



Phytochemical Analysis and *Escherichia coli* Anti-bacteria Activity of Teak (*Tectona grandis* Linn. f) Leaves Extract

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Abstract. Teak (*Tectona grandis* L.f.) is a medicinal plant that has been traditionally used by people around the forest as medicine. Teak leaves contain antibacterial compounds. This study aims to find out what compounds are contained in teak leaves extract and test antibacterial activity against *E. coli* bacteria. The methods used in this study are maceration for leaves extract, qualitative methods for phytochemical tests, and descriptive research methods with laboratory analysis techniques. The phytochemical analysis will be tested for alkaloids, flavonoids, saponins, tannins, triterpenoids, steroids, and carotenoids. And antibacterial tests against *E. coli* bacteria consisting of 7 treatments, with five constituents, namely 20%, 40%, 60%, 80%, and 100%. One positive control using chloramphenicol 0.1% and one negative control using aquades. The data obtained will be presented descriptively by looking at the color change and the shape of the liquid being tested, the data obtained will be presented in the form of pictures and tables. The results showed that teak leaves extract positively contained flavonoids flavonoid compounds, tannins, and steroids. While alkaloid compounds, saponins, triterpenoids, and carotenoids showed negative results. For the results of antibacterial tests of Teak leaf extract, using 96% ethanol solvent against *Escherichia coli* bacteria with an average value of inhibitory power at a concentration of 20% (13.73 mm), a concentration of 40% (16.36 mm), a concentration of 60% (17.43 mm), a concentration of 80% (18.66), and a concentration of 100% (19.42 mm), the higher the concentration, the greater the inhibition zone formed.

Keywords: phytochemical analysis · antibacterial · *Tectona grandis*

1 Introduction

Forests are Natural Resources that Have Many Benefits for Human Life [28, 38]. These Benefits Can Be Divided into Two, Namely, Tangible and Intangible Benefits [32]. Tangible Benefits Are Forest Benefits in the Form of Material or in the Form of Wood and Non-wood [5]. Meanwhile, Intangible Benefits Are Benefits Obtained from Forests that Cannot Be Directly Assessed by the Market System or Are in the Form of Immaterial/cannot Be Touched, Such as Natural Beauty, Microclimate, Hydrology, and so on [23].

Indonesia's Current Management of Forest Products is Directed at Developing Non-wood Forest Products [9]. Non-wood Forest Products Are Products Derived from Biological Forest Products, Both Animal and Vegetable, and Cultivation Products, Except for Wood Originating from Forests [9, 23]. Non-wood Forest Products Are Part of Forest Ecosystems that Have Various Roles for the Natural Environment and Human Life [37]. Medicinal Plants Are One of the Non-forest Products that Have the Potential to Be Developed and Used by the Community for Generations Around the Forests of the Central Sulawesi Region [11]. Medicinal Plants Are an Alternative for Communities Around the Forest Because There is Still a Lack of Access to Health Facilities [9]. One of the Medicinal Plants Used by the Local Community Regularly, Especially the People Around and Within the Forest Area, is Teak Leaves [21, 30].

Teak (*Tectona Grandis* L.f.) is a Medicinal Plant that Has Been Traditionally Used by People Around the Forest as Medicine and Dye [3, 33]. Using Young Teak Leaves as a Natural Dye Can Give a Red Color Because Teak Leaves Have Natural Pigments, Namely Anthocyanins [25]. The Results of Several Studies Stated that Teak Leaves (*Tectona Grandis* L.f) Have also Been Used by People in Several Regions in Indonesia [26]. Local People Generally Use Teak Leaves as Medicine for Several Infectious Diseases [10, 16]. Infectious Diseases Are One of the Most Common Types of Diseases Suffered by People in Developing Countries, Including Indonesia [18]. in Rural Areas, Many Infectious Diseases Are Caused by Bacteria [31]. Various Types of Bacteria Stick to Hands Daily Through Physical Contacts, Such as *Escherichia Coli*, *Salmonella Typhi*, *Staphylococcus Aureus*, and *Pseudomonas Aeruginosa* [15].

Using Teak Leaves as a Medicine for Several Infectious Diseases in Central Sulawesi Needs to Be Scientifically Proven Through Research. The Purpose of This Study Was to Determine the Content of Secondary Metabolites and the Anti-bacterial Activity of *Escherichia Coli* in Extracts of Teak Leaves Medicinal Plants.

