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RESEARCH NOTE

Antimicrobial activity of *Terminalia catappa* brown leaf extracts against *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 [version 1; referees: 1 approved]

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Abstract

The aim of this study was to determine the effects of various concentration of *Terminalia catappa* brown leaves extract which can inhibit the growth of *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. The crushed-brown leaves of *Terminalia catappa* was extracted using 95% ethanol, filtered, and evaporated. The dried *T. catappa* extract was used to identify phytochemical content qualitatively. Total phenolic and flavonoid contents were also measured quantitatively from dried extract. The dried extracts were also dissolved in sterile aquadest and serial dilutions were prepared to final concentration of 30, 60 and 90%. A disc diffusion method was used to evaluate the antibacterial activity of various concentrations of ethanol extract of brown leaves of *T. catappa*. Inhibition zone diameter was measured to determine antibacterial activity. Gentamycin sulfate and distilled water were used as positive and negative controls, respectively. Dried ethanolic extract of brown *T. catappa* leaves contained flavonoid, quinon, phenolic, triterpenoid, and tannin. A total of 208.722 mg gallic acid equivalent/g extract of total phenolic and 35.7671 mg quercetin equivalent/g extract of total flavonoid were also found in the dried extract. The inhibition zone diameters of ethanolic extracts ranged from 1.73 to 9.06 mm (*S. aureus*) and from 1.83 to 6.5 mm (*P. aeruginosa*). The higher concentration of extract, the wider the inhibition zone diameters for both bacteria. *P. aeruginosa* was more resistant to high concentrations of extract (90%) than *S. aureus*. Ethanolic extracts of the brown leaves of *T. catappa* had different antibacterial effects against *S. aureus* and *P. aeruginosa*. The higher the concentration of extract, the wider the inhibition zone diameter for both bacteria. *P. aeruginosa* was more resistant to high concentrations of ethanolic extracts of the brown leaves of *T. catappa*.

Keywords

Terminalia catappa, phytochemicals, Staphylococcus aureus, Pseudomonas aeruginosa, antibacterial

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1 **Edwin Setiawan**, Sepuluh Nopember Institute of Technology, Indonesia

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Author roles: Allyn OQ: Conceptualization, Formal Analysis, Investigation, Methodology, Resources, Validation; **Kusumawati E**: Methodology, Supervision, Writing – Original Draft Preparation; **Nugroho RA**: Data Curation, Investigation, Supervision, Validation, Writing – Review & Editing

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Introduction

Staphylococcus and *Pseudomonas* species have been identified as causative agents of disease and serious pathogens in many aquatic animals, including fish[1–4], resulting in high mortality rates in many commercially farmed fish. Among various *Staphylococcus* and *Pseudomonas* species, *Staphylococcus aureus* and *Pseudomonas aeruginosa* are known to cause disease in *Oreochromis niloticus* and *Oreochromis mossambicus*[5]. To reduce high mortality rates in farmed fish, aquaculturists and researcher used chemical agents and antibiotics to promote growth or prevent *S. aureus* and *P. aeruginosa* infection[6].

However, the use of antibiotics to prevent and cure common infectious diseases in fish is becoming increasingly limited due to environmental concern, and increasingly expensive and ineffective because of microbial resistance[7–9]. As alternatives, various plant extracts, such as those of *Boesenbergia pandurata*, *Zingiber zerumbet* and *Solanum ferox*, have been tested and used as an alternative to antibiotics[10–12]. Another potential plant extract that can be used as an antimicrobial is that of *Terminalia catappa*, which is widely distributed in tropical and sub-tropical regions, including Indonesia[13,14].

Terminalia catappa L., belonging to the family Combretaceae, is a large deciduous tree. The aqueous extract of *Terminalia catappa* leaves has been known as a folk medicine for antipyretic, hemostatic, hepatitis and liver-related diseases purposes in the Philippines, Malaysia and Indonesia[15,16]. Past research revealed that the extract of *T. catappa* leaves can be used to improve a resistance to *Aeromonas hydrophila* in *Betta* sp[17], remedy against tilapia (*Oreochromis niloticus*) parasites and bacterial pathogen[18,19]. Nevertheless, scientific literature concerning the antibacterial potency of *T. catappa* against *Staphylococcus aureus* and *Pseudomonas aeruginosa* is limited.

Thus, the aim of the study was to evaluate the effects of various concentration of *T. catappa* brown leaf extract on the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* by calculating inhibition zone diameters. The phytochemical content of the extract was also qualitatively determined and the flavonoid and phenolic concentrations in the extract was quantified.

Methods

Site and time

The research was performed from March to May 2018 at the Animal Physiology, Development and Molecular Laboratory for extracting *T. catappa* leaves. Meanwhile, assay study was done at microbiology and molecular genetic laboratory.

