Antibacterial Activity of the Leaves Extract of Tristaniopsis Merguensis Griff from the Indonesian Indigenous Plant against Staphylococcus Aureus ATCC 25923

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***Abstract*—Based on ethnopharmacology, *Tristaniopsis merguensis* Griff. is one of the indigenous Indonesian plants from the genus *Tristaniopsis,* which has the potential for biological activity, including its role as an antibacterial agent. This research aims to analyze the antibacterial activity of the extracts of the *Tristaniopsis merguensis* Griff. leaves against *Staphylococcus aureus* ATCC 25923 using the disc diffusion method with streptomycin as positive control and methanol as negative control. The chemical compounds of *T. merguensis* leaves were extracted using *n-hexane*, ethyl acetate, and methanol through the multilevel maceration method at room temperature for 2 × 24 hours. The results showed that methanol extract has the highest yield 12.7% compared to *n-hexane* and ethyl acetate, which are 0.78% and 10.58%, respectively. Phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids, and tannins in the leaf extract. Gas Chromatography/Mass Spectrometry (GC–MS) analysis of active extract confided presence of caryophilene, hexadecanoic acid, α-acarenol, 9-octadecanoic acid, ethyl iso-allocholate, seline-3,7(11)-diene, among many compounds. The results of the disc diffusion antibacterial test on the diameter of the inhibitory zone of the leaves extract showed that the ethyl acetate extract of the leaves had the greatest inhibition against the bacteria *S. aureus* at concentration of 20,000 ppm with the inhibition clear zone diameters of 9.7 mm, respectively. Based on this data, it can be concluded that the ethyl acetate of *T. merguensis* holds significant potential as a source of antibacterial compounds.**

***Keywords*—*T. merguensis*, antibacterial, *S. aureus*, patoghenic bacteria**

I. Introduction

The development of antibacterial use is currently increasing, the ability of a compound or extract to act as an antibacterial is frequently linked to its potential as an antibiotic for various diseases caused by pathogenic microorganisms. Antibacterial drugs represent a significant public health concern. A recent study estimated the global mortality burden attributed to resistance at 1.27 million deaths in 2019 [1]. Antibacterial resistance can occur through changes in the molecular structure of antibiotics, decreased penetration of antibiotics, and changes in active sites and cell adaptation due to frequent exposure to antibiotics [2]. To overcome this, searching for new antibiotic candidates from natural ingredients and designing antibiotics with new targets. According to Centers for Disease Control and Prevention (2013) reports, in 2025, it is estimated that around 23,000 people will die from infectious diseases with antibacterial resistance.

The previous research showed that the *Tristaniopsis merguensis* Griff. plants have potential biological activities. Additionally, they offer a promising solution to this issue by serving as a reservoir of natural products sought in the quest for novel compounds. Pelawan plant is a member of the *Myrtaceae* family and is widely distributed in the forests of the Bangka Belitung Islands. *T. merguensis* extracts had the highest of total phenolic content [3]. The major active compounds of the *Tristaniopsis* genus are phenolic group compounds such as flavonoids and tannins. The phenolic compounds contained in the genus *Tristaniopsis* are unique in that they have glycosylated phenol groups [4]. The mixture that has high phenolic content can have bioactivity as an antibacterial, preventive agent cancer, and toxicity [5]. The anti-bacterial activity of methanolic extract of pelawan steam had antibacterial activity against *S. aureus* and *E. coli* [6].

The compounds have successfully isolated three compounds from the dicloromethane extract of the whole plant of *Tristaniopsis coloboxus*, namely Tristaneone A and flavonoid derivatives such as eucalyptin and 8-desmethyleucalyptin [7].

The study in the stem and roots part have been conducted by many researchers, but the leaves extract still needs more exploration. For this reason, this study aimed to evaluate the antibacterial activities of different leaves extract against pathogens bacteria. The leaf of *T. merguensis* Griff was extracted with different solvents using *n-hexane*, ethyl acetate, and methanol. The antibacterial activity was investigated by the disc diffusion method and streptomycin as a positive control.