2 Method

2.1 Study Area

Teak leaves medicinal plant samples were obtained from Palolo District, Central Sulawesi Indonesia.

2.2 Research Procedure

Observation Variable. The variables observed in this study are the secondary metabolic compounds contained in teak leaves and the antibacterial activity of *E coli*.

Simplicia Preparation. Sorted healthy teak leaves, cleaned with water, and dried in direct sunlight to dry brittle. After the drying process, it was mashed with a blender and sieved to obtain a uniform sample, then weighed [11].

Extraction. Extraction using the maceration method with 96% ethanol solvent [12, 35]. The sample weighed 200 g, was put into a measuring cup, and added 96% alcohol until the volume reached 1 L (1:5 ratio), then allowed to stand for 24 h. To obtain teak leaves extract, the results of maceration were filtered until the filtrate became clear and dried using a rotary evaporator vacuum [11].

Phytochemical Analysis. The phytochemical analysis is a qualitative analysis conducted to determine the secondary metabolic components of teak leaves medicinal plant extracts. The analytical method is based on [11, 13, 27]. The phytochemical analyzes tested included: the alkaloid test, Flavonoid Test, Saponin Test, Tannin Test, Steroid/triterpenoid test, Carotenoid test [11, 13, 27].

Teak Leaves Extract Antibacterial Activity Test. The antibacterial test was carried out on E coli bacteria with the well method according to the working method [12]. The concentration of teak leaves extract used was 20%, 40%, 60%, 80%, 100%, and 0.1% chloramphenicol positive control and aquadest negative control. Each treatment was repeated three times. 1 ml of E coli bacteria suspension in 0.9% NaCl solution was taken and poured into a sterile petri dish. After that, 20 ml of nutrient agar medium was added to a petri dish containing the bacterial suspension, then homogenized and allowed to solidify. After the media is solid, in the media mixed with the bacterial suspension, wells are made using a cork bore. The wells were filled with 50 ml of teak leaves extract, 0.1% chloramphenicol and distilled water, then incubated for 24 h at 37 °C. The inhibition zone of teak leaves extract was observed by measuring the diameter of the inhibition zone formed [12, 27].

2.3 Data Analysis

The data is presented descriptively by looking at the color change and the shape of the liquid being tested. Furthermore, the data obtained from the phytochemical and antibacterial tests are presented in the form of tables and figures [12].

3 Result and Discussions

3.1 Phytochemical Analysis of Teak Leaves Ethanol Extract

The results of the phytochemical screening of teak leaves' medicinal plant extracts are presented in Table 1.

Information:

+ : Positive (Contained compound/formed color)

– : Negative (No compound/no color formed)

Table 1. Phytochemical screening of teak leaves extract.

Compound	Description	Result
Alkaloids	No orange precipitate occurs	–
Flavonoids	Formed a layer of red, yellow-orange	+
Saponins	No permanent foam formation	–
Tannins	are formed dark blue or blackish green	+
Terpenoids	No reddish-brown color is formed on the surface	–
Steroids	Formed red layer and yellow and green undercoat	+
Carotenoids	No blue color is formed on the surface	–

Flavonoid Test. Testing the flavonoid compounds' content in teak leaves' extract showed positive results. The same results in research on teak bark also showed the presence of flavonoid compounds [14]. Flavonoids can act as antifungals because they have phenolic compounds that can denature proteins and can damage cell membranes that are irreversible (irreversible) [8]. Flavonoids inhibit bacterial growth by damaging the cell wall, deactivates the action of enzymes, binds to adhesins, and damages cell membranes. Beta rings and clusters –OH in flavonoids is thought to be the structure responsible for antibacterial activity [1, 22].

Tannin Test. Tests for tannin compounds in the extract of teak leaves showed that they contained a positive tannin compound which was indicated by a change in colour to dark blue or blackish green in the sample. The results of the same study on the bark of teak bark also contain tannin compounds in teak bark [33]. Tannins are one of the secondary metabolites that have antifungal and bacterial activity [34]. In addition to tannins, several phenolic compounds in teak leaves also have anti-cancer activity. Tannin compounds can inhibit bacterial enzymes so that DNA topoisomerase and transcriptase enzymes are not formed so that the tannin compound can function as an antibacterial [19]. In addition, tannins have an antibacterial activity associated with inactivating microbial cell adhesins and inactivating enzymes and disrupting protein transport [17].