Bacterial strains and culture condition

Bacterial strains were obtained from a Microbiology Laboratory, Faculty of Pharmacy, Sumatera Utara University, Indonesia. *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 were used to investigate the antibacterial activity. Both bacteria were sub-cultured on nutrient agar and stored at 4°C until use.

Plant materials

Brown leaves of *T. catappa* were collected from a region of Mulawarman university campus, Samarinda, East Kalimantan. Leaves were dried at room temperature for 2 days, crushed, transferred into a glass container and preserved until the extraction procedure.

Extraction procedure

Approximately 1 kg of crushed leaves was soaked in 1 l of 95% ethanol for 5 days and shaken occasionally with a shaker. After 5 days, materials were filtered (Whatman No. 11 paper filter). The filtrate was evaporated using a rotary evaporator. Finally, the dried extracts were obtained and stored at 4°C in a dark bottle until use. The dried extracts were then dissolved in sterile distilled water and serial dilutions were prepared to give final concentrations of 30, 60 and 90%.

Phytochemical content

Dried extract samples were subjected to qualitative phytochemical analysis for flavonoids, quinon, alkaloids, phenolic, steroid, triterpenoid, saponins, and tannins using standard methods as previously described by Nugroho *et al.*[20]. Meanwhile, total phenolics and flavonoids were quantitatively measured, using the method described by Pourmorad *et al.*[21].

Antibacterial activity assay

The antibacterial activity of *T. catappa* brown leaf ethanolic extract was evaluated using the disc diffusion method[22]. Three replicated agar plates were used for each different concentration and both controls (distilled water and 0.1% gentamycin sulfate). A total of 10 µl extract was added to a paper disc for each concentration and controls. Each disk was then placed in agar plate which had bacterial suspension in the plates. All plates were incubated at 37°C for 24 h. The diameter of inhibition zone created by each disc was measured (in mm) using a micrometer.

Data analysis

The inhibition zone data were expressed as means ± standard error. The data were subjected to ANOVA, followed by Duncan's post hoc test to evaluate significant differences among the groups of treatments. Meanwhile, the comparison between bacteria in each concentration was performed using a t-test. All significant tests were at $P<0.05$ levels and all analysis was done using SPSS 22 (SPSS, Inc., USA). The data of the phytochemical content and the concentration of flavonoid and phenolic were analyzed descriptively.

Results and discussion

The dried extract of *T. catappa* brown leaves contained flavonoids, quinon, phenolics, triterpenoids, and tannins. There were no alkaloids, steroids or saponin found in the dried extract. Total phenolic (208.722 mg gallic acid equivalent/g extract) and total flavonoid (35.7671 mg quercetin equivalent/g extract) were

detected in the dried extract. The inhibition zone diameters of ethanolic extracts ranged from 1.73 to 9.06 mm for *S. aureus*, and from 1.83 to 6.5 mm for *P. aeruginosa*. Increasing the extract concentration increased the inhibition zone diameters for both bacteria (Figure 1). *P. aeruginosa* was more resistant to high concentrations of extract (90%) than *S. aureus* (Table 1). According to Xie *et al.*[23], flavonoids are known antibacterial agents against a wide range of pathogenic bacteria. In addition, Fu *et al.*[24] also revealed that phenolic extracts from some plants also have antibacterial effects against many kinds of bacteria. The data showing the inhibition zone diameters for both bacteria at each concentration of extract can be seen in Dataset 1[25].

Dataset 1. Inhibition zone diameters for both bacteria at different concentration of extracts and images of every repeat experiment performed

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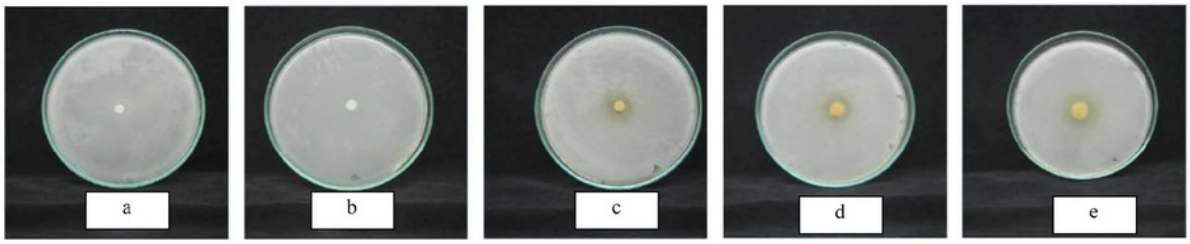
Conclusion

Ethanolic extracts of the brown leaves of *T. catappa* have potential antibacterial effects against *S. aureus* and *P. aeruginosa*, indicated by the inhibition zone formed around the extract. The inhibition zone diameter increased with increasing concentrations of *T. catappa* extract. *P. aeruginosa* exhibited more

1. *Staphylococcus aureus*



2. *Pseudomonas aeruginosa*

Figure 1. Inhibition zone of *Terminalia catappa* brown leaves ethanolic extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. (**a**) Negative control, (**b**) positive control (0.1% gentamycin sulfate), *Terminalia catappa* extract (**c**) 30%, (**d**) 60%, (**e**) 90%. Images shown are representative of n=3 repeats.

