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Phytochemical Potential of 70% Ethanol Extract of Gaharu Leaves (*Gyrinops versteegii*) Obtained by Maceration

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ABSTRACT

Agarwood (*Gyrinops versteegii*) is a medicinal plant commodity that has been used ethnopharmacologically due to its broad spectrum of pharmacological activities, which are contributed by its rich secondary metabolite content. This study was conducted to determine the phytochemical potential of 70% ethanol extracts of agarwood leaves obtained through the maceration method. The methods used in this study included extraction, in which the pegagan leaf simplisia was extracted using 70% ethanol at a ratio of 1:10 for 24 hours, then evaporated to obtain a thick extract. The extract yield obtained was 13.35%. The analysis performed included qualitative and quantitative phytochemical tests. Qualitative phytochemical screening showed that the ethanol extract of agarwood leaves (*Gyrinops verstegii*) was positive for flavonoids, tannins, saponins, polyphenols, IC50, and antioxidant capacity. Quantitative testing using the UV-Vis spectrophotometry method showed total flavonoid levels of 62.12 mgQE/g, tannins of 66.90 mgQE/g, polyphenols of 28.177 mgGAE/g, an IC_{50} value of 73.08 ppm, and antioxidant capacity of 19.13 ppm, which is classified as very strong. The results of this study indicate that 70% ethanol extract of agarwood leaves (*Gyrinops verstegii*) contains secondary metabolites that contribute to high antioxidant activity and have the potential to be further developed as a natural antioxidant agent.

Keywords: phytochemical screening; *Gyrinops verstegii*; maceration; 70% ethanol

INTRODUCTION

Agarwood leaves (*Gyrinops versteegii*) are a commodity with high economic and cultural value, which have long been used in various local traditions, including for medicinal purposes, religious rituals, and traditional ceremonies. Beyond its traditional use, G. versteegii has emerged as a significant plant in the pharmaceutical and herbal medicine industries due to its diverse profile of bioactive compounds. The presence of isolated flavonoids in these leaves exhibits very strong antioxidant activity, making it suitable for use as a natural antioxidant to mitigate oxidative stress at the cellular level. Furthermore, these flavonoids, particularly from the subgroup of isoflavones or xanthones found in the Gyrinops genus, possess phytoestrogenic properties. The classification of agarwood (*Gyrinops versteegii*) can be seen in Figure 1.

FIGURE 1: Habitus, Batang, Daun, perbungaan.

The taxonomy of agarwood is as follows[1]:
Kingdom : Plantae
Divisi : Magnoliophyta
Kelas : Magnoliopsida
Ordo : Myrtales
Famili : Thymelaeaceae
Genus : Gyrinops
Spesies : Gyrinops versteegii (Gilg) Domke

Substances that can donate electrons or act as reducing agents are called antioxidants. These compounds have a lower molecular weight that prevents the formation of free radicals. Antioxidants are substances that can inhibit oxidation by neutralizing highly reactive free radicals. Therefore, damage to cells can be limited. Antioxidants neutralize free radicals by counteracting them with a large number of electrons, which will produce neutral atoms that reach a stable state. The presence of antioxidants can prevent the body from various types of degenerative diseases and cancer. The development of many degenerative diseases is controlled by free radicals, which, due to their molecular structure, are highly reactive because they contain unpaired electrons in their outer shell. Free radicals damage macromolecules such as cell lipid membranes, DNA, and proteins, and cause oxidative stress. Various studies show that the antioxidant activity in agarwood leaves (*Gyrinops versteegii*) is classified as having an IC50 concentration <50 ppm, which is categorized as very strong [2]. Agarwood leaves can be used as an alternative measure to combat free radicals due to their role as natural antioxidant compounds. Through analysis of agarwood leaf extract, IC50 values for antioxidant activity were 11.659 µg/mL and 12.958 µg/mL. The presence of phenolics, flavonoids, and tannins is an antioxidant obtained from agarwood leaves [3].

