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Phytochemical Screening and Antibacterial Activity of Sirih Leaves (Piper betle. Linn)

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Abstract: *Sirih with its scientific name Piper betle Linn is one of the most valuable herbs from Piperaceae family. Therefore, this study was focused on examining aimed the extraction of Sirih leaves, to determine the compounds in the extracted sample and investigate their antibacterial features. The grinded Sirih leaves (Piper betle Linn) were extracted by using two different polarity of solvent namely chloroform and ethanol by using hot extraction method. The percentage yield obtained for ethanol extract was 13.10%, higher as compared to the percentage yield of chloroform extract, which was 5.32%. The ethanol extract has indicated the presence of most phytochemicals such as flavonoid, tannin, phlobatannin, terpenoid, alkaloid and phenolic compounds, with no saponin. Meanwhile, chloroform extract showed only the presence of saponin compound. Each of the crudes was also investigated for their biological activities towards four different bacteria including Salmonella typhi, Escherichia coli, Staphylococcus aureus and Bacillus subtilis by using the disc diffusion method. The result reveals that ethanol extract of Sirih leaves have high potential for being antibacterial agent.*

Keywords: Sirih leaves, Piper betle Linn, crude extracts, phytochemical screening, antibacterial activity

1. Introduction

The discovery and development of herbal medicine as an alternative medicine have led a dramatic improvement over the last several decades. The herbal medicine has been trusted since old-century for its capability in healing a universal disease (Agarwal et al., 2012). According to Deshpande and Kadam (2013), traditional herbal medicine is useful and enthusiastically accessible for major healthcare. 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. Due to the huge usage, herbal medicines are accessible in the stores and the crop is widely found in Southeast Asian countries including Malaysia (Padmapriya and Poonguzhali, 2015).

Plant-based systems play an important role in healthcare 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.

Sirih leaves with its scientific name of *Piper betle* Linn 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 (Arambewela et al., 2006). 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 (Arambewela et al., 2006). All parts of *Piper betle* Linn can be either used as vegetables and as medicine valuable for treating various kinds of diseases such conjunctivitis, headache, itches and bad breath.

The extract of Sirih leaves posses an insecticidal and antitumor activity (Gundala and Aneja, 2014), neuroprotective activity (Chan and Wong, 2014), antidiabetic and antihelmintic activity (Shah et al., 2016) and antimicrobial activity (Nouri and Nafchi, 2014). According to Banerjee and Shah (2014), the leaves extract of *Piper betle* Linn have anti proliferative and chemo preventive potential and can be used for the treatment of various ailments including human lung cancer. The pungent taste of *Piper betle* Linn show effectiveness as anti-worm agent and anti-infectious agent (Rekha et al., 2014). The ethanolic extract of *Piper betle* Linn leaves were believed to act as an antiseptic and anti-inflammatory agent (Ganguly et al., 2007) and can also be used to to relieve fever, cough, influenza, pleurisy, asthma and dyspepsia (Fazal et al., 2014). Based on the study conducted by Kanjwani et al., (2008), the methanol extract of *Piper betle* Linn show potential for immunomodulatory drug. Furthermore, study conducted by Tan and Chan (2014) found that *Piper betle* Linn has a potential as antibacterial agent due to the presence of sterol and polyphenol compound.

Therefore, the present study was design to evaluate several types of phytochemical compounds extracted from the leaves of Sirih leaves (*Piper betle* Linn) collected from the State of Negeri Sembilan, Malaysia and to investigate its antibacterial activity towards several types of bacteria.

2. Materials and Methods

2.1 Plant sample collection

An about 3.0 kg of fresh Sirih leaves (*Piper betle* Linn) (Figure 1) was collected from a wetland in Kuala Pilah, Negeri Sembilan. The Sirih leaves were plucked and washed to remove any residues. Then, they were air dried and ground to fine powder.

Figure 1: Sirih leaves (left), grinded leaves (right)

2.2 Extraction of Sirih leaves

About 60.0 g of the fine powder of Sirih 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 45^{o}C by using rotary vacuum evaporator. Each of the crude extracts was stored in a vial for further analysis.