Table 1. Inhibitory zone diameter (in mm) of *Staphylococcus aureus* and *Pseudomonas aeruginosa* after treated with different concentration of brown leaves of *T. catappa* ethanolic extract.

Bacteria	Positive control	Extract concentration		
		30%	60%	90%
Staphylococcus aureus	5.78±0,27^{a,1}	1.73±0,24^{b,1}	5.28±1,06^{a,c,1}	9.06±0,56^{d,1}
Pseudomonas aeruginosa	7.15±0,20^{a,2}	1.83±0,87^{b,1}	4.53±0,78^{c,1}	6.50±0,13^{a,d,2}

Different superscript letters in the same row indicate significantly different mean values for different treatments at $P<0.05$. Different superscript numbers in the same column indicate significantly different mean values for different treatments at $P<0.05$. The negative control was omitted as no inhibition zone was present. Positive control, 0.1% gentamycin sulfate.

resistance to high concentrations of ethanol extracts of the brown leaves of *T. catappa* than *S. aureus*.

Data availability

Dataset 1. Inhibition zone diameters for both bacteria at different concentration of extracts and images of every repeat experiment performed. DOI: https://doi.org/10.5256/f1000research.15998.d215169[25].

Grant information

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References

1. Najiah M, Aqilah NI, Lee KL, *et al.*: **Massive mortality associated with *Streptococcus agalactiae* infection in cage-cultured red hybrid tilapia *Oreochromis niloticus* in Como River, Kenyir Lake, Malaysia.** *J Biol Sci.* 2012; **12**(8): 438–442.
Publisher Full Text
2. Na-Phatthalung P, Chusri S, Suanyuk N, *et al.*: ***In vitro* and *in vivo* assessments of *Rhodomyrtus tomentosa* leaf extract as an alternative anti-streptococcal agent in Nile tilapia (*Oreochromis niloticus* L.).** *J Med Microbiol.* 2017; **66**(4): 430–439.
PubMed Abstract | Publisher Full Text
3. Saikia DJ, Chattopadhyay P, Banerjee G, *et al.*: **Time and dose dependent effect of *Pseudomonas aeruginosa* infection on the scales of *Channa punctata* (Bloch) through light and electron microscopy.** *Turk J Fish Aquat Sci.* 2017; **17**(5): 871–876.
Publisher Full Text
4. Baldissera MD, Souza CF, Santos RCV, *et al.*: ***Pseudomonas aeruginosa* strain PA01 impairs enzymes of the phosphotransfer network in the gills of *Rhamdia quelen*.** *Vet Microbiol.* 2017; **201**: 121–125.
PubMed Abstract | Publisher Full Text
5. Thomas J, Thanigaivel S, Vijayakumar S, *et al.*: **Pathogenecity of *Pseudomonas aeruginosa* in *Oreochromis mossambicus* and treatment using lime oil nanoemulsion.** *Colloids Surf B Biointerfaces.* 2014; **116**: 372–377.
PubMed Abstract | Publisher Full Text
6. Grema HA, Geidam YA, Suleiman A, *et al.*: **Multi-Drug Resistant Bacteria Isolated from Fish and Fish Handlers in Maiduguri, Nigeria.** *Int J Anim Vet Adv.* 2015; **7**(3): 49–54.
Reference Source
7. Samanidou VF, Evaggelopoulou EN: **Analytical strategies to determine antibiotic residues in fish.** *J Sep Sci.* 2007; **30**(16): 2549–2569.
PubMed Abstract | Publisher Full Text
8. Uchida K, Konishi Y, Harada K, *et al.*: **Monitoring of Antibiotic Residues in Aquatic Products in Urban and Rural Areas of Vietnam.** *J Agric Food Chem.* 2016; **64**(31): 6133–8.
PubMed Abstract | Publisher Full Text
9. He X, Deng M, Wang Q, *et al.*: **Residues and health risk assessment of quinolones and sulfonamides in cultured fish from Pearl River Delta, China.** *Aquacult.* 2016; **458**: 38–46.
Publisher Full Text
10. Hardi EH, Kusuma IW, Suwinarti W, *et al.*: **Antibacterial activities of some Borneo plant extracts against pathogenic bacteria of *Aeromonas hydrophila* and *Pseudomonas* sp.** *AACL Bioflux.* 2016; **9**(3): 638–646.
Reference Source
11. Hardi EH, Kusuma IW, Suwinarti W, *et al.*: **Short Communication: Antibacterial activity of *Boesenbergia pandurata, Zingiber zerumbet and Solanum ferox* extracts against *Aeromonas hydrophila* and *Pseudomonas* sp.** *Nusantara Bioscience.* 2016; **8**(1): 18–21.
Publisher Full Text
12. Hardi EH, Saptiani G, Kusuma IW, *et al.*: **Immunomodulatory and antibacterial effects of *Boesenbergia pandurata, Solanum ferox*, and *Zingiber zerumbet* on tilapia, *Oreochromis niloticus*.** *AACL/Bioflux.* 2017; **10**(2): 182–190.