Steroid Test. Testing of steroid compounds in the extract of teak leaves showed a positive presence of steroid compounds. This is because the formation of a red layer and the bottom layer of sulfuric acid shows yellow and green colors. The same results in the study showed positive results containing steroid compounds [2]. In contrast to research bark teak showed negative results containing steroid [14]. Steroid compounds can cause damage to bactericidal liposomes, steroids also cause steroids as antibacterials related to lipid membranes and sensitivity to steroid components which cause leakage in liposomes. Steroids can cause the integrity of the bacterial cell membrane to decrease in function causing the bacterial cells to become brittle and experience impaired function [6, 29].

3.2 Teak Leaves Extract Antibacterial Test

Research on antibacterial originating from plants is currently mostly carried out by researchers. Medicinal plants that are used by local people for antibacterial properties are teak leaves. The test results for the effectiveness of the inhibition of teak leaves extract against *E. coli* bacteria can be seen in the following (Table 2):

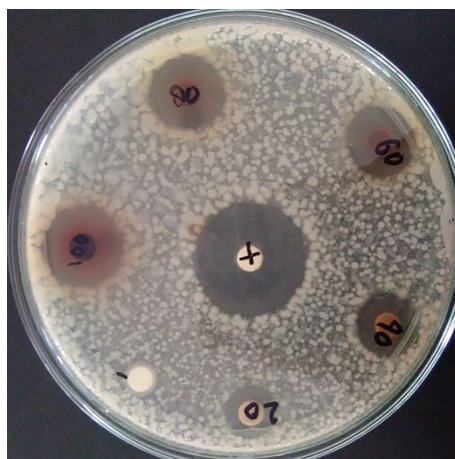
Information:

R: Replicate.

The results showed that teak leaf extract at a concentration of 20%, 40%, 60%, 80%, 100%, and 0.1% chloramphenicol was able to inhibit *E. coli* bacteria with a marked presence of an inhibition zone (clear) around the holes (Fig. 1), while the negative control using distilled water did not form an inhibition zone (clear), this was because the distilled water did not have any compounds that could inhibit the growth of the test bacteria.

Table 2. Antibacterial test of *Escherichia coli* extract of teak leaves

Treatment	inhibition zone (mm)			Mean	Category
	R1	R2	R3		
Control –	0	0	0	0	Negative
20%	12.63	13.33	15.23	13,73	Strong
40%	15.77	16.37	16.94	16,36	Strong
60%	17.25	17.55	17.5	17,43	Strong
80%	18.96	18.78	18.25	18,66	Strong
100%	19.58	19.22	19.47	19,42	Strong
Control +	19.58	29.29	29.74	29,1	Very strong

**Fig. 1.** Inhibition of teak leaves extract on the growth of *E. coli* bacteria

The comparison of the inhibition zones of teak leaves extract against *E. coli* bacteria can be seen in Fig. 2. Figure 2 explains the average diameter gives an inhibition zone at 20% concentration of 13.73 mm, 40% concentration of 16.36 mm, 60% concentration was 17.43 mm, 80% concentration was 18.66 mm, and 100% concentration was 19.42 mm, and positive control gave an inhibition zone of 29.1 mm. In contrast, the negative control did not provide an inhibition zone.

Figure 2 also shows that increasing the concentration amount affects the inhibition zone's diameter. The inhibition zone of the highest teak leaves extract was obtained when the extract was given at a concentration of 100%, but the inhibitory power produced was smaller than the positive control of 0.1% chloramphenicol. Based on these results, extract from teak leaves has inhibitory activity against *Escherichia coli* bacteria. The test results showed that different concentrations would have a significantly different effect on the effectiveness of inhibiting the growth of *E. coli* bacteria. The higher the concentration

