RESEARCH ARTICLE

REVISED Phytochemical, antioxidant and antimicrobial properties of *Litsea angulata* extracts [version 2; peer review: 3 approved]

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Latest published: 20 Mar 2019, **7**:1839 (https://doi.org/10.12688/f1000research.16620.2)

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

Open Peer Review

Referee Status: ✓ ✓ ✓

	Invited Referees 1	2	3
REVISED version 2 published 20 Mar 2019	✓ report		✓ report
version 1 published 22 Nov 2018	? report	✓ report	? report

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2 **Morina Adfa**, University of Bengkulu, Indonesia

3 **Sarifah Nurjanah**, Padjadjaran University, Indonesia

Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: Kuspradini H: Conceptualization, Data Curation, Formal Analysis, Methodology, Project Administration, Writing – Original Draft Preparation; **Wulandari I**: Formal Analysis, Investigation, Resources; **Putri AS**: Data Curation, Formal Analysis, Investigation; **Tiya SY**: Investigation, Resources; **Kusuma IW**: Conceptualization, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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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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First published: 22 Nov 2018, **7**:1839 (https://doi.org/10.12688/f1000research.16620.1)

REVISED **Amendments from Version 1**

In response to the reviewers' comments, we have updated our manuscript. A number typographical errors as pointed out by the reviewer are corrected and additions have been added on the manuscript: information on the pedoclimatic characteristic of the place where the plant are harvested, the place of determination of the *Litsea angulata*, statistical analysis on the discussion section, benefits and comparison of extracts, addition of some references, revision on the order of IC50 value in antioxidant activity, explanation about using different plant material and bacterias such as *Staphylococcus aureus* and *Streptococcus mutans*, confirmation about negative control, vehicle control and control machines in the results, clarification about why ethanol can extract more active components such as tannin, carotenoid and coumarin compared to n-hexane and ethyl acetate, revision on conclusion.

We have updated the affiliation for Indah Wulandari.

See referee reports

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 cold[1,2]. There is little information about the potency of *Litsea angulata* species. *L. angulata*, belonging to the Lauraceae family, which can be found in East Kalimantan, Indonesia, and to our knowledge, data are limited on its biological activities. The fruit seed of *L. angulata* has been reported to have antifertility properties. No previous studies report the antibacterial properties of *L. angulata* (bark, branch and leaves), while the related species such as *L. cubeba* leaf extract, *Litsea petiolata* Hook. f. leaves, and stem bark and leaf extracts of *Litsea glutinosa* (Lour.) C.B. Rob, presented antibacterial activity against *S.* aureus[3–5]. In addition essential oil of *Litsea cubeba* can also act as antibacterial against *S. mutans*[6]. Therefore the present study aimed to assess the phytochemical constituents, antioxidant, and antimicrobial of different plant part of *L. angulata*.

Methods

Sample collection

The plant material was obtained from Education Forest Laboratory of Forestry Faculty, Mulawarman University, East Kalimantan, Indonesia (0° 25'10" – 0°25'24" Southern Latitude and, 117° 14'00"-117 14'14" East longitude). The soil texture, average air temperature and relativity humidity in this location were sandy loam, 25.4°C and 91.6%, respectively[7,8]. The plant samples were identified in Dendrology and Forest Ecology Laboratory of Forestry Faculty, Mulawarman University.

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 Sruthi and Indira[9], 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 were 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 into literature by Kokate[10], Senthilmurugan[11], Harborne[12] 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.*[13]. 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 10^8 CFU ml^{-1}. The method of MIC was adopted from the method outlined by Mohsenipour and Hassanshahian, with modifications[14]. 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 alkaloid, flavonoid, tannin, terpenoid, carotenoid 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. The extraction of various phytochemicals was seen to be more effectively done by ethanol (polar) than the ethyl acetate and n-hexane (semi polar and non polar) solvents.

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: bark, ethyl acetate; leaves-bark-branch, ethanol; branch, ethyl acetate; leaves, ethyl acetate; branch and leaves, n-hexane. Raw absorbance data from which IC50 values were calculated are shown in Dataset 1[15].

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. No pink color change can be seen in the negative control, vehicle control and media control. It confirms that the media (Nutrient Broth) and solvent did not affect the antimicrobial activity.

Dataset 1. Raw data associated with this study

https://dx.doi.org/10.5256/f1000research.16620.d223677

Data include the absorbance values obtained from the DPPH scavenging assay, and the resultant IC50 values generated.

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)				
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[16–18]. The secondary metabolites extraction can be performed using solvent with different polarities, depending on the type of compound structure. Thus, it can be reported that polar solvent (ethanol) could extract more secondary metabolites such as tannin, carotenoid and coumarin than ethyl actetate and n-hexane solvent (semi-polar and non polar). It may be possibly be one of the reasons for a strong antioxidant activity shown by the ethanolic extracts of *L. angulata*. The solubility or insolubility of the active compound(s) in the solvent used for extraction can caused the differential effects on antioxidant and antibacterial[19–21]. The IC50 value was calculated using the regression linear equation, where y is 50 (percent inhibitory value) and x is amount of inhibitory concentration value in µg. Data analysis were shown in Dataset 1. According to Blois[22] sample which had an IC50 value more than 150 ppm was a weak antioxidant, 101–150 ppm was a medium antioxidant, 50–100 ppm indicated as a strong antioxidant, while lower than 50 ppm was a very strong antioxidant. Bark-ethyl acetate extract obtaining the lowest IC50 value (2.41 ppm) in comparison to another extracts, and it indicates that this extract has a 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[23].

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. It may be due to the presence of its active compound such as alkaloids, flavonoids, tannins, terpenoids, carotenoids and coumarin.

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[15].

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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Publisher Full Text

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Open Peer Review

Current Referee Status: ✔ ✔ ✔

Version 2

Referee Report 04 April 2019

https://doi.org/10.5256/f1000research.20243.r45953

✔ **Sarifah Nurjanah**
Departement of Agricultural and Biosystem Engineering, Faculty of Agro-Industrial Technology, Padjadjaran University, Bandung, Indonesia

The manuscript has been updated according to the input given, so there is no more comment required.

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.

Referee Report 20 March 2019

https://doi.org/10.5256/f1000research.20243.r45954

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

I have no other comments.

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.

Version 1

Referee Report 31 January 2019

https://doi.org/10.5256/f1000research.18164.r43839

?

Sarifah Nurjanah
Departement of Agricultural and Biosystem Engineering, Faculty of Agro-Industrial Technology, Padjadjaran University, Bandung, Indonesia

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 angulate, 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 have read this submission. I 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.

Referee Report 15 January 2019

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

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

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.

Reviewer Expertise: Natural product chemistry

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 07 January 2019

https://doi.org/10.5256/f1000research.18164.r41787

? **Mariateresa Cristani**
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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 a 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[1] should be added.

References

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Partly

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

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, however I have significant reservations, as outlined above.