International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Antimicrobial Activity of *Terminalia Catappa* L Leaves in Various Colors

Anang Juni Yastanto[1], Retno Indrati[2]

[1,2]Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia

ABSTRACT

Ketapang leaves, widely known for their antifungal properties, are traditionally extracted using organic solvents. However, this study aimed to demonstrate that it can be more accessible and practical to extract them with water. The study focused on the effect of leaf age (marked by its color) with water extraction on its antimicrobial ability. The results showed that ketapang leaf extract (*Terminalia catappa* L.) can inhibit the growth of microbes, both gram-positive, negative, and fungal growth. The effectiveness of the inhibition was higher in the inhibition of fungi. Young leaves (color light green) and old leaves (color red) are very effective in inhibiting fungal growth, higher than the dichloro control. The content of phenols and antioxidants is high compared to the other two leaf colors. At a concentration of 20% extract, it inhibited 100% of the growth of the fungus *A. oligosporus*.

Keywords: Terminalia Catappa, Leaves Color, Antimicrobia, *A. Oligosporus*

INTRODUCTION

Terminalia catappa L. belongs to the *Combretaceae*family. This plant is widely grown in tropical countries. In Indonesia, *Terminalia catappa* L., locally known as Ketapang, grows wild in the lowlands or is planted as a shade tree on the roadside or in a parking space. All parts of this plant can be used mainly for traditional medicine. For example, the leaves are used for ointments for scurvy, leprosy, and other skin diseases; fruit for treating bronchitis, colon, and diabetes; root bark for the treatment of dysentery, diarrhea, and as an antimicrobial (Nair & Chandra, 2008). The seeds can be used as a source of nutrition, energy, and vegetable oil and can be processed into various kinds of food (Ningrum, 2021). The fruit contains ascorbic acid; it tastes bitter and astringent. The bark contains tannins, while the seeds contain oil. Research by Anam et al. (2009) also showed that *Terminalia* sp. has significant potential as an herbal medicine for treating type 2 diabetes because it contains a-glucosidase inhibitor that will slow down glucose absorption, offering hope for future medicinal applications.

Among the parts of the *Terminalia catappa* plant, its leaves have been extensively studied, especially for their anti-bacterial and antifungal activity. *Terminalia catappa* leaves in fish farming have long been recognized as anti-bacterial and antifungal; until now, this method is still used (Aminah et al., 2014; Helda et al., 2018). Extraction methods usually use ethanol or methanol (Naz et al., 2007; Nadirah et al., 2013), chloroform (Putra et al., 2011), a mixture of hexane and water (Naz et al., 2007), and water (Nadirah et al., 2013). In general, the extraction method that has been published is through the process of drying the leaves, milling them into a powder, and then extracting them with a solvent through overnight soaking (Nadirah et al., 2013). In this study, fresh leaves were directly extracted using a water solvent. The goal is

to make the application easier. In addition, the influence of leaf age was also studied based on leaf color (light green, dark green, yellow, and red). Meanwhile, Nadirah (et al., 2013) divide the age of leaves into young leaves (3rd leaf from the top) and mature leaves (9th leaf from the top). This study aimed to find the effect of leaf color on the inhibition of microbial growth and the minimum concentration of inhibition.

MATERIALS AND METHODS

Material

Fresh ketapang leaves (*Terminalia catappa* L.) are taken from plants around campus. Bacteria (*Escherichia coli* and *Staphylococcus aureus*) and fungi (*Rhizopus oligosporus*) are collections from the Food Biotechnology laboratory, UGM. Media used were Mueller Hinton Agar form Merch and Potato Dextrose Agar from Oxoid. The chemicals used are analytical grade.

Preparation of leaf extract

The leaves used in the study were of various colors, including light green, dark green, yellow, and red (Figure 1). To extract the active compounds, 100g of fresh leaves were thoroughly washed and blended with 100 mL of water. The resulting extract was separated from the pulp using Whatman paper no. 42 And then centrifuged at 4000 rpm for 15 minutes to remove any precipitate. The clear extract obtained was stored at 4 ^{o}C until further use.

