باستخدام جهاز البركلاتور. تم مسح المستخلص لمعرفة المواد الكيميائية النباتية باستخدام الإجراءات القياسية ، بينما تم إجراء اختبار مضادات الأكسدة باستخدام طريقة ١,١ دايفينيل ٢ بكريلهيدرزيل (DPPH) مع المقارنة مع كورسيتين كمرجع وطريقة FRAP بالمقارنة مع ترولوكس كمعيار.

النتائج: أظهرت النتائج أن المستخلص الإيثانولي لأوراق سيناموم جافانيكم يحتوي على قلويد وفلافونويد وتانين وستيرويد. أظهر اختبار الفعالية المضادة للأكسدة أوراق سيناموم جافانيكم باستخدام طريقة DPPH IC50 = ٢٦,٩٩±٠,٢٧ جزء في المليون ونشاط مضاد للأكسدة بطريقة FRAP ١٩,٦٦±٧٧٩,٧٣ ميكرومول تروكس/جم.

الخلاصة: يمكن الاستنتاج أن أوراق سيناموم جافانيكم لها نشاط مضاد للأكسدة مرتفع تم اختباره باستخدام طريقة DPPH و FRAP. علاوة على ذلك ، تحتاج إلى مزيد من البحث ، خاصة لتحديد مركبات معينة من أوراق سيناموم جافانيكم.