II. Literature Review

Tristaniopsis merguensis is one of the members of the Myrtaceae family. Resistant plants are widely distributed in the forests of the Bangka Belitung Islands [8]. The medicinal properties of this plant are extensive, yet information on the secondary metabolites of *T. merguensis* remains limited, particularly in Indonesia. The plant *T. merguensis* exhibits a diverse range of secondary metabolites such as flavonoids, tannins, saponins, steroids, and triterpenoids, with one of them belonging to the flavonoid group, demonstrating antibacterial properties [9].

The research by Brophy and Goldsack (1999) [10] reported that *Tristaniopsis* plants from the *Myrtaceae* genus contain essential oils. *T. colinna* contains compounds such as *α-pinene* (35.9%), *myrcene* (5.6–29.3%), and *cubenol* (0–16.6%). *T. exiliflora* contains *β-caryophyllene* and

δ-cadinene (16.4%). *T. laurina* contains *α-pinene* (79.4%), *limonene* (13%), and *globulol* (9.7%). *T. neriifolia* contains *α-pinene* (24.4%), *α-eudesmol* (17.8%), *β-eudesmol* (17.2%), and *γ-eudesmol* (28%).

Panagan and Syarif (2009) [11] reported that the liquid smoke from the pyrolysis of *T. abavata* wood can inhibit the growth of *E. coli* bacteria. The ethanol extract of *Tristaniopsis whiteana* leaves contains compounds of flavonoids, alkaloids, tannins, phenols, and terpenoids. These compounds also exhibit bioactivity as antibacterial agents [12].

III. Materials and Methods

The sample *T. merguensis* Griff was collected from Bangka Belitung Island, Indonesia. The specimen was determined and deposited (No. 0262/S. Tb/II/2023) at the Laboratory of Plant Systematics, Universitas Gadjah Mada, Special Region of Yogyakarta, Indonesia. The solvents used for extraction are *n-hexane*, ethyl acetate, and methanol purchased from SmartLab. The materials used for the antibacterial tests are nutrient agar, nutrient broth obtained from Merck, paper blank disc, and streptomycin from Oxoid. Instrumentation used multimode microplate reader (SPARK TECAN), laminar air flow, incubator, and autoclave Tutt-nauer.

A. Extraction of Sample

The fresh sample of *T. merguensis* leaves (1160 g) was cut into small pieces. The samples were maceration using *n-hexane*, ethyl acetate, and methanol as a solvent for 2 × 24 hours. All extracts were evaporated to give a residue of leaf extract of *n-hexane* (33.04 g), extract of ethyl acetate (444.38 g), and extract of methanol (12.17 g). All of the extract was tested using phytochemical screening [13] (Harborne, 1998).

B. Analysis of Extract Using Gas Chromatography Mass Spectrometry (GC-MS)

Analysis of extract for identification of its components was carried out using Shimadzu GC–MS ISQD (Thermo Scientific ISQ LT Single Quadropole Mass Spectrometer). The column used for the analysis was a 30 mm × 0.25 µm × 0.25 mm i.d. HP-5MS UI. Onemicrolitre of the solution prepared by dissolving one milligram of extract in one millilitre of ethyl acetate and was injected into the GC–MS system in the split mode (split ratio 1:5) using autosampler. Helium with a flow rate of 1.4 mL/minute act as the carrier gas. For two minutes, a temperature of 60 °C was maintained in the column. The final temperature of 280 °C was then held for one minute after being set to 240 °C at a rate of 4 ◦C/minute. The optimal temperatures for the injector and detector were 230 and 250 °C, respectively. Ionisation energy, 70 eV; ion source temperature, 200 °C; solvent delay, 5.0 min; scan range, 40 to 500, were the MS operating parameters. The components were recognised based on a match between their retention indices and mass spectral libraries (Chromeleon 7).