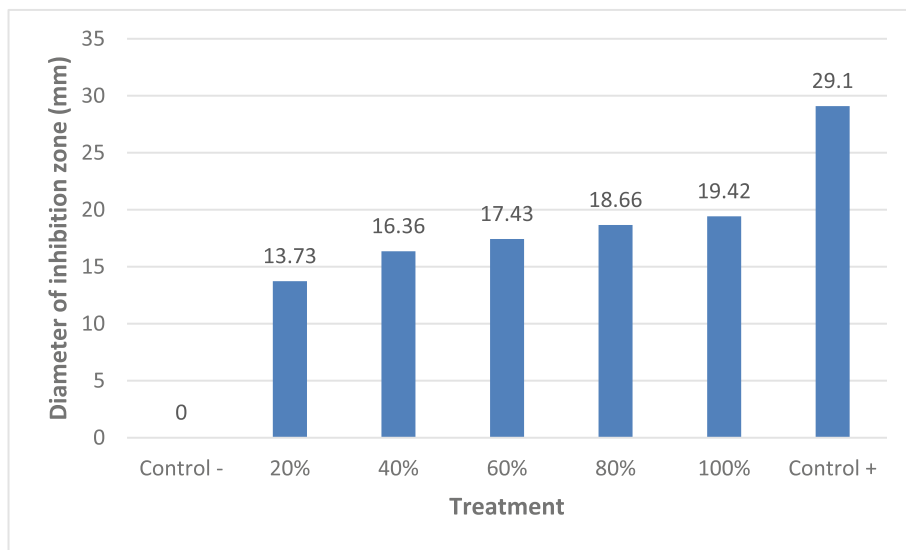


Fig. 2. Diameter of inhibition zone (mm) teak leaves extract

of teak leaf extract, the greater the ability to inhibit the growth of *E. coli* bacteria. This is because the higher the concentration of the extract, the higher the amount of secondary metabolites that function to inhibit bacteria [7].

The diameter of the inhibition zone formed has a value of 0–5 mm, then the antibacterial power is weak. If the inhibition zone formed is 5–10 mm, then the antibacterial power is moderate, while at 10–20 mm, the antibacterial power is strong, and > 20 mm, its antibacterial power is very strong. Based on this, it can be seen that teak leaves extract has a strong inhibitory power because it has an inhibitory zone of 19.42 mm at a concentration of 100%, and 3% chloramphenicol is categorized as very strong because it has an inhibition zone of 29.1 mm [4].

Based on the results of research carried out through phytochemical screening tests, data are obtained from compounds in the extract of teak leaves, namely flavonoids, tannins, and steroids. Flavonoids have a mechanism of inhibiting bacterial growth, namely by forming complex compounds against extracellular proteins that disrupt the integrity of the bacterial cell membrane [7, 8, 15, 29]. The mechanism of tannins as an antibacterial is that tannins have chelating properties that can shrink the walls or bacterial cell membranes and interfere with their permeability so that bacterial growth will be inhibited. Steroids can interfere with forming membranes and cell walls by damaging their lipophilic groups, so bacterial cells are fragile and lysed [6, 20], [324].

4 Conclusion

The results of phytochemical analysis of the ethanolic extract of teak leaves (*Tectona grandis* Linn. f), using 96% ethanol as solvent, were positive for flavonoids, tannins, and steroids. At the same time, alkaloids, saponins, triterpenoids, and carotenoids were

negative. Antibacterial test results of teak leaves extract, using 96% ethanol as a solvent against *Escherichia coli* bacteria with an average value of inhibition at a concentration of 20% (13.73 mm), 40% concentration (16.36 mm), 60% concentration (17.43 mm), 80% concentration (18.66), and 100% concentration (19.42 mm), the higher the concentration, the larger the inhibition zone formed.

Acknowledgment. We thank you the Dean of the Faculty of Forestry, Tadulako University for the research funding support that has been given to us through contract number 456.k/UN28.2/PL/2021, and the students of the Faculty of Forestry, Tadulako University who have helped us in sampling and testing in the laboratory.