Identifying the phytochemical content of agarwood leaves (*Gyrinops versteegii*) is an important first step in supporting the development of its use. Phytochemical screening is the initial stage for identifying the general content of secondary metabolites in natural materials. Qualitative testing is usually carried out using color tests with specific reagents. The selection of solvents and extraction methods is a determining factor, because unsuitable solvents can inhibit the maximum extraction of active compounds [4]. The selection of extraction solvents generally uses the principle of like dissolves like, where nonpolar compounds will dissolve in nonpolar solvents, while polar compounds will dissolve in polar solvents. This affects the chemical content that can be extracted [5]. Therefore, the purpose of this study was to determine the phytochemical potential of 70% ethanol extract of agarwood leaves (*Gyrinops versteegii*) obtained through the maceration method.

RESEARCH METHOD

The methods used in this study were qualitative and quantitative chemical analysis methods. The preparation of simplisia, extraction, and phytochemical screening were carried out at the Integrated Biomedical Laboratory of Udayana University, Denpasar. The equipment used in this study included: glass maceration vessels, rotary evaporators, Buchner funnels, Erlenmeyer flasks, water baths, test tubes, analytical scales, dropper pipettes, beakers, filter paper, and UV-Vis spectrophotometers. The materials used were 70% methanol, distilled water, agarwood leaves (*Gyrinops versteegii*) obtained from Bali Tangi, Denpasar City, Bali Province, and materials/reagents for phytochemical testing (flavonoids, tannins, polyphenols, IC50, and antioxidant activity).

Simplisia Manufacturing Procedure

A total of 4 kg of fresh agarwood leaves (*Gyrinops versteegii*) were collected and then wet sorted to separate dirt and foreign objects. This process involved washing the leaves with running water to clean the surface of the leaves from soil, dust, or dirt that stuck to them. Next, drying and dry sorting. The dried simplisia is reduced to a coarse powder using a blender and sieved with a 40 and 80-mesh sieve. Then it is weighed, and the weight is recorded. In this study, 654 grams of simplisia were obtained.

Maceration Extraction

The extraction process was carried out using crude drugs with the maceration method using 70% methanol solvent. Maceration was carried out by adding one part of crude drugs into a maserator with ten parts of solvent (1:10), soaked for 6 hours while stirring occasionally, then left for 24 hours. After that, the solution is filtered using filter paper to separate the filtrate. The obtained gotu kola leaf extract filtrate is then concentrated through an evaporation process using a rotary evaporator at a temperature of 40 °C and a pressure of 175 mbar to separate the solvent and obtain a solvent-free liquid extract. After evaporation, the concentrated filtrate was weighed using a porcelain dish to calculate the weight of the concentrated extract produced. In this study, 87.33 grams of concentrated extract were obtained. Next, the yield value obtained was determined as the percentage by weight (w/w) between the yield and the crude drug [6]. The yield value of agarwood leaf extract (*Gyrinops versteegii*) was 13.35%.

Phytochemical Screening

The stock solution was prepared at a concentration of 1000 ppm (10 mg of extract dissolved in 10 ml of ethanol).

- **Flavonoids**

1mL of extract is placed in a test tube, then a few drops of 10% NaOH reagent are added. If positive, the solution will turn orange or orange-red. Next, 1 mL of extract is added to 0.1 grams of magnesium powder and 5 drops of concentrated HCl. If the solution turns orange, the extract is positive for flavonoids.

- **Tannins and Polyphenols**

1 mL of extract is placed in a test tube, then 10% FeCl3 is added. If a dark blue or blackish green color appears, this indicates the presence of tannins.

- **IC50 and Antioxidant Activity**

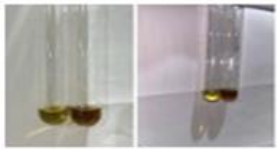
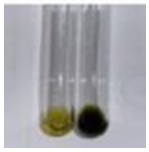
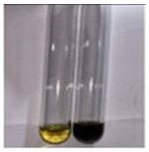
This is done using the DPPH method by preparing a 0.1 mM DPPH solution in methanol, which is stored in dark conditions. The extract is made into a stock solution and then diluted into several concentration variations (e.g., 10, 25, 50, 75, and 100 ppm). A total of 1 mL of each extract concentration was mixed with 1 mL of DPPH solution, shaken homogeneously,

then incubated for 30 minutes at room temperature in the dark. After incubation, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm, with a control consisting of a mixture of 1 mL of DPPH and 1 mL of methanol without extract. The IC_{50} value was determined as the concentration capable of inhibiting 50% of DPPH free radicals.

