PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF KADUK (*PIPER SARMENTOSUM*)

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Abstract

Piperaceae species especially Kaduk (Piper Sarmentosum) is one of the most valuable herbs. Therefore, this study was focused on examining aimed the extraction of kaduk leaves, to determine the compounds in the extracted sample and investigate their antibacterial features. The grinded kaduk leaves (Piper sarmentosum) were extracted by using two different solvent namely chloroform and ethanol by using hot extraction method. The percentage yield obtained for ethanol extract was 10.5%, higher as compared to the percentage yield of chloroform extract, which was 8.92%. The ethanol extract has indicated the presence of most phytochemicals such as flavonoid, tannin, terpenoid, alkaloid and phenolic compounds. Meanwhile, chloroform extract showed only the presence of saponin compounds. Each of the crudes was also investigated for their biological activities towards four different bacteria by using the disc diffusion method. The result reveals that ethanol extract of kaduk leaves have potential for being antibacterial against gram positive bacteria.

Keyword: Kaduk leaves, Piper sarmentosum, crude extracts, phytochemical screening, antibacterial activity.

Introduction

Herbal medicine is an alternative medicine that has been used by people over the last several decades. According to Schulte (1999), a study conducted by National Institutes of Health indicated that the World Health Organization (WHO) has estimated 4 billion people as equal to 80% of the world's population use herbal medicine as their main healthcare product. There are some reasons why people all over the world believe in herbal medicines as their primary source of healthcare. One of the reasons is people today show their dissatisfaction towards conventional treatment methods as they believe that these methods are ineffective. Besides, people believe that herbal medicines are safe, easily available, show lesser side effects and have better compatibility with the human body as compared to the conventional treatment methods. Herbal medicines are also widely used for age-related disorders such as memory loss and immune disorders in which there are no modern medicines that can be used to treat these kinds of diseases. Therefore, it is recommended that the government should take efforts in integrating herbal medicines into the modern healthcare system (Adjei, 2013).

Plant-based systems play an important role in health care as it can be the basic of herbal medicines. Most parts of the plant such as leaves, roots and fruits contain medicinal properties to treat various diseases. For example, the leaves of *Peperomia pellucia* was used in the treatment of hypertensive (Fasola & Adebove, 2015). Other than that, the study carried out by Camposano et al. (2016) revealed anti-angiogenic activity of *Peperomia pellucia*. In addition,

ethanol extract of *Piper crocatum*, another species of Piperaceae show potential as anti-imflammatory agent (Laksmitawati et al., 2017). Compounds isolated from *Piper tuberculatum* show antiplasmodial and antileishmanial activities (De Souza Oliveira et al., 2018). Piplartine, an amide alkaloid found in several Piperaceae species, has shown has potential anticancer properties (Bezerra et al., 2015; Raja Mazlan et al., 2018) and benefits in the treatment of the parasitic infection (Campelo et al., 2018). The other plants from Piperaceae family are also widely used for the treatment of rheumatism, toothache, epilepsy, stomach-ached, anxiety disorder and found active as anti-diabetic, anti-ulcer, diuretic, and local anesthetic agents (Durant-Archibold et al., 2018). All these activities of Piper plants on neglected tropical diseases are very important for pantropical regions, which are the natural habitats of these plants.

The focus of this paper is *Kaduk* with its scientific name *Piper sarmentosum* is one of the members of Piperaceae family. It is terrestrial herb with erect and slender branchlets. Usually, it is found in damp open spaces, riverbanks cleared and cultivated lands (Chieng et al., 2008). It has tender, bright green, ovate to sub orbicular leaves with distinct veins. It is also widely distributed throughout India and South East Asian region (Hussain et al., 2008). All parts of *Piper sarmentosum* can be either used as vegetables, commercial and as medicine valuable for treating various kinds of diseases.