C. Antibacterial Assay

Antibacterial activity of sample tested by disc diffusion dilution against Staphylococcus aureus ATCC 25923 from Faculty of Medicine, Universitas Gadjah Mada. Inoculant made by growth bacteria in Nutrien Broth for 24 hours and diluted until the bacteria concentration was 0.1 OD (1 × 108 CFU/mL) measurement using multimode microplate reader. The disc diffusion assay was carried out by spreading the inoculant at Nutrien Broth agar. Methanol was used as negative control; streptomycin disc 10 µg was used as positive control. The extract of the sample with concentrations of 5000, 10,000, and 20,000 ppm was added for 10 µL to obtain a 10 µg sample on the disc. The inoculant was incubated for 24 hours at 37 °C [14]. The active extract as antibacterial was determined by calculating the clear zone of inhibition.

IV. Result and Discussion

Determination was conducted at the Systematics Plant, Universitas Gadjah Mada, and conducted that the sample used was *Tristaniopsis merguensis* Griff. or pelawan for the local name. *T. merguensis* extraction with the multilevel maceration method, the yield of extract of *n-hexane* at 0.78%, extract of ethyl acetate at 10.5%, and extract of methanol at 12.17%. Variations in the yield of extract in *T. merguensis* can be caused by several factors, including the size of the simplicia and the content of secondary metabolites.

Phytochemical screening was carried out to overview the class of compounds contained in the leaf extract of *T. merguensis* The secondary metabolites phytochemical screening of *T. merguensis* with different solvents of the leaf is shown in Table 1. The results of the phytochemical screening test showed that *T. merguensis* leaf extract from the different solvents contained alkaloids, flavonoids, terpenoids, tannins, steroids, and phenolic compounds.

Table 1. The phytochemical screening of various solvents of extracts *T. merguensis*

No	Secondary Metabolites	Reagent	*n-hexane*	ethyl acetate	methanol
1.	Alkaloids	Dragendrof	+	+	+
2.	Flavonoids	$AlCl_3$ 5%	+	+	+
3.	Tannins	$FeCl_3$ 5%	−	−	+
4.	Terpenoids	CH_3COOH/H_2SO_4	+	+	+
5.	Saponins	H_2O	−	−	−

As shown in Table 1, the three extracts of *T. merguensis* such as n-hexane, ethyl acetate, and methanol extracts contained flavonoids and alkaloids. Meanwhile, only the methanol extract contains tannin. Phytochemical analysis of ethyl acetate and methanolic extract of *T. merguensis* showed the presence of alkaloids, terpenoids, steroids, flavonoids.

Based on this data it can be concluded that many polar compounds are extracted in the polar solvent methanol such as tannins. Methanol is a polar solvent, this is the same as flavonoid compounds and phenol, both of which have antibacterial properties. Because the properties of the solvent and the dissolved compound are the same, it will make it easier for the solvent to dissolve the compound from leaf of *T. merguensis*. *T. merguensis* stem extract also contains flavonoids, alkaloids, terpenoids, and steroids [6]. Secondary metabolites of the genus *Tristaniopsis* contain tannins 1.04%, flavonoids 0.03%, and saponins 0.95% [15]. The difference in results obtained could be due to several factors, one of which is the solvent used. The effectiveness of extraction is influenced by the level of solubility of the material with the solvent used [16]. The majority of the secondary metabolite of the genus Tristaniopsis is phenolic compounds such as tannins and flavonoids. Phenolic compounds generally have

certain biological activities. *T. merguensis* stem extract also contains flavonoids for all types of extracts [3].

GC–MS analysis of active extract detected presence of among a total of 74 compounds (Fig. 1) (Tables 2).

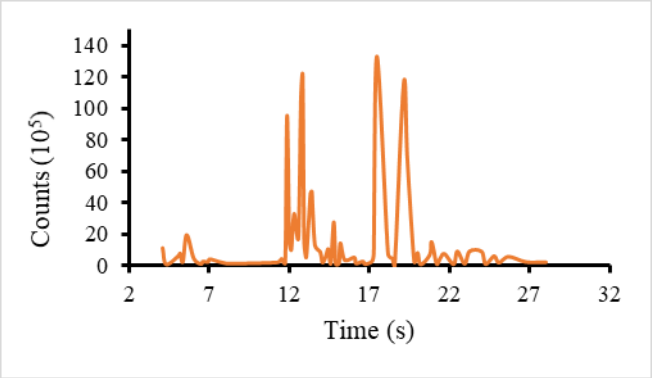


Fig. 1. GC–MS Chromatogram of ethyl acetate extract of *T. merguensis*.