RESULTS AND DISCUSSION

This study used 4 kg of fresh agarwood leaves (*Gyrinops versteegii*) and produced 654 g of simplisia. The crude extract was then extracted using the maceration method with 70% ethanol at a material: solvent ratio (1:10) for 24 hours to obtain a thick extract of agarwood leaves (*Gyrinops versteegii*) weighing 87.33 g. Qualitative and quantitative phytochemical tests were then conducted. The results of the qualitative phytochemical screening of the agarwood leaf extract (*Gyrinops versteegii*) can be seen in Table 1.

TABLE 1: Qualitative Phytochemical Screening Results.

No.	Parameter	Method/unit	Result		Interpretation
Quantitative Test					
1	Flavonoids	HCl, Magnesium Powder, NaOH		Orange Orange	Positive
2	Tannins	$FeCl_3$ 1%		Dark Green	Positive
3	Polyphenols	$FeCl_3$ 1%		Dark Green	Positive

In this study, the antioxidant value of agarwood leaf extract (*Gyrinops versteegii*) was found to be 19.13 ppm. This is in line with the study by [7], which obtained an antioxidant capacity value of 17.14 ppm. The study by [8] evaluated several comparisons of antioxidant ethanol extracts from agarwood leaves IC50: 16.45 ppm, ethyl acetate fraction: 47.69 ppm, N-hexane fraction: 57.24 ppm, and water fraction: 76.95 ppm. Variations in antioxidant values between studies can be influenced by biological factors, such as plant age, soil fertility, growing altitude, and sunlight intensity, as well as extraction factors including method, solvent type, extraction duration, temperature, and pressure, which affect mass transfer and compound solubility.

Qualitative analysis results show that the ethanol extract of agarwood leaves (*Gyrinops versteegii*) contains flavonoids, tannins, polyphenols, IC50, and high antioxidant capacity. This is in line with research by [9], which states that phytochemical screening of agarwood leaves (*Gyrinops versteegii*) shows the presence of flavonoids, tannins, polyphenols, glycosides, and triterpenoids. Bioactive compounds such as alkaloids, phenols, flavonoids, saponins, tannins, and terpenes are thought to synergistically enhance the activity and phagocytic capacity of macrophage cells as immunomodulators.

TABLE 2: Quantitative Phytochemical Test Results.

No.	Parameter	Method	Result	Interpretation
Quantitative Test				
1	Flavonoids	Spectrophotometry (mgQE/g)	62,12	High
2	Tannins	Spectrophotometry(mgQE/g)	69,90	High
3	Polyphenols	Spectrophotometry(mgGAE/g)	28,17	High
4	IC_{50}	Spectrophotometry(ppm)	73,08	Strong
5	Antioxidant Capacity	Spectrophotometry(ppm)	19,13	Very Strong

Based on Table 2, quantitative analysis using spectrophotometry showed that the total flavonoid content of the ethanol extract of agarwood leaves (*Gyrinops versteegii*) was 62.12mgQE/g. These results are in line with the research by [10], which found that solvent polarity (the ratio of ethanol to water) significantly affects the total phenol and antioxidant activity of agarwood leaves (*Gyrinops versteegii*). Ethanol is a highly effective polar solvent for extracting most of the above secondary metabolites compared to non-polar solvents.

Solvent concentration affects flavonoid yield because flavonoids are generally semi-polar. The presence of water in ethanol increases the polarity of the solvent, thereby helping the solubility and release of flavonoids from the crude drug matrix. In addition, smaller crude drug particle sizes (±180 µm) have been reported to increase total flavonoid content by up to 1.28% w/w EK. This increase occurs due to a larger contact surface area, accelerating solvent penetration and facilitating the diffusion of compounds in the solvent. Conversely, large particle size can reduce extraction efficiency due to higher mass transfer resistance [5].