The methanol extract of *P. sarmentosum's* leaves were believed to act as antimalarial and can also be used to relieve fever, cough, influenza, pleurisy, asthma, abdominal pain and bacterial infection (Zaidan et al., 2006). The leaves of methanolic extract of *Piper sarmentosum* possess a similar protective effect against stressinduced gastric lesions as omeprazole (Azlina et al., 2016). Some of the phytochemical compounds found in methanol extract of *P. sarmentosum* can be used to reduce cancer risk (Murakami et al., 1995). Furthermore, several amide compounds that found in the hexane and methanol extracts of *P. sarmentosum* indicated the antituberculosis and antiplasmodial activities. *Piper sarmentosum* also exhibits antioxidant properties. A study conducted by Chanwitheesuk et al. (2005) had indicated the presence of another antioxidant compounds namely as vitamin C and vitamin E, xanthophylls, tannins and carotenes. Natural antioxidants from *P. sarmentosum* also play an important role in the chemoprevention of diseases and aging. A study carried out by Zainudin et al. (2015) found that *P. sarmentosum* have potential as anti-hypertension agent. In addition, Chan and Wong (2015) reported *P. sarmentosum* had showed potential as antifungal, anti-amoebic, antituberculosis, cytotoxic, anti-dengue, neuromuscular-blocking, hypoglycemic, and atherosclerosis.

Based on the study conducted by Zaidan et al. (2006), the methanol extract of *P. sarmentosum* leaves show potential as antibacterial agent against Gram-negative bacteria which was *Pseudomonas aeruginosa* and Gram-positive bacteria which was *Staphylococcus aureus* and *methicillin-resistant Staphylococcus aureus* (MRSA). According to Sharifah et al. (2016), *kaduk* has a potential as antibacterial activity due to the presence of flavonoids and alkaloids compound.

Therefore, this study was carried out to screen several types of phytochemical compounds extracted from the leaves extracts of kaduk (*Piper sarmentosum*) collected from state of Perak, Malaysia and to investigate its antibacterial activity towards several types of bacteria.

Materials and Methods

Plant sample collection

3.0 kg of fresh *kaduk* (*Piper sarmentosum*) (**Figure 1**) was collected from a wetland in Beruas, Perak. The leaves of *kaduk* were plucked and washed to remove any residues. Then, they were air dried and ground to fine powder.

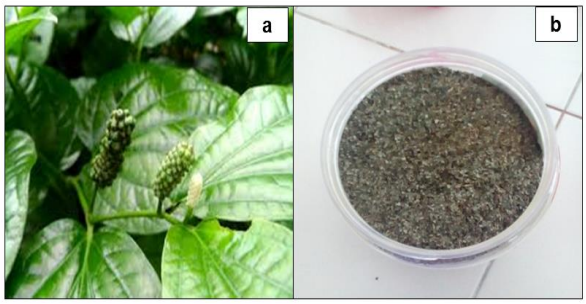

Figure 1 *Piper Samentosum* (a) leaves (b) grinded leaves

Extraction of kaduk's leaves

About 60.0 g of the fine powder of kaduk's leaves was extracted continuously with 500 mL two solvents of different polarity which were chloroform (semi-polar solvent) and ethanol (polar solvent). The extraction process was applied by using Soxhlet apparatus method with continuous heat extraction for 8 - 12 hours. Then, the extracts were concentrated under reduced pressure at 45ºC by using rotary vacuum evaporator. Each of the crude extracts was stored in a vial for further analysis

Phytochemical screening analysis

Phytochemical screening on the crude extract was done to identify several constituents such as flavanoid, phenolic, tannin, saponin, terpenoid and alkaloid through several tests (Method for the tests was modified from Alfallous et al. (2017) and Gul et al. (2017).

Flavonoid test

About 5 mL of ammonia solution (10% NH_4OH) was added to a portion of each of the crude extracts followed by a few drops of concentrated sulfuric acid (H_2SO_4). A yellow coloration of solution indicates the presence of flavonoid compounds.