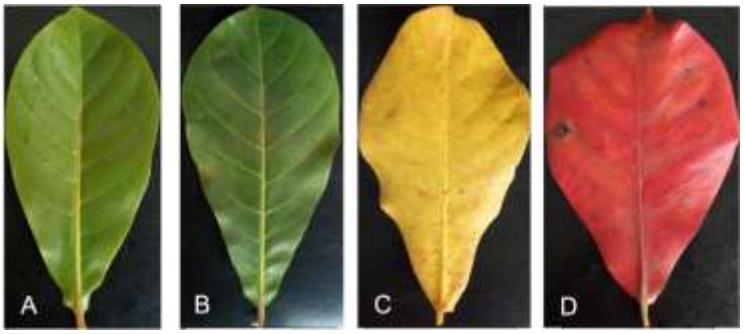

Figure 1. Changes in leaf color from young to late fall. A, young leaves (light green); B, dark green; C, old leaves (yellow); D, old leaves (red).

Growth inhibition analysis

Bacteria growth inhibition was analyzed using the method of well diffusion using Mueller Hinton Agar media, with a well diameter of 5 mm (20 ml leaf extract). Incubation was carried out at 37 ^{o}C for 24 h. Each petri dish contained 10^{6}CFU bacterial cells inoculated by the pour plate method. The resulting clear zones indicating growth inhibition were measured

Fungi growth inhibition was measured using a medium of Potato Dextrose Agar containing leaf extract (10 mL/Petri dish). Twenty mL of fungal spore suspension (105/mL) was inoculated in a well 5 mm in diameter (volume 20 mL) and then incubated at 30 ^{o}C for 4 d. Growth diameter was measured daily. As a control, 100 ppm Chloramphenicol (for bacteria) or 100 ppm Dichloran (for fungi) were used.

In the same way the minimal concentration of leaf extract (0–50%), which inhibits the growth of the fungus *R. oligosporus*, was studied with various incubation times of 0–4 days. Every day the diameter of the colony was measured. The percentage of inhibition was calculated with the following formula:

$$\% \text{ Inhibition} = (Ac - At)/Ac \times 100$$

Where Ac = control absorbance and At = test absorbance. The MIC was defined as the lowest concentration at which no fungal growth was observed.

Phenolic compound

The phenolic compound was measured using the method of Khoddami et al. (2013), and the results were compared with the standard curve of phenol. All tests were performed in triplicate.

Antioxidant activity

The antioxidant activity was analyzed by the free radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Christodoulou et al., 2022). All tests were performed in triplicate.

RESULT AND DISCUSSION

Inhibition of microbial growth

Changes in leaf color indicate the age of the leaves, starting from light green to dark green, getting older yellow and red before falling off. Ketapang leaf extract showed a clear solution, but the young leaf extract looked slightly cloudy. All extracts obtained were free from bacterial or fungal contamination; this indicates that the solution contains compounds that can inhibit or kill microbes. Tests on inhibiting the growth of Staphylococcus aureus bacteria showed that all types of leaves could inhibit growth, although light green and red leaf types inhibited more strongly (Table 1). On the other hand, the inhibition test on Escherichia coli bacteria, light green leaves, and red leaves did not show any inhibition. All these results were still below the inhibition of chloramphenicol (100 ppm). In other words, the ketapang extract was ineffective in inhibiting the growth of gram-positive and gram-negative bacteria. This result differed from the report of Nadirah et al. (2013), who found that the aqueous extract of ketapan leaves effectively inhibited the growth of *Vibrio parahemolyticus*. However, this study is also in line with the results of research by Nair and Chanda (2008), which states that the extraction of ketapang leaves with water is less effective in inhibiting bacterial growth. As stated by Cock (2015) that non-polar components are responsible for inhibiting bacteria. Extraction using organic solvents such as acetone and methanol is very effective in inhibiting (bacteriostatic)/killing (bactericidal) the growth of bacteria (*Staphylococcus aureus*, *Proteus vulgaris*, *Salmonella typhi*, *Pseudomonas aeroginosa*, and *Bacillus subtilis*) (Raveesha, 2021).

Table 1. Inhibition of ketapang leaf extract (*Terminalia catappa* L.) on the growth of *Escherichia coli* and *Staphyllus aureus bacteria*, and the fungus *Rhizopus oligosporus*.