References

1. Al Aboody, M. S., Mickymaray, S.: Anti-fungal efficacy and mechanisms of flavonoids. *Antibiotics*, 9(2), 45 (2020).
2. Astiti, N. P. A.: Efektivitas ekstrak daun jati (*Tectona grandis* L. F) dalam menghambat pertumbuhan jamur *Hormiscium* sp. *Jurnal bumi lestari*, 15(1), 66–70 (2015).
3. Charoensit, P., Sawasdiapol, F., Tibkawin, N., Suphrom, N., Khorana, N.: Development of natural pigments from *Tectona grandis* (teak) leaves: Agricultural waste material from teak plantations. *Sustainable Chemistry and Pharmacy*, 19, 100365 (2021).
4. Davis, W. W., Stout, T. R.: Disc Plate Method of Microbiological Antibiotic Assay: II. Novel Procedure Offering Improved Accuracy I. *Applied Microbiology*, 22(4), 666 (1971).
5. de Mello, N. G. R., Gulinc, H., Van den Broeck, P., Parra, C.: Social-ecological sustainability of non-timber forest products: A review and theoretical considerations for future re-search. *Forest Policy and Economics*, 112, 102109 (2020).
6. Doğan, A., et al.: An investigation of antibacterial effects of steroids. *Turkish Journal of Veterinary & Animal Sciences*, 41(2), 302–305 (2017).
7. Egra, S., et al.: Aktivitas antimikroba ekstrak bakau (*Rhizophora mucronata*) dalam menghambat pertumbuhan *Ralstonia solanacearum* penyebab penyakit layu. *Agrovigor: Jurnal Agroekoteknologi*, 12(1), 26–31 (2019).
8. Friedman, M.: Antibacterial, antiviral, and antifungal properties of wines and winery by-products in relation to their flavonoid content. *Journal of agricultural and food chemistry*, 62(26), 6025–6042 (2014).
9. Giesen, W.: Utilising non-timber forest products to conserve Indonesia's peat swamp forests and reduce carbon emissions. *Journal of Indonesian Natural History*, 3(2), 17–26 (2015).
10. Hapid, A., Ramlah, S.: Potensi Tumbuhan Obat Di Kawasan Hutan Taman Nasional Lore Lindu (Studi Kasus Desa Namo Kecamatan Kulawi, Kabupaten Sigi). *ForestSains* 14(1), 24–32 (2016).
11. Hapid, A., Napitupulu, M., Zubair, M. S.: Ethnopharmacology and antioxidant activity studies of woody liana original wallacea. *International Journal of Design and Nature and Ecodynamics*, 16(5), 495–503 (2021).
12. Hapid, A., Napitupulu, M., Zubair, M. S.: Phytochemical Screening, GC-MS Analysis, Toxicity and Antimicrobial Properties of Extracts Outer Shell *Poikilospermum suaveolens* (Blume) Merr. *International Journal of Research and Innovation in Applied Science*, 06(09), 111–117 (2021).
13. Harborne, J. B.: *Metode fitokimia*. Penerbit ITB, Bandung (1987).