Phenolic test

About 2 mL of each of the crude extracts was added with 4 – 5 drops of ferric chloride (10% $FeCl_3$) solution. Formation of bluish black solution showed a positive test for phenolic compounds.

Tannin test

About 2 mL of each of the crude extracts was added with 2% lead acetate. A green or blue-black coloration indicated the presence of tannin compounds.

Saponin test

About 2 mL of each of the crude extracts was mixed with 1 mL of distilled water and it was shaken vigorously for a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and shaken again vigorously. The formation of emulsion indicated the presence of saponin compounds.

Terpenoid test

About 5 mL of each of the crude extracts was treated with 3 mL of concentrated sulfuric acid (H_2SO_4) and the reddish-brown coloration of interface indicated the presence of terpenoid compounds.

Alkaloid test

A volume of 2 mL of each of the crude extracts was added with 6 drops of Wagner's reagent and the presence of orange precipitate indicated the presence of alkaloid compounds.

Antibacterial activity

Antibacterial activity of the crude extracts was investigated using disc diffusion method. About 5 mg of each of the crude extracts was weighed and they were dissolved in 1 mL of DMSO (dimethyl sulfoxide) solvent for antibacterial activity.

The disc diffusion method was carried out on two types of Gram-positive bacteria namely *Bacillus subtilis* and *Staphylococcus aureus* and two types of Gram-negative bacteria which were *Escherichia coli* and *Salmonella typhimurium*. Meanwhile, the positive control used was streptomycin sulphate and the negative control used was DMSO solvent. The bacteria cultures were adjusted to 0.5 McFarland standard to lawn the agar plates evenly by using a sterile swab. Then, the plates were dried for 15 minutes. The crude extracts were loaded on Whatman filter paper disks (6.0 mm) and evenly placed on the agar surface that was previously inoculated with suspensions of bacteria to be tested. All determinations were made in duplicates.

Result and Discussion

Extraction of the leaves of Piper sarmentosum

The dried and ground leaves of kaduk (*Piper sarmentosum*) were extracted by using two different types of solvent which were chloroform and ethanol. The extracts were concentrated until they became crudes. Each of the crude extracts was stored in pre-weighed vials and left in a fume cupboard until they dried. The crude extracts were reweighed and kept until further analysis. The percentage yield for each of the crudes was calculated and presented in **Table 1**.

Table 1 Percentage yield of each of the crude extracts

Type of Crude Extract	Weight of sample (g)	Weight of crude (g)	Percentage of Crude Extract (%)
Chloroform	61.27	5.46	8.92
Ethanol	61.27	6.43	10.50

The weight of crude and percentage yield obtained for ethanol extract was 6.43 g and 10.50%, respectively. As for the chloroform extract, the weight of crude and percentage yield obtained was 5.46 g and 8.92%, respectively. The ethanol extract with the highest extraction weight and percentage yield shows that more compounds of kaduk leaves can be extracted using polar solvent.

Based on a study conducted by Hussain et al. (2008), the ethanol extract of *Piper sarmentosum* showed the highest percentage yield of crude due to the highest percentage of total protein and total polysaccharides that have been measured. Furthermore, the methanol extract of a *Piper* species showed the highest percentage yield of crude extract which was 12.56% compared to other extracts such as chloroform and petroleum ether (Ahmad et al., 2015).

Phytochemical screening analysis

In this study, both extracts were used in several phytochemical screening tests to determine some of the active compounds such as flavonoids, tannins, saponins, terpenoids, alkaloids and phenolics. The ethanol extract showed positive test for all the active compounds except for saponins. Meanwhile, chloroform extract showed positive test only for saponin compounds and

the rest were negative. **Table 2** below shows the summaries of results from the phytochemical screening analysis.

