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Phytochemical, Antibacterial and Antioxidant Activities of *Schefflera elliptica* Leaves

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Abstract

Schefflera elliptica or locally called *kayu tulak*, is one of the Balinese herbal plants that have traditionally be used to reject (*tulak*) negative influences that exist in the human body. Althoug, *S. elliptica* has been routinely used as a part of a ritual in Bali, only a limited study has been reported on its bioactivity. This study was designed to analyze the phytochemical content, antibacterial activity, and antioxidant activity of *S. elliptica*. In this study, simplisia of *S. elliptica* was extracted using n-hexane and ethyl acetate solvents, then the viscous extracts of the two solvents were carried out for phytochemical tests, antibacterial activity tests with the Kirby-Bauer method and antioxidant activity tests with based on DPPH method. Phytochemical screening showed that the n-hexane extract contains active compounds in the form of phenols and steroids while ethyl acetate contains active compounds in the form of phenols, tannins, and steroids. Antibacterial screening showed ethyl acetate extract of *S. elliptica* displayed a diameter zone of inhibition of 10.72±0.71 mm against *Staphylococcus aureus*, 12.17±2.80 mm against *Streptococcus mutans*, 12.40±1.65 mm against *Escherichia coli* and 15.20±2.44 mm against *Klebsiella pneumoniae*. The DPPH analysis showed percentages of 61.17% and 67.42% from n-hexane and ethyl acetate extracts respectively, which indicated the antioxidant properties of *S. elliptica*. Overall, this research provides a preliminary report on the bioactivity potential of *S. elliptica* mainly in term of antibacterial and antioxidant properties which open up possibilities for future drug development.

Keywords: antibacterial; antioxidant; phytochemical; *schefflera elliptica*.

INTRODUCTION

Indonesia is one of the countries with high plant diversity. The biological diversity of flora in Indonesia, especially seeded plant species reaches 30,000–40,000 types, or equivalent to 15.5% of the total number of plants in the world (Widjaja *et al.*, 2014). Furthermore, more than 2,039 plant species are categorized as medicinal plants (Zuhud, 2009). Among many islands in Indonesia, Bali is one of the islands with rich biological diversity and Balinese people have applied herbal plants for traditional medicine (Sutomo and Iryadi, 2019). In general, information about traditional Balinese medicine has been recorded in a manuscript called *Lontar Usada Bali*, which explains the function of each herbal function and the procedure to use it (Oktavia *et al.*, 2017).

Schefflera elliptica or locally called *kayu tulak* is one of the types of local Balinese plants listed in *Usada Tiwang* (Arsana *et al.*, 2020). This plant is commonly found in the traditional Balinese offerings called *banten byakala* has a function to reject (*tulak*) any impurities or negative influences that exist in the human body (Puspa *et al.*, 2019). More specifically, the leaf part of kayu tulak is used as a repellent for disasters or bad luck in human body (Hanum, 2011).

Schefflera elliptica leaves can also traditionally be used as a remedy for skin diseases and fractures (Sivaperuman *et al.*, 2018). Methanol and ethyl acetate extracts of *S. elliptica* leaves have also been reported to have antibacterial activity against *Staphylococcus aureus* (Purwantoro *et al.*, 2009). However, apart from antibacterial against *S. aureus*, no other studies have been published on the antibacterial activities of *S. elliptica* extracts against other Gram-positive and Gram-negative bacterial species. In addition, rather limited information is available on other aspects of the bioactivities of *S. elliptica* e.g. phytochemicals and antioxidant activities.

This present study aimed to assess the bioactivities *S. elliptica* leaves focusing on three main aspects namely phytochemical, antibacterial, and antioxidant bioactivities. The obtained information is expected to give further knowledge on the bioactivities of S. *elliptica* which could be the basis to develop the ethnomedicine and drug development purposes of the plant.

METHODS

Sample Collection and Determination

Leaves samples of *S. elliptica* plant were obtained from Gerih Village, Bali, Indonesia on February 2022 (Figure 1). Samples were selected by taking 3-5 leaves of *S. elliptica* which were calculated from the leaflets, provided that it was in full bloom, fresh, without hollow, and free of insect infections. Plant determination was performed by sending fresh and dried vouchers to the Characterization Laboratory of the Botanical Garden "Eka Karya" Bali – National Research and Innovation Agency (BRIN), Candikuning, Tabanan, Bali. The purpose of plant determination is to obtain a clear identity of the plant under study and avoid errors in collecting the main research material.

