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Phytochemical test and identification of active compounds with LC-MS/MS in green meniran leaf (Phyllanthus niruri Linn)

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ABSTRACT

Background: Green meniran plant (Phyllanthus niruri Linn) is traditionally believed to treat many diseases, such as reducing fever, diarrhea, cough with phlegm, inflammation, malaria, and acne. This research aims to determine the class of compounds and active compounds in the ethanol extract of green meniran leaf.

Methods: An experimental study was conducted using phytochemical testing includes qualitative and quantitative tests to identify the active compounds in green meniran leaf (Phyllanthus niruri Linn) by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). Crude powder packaging of green meniran leaf was conducted at Materia Medika Herbal Laboratory, Malang, Indonesia. The ethanol extraction from green meniran leaf was conducted at Pharmacology Laboratory, Faculty of Mathematics and Natural Sciences, Udayana University.

Results: Based on the results of the phytochemical qualitative test, the green meniran leaf extract was positive for flavonoids, saponins, steroids, terpenoids, alkaloids, phenols, tannins, and glycosides. From quantitative phytochemical tests, we found the levels of phenolic, flavonoid, tannin 5.13%, 3.30%, 5.01%, respectively and 28.212.67 mg/kg chlorophyll. Identification using LC-MS/MS showed the suspected compounds Apigenin-8-C-glucoside from the glycoside group and 5,6,7-trimethoxyflavone from the flavonoid group.

Conclusion: Green meniran leaf contains flavonoids, saponins, steroids, terpenoids, alkaloids, phenols, tannins, and glycosides with the active compounds Apigenin-8-C-glucoside and 5,6,7-trimethoxyflavone.

Keywords: green meniran leaf, active compounds, LC-MS/MS, phytochemical testing.
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INTRODUCTION

Traditional medicines are generally medicines formulated and developed traditionally based on experience from generation to generation.[1] The development of studies on the benefits of plants in treating various diseases has led to increased public confidence in the use of plants as ingredients in traditional medicine. Researchers are currently competing with each other to develop traditional medicines as standardized medicines. Traditional medicinal ingredients can be obtained from plants that are around it.[2]

One type of plant that is commonly seen is green meniran (Phyllanthus niruri Linn). Phyllanthus niruri Linn is a type of plant from the Euphorbiaceae family and the Phyllanthus genus.[3] Green meniran has traditionally been able to treat many diseases such as reducing fever, diarrhea, cough with phlegm, inflammation, malaria, and acne.[4] People generally consumed this by boiling it in the water. Scientifically this plant has also shown to have properties as a strong antioxidant and immunomodulator that can increase the immune system or the body's immune system that accelerate the healing process.[5,6]

Many scientific studies on meniran plants have been carried out to determine their properties as a standardized medicinal substance. Several studies have reported that the green meniran leaf (Phyllanthus niruri Linn) is active as an antimicrobial, which can inhibit the Escherichia coli development Staphylococcus aureus, Candida albicans, and Shigella dysenteriae.[7-9] According to Jansen et al. which explained that the extract of green meniran (Phyllanthus niruri L) has potential as a fever-lowering drug because it shows an antipyretic effect in Wistar rats with DPT-HB vaccine-induced fever.[10] Other studies have also reported that herbal decoctions containing meniran herbs affect lowering blood pressure in patients with hypertension; meniran root extracts have shown a lowering activity of blood glucose, which can be an antidiabetic and reduce cholesterol levels.[11,12]

Based on the background above, there are many benefits of green meniran (Phyllanthus niruri L.), but there are still

Table 1. Phytochemical qualitative test results

Compound Group	Reagent	Alteration	Result
Flavonoid	Oxalic acid and boric acid reagent, 366 nm UV fluorescence	Yellow fluorescence	+
Saponin	Aquades-HCl	Foam is stable	+
Steroid	Liebermann-Burchard	Bluish-green	+
Terpenoid	Liebermann-Burchard	Brown ring appearance	+
Alkaloid	Meyer	White sediment	+
	Bouchard	Blackish-brown sediment	
	Dragendrof	Orange sediment	
	Wagner	No sediment	
Phenol	$FeCl_3$	Blackish-blue	+
Tannin	Pb acetate 10%	White sediment	+
Glycosides	Liebermann-Burchard	Bluish-green	+

nm = nanometer, UV = ultraviolet, HCl = hydrochloride, $FeCl_3$ = ferric chloride

few studies that reported their chemical component contents. Hence the authors intend to identify the content of active compounds in green meniran leaf (Phyllanthus niruri L).