Table 2 Pyhtochemical screening analysis on the crude extracts

Phytochemical compounds	Observation	Chloroform extract	Ethanol Extract
Flavonoids	Yellow	-	+
Tannins	Green/blue-black	-	+
Saponins	Emulsion	+	-
Terpenoids	Reddish brown	-	+
Alkaloids	Orange percipitate	-	+
Phenolics	Bluish black	-	+

(+ : present, - : absent)

Previous study showed that the most of major compounds extracted from higher polarity of solvent like methanol or ethanol. According to Miean & Mohamed (2001), methanol extract of *Piper sarmentosum* show the presence of major compound of flavonoids such as quercetin, myricetin and apigenin. A study by Chieng et al. (2008) stated that the main compounds identified in the leaves oil of *Piper sarmentosum* were sesquiterpenoids including *β*-caryophyllene, spathulenol, myristicin and (*E,E*)-farnesol.

Other phytochemicals that were identified in the methanol extract of *Piper sarmentosum* was a group of phenolic compounds such as 1-allyl-2,6-dimethoxy-3,4-methylenedioxybenzene, 1-allyl-2,4,5-trimethoxybenzene, 1-(1-*E*-propenyl)-2,4,5-trimethoxybenzene and 1-allyl-2-methoxy-4,5-methylenedioxybenzene (Masuda et al., 1991). Furthermore, Fernandez et al. (2012) had indicate the presence of tannin and alkaloid compounds in methanol extract of *Piper sarmentosum* through their study. They also found that no saponin compounds in the polar extract of *Piper sarmentosum*.

Antibacterial activity test

In this study, the antibacterial activity of ethanol and chloroform extracts of *Piper sarmentosum* was carried out on four types of bacteria which were *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Salmonella typhimurium*. The method used was the disc diffusion method. The diameters of the inhibition zones for all tested bacteria are listed in **Table 3**.

Table 3 Zone of inhibition zone (mm) of crude extracts against four bacteria

Type of bacteria	Chloroform extract	Ethanol extract	Positive control	Negative control
Escherichia coli	6.0	6.0	21.0	6.0
Salmonella typhimurium	8.0	8.0	18.0	6.0
Bacillus subtilis	8.0	11.0	21.0	6.0
Staphylococcus aureus	7.0	9.0	16.0	6.0

(6 mm is a diameter for the disc)

From the result obtained, both extracts showed the antibacterial activity towards all types of

bacteria except for *E. coli*. This result is similar to the findings of a study conducted by Voravuthikunchai et al. (2004) which stated that there was no inhibition zone for both aqueous and ethanol extracts against *E. coli*. In addition, the study by Atiax et al. (2011) indicates that the antibacterial activity of the isolated compounds of *Piper sarmentosum* had shown positive result for Gram-positive bacteria which were *B. subtilis* and *S. aureus* while there was no zone of inhibition observed towards Gram-negative bacteria which were *E. coli* and *P. aeruginosa*.

Conclusion

This study show that the ethanol extract has higher weight of crude as well as higher percentage yield as compared to the chloroform extract. The percentage yield obtained for ethanol extract was 10.50 % while the percentage yield obtained for chloroform extract was 8.92 %. Thus, ethanol extract has the highest extraction weight and percentage yield shows that more compounds of kaduk leaves can be extracted using polar solvent.

In phytochemical screening analysis, some of the active compounds are tannins, flavonoids, saponins, terpenoids, alkaloids and phenolics had been determined through several tests. The ethanol extract showed positive test for all the active compounds except for saponins. Meanwhile, chloroform extract showed positive test only for saponin compounds. High percentage yield of ethanol may contribute to the present of the most phytochemicals in the crude extracts.

For the antibacterial activity, both extracts have shown their activities towards several bacteria such as *Bacillus subtilis, Salmonella typhimurium* and *Staphylococcus aureus*, except for *Escherichia coli.* Ethanol extract reveal the potential as antibacterial agent against *Bacillus subtilis* with 11.0 mm diameter of inhibition zone.

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Conflict of interests

Author declare there is no conflict of interest.

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