Phytochemicals Screening and Antibacterial Activity of *Teijsmania dendron Holrungii* from West Papua

Sabir Sumarna¹, Muhammad Fajar Islam²*, Aslan Irunsa³, Maria J. Sadsoeitoeboen⁴, Kornela Irianti Mandatjan⁵

¹ Department of Chemistry, Papua University, Indonesia, s.sumarna@unipa.ac.id
² Department of Chemistry, Papua University, Indonesia, m.fajar@unipa.ac.id
³ Department of Biotechnology, Institut Teknologi Kelautan Buton, Indonesia, aslan.irunsah@gmail.com
⁴ Department of Biology, Papua University, Indonesia, ritasadsoeitoeboen@gmail.com
⁵ Microbiology laboratory, Papua University, Indonesia, drimys.alexandria@gmail.com

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**ABSTRACT**

Papua is an island with the highest flora biodiversity in the world. To date, the phytochemical content and antibacterial bioactivity of the *Teijsmania dendron holrungii* plant from West Papua have never been reported. In this study, phytochemical screening and bioactivity tests of *T. holrungii* bark ethanol extract against *E. coli* and *S. aureus* bacteria were carried out. The results showed that *T. holrungii* bark ethanol extract contained alkaloids, flavonoids, steroids, and tannins. The inhibitory activity of bacterial growth increased with an increase in concentrations (w/v) of 5%; 10%; 20%; 40%; and 80%, with inhibitory zones of 14.5 mm, 17 mm, 18 mm, 20 mm, and 22 mm for *E. coli* bacteria and 4.5 mm, 5.5 mm, 6 mm, 7.5 mm, and 9.5 mm for *S. aureus* bacteria.

Keywords: Antibacterial, Secondary metabolites, Phytochemicals, Teijsmania dendron holrungii

**INTRODUCTION**

Natural products have historically been a source of antibacterial medicinal ingredients. Various bioactive secondary metabolites have been found, from both terrestrial and marine sources. Many of these natural products are current drug candidates. The medicinal uses of plants have been well documented for thousands of years. Plants have evolved and adapted over millions of years to survive the onslaught of bacteria, insects, fungi, and weather by producing unique and chemically diverse secondary metabolites. According to the World Health Organization...
(WHO), 80% of people still rely on traditional plant-based medicines for health care, and 80% of 122 plant-based medicines are related to ethnopharmacological purposes. Knowledge of traditional medicine (complementary or alternative herbal products) has encouraged further investigation of the potential of medicinal plants to isolate many natural products into pharmacologically useful drugs (Dias et al., 2012).

The content of phytochemicals in medicinal plant parts can cure various acute and chronic diseases, such as coronary heart disease, diabetes, liver, high blood pressure, and cholesterol. Based on their function in plants, phytochemicals consist of primary and secondary metabolites. Primary metabolites are needed for the survival of plants, such as carbohydrates, amino acids, proteins, fats, purines, and pyrimidines, which are the main composition of nucleic acids (DNA). In contrast, secondary metabolites are chemical residues produced by plant cells through their derivative metabolic pathways to produce chemical components that are antiviral, antifungal, and antibiotic, which function to protect plants from other organisms or pathogens (Rabizadeh et al., 2022).

The main phytochemical components in medicinal plants are tannins, alkaloids, saponins, glycosides, steroids, terpenoids, and flavonoids. Phytochemical screening of leaves, roots, bark, and fruit of medicinal plants has been carried out to identify and isolate the content of secondary metabolites and their bioactive properties (Agidew, 2022). The results show that phenolic and flavonoid compounds have anti-inflammatory, antimalarial, antidepressant, antidiabetic, cytotoxic, antitumor, antimicrobial, and antioxidant properties. In addition, steroids and their derivatives from medicinal plants have antibacterial and insecticidal properties (Ndezo Bisso et al., 2022).

Papua is the largest tropical island in the world and is home (habitat) to several ecosystems on this Earth that are still preserved and globally recognized as a center of biodiversity and culture. A total of 13,634 plant species have been inventoried, of which 68% are endemic from 1,742 genera and 264 families. This shows that Papua is an island with the most biodiversity of flora in the world that has not been optimally managed and used. Plant endemism in the New Guinea region is very high, the only island group in Malesiana that has more endemic species, around 68% of the total species (9,301 species) (Cámara-Leret et al., 2020).

Angiosperms have a higher species endemism (71%) than ferns and lycophytes (46%) or gymnosperms (41%). Eight angiosperm families account for 50% of all endemic species: Orchidaceae (2,464 endemic species), Rubiaceae (669), Ericaceae (431), Arecaceae (257), Myrtaceae (255), Gesneriaceae (218), Apocynaceae (196) and Lauraceae (195). Families with the highest proportion of endemism are Ricaceae (98% endemic species), Gesneriaceae (96%), and Zingiberaceae (95%). All Vaccinium (Ericaceae) species in the New Guinea region are endemic and more than 95% of the species of Begonia (Begoniaceae), Cyrtandra (Gesneriaceae), Glomera (Orchidaceae), Psychotria (Rubiaceae), Rhododendron (Ericaceae), Saurauia (Actinidiaceae) and Taeniophyllum (Orchidaceae) are also endemic. 61 plant species endemics to New Guinea and 164 species (1–17 species per genus) or 2% of endemic species. However, molecular research is urgently needed to test the endemic monophyline species because there is no phylogenetic data (Cámara-Leret et al., 2020).