METHODS

An experimental study was conducted to identificate the active compounds in green meniran leaf (Phyllanthus niruri Linn) using phytochemical testing includes qualitative and quantitative testing by LC-MS/MS. At first, sample preparations and extractions were done. Green meniran leaf samples (Phyllanthus niruri L.) were obtained from sugarcane farmers in Batu, Malang. Crude powder packaging was conducted at Materia Medika Herbal Laboratory, Malang, Indonesia. The ethanol extraction from green meniran leaf was conducted at Pharmacology Laboratory, Faculty of Mathematics and Natural Sciences, Udayana University. The fine meniran powder then extracted using the maceration technique for three days; continue with 1.5 liters of 96% ethanol maceration and stir for three days until the liquid extract of green meniran leaf was obtained using a rotary evaporator.

Once a liquid extract of green meniran leaf was obtained, a phytochemical qualitative test was conducted. The phytochemical qualitative test used 10 ml meniran green leaf extract dissolved in 10ml of 96% ethanol. Phytochemical qualitative test including flavonoid tets, saponin test, steroid and terpenoid test, alkaloid test, phenol test, tannin test and glycoside test. Furthermore, we also did a quantitative test to determine each bioactive compound's level in green meniran leaf. Determination of phenol and flavonoid levels calculated using regression equation formula $y = ax + b$.[13] Tannin level was read with a spectrophotometer at λ 725 nm, using a standard curve of tannic acid (0-100 mg/L).[14] Total chlorophyll level was measured directly on the absorbance of the supernatant at 645 and 663 nm. The last is the identification of active compounds in green meniran leaf extract (Phyllanthus niruri L.) were analyzed by LC-MS/MS XEVO G2-S QTOF. Identification is made by comparing the spectrum of the analysis results to the spectrum of standard compounds in the database.

RESULT

Result of Phytochemical Qualitative Test

The qualitative phytochemical test is a preliminary test that can provide information about the group of compounds contained and the potential for samples of natural substances as medicinal substances.[15] The phytochemical test results on the green meniran leaf extract positive for containing flavonoids, saponins, steroids, terpenoids, alkaloids, phenols, tannins, and glycosides, as shown in Table 1.

Result of Phytochemical Quantitative Test

In the phytochemical quantitative test, we determine the levels of each compound. Phenol, flavonoid and tannin level was determined using a UV-Vis UV-Vis spectrophotometric method. The measurement results show the phenol level of green meniran leaf was 5,134.55 mg/100 g GAE or 5.13%. In comparison, the flavonoid level was 3,300.22 mg / 100g QE or as much as 3.30%. The level of tannin was 5.013.44 mg/100g TAE, equivalent to 5.01%. The last is chlorophyll level. Chlorophyll is one of the plant components that have a very important role in forming substances needed by plants to grow and develop. Chlorophyll is the main part that can absorb light, which is useful as a catalyst for the photosynthesis process. Chlorophyll inside green meniran leaf extract also measured using the UV-Vis spectrophotometric method. Based on the measurement results, the total chlorophyll content was 28,212.67 mg/kg.

Identification of Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS)

The process of identifying the content of the active compounds in green meniran leaf extract using the Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS) method and compound interpretation using the MassLynx V4.1 program. The data obtained in the analysis using LC-MS / MS

Table 2. LC-MS/MS identification results

Retention Time (minute)	Molecular Formula	Ion Mass (m/z)		Alleged Compound	Compound Group
		Precursor Ion	Product Ion		
4.92	$C_{21}H_{20}O_{10}$	433.1179	415.1068; 313.0741; and 283.0633	Apigenin-8-C-glucoside	Glycosides
9.84	$C_{18}H_{16}O_5$	313.1074	297.0764; 283.0606; 269.0811; and 255.0655	5,6,7-trimethoxyflavone	Flavonoid

in the form of mass spectra and molecular weight of the estimated compounds. After obtaining information on the sample's mass spectra and molecular weight, mass spectrum matching was carried out with the Chemspider, HMDB, and Massbank databases. The results of the LC-MS/MS identification obtained can be seen in Table 2.

