



## RESEARCH NOTE

# Antimicrobial activity of *Terminalia catappa* brown leaf extracts against *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 [version 1; referees: 2 approved, 1 not approved]

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**v1** First published: 04 Sep 2018, 7:1406 (doi: [10.12688/f1000research.15998.1](https://doi.org/10.12688/f1000research.15998.1))  
Latest published: 04 Sep 2018, 7:1406 (doi: [10.12688/f1000research.15998.1](https://doi.org/10.12688/f1000research.15998.1))

## 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

## Open Peer Review

Referee Status:   

	Invited Referees		
	1	2	3
<b>version 1</b>			
published 04 Sep 2018	report	report	report

- Edwin Setiawan**, Sepuluh Nopember Institute of Technology, Indonesia
- Md Fakhruddin**, International Research Center for Medical Sciences, Kumamoto University, Japan
- Cimi Ilmiawati** , Andalas University, Indonesia

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Comments (0)

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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

**Competing interests:** No competing interests were disclosed.

**Grant information:** The author(s) declared that no grants were involved in supporting this work.

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**How to cite this article:** Allyn OQ, Kusumawati E and Nugroho RA. **Antimicrobial activity of *Terminalia catappa* brown leaf extracts against *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 [version 1; referees: 2 approved, 1 not approved]** *F1000Research* 2018, 7:1406 (doi: [10.12688/f1000research.15998.1](https://doi.org/10.12688/f1000research.15998.1))

**First published:** 04 Sep 2018, 7:1406 (doi: [10.12688/f1000research.15998.1](https://doi.org/10.12688/f1000research.15998.1))

## Introduction

*Staphylococcus* and *Pseudomonas* species have been identified as causative agents of disease and serious pathogens in many aquatic animals, including fish<sup>1-4</sup>, 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*<sup>5</sup>. 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<sup>6</sup>.

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<sup>7-9</sup>. 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<sup>10-12</sup>. 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<sup>13,14</sup>.

*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<sup>15,16</sup>. Past research revealed that the extract of *T. catappa* leaves can be used to improve a resistance to *Aeromonas hydrophila* in *Betta* sp<sup>17</sup>, remedy against tilapia (*Oreochromis niloticus*) parasites and bacterial pathogen<sup>18,19</sup>. 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.*<sup>20</sup>. Meanwhile, total phenolics and flavonoids were quantitatively measured, using the method described by Pourmorad *et al.*<sup>21</sup>.

### Antibacterial activity assay

The antibacterial activity of *T. catappa* brown leaf ethanolic extract was evaluated using the disc diffusion method<sup>22</sup>. 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

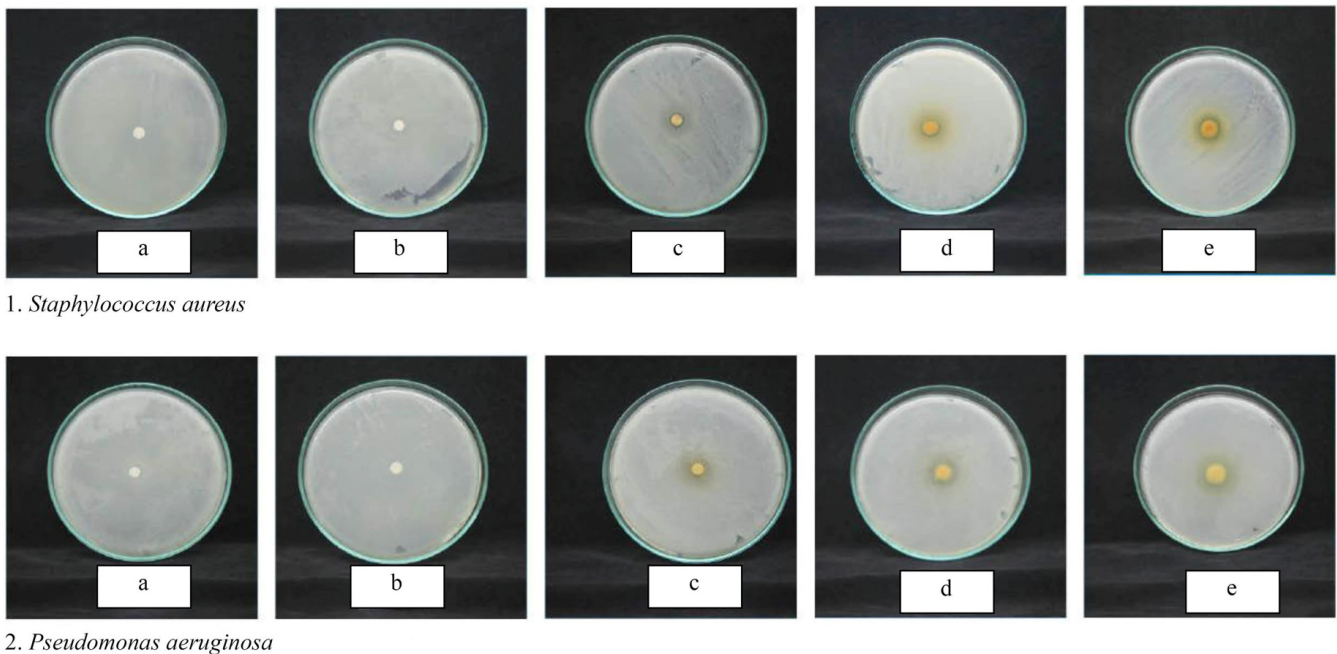
detected in the dried extract. The inhibition zone diameters of ethanolic extracts ranged from 1.73 to 9.06 mm for *S. aureus*, and from 1.83 to 6.5 mm for *P. aeruginosa*. Increasing the extract concentration increased the inhibition zone diameters for both bacteria (Figure 1). *P. aeruginosa* was more resistant to high concentrations of extract (90%) than *S. aureus* (Table 1). According to Xie *et al.*<sup>23</sup>, flavonoids are known antibacterial agents against a wide range of pathogenic bacteria. In addition, Fu *et al.*<sup>24</sup> 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<sup>25</sup>.

