Phytochemical, antioxidant and antimicrobial properties of Litsea angulata extracts [version 1; peer review: 1 approved, 2 approved with reservations]

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Abstract
Background: Litsea angulata is a plant species belonging to Lauraceae family that is distributed throughout Indonesia, Malaysia, and New Guinea. The seeds have been traditionally used by local people in Kalimantan, Indonesia for the treatment of boils; however, there is no information about the potency of its branch, bark and leaves yet. This study aimed to determine the antioxidant, antimicrobial activity as well as the phytochemical constituent of Litsea angulata branch, bark, and leaves.

Methods: Extraction was performed by successive maceration method using n-hexane, ethyl acetate, and ethanol solvent. Antioxidant activity was evaluated by DPPH radical scavenging assay. The antimicrobial activity using the 96 well-plate microdilution broth method against Staphylococcus aureus and Streptococcus mutans.

Results: Based on the phytochemical analysis, it showed that extract of L. angulata contains alkaloids, flavonoids, tannins, terpenoids, and coumarin. The results showed that all extracts of plant samples displayed the ability to inhibit DPPH free radical formation and all tested microorganisms.

Conclusions: L. angulata contains secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, carotenoids, and coumarin. The antioxidant activity on different plant extracts was a range as very strong to weak capacity. All extracts in this study could inhibit the growth of S. aureus and S. mutans.

Keywords
Litsea angulata, maceration, phytochemical, antioxidant, antimicrobial
Introduction
Many plants species from the genus Litsea are a potential source of biologically active compounds and are used as traditional medicines such as antispasmodic, wound healing, relieving rheumatism, and colds. There is little information about the potency of Litsea angulata species. L. angulata belongs to the Lauraceae family, which can be found in East Kalimantan, Indonesia, and to our knowledge, data are limited on its biological activities. Therefore, the present study aimed to assess the phytochemical constituents, antioxidant, and antimicrobial of different plant parts of L. angulata.

Methods
Preparation of plant extracts
The plant material was obtained from Education Forest Laboratory of Forestry Faculty, Mulawarman University, East Kalimantan, Indonesia. Three different plant parts of L. angulata (bark, branch, and leaves) were separated, ground and extracted. The successive maceration extraction method was adopted from Suthi and Indira, with the solvents modified. About 50 g of the air-dried powder of each plant material was extracted individually with one of the following solvents: n-hexane, ethyl acetate, and 96% ethanol. The extracts were filtered and concentrated under vacuum using a rotary evaporator until the solvent was completely evaporated. In total, nine different extracts were produced, with each solvent used to produce extract from each plant part (branch, n-hexane; branch, ethyl acetate; branch, ethanol; bark, n-hexane; bark, ethyl acetate; bark, ethanol; leaves, n-hexane; leaves, ethyl acetate, and leaves, ethanol extracts).

Phytochemical screening
A total of 60 mg of each extract was dissolved individually in 1 ml solvent that used for extraction and the solutions were used to test for qualitative phytochemical tests. The tests were done according to the standard procedures described in literature by Kokate, Senthilmurugan, Harborne, to detect the following bioactive compounds: alkaloids, flavonoids, saponins, tannins, terpenoids, steroids, carotenoids, and coumarin.

DPPH free radical scavenging assay
The DPPH assay was performed as described by Kuspradini et al. Various concentrations of samples of each extract (12.5, 25, 50, and 100 ppm) in 96% ethanol were added with DPPH. After 20 minutes, the absorbance of the resulting solution and the blank were recorded. Ascorbic acid was used as a positive control. The absorbance was recorded spectrophotometrically at a wavelength of 517 nm. DPPH free radical scavenging activity was stated as % inhibition = (1 – Absorbance of sample/Absorbance of control) × 100. To inhibitory activity, half-maximal inhibitory concentration (IC50) values were calculated.

Determination of antibacterial activity
The minimum inhibitory concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of the samples were assessed against Staphylococcus aureus and Streptococcus mutans using the 96-well microdilution and solid medium, respectively. The bacterial concentration in the inoculum was standardized at 0.5 McFarland turbidity scale, equivalent to 10⁶ CFU ml⁻¹. The method of MIC was adopted from the method outlined by Mohsenipour and Hassanshahian, with modifications. A stock solution was prepared by dissolving 5 mg extracts in 1 ml of 40% ethanol. A total of 50 μl stock solution were serially diluted twofold in 40% ethanol to achieve the range of test concentrations (1250, 625, 312.5 and 156.25 ppm), which were added to wells of a 96-well microplate. Next, 100 μl sterile nutrient broth culture medium (NB) and 50 μl of the culture of the respective organism were added into each well. The inoculated microplates were incubated at 37°C for 24 h.