DISCUSSION

In this study, we used the extract derived from dried simplicia of green meniran leaf (Phyllanthus niruri L.) with a size of 60 mesh. According to Malinda and Guntarti, the simplicia particles' size affects the amount of bioactive content that the solvent can draw. This is because the smaller the sample enlarges the outer surface. The large sample surface area will increase the contact between the sample and the solvent used in the extraction process to make the extraction process more optimal. A good sample size to use is 50 - 60 mesh.[16]

The extraction process uses the immersion method (maceration) with 96% ethanol as the solvent. Maceration is a simple and effective method for extracting secondary metabolites in green meniran simplicia at room temperature. The ethanol solvent is commonly used in the extraction process because it can extract all secondary metabolites from polar to non-polar groups. This is because ethanol solvent has a hydroxy group (-OH) as a polar group and an ethyl group ($-C_2H_5$) as a non-polar group. Also, ethanol solvent is an organic solvent with a low boiling point of 78°C so that it is easily separated from the extraction. The extraction process produces a blackish green ethanol crude extract. The ethanol extract of meniran leaf was then tested qualitatively for phytochemicals.[17]

In the phytochemical qualitative test, we used boric acid and oxalic agent to identification flavonoid compounds.[18] The saponin compound was tested by adding water and shaking it vertically after the foam appeared, then diluted HCl was added. The appearance of foam on the surface of the solution indicates the presence of saponin group compounds. Foam is produced from the hydrolysis reaction of saponins. The hydrolysis reaction of saponins in the presence of a match causes the hydrophilic saponin groups to bind to water and the hydrophobic groups to bind to the air to cause foam. The addition of HCl aims to determine the stability of the foam.[19] The group of steroids and triterpenoids was tested using Liebermann-Burchard reagent. Examination of steroids and