Two antibacterial compounds, ferruginol, and trans communication acid, have been isolated from Pauacedrus papuana leaves by (Agusta et al., 2022). Sadsoeitoenboen and Kilmaskoossu (2010) reported that the Moorish community on Arui Island, Nabire Regency, has used 39 species from 30 plant families as traditional medicines, including drugs for malaria, diabetes, chickenpox, measles, kidney stones, high blood pressure, rheumatism, and toothache. Another plant that has medicinal properties is akway wood (Drimys beccariana Gibbs), in which the
methanol extract of akway leaves and bark has antibacterial activity (Parubak, 2010). Antibacterial activity has also been reported by Restianti et al., (2020) against *E. coli* and *S. aureus* from n-hexane, ethyl acetate, and methanol extracts of Suruhan plants (*Peperomia pellucida* L. Kunth) and methanol extracts (Siahaan et al., 2021) Fruit Black (*Haplolobus monticola*) from wondama bay.

*Teijsmania dendron holrungii* is spread across the islands of Kalimantan, Sulawesi, Maluku, and Papua, and grows in primary or secondary forests along rivers and swamps (de Kok et al., 2009). The Maybrat people in West Papua use the leaves of the *T. holrungii* plant as an antidote to snake venom (Hara et al., 2009). To date, studies on the content of secondary metabolites and antibacterial tests of *T. holrungii* have never been reported. Phytochemical screening and antibacterial bioactivity tests have been carried out on this plant to determine the secondary metabolites contained in the ethanol extract of *T. holrungii* stem bark and how these compounds inhibit *Escherichia coli* and *Staphylococcus aureus* bacteria.

**METHODOLOGY**

**Sampel Preparation**

The object of this study was the bark of the *T. holrungii* tree taken from Mount Meja, Amban Manokwari Barat. The sample was then dried and ground to obtain a powder form. *T. holrungii* bark powder was macerated with 70% ethanol for 3 x 24 h, and the macerated ethanol was separated by filtering using ordinary filter paper. The macerated ethanol extract of *T. holrungii* stem bark was concentrated using a rotary evaporator EYELA N-1000. The result of evaporation was a dry extract of *T. holrungii* bark, which was used for further testing.

**Phytochemical Screening**

Phytochemical screening was intended to observe the secondary metabolites contained in the ethanol extract of *T. holrungii* stem bark. Tests for the content of alkaloids, flavonoids, steroids, triterpenoids, tannins, and saponins were carried out on extract samples referring to Harbone's standard phytochemical screening method (1998).

**Antibacterial Bioactivity Test**

The antibacterial activity of the ethanol extract of *T. holrungii* stem bark was carried out against gram-positive *S. aureus* bacteria and gram-negative *E. coli* bacteria. Antibacterial activity testing used the agar diffusion method using chloramphenicol as the positive control. The dry extract of *T. holrungii* bark was dissolved in DMSO with several concentration variations, namely 5; 10; 20; 40; and 80%. Extracts with various concentrations were then soaked with paper disks and contacted with the test bacteria that had been inoculated on nutrient agar media. The measurement of the diameter of the inhibition zone was carried out after incubation for 24 h (Hasan et al., 2022).

**RESULTS AND DISCUSSION**

The ethanol extract of *T. holrungii* stem bark from the Gunung Meja Region, was tested for its phytochemical content (Table 1). The results of stem bark screening of *T. Holrungii* contained secondary metabolites of alkaloids, flavonoids, steroids, and tannins, while the saponin and triterpenoid tests showed negative results. The alkaloid test results on the ethanol extract of *T. Holrungii* showed positive results, as indicated by the formation of white precipitate when reacted with Mayer, Wagner, and Dragendorff reagents. The precipitate formed as a result of
the reaction between K⁺ ions from the reagent and N atoms in the alkaloids produced a potassium-alkaloid complex.