Sampel	Clear Zone (mm) [2)]		Diameter Growth (mm) [3)]
	E.coli	*S.aureus*	*R. oligosporus*
Control[1)]	40.4	40	50.7
Light green	0	10.5	0
Dark green	6.3	8	0
Yellow	9.7	7.1	0
Red	0	11.8	0

Note: 1) control: chlorampenicol 100 ppm for bacteria and dichloran 100 ppm for fungi; 2) clear zone, indicating inhibition zone (the higher the number, the more inhibition); 3) diameter growth, indicating growth inhibition (the lower the number, the more inhibition). Fungal growth on media without the addition of dichlorine or leaf extract had a diameter of 91.5 mm.

Table 1 also shows the growth inhibition of the fungus *Rhizopus oligosporus*. All types of leaves (50% concentration, 5 g/mL) inhibited 100% of fungal growth (zero diameter growth). The control in the form of 100 ppm dichlorane showed a diameter of 50.7 mm or only inhibited 44.6%. From these results, ketapang leaves extracted with water were very effective in inhibiting the growth of fungi. The same results were shown in ketapang extract in ethanol, which could inhibit the growth of *Candida*spp (Terças et al., 2017). Previous studies showed that the most polar fraction obtained from the leaves of *T. catappa* was effective against fungi (Jagessar & Alleyne, 2011). The extraction of ketapang leaves was generally done using dry leaves that have been ground into a powder and then extracted. This study directly extracted fresh leaves with water, making it easier to prepare.

Phenol and antioxidant levels

The levels of phenol and antioxidants in young (light green) and red leaves were higher than in other types of leaves (Table 2). This may cause the leaf extract to inhibit the growth of S. aureus more strongly. Further chemical analysis showed that *ketapang* leaf extract contains tannins and favonoids (Purwani et al., 2015), as well as other antioxidants such as punicalagin, punicalin, terfluvina A and B, khebulis acid, benzoic acid, coumaric, and their derivatives (Chyau et al., 2006; Kinoshita et al., 2007).

Table 2. Phenol and antioxidant levels of ketapang leaf extract (*Terminalia catappa* L) in various leaf colors.

Leaf Extract	Total phenol (mg/100 mL)	Antioxidant (%)
Light green	619.74 ± 8.06	63.92 ± 1.46
Dark green	448.73 ± 8.06	43.30 ± 1.46
Yellow	528.55 ± 8.08	55.67 ± 1.46
Red	1,349.0 ± 8.06	72.16 ± 1.46

Note: Values are mean ± standard deviation in triplicates.

Minimum inhibitory level

The effect of the concentration of ketapang leaf extract on the growth of the fungus *R. oligosporus* was analyzed at an extract concentration of 0-50%. The results can be seen in Figure 2. The higher the leaf extract concentration, the greater the inhibition on the growth of *A. oligosporus*. Young leaves that are light green in color and old leaves that are red are the most effective in inhibiting growth. At a concentration of 10% incubation for 4 days, it inhibited 35.81 and 39.07, respectively, compared to growth without leaf extract (Table 3). Concentration above it causes the fungus not to grow at all. This result differed from dark green and yellow leaf extracts, each of which were slightly less inhibitory, and inhibited 100% growth at 30% leaf concentration. This may be related to the levels of phenols and antioxidants (Table 3), which showed high levels in light green and red leaves.