Sample Preparation and Extraction

Two kilograms of *S. elliptica* leaves were washed in running tap water to remove debris. Subsequently, leaves samples were drained, and dried using an oven at 40ºC. The dried simplisia was dried and were sorted by separating foreign objects that occurred during drying. The dried simplisia was turned into powder using a blender and was sieved with a 60-mesh sieve. Finally, the powder was stored in a clean glass jar to prevent contamination and other impurities before extraction.

Two types of crude extracts were prepared using two different chemical solvents namely ethyl acetate (Smart-Lab) dan n-hexane (Merck). For each of solvent, 100 gram of dry powder leaves of *S. elliptica* was macerated with a ratio of 1:5 (w/v) (Wijaya and Indraningrat, 2021). Maceration was carried out for 24 hours and each mixture was stirred every five minutes with a time span of six hours. For each of the solvent, remaceration was carried out after 24 hours. Subsequently, after maceration and remaceration were completed, each macerate was separated from the residue using a vacuum filter, followed by evaporation in a rotary evaporator (Ika RV 10, Germany) at a speed of 100 rpm at a temperature of 40ºC.

Figure 1. *S. elliptica* plant (left), and *S. elliptica leaves* (right).

Phytochemical Screening

Each the ethyl acetate and n-hexane *S. elliptica* crude extracts was tested to detect the presence of a group of compounds based on the following methods.

Phenol

One mL of each S. *elliptica* extract was transferred into a test tube followed by the addition of 2 – 3 drops of iron (III) chloride (FeCl3) 5%. The presence of phenols was indicated by a blue-black color (Friany *et al*., 2017).

Flavonoids

One mL of each *S. elliptica* extract was transferred into a test tube. Subsequently, 2 mg of magnesium powder and 3 drops of concentrated HCl were added to the tube. The mixture was shaken and the formation of a red, yellow, or orange color on the solution indicated the presence of flavonoids (Purwati *et al*., 2017).

Tannins

One mL of each *S. elliptica* extract was mixed with a few drops of 10% iron (III) chloride ($FeCl_3$) solution. A dark blue or greenish-black color indicates the presence of tannins in a solution (Baud *et al*., 2014).

Alkaloids

Two mL of each *S. elliptica* extract was mixed with 3 drops of concentrated HCl and 5 drops of Mayer reagent. A white precipitate indicates that a sample contains alkaloids (Ergina and Pursitasari, 2014).

Steroid/Terpenoid

Two mL of each *S. elliptica* extract were mixed with *Liebermann Burchard* reagent, a mixture of concentrated HCl and concentrated H_2SO_4. Positive results were indicated by the presence of a red-orange color for triterpenoids and blue for steroid (Sangi *et al*., 2008; Ergina and Pursitasari, 2014).

Saponin

Two mL of each *S. elliptica* extract were mixed with 10 mL of aqua dest. The mixture was shaken for 1 minute and subsequently, two drops of HCl 1 N was added. The presence of a stable foam for approximately 7 minutes indicated that the mixture contains saponins (Mondong *et al*., 2015).

Antibacterial Activity Screening

For each ethyl acetate and n-hexane extract, paper discs with a diameter of 6 mm were prepared. Each paper disc was soaked into a viscous extract and allowed to dry for 15 minutes until the extract was evenly absorbed. Paper discs containing extracts were transferred into Luria Bertani agar which already contained one of the following test bacteria namely *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* FNCC 0405, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603. Triplicate paper discs of

each extract were used for each of the tested bacteria. Nalidixic Acid (Oxoid) paper discs were used as a positive control, while ethyl acetate and n-hexane were used as negative controls. Antibacterial activities were calculated based on the triplicate average zone of inhibition (ZOI) that were formed on each of the lawn bacterial species.

Antioxidant Assay

The radical inhibition activity of the sample was carried out based on its inhibition against free radicals *1, 1-diphenyl-2-picrylhydrazyl* (DPPH) (Pangestuty, 2016). Sample absorbance was measured with a UV-Vis spectrophotometer. The magnitude of antioxidant activity was measured by the following formula:

$$\text{Antioxidant Activity (AA\%)} = \frac{Abs\ control - Abs\ sample}{Abs\ control}\ x\ 100$$

RESULT AND DISCUSSION

Phytochemical Screening Results

Phytochemical screening is a preliminary test and qualitative analysis that can be used as initial information about a group of compounds present in a plant. In this research, phytochemical screening of n-hexane and ethyl acetate extracts of *S. elliptica* were summarized in Table 1. In general, there were discrepancies in the presence of specific groups of compounds by comparing the phytochemical constituents of each extract.