Reference Source
13. Hyttel P, Sinowatz F, Vejlsted M: **Essentials of Domestic Animal Embryology.** Saunders/Elsevier. 2010.
Reference Source
14. Hyttel P, Sinowatz F, Vejlsted M, *et al.*: **Essentials of domestic animal embryology.** Elsevier Health Sciences UK. 2009.
Reference Source
15. Meena K, Raja TK: **Immobilization of *Saccharomyces cerevisiae* cells by gel entrapment using various metal alginates.** *World J Microbiol Biotechnol.* 2006; **22**(6): 651–653.
Publisher Full Text
16. Vučurović VM, Razmovski RN: **Sugar beet pulp as support for *Saccharomyces cerivisiae* immobilization in bioethanol production.** *Ind Crops Prod.* 2012; **39**: 128–134.
Publisher Full Text
17. Nugroho RA, Manurung H, Nur FM, *et al.*: ***Terminalia catappa* L. extract improves survival, hematological profile and resistance to *Aeromonas hydrophila* in *Betta* sp.** *Arch Pol Fisheries.* 2017; **25**(2): 103–115.
Publisher Full Text
18. Goh CS, Tan KT, Lee KT, *et al.*: **Bio-ethanol from lignocellulose: Status, perspectives and challenges in Malaysia.** *Bioresour Technol.* 2010; **101**(13): 4834–41.
PubMed Abstract | Publisher Full Text
19. Öztop HN, Öztop AY, Işikver Y, *et al.*: **Immobilization of *Saccharomyces cerevisiae* on to radiation crosslinked HEMA/AAm hydrogels for production of ethyl alcohol.** *Process Biochem.* 2002; **37**(6): 651–657.
Publisher Full Text
20. Nugroho RA, Manurung H, Saraswati D, *et al.*: **The effects of *Terminalia catappa* leaf extract on the haematological profile of ornamental fish *Betta splendens*.** *Biosaintifika: Journal of Biology and Biology Education.* 2016; **8**(2): 241–248.
21. Pourmorad F, Hosseinimehr SJ, Shahabimajd N: **Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants.** *Afr J Biotechnol.* 2006; **5**(11): 1142–1145.
Reference Source
22. Reddy PS, John MS, Devi PV, *et al.*: **Detection of vancomycin susceptibility among clinical isolates of MRSA by using minimum inhibitory concentration method.** *Int J Res Med Sci.* 2015; **3**(6): 1378–1382.
Publisher Full Text
23. Xie Y, Yang W, Tang F, *et al.*: **Antibacterial activities of flavonoids: structure-activity relationship and mechanism.** *Curr Med Chem.* 2015; **22**(1): 132–49.
PubMed Abstract | Publisher Full Text
24. Fu L, Lu W, Zhou X: **Phenolic Compounds and *In Vitro* Antibacterial and Antioxidant Activities of Three Tropic Fruits: Persimmon, Guava, and Sweetsop.** *Biomed Res Int.* 2016; **2016**: 4287461.
PubMed Abstract | Publisher Full Text | Free Full Text
25. Allyn OQ, Kusumawati E, Nugroho RA: **Dataset 1 in: Antimicrobial activity of *Terminalia catappa* brown leaf extracts against *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853.** *F1000Research.* 2018.
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Referee Report 20 September 2018

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✔ **Edwin Setiawan**
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The study is designed appropriately and and technical procedure has been taken sufficiently to answer the research question. Furthermore, methods and analyses process also sufficient to be replicated by readers. In addition, statistical method that used is also appropriate. They author need to highlighted and emphasized on the results and discussion with expanding their discussion with comparing another result that similar to this study.

In the result and discussion part, it is better to compare phenolic and flavonoid content of Terminalia catappa to other plant extract that close to Terminalia catappa if possible. Or in other words, comparing other similar research that used plant bioactive compound for antibacterial to research. Therefore, this result and discussion part could be expanded comprehensively.

In addition, simple and short explanation on how the mechanism flavonoid and phenolic inhibit bacterial growth can be added in this part.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

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