At 1 hour before the end of incubation, the bacterial growth was confirmed by adding 0.01% solution of 2,3,5-triphenyl tetrazolium chloride (TTC, Merck, Germany) (50 μl) and the plate was incubated for another hour. The viable bacterial cells reduced the yellow TTC to pink. The inhibition of growth was visually detected when the solution in the well remained clear after incubation with TTC. Positive controls (bacteria + NB + chloramphenicol), negative controls (bacteria and NB), vehicle controls (bacteria + NB + solvent), and media controls (NB) were included in each test. MBC was determined by inoculating the assay from the wells showing no microbial growth onto the surface of nutrient agar medium on the petri dish. The petri dishes were incubated for 24 h at 37°C and subjected to visual inspection. MBC was considered as the lowest concentration where there was no resumption of bacterial growth.

Statistical analysis
All experiments were conducted three times. Regression analysis was used to calculate IC50 values of antioxidant. All statistical analyses used Microsoft Excel 2010 software.

Results
Phytochemical screening
The result of phytochemical screening showed that L. angulata contain alkaloids, flavonoids, tannins, terpenoids, carotenoids and coumarin (Table 1). It can be shown that the ethanolic extract of the L. angulata showed more number of secondary metabolites when compared with other extracts.

Antioxidant activity
All extracts could inhibit DPPH radical scavenging activity (Table 2). The IC50 values with regards to different used solvents and plant parts were, in increasing order, as follows: leaves, ethanol; bark, ethanol; branch, ethanol; branch, ethyl acetate; bark, ethyl acetate; branch, n-hexane; leaves and bark, n-hexane. Raw absorbance data from which IC50 values were calculated are shown in Dataset 1.

Antimicrobial activity
All extracts could inhibit the growth of S. mutans and S. aureus and showed the MIC value at 156.25 ppm concentration (Table 3). The MBC value could not detect in the range of 156.25–1250 ppm concentration. It is indicated that the MBC value in this study was higher than 1250 ppm.
### Table 1. Secondary metabolites in *L. angulata* extracts.

| Solvent   | Part  | Alkaloid | Flavonoid | Saponin | Tannin | Terpenoid | Steroid | Carotenoid | Coumarin |
|-----------|-------|----------|-----------|---------|--------|-----------|---------|------------|----------|
| n-Hexane  | Bark  | +        | +         | -       | -      | +         | -       | -          | -        |
|           | Branch| +        | -         | -       | +      | -         | -       | -          | -        |
|           | Leaves| +        | -         | -       | +      | -         | -       | -          | -        |
| EtOAc     | Bark  | +        | -         | -       | +      | -         | +       | -          | +        |
|           | Branch| +        | -         | -       | +      | -         | -       | -          | -        |
|           | Leaves| +        | -         | -       | +      | -         | +       | +          | +        |
| EtOH      | Bark  | +        | -         | -       | +      | +         | -       | +          | +        |
|           | Branch| +        | -         | -       | +      | -         | +       | +          | +        |
|           | Leaves| -        | +         | +       | +      | -         | +       | +          | +        |

### Table 2. Half-maximal inhibitory concentration (IC50) values of *L. angulata* extracts on DPPH free radical.

| No. | Solvent   | Plant Part | IC50 (ppm) |
|-----|-----------|------------|------------|
| 1   | n-hexane  | Bark       | 76.12      |
|     |           | Branch     | >100       |
|     |           | Leaves     | >100       |
| 2   | Ethyl acetate | Bark       | 2.41       |
|     |           | Branch     | 52.75      |
|     |           | Leaves     | >100       |
| 3   | Ethanol   | Bark       | 14.69      |
|     |           | Branch     | 26.81      |
|     |           | Leaves     | 14.58      |

### Table 3. Minimum inhibitory and bactericidal concentrations of the *L. angulata* extracts.

#### MIC (ppm)

| Solvent | Part       | *S. mutans* | *S. aureus* | Control + |
|---------|------------|-------------|-------------|-----------|
| n-Hexane| Bark       | 156.25      | 156.25      | 100       |
|         | Branch     | 156.25      | 156.25      | 100       |
|         | Leaves     | 156.25      | 156.25      | 100       |
| Ethyl acetate | Bark       | 156.25      | 156.25      | 100       |
|         | Branch     | 156.25      | 156.25      | 100       |
|         | Leaves     | 156.25      | 156.25      | 100       |
| Ethanol | Bark       | 156.25      | 156.25      | 100       |
|         | Branch     | 156.25      | 156.25      | 100       |
|         | Leaves     | 156.25      | 156.25      | 100       |