Alkaloids tests showed clean deposits were formed in both extracts. This result indicated the absence of alkaloids in ethyl acetate and n-hexane. A positive result was indicated by the presence of white deposit (Ergina and Pursitasari, 2014)

Table 1. Phytochemical composition of *S. elliptica* extracts.

Test	Extract	
	N-hexane	Ethyl Acetate
Alkaloid	-	-
Flavonoid	-	-
Tannin	-	+
Phenol	+	+
Saponin	-	-
Steroid	+	+
Terpenoid	-	-

The presence of flavonoids in n-hexane and ethyl acetate extracts was tested using two methods, namely by adding concentrated Magnesium (Mg) + Hydrochloric Acid (HCl) and using H_2SO_4. Positive signs were indicated by a change of color in the mixture to red or orange. In this study, n-hexane and ethyl acetate samples did not change color to red or orange, so it was concluded that flavonoids were absent in both extracts (Friany *et al*., 2017; Purwati *et al*., 2017).

A positive test of tannin was indicated by the formation of blackish-green color (Baud *et al*., 2014). The occurrence of this green color change is due to the reaction between Fe metals and tannins to form complex compounds due to the presence of coordination covalent bonds between metal ions or atoms and non-metallic atoms (Effendy, 2007). Our result indicated that tannin was present in ethyl acetate extract based on the color change that was observed. Meanwhile, no color change was observed from the n-hexane extract. This could happen mainly because n-hexane is a solvent that has non-polar properties while tannins are polar compounds and tend to dissolve in polar or semi polar solvents (Muthmainnah, 2017).

The presence of phenol was screened by adding 10% of FeCl3 reagent and positive signs were indicated by a color change to blackish-green (Friany *et al*., 2017). Color changes to green-black were observed from both *S. elliptica* extracts to indicate both extracts contained phenol compounds. The discoloration of n-hexane and ethyl acetate extracts of *S. elliptica* was because phenol compounds can dissolve in polar solvents as well as non-polar solvents (Wongso, 2014).

In the saponin test, n-hexane and ethyl acetate extracts of *S. elliptica* were heated followed by addition of 10 ml of aquadest. The mixture was shaken strongly and a positive sign was indicated by the presence of a stable foam of 1-10 cm high which was stable after the addition of 1 drop of HCl 1N (Mondong *et al*., 2015). The appearance of foam was present because saponin compounds have physical properties that are easily hydrolyzed in water so that saponin compounds will cause foam when shaken. In both n-hexane and ethyl acetate extracts of *S. elliptica* the saponin test did not show positive results because the foam formed after shaking only lasted for a few seconds.

The presence of steroids and terpenoids were screened by the addition of Liebermann-Burchard reagents. The positive result for steroid compounds was indicated by the color change to blue or purple while for triterpenoid compounds was indicated by a brownish-red color (Sangi *et al*., 2008; Ergina and Pursitasari, 2014). The test results showed that n-hexane and ethyl acetate of *S. elliptica* extracts underwent a blue color change so that they were positive for containing steroid compounds.

Antibacterial Activities Screening

The antibacterial screening showed that the crude extractof n-hexane displayed lower antibacterial activity against test bacteria compared to the crude extract of ethyl acetate (Figure 2, Table 2). Ethyl acetate extract has an average ZOI of 10.72±0.71 mm against *S. aureus*. Ethyl acetate extract had an average ZOI of 12.17±2.80 mm against *S. mutans*. Meanwhile, in the ethyl acetate extract has an average ZOI of 12.40±1.65 mm against *E. coli* and an average ZOI of 15.20±2.44 mm against *K. pneumoniae*.