Table 2. GC-MS analysis of ethyl acetate extract of *T. merguensis*

Compounds	Retention Time	Probability
Caryophilene	11.88	8.16
Hexadacanoic acid	18.46	11.36
α-acarenol	14.79	2.73
9-Octadecanoic acid	19.17	9.96
Ethyl iso-allocholate	21.45	0.44
Selina-3,7(11)-diene	13.42	4.04

GC–MS analysis of extract detected presence of Caryophilene, hexadecanoic acid, α-acarenol, 9-octadecanoic acid, ethyl iso-allocholate, seline-3,7(11)-diene. GC–MS analysis of extract detected presence of terpenoid groups according to Brophy and Goldsack (1999) [10] the genus *Tristaniopsis* contained the major compound terpenoid groups and essential oil.

Evaluation for antibacterial activity of the extract was conducted by testing against pathogenic oral bacteria of *S. aureus ATCC 25923* using disc diffusion methods for inhibition zone values. The antibacterial activity of the different solvents n-hexane, ethyl acetate, and methanol extracts of *T. merguensis* plant against pathogenic bacteria is presented in Fig. 2. The highest antibacterial activity against *S. aureus ATCC 25923* was shown in the extract of ethyl acetate and methanol, while inhibition zone 9.7 mm at a concentration of 20,000 ppm indicated a very strong interpretation. Compared to the methanolic extract at 8.4 mm, the extract of ethyl acetate was more active than the streptomycin as positive control at 9 mm. All extracts of *T. merguensis* have an inhibition zone except for n-hexane has no clear zone inhibition. The ethyl acetate extracts of the leaves showed better antibacterial activity compared to other parts of the plant. The findings from this study, following the CLSI protocol's guidelines, delineate susceptibility categories for bacteria based on the inhibition zone method: susceptible (≤ 20 mm), intermediate (15–19 mm), and resistant (≤ 14 mm). Within the spectrum of inhibited bacteria, the majority exhibit susceptibility solely to the ethyl acetate extracts, suggesting that numerous active compounds are soluble in semi-polar organic solvents.

As shown in Fig. 2, the ethyl acetate extract of *T. merguensis* leaves was able to inhibit the growth of *S. aureus* bacteria from the lowest to the highest concentration. The wider diameter of the inhibition zone formed proves the strength of the bioactive compound in inhibiting bacterial growth [17]. Factors that also influence the increase in inhibitory diameter are due to the concentration of antimicrobial agents which increases with each concentration. Apart from that, the ability of antibacterial activity in leaf extract of *T. merguensis* Griff. is because the leaves of plant positively contain other secondary metabolite compounds such as flavonoids, saponins, alkaloids, tannins, phenolics, triterpenoids, steroids and glycosides. The methanolic extract of *T. merguensis* stem had the potential antibacterial activity against *S. aureus* and *E. coli* [6].