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

<http://dx.doi.org/10.5256/f1000research.15998.d215169>

### 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



**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%
<i>Staphylococcus aureus</i>	5.78±0,27 <sup>a,1</sup>	1.73±0,24 <sup>b,1</sup>	5.28±1,06 <sup>a,c,1</sup>	9.06±0,56 <sup>d,1</sup>
<i>Pseudomonas aeruginosa</i>	7.15±0,20 <sup>a,2</sup>	1.83±0,87 <sup>b,1</sup>	4.53±0,78 <sup>c,1</sup>	6.50±0,13 <sup>a,d,2</sup>

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><sup>25</sup>.

## Grant information

The author(s) declared that no grants were involved in supporting this work.

## Acknowledgments

The authors thank to Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, East Kalimantan. The appreciation goes to all of our students who helped the authors during the trial.

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# Open Peer Review

Current Referee Status:



Version 1

Referee Report 23 October 2018

doi:10.5256/f1000research.17473.r37962



**Cimi Ilmiawati** 

Division of Environmental Toxicology, Department of Pharmacology, Faculty of Medicine, Andalas University, Padang, Indonesia

Comments:

This study contributes information on the potential of *T. catappa* leaves extract as an antibacterial alternative to address antibacterial resistance problems in fish farming.

### Methods

Site and time: Please specify the affiliation of the laboratory (whether it belongs to a department in a university or else).

### Plant materials

Please correct the writing of Mulawarman University.

How to preserve the crushed leaves? How long the preservation until the extraction?

### Phytochemical contents

What is the purpose of qualitative and quantitative phytochemical analysis in relation to the study objectives? Please elaborate in the introduction part.

### Results

1. The inhibition zone in Figure 1 for the positive control is not very clear, therefore a zoom in figure is required to judge the result.
2. I was expecting to see three discs in each plate of treatment and this experiment shall be replicated three times, before analyzing the data and arriving into a conclusion. Please comment on your replication method.

**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.

**Referee Expertise:** Pharmacology, toxicology, molecular endocrinology

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Referee Report 23 October 2018

doi:[10.5256/f1000research.17473.r37961](https://doi.org/10.5256/f1000research.17473.r37961)



**Md Fakruddin**

Laboratory of stem cell stress, International Research Center for Medical Sciences, Kumamoto University, Kumamoto, Japan

1. In the title, it should be mention ethanol extract, as the authors only evaluate ethanol extract of the leaves.
2. In the figure 1, the quality is poor, so it seems the positive control do not have any inhibition zone!
3. Only one strain of each bacteria was tested, it would be more authentic if more types of strains as well as some wild or environmental strains were tested.
4. MIC/MBC of the extract against these bacteria should be determined.
5. Data of this study should be compared with similar data published already. It is understood, that there may be no data of this particular species, but other species of terminalia can be consulted.

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

Partly

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

No

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

Partly

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

Not applicable

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

Partly

**Are the conclusions drawn adequately supported by the results?**

No

**Competing Interests:** No competing interests were disclosed.

**I have read this submission. I believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.**

Referee Report 20 September 2018

doi:[10.5256/f1000research.17473.r37963](https://doi.org/10.5256/f1000research.17473.r37963)

**Edwin Setiawan**

Department of Biology, Faculty of Natural Sciences, Sepuluh Nopember Institute of Technology, Surabaya, Indonesia

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.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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