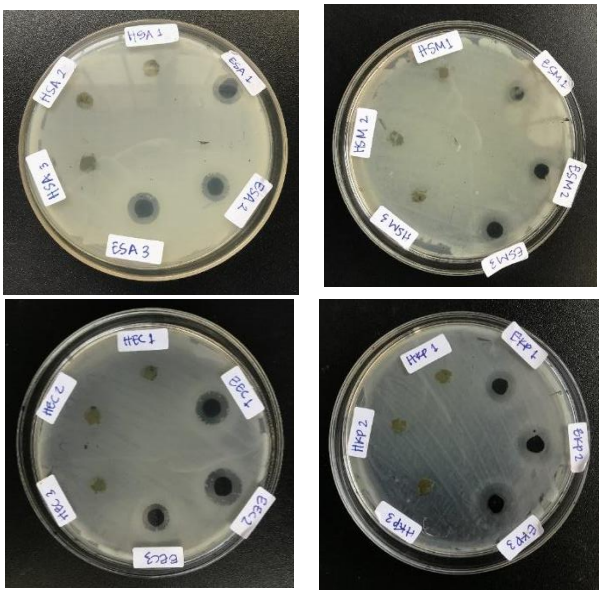

Figure 2. Antibacterial activities of n-hexane and ethyl acetate extract againts bacteria. Annotations: H = n-hexane, E= Ethyl acetate; SA = *Staphylococcus aureus* ATCC 25923; SM = *Streptococcus mutans* FNCC 0405; EC= *Escherichia coli* ATCC 25922; KP=*Klebsiella pneumoniae* ATCC 700603

Table 2. Antibacterial activities of *S. elliptica* extract against testing bacteria. Diameter zone of inhibition of each treatment (ethyl acetate, n-hexane, positive and negative controls) were calculated from triplicate samples.

Bacterial Strains	Samples	Solvents	ZOI (mm)
S. aureus	*S. elliptica*	n-Hexane	-
		Ethyl acetate	10,72±0,71
	+ control	Nalidixic acid	15,34±0,03
	- control	n-Hexane	-
		Ethyl acetate	-
S. mutans	*S. elliptica*	n-Hexane	-
		Ethyl acetate	12,17±2,80
	+ control	Nalidixic acid	16,29±0,12
	- control	n-Hexane	-
		Ethyl acetate	-
E. coli	*S. elliptica*	n-Hexane	-
		Ethyl acetate	12,40±1,65
	+ control	Nalidixic acid	16,58±0,04
	- control	n-Hexane	-
		Ethyl acetate	-
K. pneumoniae	*S. elliptica*	n-Hexane	-
		Ethyl acetate	15,20±2,44
	+ control	Nalidixic acid	15,50±0,13
	- control	n-Hexane	-
		Ethyl acetate	-

When compared to the results of the study of Purwantoro *et al*, (2009) which stated that the ethyl acetate extract of *S. elliptica* at a concentration of 50 µg/ml, 100 µg/ml, and 200 µg/ml had a ZOI with an average of 7.5 mm against *S. aureus* and *E. coli* bacteria, the crude extract of ethyl acetate with a concentration of 100% had a bigger ZOI against *S. aureus* and *E. coli* bacteria. The difference in inhibitory power can occur due to the difference in the concentration of the extract where the higher the concentration of the extract, the higher the inhibitory power will be. The same thing is also stated by (Zuhud *et al.*, 2001) who mentioned that the higher the concentration of the extract, the more the amount of antimicrobial compounds released will be, thus facilitating the penetration of compounds into cells.

Davis and Stout, (1971) classified the diameter zone of inhibition into four categories, namely weak, medium, strong, and very strong. Zones of inhibition with a diameter of ≤5 mm are categorized as weak, 6-10 mm are categorized as medium, 11-20 mm are categorized as strong, and above 20 mm are categorized as very strong. Based on these categories, the ZOI formed by ethyl acetate extracts of *S. elliptica* could be considered as a strong activity. However, the observed antibacterial activity was still lower compared to positive control nalidixic acids. Nevertheless, the observed antibacterial activity from ethyl acetate extracts provides a valuable insight on bioactive compounds that present in *S. elliptica* leaves.

The presence of antibacterial activities in the ethyl acetate extract of *S. elliptica* may occur due to the content of its secondary metabolites. The mode of action of phenol compounds is in general by denaturing cell proteins. Hydrogen bonds formed between phenol compounds could damage protein layers in cell structures (Bontjura *et al*., 2015) Meanwhile, tannin was also reported to display have antibacterial activity against Gram-positive and Gram-negative bacteria by entering the cell wall (Kaczmarek, 2020). Tannin forms hydrogen bonds with bacterial cell's proteins and subsequently hydrogen bonds formed between tannins and proteins will disrupt bacterial cell walls (Mailoa *et al.*, 2014).