2.3 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).

2.3.1 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.

2.3.2 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.

2.3.3 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.

2.3.4 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.

2.3.5 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.

2.3.6 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.

2.4 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 typhi*. 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.

3. Result and Discussions

3.1 Extraction of the leaves of Piper betle Linn

The dried and grounded sirih leaves (*Piper betle* Linn) 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	60.08	3.19	5.32
Ethanol	60.08	7.87	13.10

The weight of crude and percentage yield obtained for ethanol extract was 7.87 g and 13.10%, respectively. As for the chloroform extract, the weight of crude and percentage yield obtained was 3.19 g and 5.32%, respectively. The ethanol extract with the highest extraction weight and percentage yield shows that more compounds of Sirih leaves can be extracted using polar solvent. Based on a study conducted by Ahmad et al. (2015), 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 due to the properties of polar solvent that can extract out

most major phytochemical compound. A study by Annegowda et al. (2013) indicated that the ethanolic extract of Sirih yielded of about 9.1% of extract with maceration, 10.25% with Soxhlet extraction.

3.2 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, phlobatannins, 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: Phytochemical screening analysis on the crude extracts

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

(+ : present, - : absent)

This finding is similarly to previous finding. According to Syahidah et al. (2017), the methanolic extract of *Piper betle* Linn was analysed for the presence of active phyto-constituents such as alkaloids, flavonoids, phenols, tannins, saponins, glycosides, terpenoids and steroids. It is important to highlight that the type of diluent used was the main factor that could influence in variation of phyto-constituents being extracted. For example, a study by Chakraborty and Shah (2011) on several extracts of *Piper betle* Linn using methanol, petroleum ether, and ethyl acetate produced different results in which all the tested solvents had indicated the presence of flavonoids, tannins, sterols and phenol, but lack of alkaloids. Previous study showed that the most of major compounds extracted from higher polarity of solvent like methanol or ethanol. In comparison, lower polarity of solvent, such as petroleum ether and chloroform, there were found incapable to extract more than two phytochemicals tested (Saini et al., 2016). These results might be explained by the fact that phytochemical compounds were more soluble in moderate polar organic solvent as compared to non-polar organic solvent (Cowan 1999).

3.3 Antibacterial activity test

In this study, the antibacterial activity of ethanol and chloroform extracts of Sirih leaves was carried out on four types of bacteria which were *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Salmonella typhi*. 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 tested bacteria

Type of bacteria	Zone of inhibition (mm)			
	Chloroform extract	**Ethanol extract**	**Positive control**	**Negative control**
Salmonella typhi	9.0	14.0	15.0	6.0
Escherichia coli	11.0	13.5	19.0	6.0
Bacillus subtilis	8.0	10.5	27.5	6.0
Staphylococcus aureus	7.0	8.5	17.0	6.0

(6 mm is a diameter for the disc)

From the result obtained, ethanol extract shows highest antibacterial properties as compared to chloroform extracts against all types of tested bacteria. The finding was similar to the study by Syahidah et al. (2017) which illustrated that the *Piper betle* Linn methanolic extract seemed to be sensitive to both Gram-positive and Gram-negative bacteria. In addition, according to Nalina and Rahim (2007), phytocompounds will demonstrated their antibacterial action by interrupting the bacterial plasma cell membrane and rendering them more permeable. Therefore, those phytochemicals that show inhibition of the bacteria growth, can be concluded to have potential as antibacterial agent.

4. Conclusion

As conclusion, from the result obtained, 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 13.10 % while the percentage yield obtained for chloroform extract was 5.32 %. Thus, ethanol extract has the highest extraction weight and percentage yield shows that more compounds of Sirih leaves (*Piper betle* Linn) 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, ethanol extract reveals the potential as antibacterial agent against all tested bacteria, with highest inhibition diameter zone was 14.0 mm against *Salmonella typhi*.

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Conflict of interests
Author declare there is no conflict of interest.

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