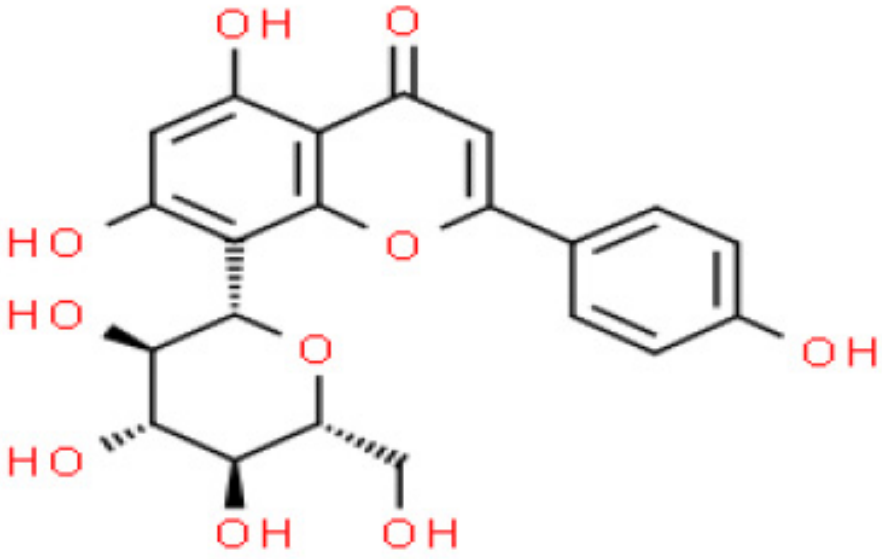

Figure 1. Chemical structure of Apigenin-8-C-glucoside compound

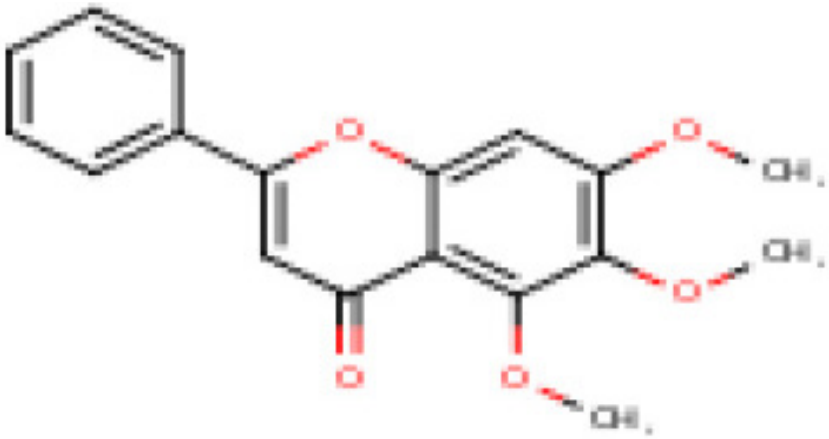

Figure 2. Chemical structure of the 5,6,7-trimethoxyflavone compound

terpenoids based on the ability of discoloration caused by acid anhydrous and concentrated H_2SO_4. A positive result in the steroid group was indicated by a change in the solution to bluish-green and positive triterpenoid with a brown ring formation.[15]

The test for alkaloid group compounds is based on the formation of deposits due to covalent bonds' coordination between the free electrons on the N atom and the positive ions in the reagent. The reagents used were the Meyer reagent, Bouchardat reagent, and Wagner's reagent. Those three reagents showed that the green meniran leaf samples were positive for alkaloid class compounds.[15,19] Testing for phenolic compounds is determined by reacting the sample with $FeCl_3$ reagent. The iron ion (III) will coordinate with the hydroxy group on phenolic compounds to form complex compounds. The positive results of the phenolic test can be seen from the formation of complex blackish-blue compounds.[17] Tannins are polyphenolic compounds which when tested with Pb-acetate reagent, will produce a white residue. This happens because the Pb^{2+} ion in the reagent will form a coordination bond with the tannin compound's polyhydroxy group to form a complex white precipitate.[20]

Glycosides are compounds that contain sugar (glycans) and non-sugar compounds (aglycones). These glycoside compounds include phenolics, flavonoids, and steroids, which are highly polar.[21] The glycoside test results showed that the green meniran leaf extract positively contained a group of glycoside compounds. This is indicated by the change in the color of the sample solution to a bluish-green. This change is caused by the reaction of glycosides with concentrated sulfuric acid in acetic anhydrous.[19]

From the identification of bioactive compounds in green meniran leaf extract using LC-MS/MS, the compounds identified in the green meniran leaf extract are Apigenin-8-C-glucoside (Figure 1) from the glycoside group and 5,6,7-trimethoxyflavone (Figure 2) from the flavonoid group. These compounds' content was strengthened by the results of qualitative phytochemical tests, which produced positive results for the glycoside and flavonoid compounds. Research conducted by He et al. explained that the Apigenin-8-C-glucoside (Vitexin) compound is one of the active compounds that has been widely used as an ingredient in traditional medicine in China has been studied as an antioxidant, anticancer, anti-inflammatory, antihyperalgesic, and neuroprotective effects.[22] In addition, according to Yadav et al., plants containing 5,6,7-trimethoxyflavone compounds are also reported to have anti-inflammatory, cardioprotective, hepatoprotective, anthelmintic, antifungal, antioxidant, antimicrobial activity, cytotoxic activity, antimicrobial, anti-fertility, antipyretic, and insecticide. From each clinical trial, it was found that the majority of bioactive components in green meniran leaf were glycoside and flavonoid. Both bioactive compounds can be used to eliminate bacteria in canal root treatment. They also can act as immunomodulators and anti-inflammation for periodontitis cases with the chronic application. [23]

CONCLUSION

Based on the phytochemical qualitative test results, the green meniran leaf extract was positive for the flavonoids, saponins, steroids, terpenoids, alkaloids, phenols, tannins, and glycosides. The quantitative phytochemical test resulted in phenols' content, flavonoids, tannins, respectively 5.13%, 3.30%, 5.01%, and chlorophyll content of 28.212,67 mg/kg and containing the suspected compounds Apigenin-8-C-glucoside and 5,6,7-trimethoxyflavone from the results of the LC-MS/MS identification. Green meniran leaf has its role as antibacterial, anti-inflammation and strong immunomodulator for periodontitis cases with the chronic application.

CONFLICT OF INTEREST

The authors declare that there is no competing interest regarding the manuscript.

ETHICAL CONSIDERATION

This research was conducted based on the ethical conduct of research from the Ethics Committee of Udayana University.

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AUTHOR CONTRIBUTION

All of the authors equally contributed to the study from the conceptual framework, data gathering, and data analysis until interpreting the study results on publication.

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