14. Hutabarat, F. V., Diba, F., Sisillia, L.: Daya Hambat Ekstrak Kulit Jati (*Tectona grandis* Linn F) terhadap Pertumbuhan Jamur Pelapuk Kayu *Schizophyllum commune* Fries. *Jurnal Hutan Lestari*, 7(3), 1- 9 (2019).
15. Ichor, T., Aondoakaa, E. M., Ebah, E. E.: Comparative Studies on the Antibacterial Activity of Alcohol-Based Hand Sanitizers Against Bacteria Isolates from the Hands of Undergraduate Students of University of Agriculture. *J Clin Case Rep*, 8(7), 1–6 (2018).
16. Irsyad, M. N., Jumari, J., Murningsih, M.: Studi Etnobotani Masyarakat Desa Sukolilo Kawasan Pegunungan Kendeng Pati Jawa Tengah. *Bioma Berk. Ilm. Biol.* 15(1), 27–34 (2013).
17. Javed, B., Nawaz, K., Munazir, M.: Phytochemical analysis and antibacterial activity of tannins extracted from *Salix alba* L. against different gram-positive and gram-negative bacterial strains. *Iranian Journal of Science and Technology, Transactions A: Science*, 44(5), 1303–1314 (2020).
18. Kitagawa, K., et al.: International comparison of causative bacteria and antimicrobial susceptibilities of urinary tract infections between Kobe, Japan, and Surabaya, Indonesia. *Japanese Journal of Infectious Diseases*, 71(1), 8–13 (2018).
19. Liu, M., Feng, M., Yang, K., Cao, Y., Zhang, J., Xu, J., et al.: Transcriptomic and metabolomic analyses reveal antibacterial mechanism of astringent persimmon tannin against Methicillin-resistant *Staphylococcus aureus* isolated from pork. *Food chemistry*, 309, 125692 (2020).
20. Moghimipour, E., Handali, S.: Saponin: Properties, Methods of Evaluation and Applications. *Annual Research & Review in Biology*, 5(3), 207–220 (2015).
21. Nidavani, R. B., Mahalakshmi, A. M.: Teak (*Tectona grandis* Linn.): a renowned timber plant with potential medicinal values. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(1), 48–54 (2014).
22. Nugraha, A. C., Prasetya, A. T., Mursiti, S.: Isolasi, Identifikasi, Uji Aktivitas Senyawa Flavonoid sebagai Antibakteri dari Daun Mangga. *Indonesian Journal of Chemical Science*, 6(2), 91–96 (2017).
23. Pandey, A. K., Tripathi, Y. C., Kumar, A.: Non timber forest products (NTFPs) for sus-tained livelihood: Challenges and strategies. *Research Journal of Forestry*, 10(1), 1–7 (2016).
24. Pitopang, R., Umrah, U., Harso, W., Nurainas, N., Zubair, M. S.: Some botanical aspects of *Etlingera flexuosa* (Zingiberaceae) from Central Sulawesi (Indonesia) and its antifungal activity. *Biodiversitas Journal of Biological Diversity*, 21(8), 3547–3553 (2020).
25. Pramananda, V., Fityay, T. A. H., Misran, E.: Anthocyanin as natural dye in DSSC fabrication: A review. in *IOP Conference Series: Materials Science and Engineering*, vol. 1122, no. 1, p. 12104 (2021).
26. Puspita, A. A., Sachari, A., Sriwarno, A. B.: Knowledge from Javanese Cultural Heritage: How They Manage and Sustain Teak Wood. *Current Biochemistry*, 5(3), 1–10 (2018).
27. Puspita, P. J., Safithri, M., Sugiharti, N. P.: Antibacterial activities of sirih merah (*Piper crocatum*) leaf extracts. *Current Biochemistry*, 5(3), 1–10 (2018).
28. Seidl, R., Spies, T. A., Peterson, D. L., Stephens, S. L., Hicke, J. A.: Searching for resilience: addressing the impacts of changing disturbance regimes on forest ecosystem services. *Journal of applied ecology*, 53(1), 120–129 (2016).
29. Shinde, A. B., Mulay, Y. R.: Phytochemical analysis and antibacterial properties of some selected Indian medicinal plants. *International Journal of current Microbiology and Applied sciences*, 4(3), 228–235 (2015).
30. Sree, D. S., Sankar, N. R.: Evaluation of thirteen medicinal plant extracts against teak (*Tectonic grandis*) leaf skeletonizer *Eutectona machoeralis* Walk. *Biomed*, 3(1), 33–35 (2008).
31. Sudheesh, P. S., Al-Ghabshi, A., Al-Mazrooei, N., Al-Habsi, S.: Comparative patho-genomics of bacteria causing infectious diseases in fish. *International journal of evolutionary biology* 2012, 1-6 (2012).

32. Suharti, S., Darusman, D., Nugroho, B., Sundawati, L.: Economic valuation as a basis for sustainable mangrove resource management: A case in East Sinjai, South Sulawesi. *Jurnal Manajemen Hutan Tropika*, 22(1), 13 (2016).
33. Suryanti, V., Kusumaningsih, T., Marliyana, S. D., Setyono, H. A., Trisnawati, E. W.: Identification of active compounds and antioxidant activity of teak (*Tectona grandis*) leaves. *Biodiversitas Journal of Biological Diversity*, 21(3), 946-952 (2020).
34. Wafa, N., Sofiane, G., Mouhamed, K.: The antioxidant and antimicrobial activities of flavonoids and tannins extracted from *Phlomis bovei* De Noé. *European Journal of Experimental Biology*, 6(3), 55–61 (2016).
35. Wolde, T., Kuma, H., Trueha, K., Yabeker, A.: Anti-bacterial activity of garlic extract against human pathogenic bacteria. *J Pharmacovigil*, 6(253), 2–8 (2018).
36. Zahro, L., Agustini, R.: Uji efektivitas antibakteri ekstrak kasar saponin jamur tiram putih (*pleurotus ostreatus*) terhadap *Staphylococcus aureus* dan *Escherichia coli*. *UNESA Journal of Chemistry*, 2(3), 120–129 (2013).
37. Zhu, L., Lo, K.: Non-timber forest products as livelihood restoration in forest conservation: A restorative justice approach. *Trees, Forests and People*, 6, 100130 (2021).
38. Zulkaidhah, Z., Malik, A., Hapid, A., Hamka, H., Ariyanti, A., Rahman, N.: The diversity of termite species on natural forest and agroforestry land in Sulawesi tropical forests in Indonesia. *Annals of Silvicultural Research*, 46(2), 141–147 (2021).

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