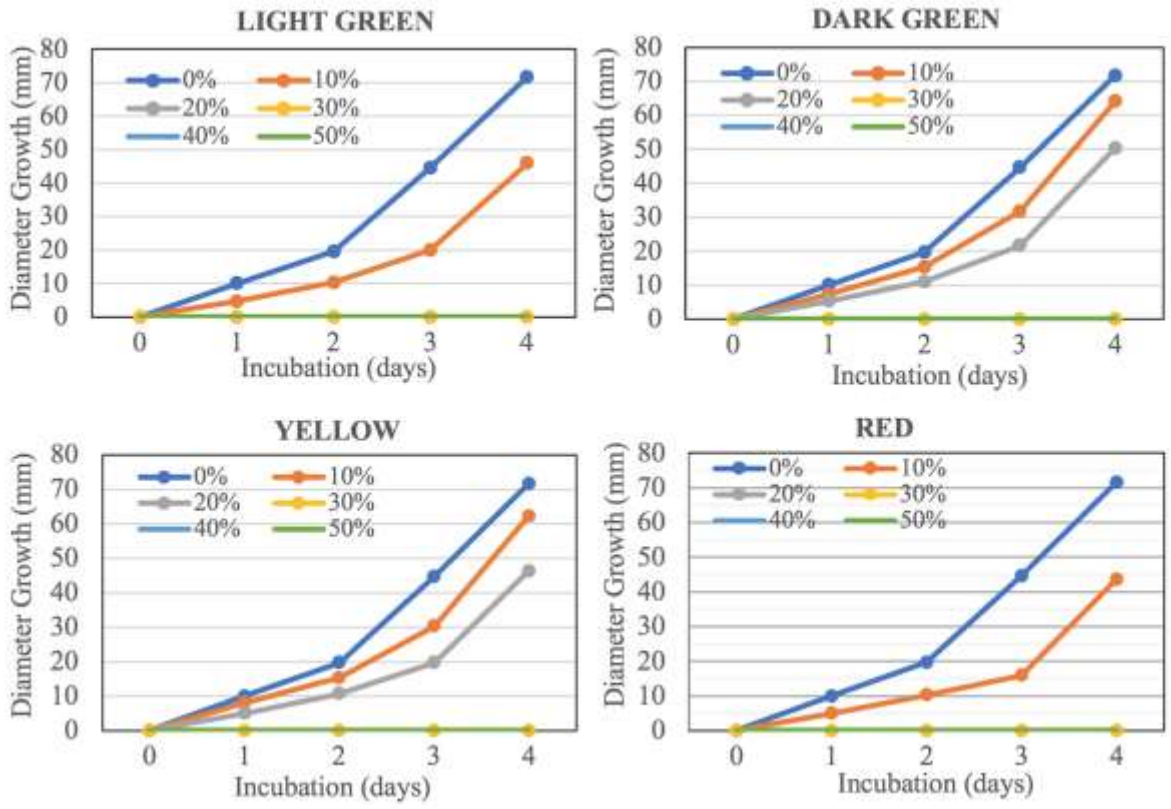

Figure 2. Fungal growth (in colony diameter) at various concentrations of leaf extract. Values are mean ± standard deviation in triplicates.

Table 3. Inhibition of growth of *A. oligosporus* at various concentrations of extracts and various ketapang leaf (*Terminalia catappa* L.) colors.

Leaf Extract (%)	Growth inhibition of A. oligosporus (%)			
	Light Green	Dark Green	Yellow	Red
10	35.81	10.70	13.02	39.07
20	100.00	29.77	35.35	100.00
30	100.00	100.00	100.00	100.00
40	100.00	100.00	100.00	100.00
50	100.00	100.00	100.00	100.00

CONCLUSION

Ketapang leaf extract (*Terminalia catappa* L.) can inhibit the growth of microbes, both gram positive, negative, and fungal growth. The effectiveness of the inhibition was higher in the inhibition of fungi. Young leaves that are light green and old leaves that are red are very effective in inhibiting fungal growth, higher than the dichloro control. This is because the content of phenols and antioxidants is high compared to the other two leaf colors. At a concentration of 20% extract, it inhibited 100% of the growth of the fungus *A. oligosporus*.

ACKNOWLEDGEMENT

The authors thank to the Faculty of Agricultural Technology, Universitas Gadjah Mada for facilitating this research.