The ratio of crude drug to solvent also affects the flavonoid content. A ratio of 1:5 produced the highest flavonoid content compared to ratios of 1:10 or 1:20. This condition is thought to be related to changes in the concentration gradient between the matrix and the solvent; at too large a solvent volume, the effective concentration gradient can decrease, thereby reducing the diffusion rate. In addition, extracts that are too dilute can reduce the intensity of contact between the solvent and the matrix, thereby reducing the mass transfer rate of semipolar flavonoids. The use of excessive solvent also has the potential to cause over-washing, which can alter the distribution of dissolved compounds during the separation process, thereby reducing the yield of target compounds in the final extract [5].

The tannin content of agarwood leaves (*Gyrinops versteegii*) in this study was 69.90 mgQE/g. This is significant in relation to the results of the study by [11], based on qualitative data showing a strong positive reaction (+++) to the tannin test on 70% ethanol extract of agarwood leaves (*Gyrinops versteegii*). The results of the study by [11] also reported similar results, namely that the total tannin content in the ethanol extract of agarwood leaves was in the range of 1.5% to 4.2% or equivalent to 15-42 mg/g of extract. This figure shows that tannin is the second largest secondary metabolite after flavonoids. However, the total tannin content in the study by [12] differs from several other studies using 96% ethanol solvent, which obtained a fairly stable figure of ~3.8% of the dry extract weight. Based on this description, it can be concluded that the use of 70% ethanol solvent is more effective than 96%.

Gyrinops versteegii polyphenols in this study yielded 28.1774923 mgGAE/g, which is classified as high antioxidant potential. This differs from the results of [14], who obtained a total phenol content of agarwood leaf extract of 14.81% GAE, which is classified as moderate, due to the use of 85% methanol as a solvent. The results of the study by [15], describe different results, where the phenol content obtained was 12.26% and 19.73% in young and old agarwood leaves (*Gyrinops versteegii*). These differences may occur due to differences in extraction methods, solvent types, plant varieties, and environmental conditions.

This study obtained IC50 and antioxidant capacity values of 73.08 ppm and 19.13 ppm, which are classified as strong and very strong, respectively. These results are in line with the study by [13], which obtained an IC50 value of 31.6304 µg/mL, classified as very high. Another study conducted by [10], showed that the antioxidant activity of agarwood leaves (*Gyrinops versteegii*) was the highest in the very strong category, marked by an EC_{50} value of 14.46 µg/mL. Furthermore, ethyl acetate extract showed antioxidant activity in the moderate category with an EC_{50} value of 121.26 µg/mL. Meanwhile, n-hexane extract did not show significant antioxidant activity, as reflected in the high EC_{50} value of 486.24 µg/mL.

The antioxidant activity of agarwood leaves (*Gyrinops versteegii*) has been reported to be related to their cytotoxic effects. Chloroform extract of agarwood leaves (*Gyrinops versteegii*) showed cytotoxic activity with an IC_{50} value of 22.71± 1.31 µg/mL. This IC_{50} value indicates strong cytotoxic potential because it is below 100 µg/mL. The presence of phenolic and flavonoid compounds contributes to cytotoxic activity through oxidative stress mechanisms, apoptosis induction, or inhibition of cancer cell proliferation [16]. In addition, the UV protection activity of agarwood leaves (*Gyrinops versteegii*) varies depending on the type of solvent. Methanol extract shows ultra-effectiveness with an SPF value exceeding 15, while ethyl acetate extract falls into the maximum category (SPF 8-15), and n-hexane extract is at a moderate level (SPF 4-6) [10].

CONCLUSION

This study shows that 70% ethanol extract of agarwood leaves (Gyrinops versteegii) obtained through the maceration method has significant potential. It can be concluded that 70% ethanol extract of gotu kola leaves contains secondary metabolites that contribute to high antioxidant activity and have the potential to be further developed as a natural antioxidant agent in the prevention and treatment of diseases related to oxidative stress.

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