Table 1. Phytochemical screening of ethanol extract of *t. holurungii* bark

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Reagent</th>
<th>Result</th>
<th>Blank</th>
<th>Indicator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>Dragendorff</td>
<td>+</td>
<td>Blank Indicator</td>
<td>There was a white precipitate</td>
</tr>
<tr>
<td>Mager</td>
<td>+</td>
<td>There was a white precipitate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wagner</td>
<td>+</td>
<td>There was a white precipitate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonoid</td>
<td>NaOH</td>
<td>+</td>
<td>Blank Indicator</td>
<td>Color changed to orange</td>
</tr>
<tr>
<td>Shinoda</td>
<td>+</td>
<td>Color changed to red</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saponin</td>
<td>aqudest</td>
<td>-</td>
<td>Blank Indicator</td>
<td>No permanent foam was formed</td>
</tr>
<tr>
<td>Steroid/Triterpenoid</td>
<td>Libermann Burchard</td>
<td>+</td>
<td>Blank Indicator</td>
<td>Color changed to green</td>
</tr>
<tr>
<td>Tannin</td>
<td>FeCl₃</td>
<td>+</td>
<td>Blank Indicator</td>
<td>Color changed to blackish green</td>
</tr>
</tbody>
</table>
The flavonoid test also provided positive results using the Shinoda test method and the reaction with 10% NaOH, in which the color of the sample changed to yellow/orange. The reaction mechanism that occurred was the hydrolysis of flavonoid glycosides from plant extracts by concentrated HCl to form flavonoid aglycones. Mg reacts with HCl to form Mg$^{2+}$ ions and H$_2$ gas (bubbles in the mixture). Flavonoids then formed a complex with Mg$^{2+}$ ions causing a color change. NaOH reacted with flavonoids to form red quinoid compounds or yellow acetophenone.

Table 2. Bioactivity of T. holrungii Bark Ethanol Extract

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>E. coli</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>14.5</td>
<td>4.5</td>
</tr>
<tr>
<td>10</td>
<td>17</td>
<td>5.5</td>
</tr>
<tr>
<td>20</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>40</td>
<td>20</td>
<td>7.5</td>
</tr>
<tr>
<td>80</td>
<td>22</td>
<td>9.5</td>
</tr>
<tr>
<td>Control (+)</td>
<td>27.5</td>
<td>26</td>
</tr>
</tbody>
</table>

The ethanol extract of T. holrungii stem bark positively contained tannin compounds. The tannin compound in the sample binds to Fe$^{3+}$ to form a complex, causing the color to turn green or blue-black depending on the type of tannin compound. Steroid and triterpenoid detection was carried out by the Libermann-Burchard reagent with acetic anhydride and concentrated sulfuric acid. Acetic anhydride reacted with concentrated sulfuric acid to form a carbocation, then the O atom on the hydroxyl group of the terpenoid compound reacted with the carbocation to produce a brownish-red color change. Dehydration of steroid compounds due to the addition of concentrated sulfuric acid, further oxidation occurred causing the formation of conjugated double bonds resulting in a change in color to bluish-green. The test result was positive for steroids and negative for triterpenoids. The saponin test does not provide a positive result because no permanent foam is formed for 10 min.

The results of the antibacterial test of T. Holrungii stem bark showed activity in inhibiting the growth of the test bacteria (Table 2). The inhibition power of the ethanol extract of T. Holrungii stem bark increased with the increasing concentration of the extract. Based on a comparison of the diameter of the inhibition zone of the sample extract and chloramphenicol as a positive control, the ethanol extract of T. holrungii stem bark was more sensitive in inhibiting the growth of E. coli bacteria than S. aureus bacteria. The inhibition activity of bacterial growth from the ethanol extract of T. Holrungii stem bark is caused by the content of secondary metabolites, namely alkaloids, flavonoids, steroids/terpenoids, and tannins.

Most of the alkaloids that are antibacterial and antifungal have a quinoline or indole basic framework, with a target mechanism of inhibition of alkaloid compounds through cross-linking with DNA or protein molecules that cause genetic mutations, topoisomerase inhibition, and damage to the cytoplasmic membrane in bacterial and fungal cells (Herrera et al., 2022; Alghazeer et al., 2022; Sulaiman et al., 2022). Flavonoid compounds have been identified as polyphenolic compounds with antibacterial activity through various mechanisms. According to research results, flavonoids can inhibit nucleic acid synthesis, damage the cytoplasmic membrane, and inhibit bacterial cell metabolism. Flavonoids can also reduce adhesion and biofilm formation, porins in cell membranes, membrane permeability, pathogenicity, and inhibit cell proliferation, all of which are essential for bacterial growth (Baker, 2022; Shamsudin et al., 2022; Wronska et al., 2022).
The mechanism of steroid antibacterial activity through interactions with lipid membranes results in damage to liposomes, thereby inhibiting the entry of nutrients into bacterial cells (Nadaraia et al., 2019; Felisbino et al., 2021; Sharaf et al., 2022). Similarly, tannin compounds inhibit bacterial growth through interactions with cell membranes, causing changes in the structure of cell membranes and damaging membrane function in bacteria (Carvalho et al., 2018; Maisetta et al., 2019; Olchowik-Grabarek et al., 2022).

CONCLUSION

The results of the phytochemical screening showed that the ethanol extract of T. holrungii stem bark contained secondary metabolites of alkaloids, flavonoids, steroids, and tannins. The ethanol extract of T. holrungii stem bark could inhibit the growth of E. coli and S. aureus bacteria. Thus, it had potential as an antibiotic drug. Based on the results of this study, T. holrungii stem bark extract had an excellent opportunity to isolate and characterize compounds that acted as raw materials for new drugs.

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REFERENCES