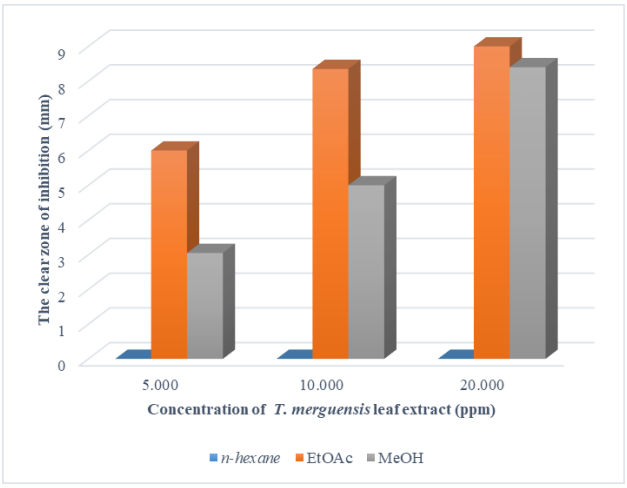
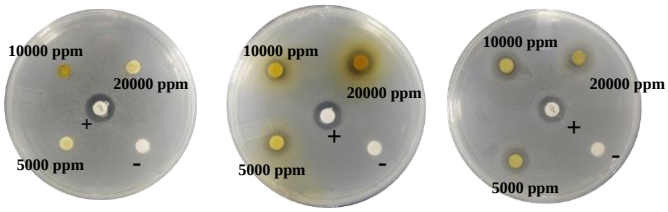


Fig. 2. The measurement result of the inhibition zone diameter against *S. aureus*.

The potential of the ethyl acetate extract as an antibacterial agent due to the presence of flavonoid compounds revealed in the phytochemical screening. Flavonoids are known for their antibacterial properties. The mechanism of action of flavonoids as antibacterials can be categorized into three aspects. First, they inhibit nucleic acid synthesis, wherein the A and B rings of flavonoids play a crucial role in intercalation or hydrogen bonding. This process involves accumulating nucleic acid bases, ultimately impeding the formation of DNA and RNA. Consequently, the interactions with flavonoids cause damage to the cell wall permeability [18].

Secondly, flavonoids hinder the function of the cell membrane by forming complex compounds with extracellular and dissolved proteins, leading to membrane damage and the release of intracellular compounds. Lastly, they impede energy metabolism by restraining bacterial oxygen utilization. This inhibition occurs by preventing energy formation in the cytoplasmic membrane and hindering bacterial motility, both of which are essential for antimicrobial activity and extracellular proteins [18]. Fig. 3 provides a visual representation of the antibacterial test.

As shown in Table 2, of the ethyl acetate extract as an antibacterial agent due to the presence of terpenoid compounds revealed in the phytochemical screening. The mechanism of terpenoids as antibacterials involves reacting with porins (transmembrane proteins) on the outer membrane of bacterial cells, forming strong polymer bonds that lead to the damage of porins. The damage to porins, which serve as the entry and exit gates for compounds, reduces the permeability of the bacterial cell wall, causing the bacteria to be deprived of nutrients. As a result, bacterial growth is inhibited or the bacteria die [19].



(a) (b) (c)

Fig. 3. (a) Antibacterial activity of n-hexane extract, (b) ethyl acetate extract and (c) methanolic extract.

Based on Fig. 3, the inhibition zone is formed at the lowest concentration of 5000 ppm to the highest concentration of 20,000 ppm for ethyl acetate and methanol extract. The concentration used is a factor that also influences the size of the inhibition zone that forms around the paper disc that has been filled with extract. The wider diameter of the inhibition zone formed proves the strength of the bioactive compound in inhibiting bacterial growth [11].

V. Conclusion

The result of the phytochemical screening test showed that *n-hexane*, ethyl acetate, and methanol extract of the leaf of *T. merguensis* had positive results in the flavonoids and terpenoid groups. GC-MS analysis of ethyl acetate as active extract presence of caryophilene, hexadecanoic acid, α-acarenol, 9-octadecanoic acid, ethyl iso-allocholate, seline-3,7(11)-diene. Antibacterial test based on diameter measurements inhibition zone indicates ethyl acetate extract had antibacterial activity against *S. aureus ATCC 25923* has the clear zone diameter of 9.7 mm, in the resistant to moderate category.

Conflict of Interest

The authors declare no conflict of interest.

Author Contribution

Taufik Hidayat: Conducted the research, validation, formal analysis, investigation, and writing the original draft.

Respati Tri Swasono: Validation, formal analysis, writing-review and editing.

Winarto Haryadi: Conceptualization, validation, formal analysis, writing-review, and editing.

Boima Situmeang: conducted the research especially in antibacterial activity test and investigation the result.

All authors had approved the final version.

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