#### MBC (ppm)

| Solvent | Part       | *S. mutans* | *S. aureus* | Control + |
|---------|------------|-------------|-------------|-----------|
| n-Hexane| Bark       | >1.250      | >1.250      | >1.250    |
|         | Branch     | >1.250      | >1.250      | >1.250    |
|         | Leaves     | >1.250      | >1.250      | >1.250    |
| Ethyl acetate | Bark       | >1.250      | >1.250      | >1.250    |
|         | Branch     | >1.250      | >1.250      | >1.250    |
|         | Leaves     | >1.250      | >1.250      | >1.250    |
| Ethanol | Bark       | >1.250      | >1.250      | >1.250    |
|         | Branch     | >1.250      | >1.250      | >1.250    |
|         | Leaves     | >1.250      | >1.250      | >1.250    |
Discussion

Plant extracts have been reported to have numerous biological activities due to their phytochemical contents, which contribute significantly towards the antioxidant and antimicrobial activities such as flavonoids, tannins, and terpenoids. The solubility or insolubility of the active compound(s) in the solvent used for extraction can cause differential effects on antioxidant and antibacterial. According to Blois, samples which had an IC50 value more than 150 ppm were considered weak antioxidants, 50-100 ppm indicated as a strong antioxidant, while lower than 50 ppm was a very strong antioxidant. Antimicrobials are considered as bactericidal if the MBC is not more than four times higher than the MIC, and MBC value is always equal or higher than MIC.

Conclusions

*L. angulata* can be used as a source of natural antioxidant to prevent damage associated with DPPH free radicals and antibacterial to inhibit the growth of *S. mutans* and *S. aureus* bacteria.

Data availability

Dataset 1. Raw data associated with this study. Data include the absorbance values obtained from the DPPH scavenging assay, and the resultant IC50 values generated. DOI: https://doi.org/10.5256/f1000research.16620.d223677.

Grant information

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Sarifah Nurjanah

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This is an interesting manuscript containing new information on the potential of Litsea angulata. The paper is well written and provides valuable data. However, there are some suggestions to improve.

1. Introduction:
   - Not only include the reason for exploring the potential of Litsea angulata, but also why should it be seen from each part of the material (bark, branch and leaves), is there any previous research that each part of the plant has different active ingredients?
   - It should also be written the reasons for using Staphylococcus aureus and Streptococcus mutans.

2. Results:
   - An increase of IC50 value in antioxidant activity written in succession leaves, ethanol; bark, ethanol; ethanol branch; branch, ethyl acetate; bark, ethyl acetate; branch, n hexane; leaves and bark, n hexane; it should be bark, ethyl acetate; leaves, ethanol; bark, ethanol; ethanol branch; bark, ethyl acetate; branch, ethyl acetate; bark, n hexane; leaves ethyl acetate; branch and leaves n hexane.
   - In the written method there are several controls used, namely positive control, negative control, vehicle control and control machines, but in the results only positive controls are written, what are the other controls?

3. Discussion:
   - Need clarification as to why ethanol can extract more active components such as tannin, carotenoid and coumarin compared to n hexane and ethyl acetate.
   - Is there a relationship between antioxidant properties and antibacterial with component ingredients (phytochemical assessment results).

4. Conclusion:
   - In conclusion section, only conclude about antioxidant activity and antibacterial activity. It should be mentioned also the conclusion of phytochemical assessment.

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?
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 confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 15 January 2019

https://doi.org/10.5256/f1000research.18164.r41035

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Morina Adfa
Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Bengkulu, Bengkulu, Indonesia

Manuscripts entitled Phytochemicals, antioxidants and antimicrobial properties *Litsea angulata* extract has been written well. Revise in the keyword *Litsea angulata* in italic format, add your information where the species (*Litsea angulata*) was determined. Please make sure the alkaloids contained in *n*-hexane extract because positive false in phytochemical tested are often reported. Authors wrote that all statistical analyses used Microsoft Excel 2010 software, but in the results and discussion, we cannot find the data with statistical analysis. The authors may add the data with statistical analysis or they can explain in the discussion because in the method they used the statistical analysis for data analysis. The authors may add the data with statistical analysis or they can explain in the discussion because in the method they used the statistical analysis for data analysis.

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

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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.

Reviewer Expertise: Natural product chemistry

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

Reviewer Report 07 January 2019
https://doi.org/10.5256/f1000research.18164.r41787

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Mariateresa Cristani
Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy

I like the concept of this manuscript, and it could be interesting, but I think it needs some additional work. The only DPPH test is not enough to demonstrate the antioxidant activity and it is an easy test. Phytochemical screening is already known so it would be interesting at least HPLC analysis. The discussion could be a bit deeper explaining the benefits of extracts and comparing better which extracts is more useful and why. It would have been interesting to have information on the pedoclimatic characteristic of the place where the plant are harvested. The description of experimental part is for the most part good and clear. The written English needs to be better. The following reference which appeared recently in Natural Products Research, 2018 should be added.

References
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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?**
Partly

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Yes

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**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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