Antibacterial screening showed that the zone of inhibition of ethyl acetate extract of *S. elliptica* against Gram-positive bacteria (*S. aureus* and *S. mutans*) was smaller compared to Gram-negative bacteria (*E. coli* and *K. pneumoniae*). Such discrepancies could probably happen because the cell wall of Gram-negative bacteria is thinner compared to Gram-positive bacteria so the protein structure in the cell wall of Gram-negative bacteria was damaged by the presence of tannin compounds (Mailoa *et al.*, 2014). Furthermore, Gram-positive bacteria have a thick and rigid peptidoglycan layer while Gram-negative bacteria have a thinner peptidoglycan layer (Sudarmi *et al*., 2017).

The absence of ZOI observed in n-hexane extracts even though qualitatively the extract contained phenol compounds could be influenced by differences of the polarity of solvents in extraction which affecting the total content of bioactive compounds in the extract (Santoso *et al.*, 2012). The total content of phenol compounds according to Hidayah *et al*., (2017) has an influence on antibacterial activity as the higher the levels of oxidized phenol compounds, the stronger the antibacterial activity

will be. The extract of n-hexane *S. elliptica* did not display a zone of inhibition power even though it qualitatively contained steroid compounds. Such a condition could happen because lipids have large sizes of molecules which interfere with the diffusion process and consequently, n-hexane extracts are unable to inhibit bacterial growth (Naufalin *et al.*, 2005).

Antioxidant Activities Test Results

Antioxidant activities were analyzed based on the 2,*2-diphenyl-1- picrilhydrazyl* (DPPH) method. In theory, the higher the concentration of antioxidant activity added to the DPPH solution, the more the absorbance value will decrease (Sapri *et al*., 2013). The absorbance value of n-hexane and ethyl acetate *S.elliptica* samples (Table 3) showed that the antioxidant activities (AA) of n-hexane extract was 61.17% while for ethyl acetate extract was 67.42%. According to Rahmawati (2004) in (Parwata *et al*., 2009) the inhibition value of 0% means that an extract has no antioxidant activity and the inhibition value of 100% means total dampening. An extract could be classified as an active antioxidant when its inhibition percentage is more than or equal to 50%. Therefore, based on this criteria, crude extracts of n-hexane and ethyl acetate of *S. elliptica* are classified as active antioxidants.

Table 3. Antioxidant percentages of *S. elliptica* crude extracts.

Sample	Crude extracts	Concentrations	AA%
S. elliptica	N-Heksan	1000 ppm	61,17
	Etil Asetat	1000 ppm	67,42

Factors that affect the absorbance are the type of solvent, the pH of the solution, the temperature, the high concentration of the solution, and the presence of a disruptive substance. The amount of antioxidant activity of n-hexane and ethyl acetate extracts from *S. elliptica* is due to the content of secondary metabolites such as phenols, steroids, and tannins in the extract. Tannins are compounds composed of polyphenols that have free radical capture activity. The more tannin content contained in the extract, the more antioxidant activity (Malangngi *et al*., 2012). According to (Amarowicz, 2007) tannins not only function as primary antioxidants but also function as secondary antioxidants. Phenolic compounds have the ability to contribute hydrogen atoms or electrons to free radicals. This process converts phenols into phenoxyl radicals that can stabilize themselves so that there is no radical formation reaction (Pangestuty, 2016; Diniyah and Lee, 2020).

CONCLUSIONS

In conclusion, this study confirmed antibacterial and antioxidant activities from *S. elliptica* crude extracts. In addition, *S. elliptica* leaves contain phytochemicals in the form of phenol compounds, tannins, and steroids. The selection of solvents seems to play an important role in extracting bioactive compounds from *S. elliptica* leaves. In terms of antibacterial activity, ethyl acetate which is a semipolar solvent seems more suitable to extract the active antibacterial substances compared to n-hexane which is a non-polar solvent. Further studies should be focused to explore the ideal solvent for chemical extraction. Comparison of polar, semipolar, and non-polar solvents for extraction of *S. elliptica* should also be done to provide an ideal comparison of bioactivities. In addition, antibacterial screening should also be expanded against multi-drug resistance bacteria and fungi. Further studies should also explore other aspects of bioactivity screenings such as cytotoxicity, anti-larvicide, and anticancer tests of *S. elliptica* to elucidate possible untapped bioactivities of the plant.

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