REFERENCES

1. Aminah, Prayitno, S.B., and Sarjito. 2014. The Soaking Effect of Ketapan Leaves (*Terminalia cattapa*) Extract Toward Survival Rate and Histological Liver of Carp Infected by *Aeromonas hydrophila*. Journal of Aquaculture Management and Technology, 3 (4): 118-125.
2. Anam, K., Widharna, R.M., and Kusrini, D. 2009. A-Glucosidase inhibitor activity of *Terminalia* species, IJP-International Journal of Pharmacology, 5 (4): 277-280
3. Chyau C. C., Ko P. T., Mau J. L. (2006). Antioxidant properties of aqueous extracts from Terminallia catappa leaves. LWT–Food Sci. Technol. 39, 1099–1108. 10.1016/j.lwt.2005.07.016
4. Christodoulou, M.C., Palacios, J.C.O., Hesami, G., Jafarzadeh, S., Lorenzo, J.M.,Domínguez, R., Moreno, A., and Hadidi, M. 2022. Spectrophotometric Methods for Measurement of Antioxidant Activity in Food and Pharmaceuticals. Antioxidants 11(11), 2213; https://doi.org/10.3390/antiox11112213
5. Cock, I.E. 2015. The medicinal properties and phytochemistry of plants of the genus Terminalia (Combretaceae). Inflammopharmacology. 23(5):203-29. doi: 10.1007/s10787-015-0246-z.
6. Helda, Y., Harpeni, E., and Supono. 2018. Application of Ketapan Leaf Extract (*Terminalia catappa* L.) Against Vaname Shrimp (*Litopenaeus vannamei*) Infected with White Feces Disease (WFD). Jurnal Sains Teknologi Akuakultur, 2 (2): 7-15. [in Indonesian].
7. Jagessar, R.C. and Alleyne, R. (2011). Antimicrobial potency of the aqueous extract of leaves of *Terminalia catappa*. Acad. Res. Int. 1, 362–371.
8. Khoddami A., Wilkes, M.A., and Thomas H. Roberts, T.H. 2013. Techniques for Analysis of Plant Phenolic Compounds. Molecules. 2013 Feb; 18(2): 2328–2375. doi: 10.3390/molecules18022328
9. Kinoshita S., Inoue Y., Nakama S., Ichiba T., Aniya Y. (2007). Antioxidant and hepatoprotective actions of medicinal herb, Terminalia catappa L. from Okinawa Island and its tannin corilagin. Phytomedicine 14, 755–762. 10.1016/j.phymed.2006.12.012
10. Lee, K.W., Kim, Y.J., Lee, H.J., and Lee, C.Y. 2003. Cocoa Has More Phenolic Phytochemicals and a Higher Antioxidant Capacity than Teas and Red Wine. Journal of Agricultural and Food Chemistry, 51 (25): 7292-7295 doi:10.1021/jf0344385
11. Nadirah, M., Wee, T.L., and Najiah, M. 2013. Differential responses of Vibrio sp. to young and mature leaves extracts of *Terminalia catappa* L. International Food Research Journal, 20: 961-966.
12. Nair, R. and Chanda, S. 2008. Antimicrobial Activity of *Terminalia catappa*, *Manilkara zapota* and *Piper betel* Leaf Extract. Indian J Pharm Sci. 2008;70(3):390-393. Doi:10.4103/0250-474X.43012
13. Naz, S., Ahmad, S., Rasool, S.A., Siddiqi, R., and Sayeed, S.A. 2007. In vitro Antibacterial Activity of the Extracts Derived from *Terminalia catappa*. Research Journal of Microbiology, 2: 180-184.
14. Ningrum, L.W. 2021. Distribution of *Terminalia catappa* L. plant species and their potential seeds in the Purwodadi Botanical Gardens. Prosiding Biologi Achieving the Sustainable Development Goals woth Biodiversity in Confronting Climate Change. Gowa. http://journal.uin-alauddin.ac.id/index.php/psb. [in Indonesia].

15. Purwani, K.I., Alami, N.H., Nurhatika, S., Marcilia, S.N., and Arifiyanto, A. (2015). In Vitro Potential Test of Ketapang (*Terminalia catappa*) Leave Extract against *Aeromonas salmonicida*. J. Appl. Environ. Biol. Sci., 5 (7): 1-6.
16. Putra, C.D.P., Fachriyah, E., and Kusrini, D. 2011. Isolation, Identification and Toxicity Test of Steroid Compounds in Chloroform Extract of Ketapan Leaves (*Terminalia catappa* Linn). Jurnal Kimia Sains dan Aplikasi, 14 (1): 4 – 7. [In Indonesia].
17. Raveesha, K. A. (2021). Antibacterial Activity and Time-kill Assay of Terminalia catappa L. and Nigella sativa L. against Selected Human Pathogenic Bacteria. Journal of Pure and Applied Microbiology, 15(1), 285+.
18. https://link.gale.com/apps/doc/A674465463/AONE?u=anon~888be2f4&sid=googleScholar&xid=3ba50d85
19. Terças, A. G., Monteiro, A. S., Moffa, E. B., Dos Santos, J., de Sousa, E. M., Pinto, A., Costa, P., Borges, A., Torres, L., Barros Filho, A., Fernandes, E. S., & Monteiro, C. A. (2017). Phytochemical Characterization of Terminalia catappa Linn. Extracts and Their antifungal Activities against Candida spp. Frontiers in microbiology, 8, 595. https://doi.org/10.3389/fmicb.2017.00595