Antioxidant and antibacterial activity of *Litsea garciae*

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**Abstract.** *Litsea garciae* is an evergreen tree growing up to 26 meters tall and useful tropical plant species. This plant have medicinal uses. The lightly burned bark can be used to cure caterpillar sting. The purpose of this study was to analyze the characteristics of secondary metabolite compounds, total phenolic and flavonoid content, antioxidant and antibacterial activity from leaf, bark and branch of *Litsea garciae* plant extracts. Antioxidant activity was determined by using free radical method (1,1-diphenyl 2-picrylhydrazyl). Antibacterial activity against *Propionibacterium acnes* was assayed by 2,3,5-triphenyl tetrazolium chloride method. The sample extracts were obtained using a successive maceration method with n-hexane, ethyl acetate, and ethanol solvent. The result of phytochemical analysis on *Litsea garciae* extract positive contained several secondary metabolite compounds. Among the three sample extracts, the highest of total phenol content present in all three parts of ethanol extract with a value of 0.9-1.0 μg/mg GAE. The highest total flavonoid content was 10.1 μg/mg CE. The highest antioxidant activity was found in ethyl acetate stem extract (86% ± 0.00) at 100 ppm concentration, with IC₅₀ at 41.54 ppm. The present work showed that *L. garciae* ethanol extract has potential to inhibit the growth of *P. acnes* bacteria.

1. Introduction

Lauraceae (Medang-medangan) is a large plant and can grow not only in the tropics but can also grow in subtropics. In addition to containing essential oils, Lauraceae has been known to also contain several classes of other secondary metabolite compounds such as alkaloids, phenylpropanoids, flavonoids, benzyl-esters, and alkane-alkene derivatives [1]. *Litsea*, an important genus of the Lauraceae family, is often found in areas such as tropical and subtropical Asia, Australia, and from North America to South American subtropical [2]. Studies of literature related to biological activity indicate that the secondary metabolite compounds contained in the Lauraceae plants indicate the presence of insecticide and cytotoxic activities. Bioactive compounds that have insecticidal activity, among others, from the alkaloids, terpenoids, and flavonoids [3]. The solvents of ethanol and other alcohols are usually used to extract the secondary metabolite compounds of the plant. This solvent is able to increase the permeability of the cell wall and penetrate into the cell to extract the secondary metabolites more and more widely than the extraction with the n-hexane solvent. [4].

Due to rapid increase of antibiotic resistance in our region, it is interesting to determine whether their traditional uses are supported by actual pharmacological effects or merely based on folklore. This study was conducted to determine phytochemicals components, antioxidant and antibacterial activity of *L. garciae* from three different extract.
2. Methods

2.1 Plant material and sample
The branch, bark, and leaf of **Litsea garcia** were collected from Samarinda, East Kalimantan. Research was conducted in Forest Products Chemistry Laboratory of Mulawarman University, Samarinda.

2.2 Maceration
The sample (50 grams) was extracted for 1x24 hours, using successive maceration methods with three solvents which has different polarity (n-hexane, ethyl acetate, and 96% ethanol). The sample were filtered to continue the next process.

2.3 Phytochemical screening
Phytochemical analysis was performed on **L. garciae** extract, for the determination of chemical elements including alkaloids, flavonoids, steroids, terpenoids, tannins, saponins, carbohydrates, coumarin, and carotenoids. The procedure for analyzing each of the bioactive compounds is described by Harborne[5]Kokate[6] Senthilmurugan, [7]

2.4 Determination of Total Phenolic Content (TPC)
Total phenolic content was determined using Folin-Ciocalteu reagent according to the method described by Velioglu et al [8]. Extract (100 µL) was mixed with 0.25 mL of Folin-ciocalteu reagent and 2.5 ml distilled water and then 1.25 ml sodium carbonate (60g/L). After 60 min at room temperature, absorbance was measured at 760 nm using spectrophotometer. Standards of gallic acid (Sigma-Aldrich) in the concentration range 10 to 100 µg/mL were run with the test samples, from which a standard curve was plotted. Result was expressed as mg gallic acid equivalents (GAE)/g of dried sample.

2.5 Determination of Total Flavonoid Content (TFC)
Total flavonoid content was determined by using colorimetric method described by Dewanto et al [9] with slight modification. Briefly, 0.1 µL of extract was mixed with 2.5 mL of distilled water in a test tube followed 1.5 mL of a 2% AlCl₃·6H₂O (Sigma-Aldrich) solution was added and allowed to stand for another 20 min. The mixture was mixed well with vortex, and the absorbance measured immediately at 420 nm using spectrophotometer. Standards of rutin (Sigma-Aldrich) in the concentration range 10 to 100 µg/mL were run with the test samples, from which a standard curve was plotted. Results were expressed as mg rutin equivalents (RE)/g of dried sample.

2.6 DPPH free radical scavenging Assay
The antioxidant potential of **L. garciae** extract assessed by measuring the scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The DPPH assay was performed as described by Shimizu et al [10]. The samples were mixed with 33 µl of 467 µl 96% ethanol and filled up with 500 µl DPPH solutions, to a final volume 1 ml. The absorbance of the resulting solution and the blank were recorded after 20 minute at room temperature. Ascorbic acid was used as a positive control. Scavenging of DPPH free radical was recorded spectrophotometrically at 517 nm and the free-radical scavenging activity was calculated as follows:

\[
\% \text{ Scavenging Effect} = \left(1 - \frac{Abs_{\text{Sample}}}{Abs_{\text{Control}}} \right) \times 100\%
\]
Determination Antibacterial activity

Minimum Inhibitory concentration (MIC) was determined in the plant extracts that showed some efficacy against the tested isolates. Extracts were tested against the isolates for their inhibitory activity, using a common broth microdilution method in 96 multiwell microtiter plates, in duplicate, as reported by [11] with slight modification. For susceptibility testing, 50 μl of Mueller-Hinton broth was distributed from the second to the twelfth test wells extracts from each plant part were dissolved in 96 ethanol to reach a final concentration 1.250 μg/ml. Inoculated plates were incubated at 37°C for 24 h. One hour before the end of incubation 50 μl of a 0.01% solution of 2, 3, 5- triphenyl tetrazolium chloride (TTC) was added to the wells and the plate was incubated for another hour. Since the colorless tetrazolium salt is reduced to a red colored product by biologically active organisms, the inhibition of growth can be detected when the solution in the well remains clear after incubation with TTC. The lowest concentration of each extract showing no visible growth was recorded as the minimum inhibitory concentration (MIC). Inoculated and uninoculated wells of plant extract-free broth was included (the first controls of the adequacy of the broth to support the growth of the organism; the second is a check of sterility). Chloramphenicol was used as positive controls.

Results and discussion

3.1 Phytochemical screening

Identification of secondary metabolite content is an important first step in the search for new bioactive compounds from natural ingredients that can be a for new drug synthesis or prototype of drugs.

Table 1. Phytochemical screened of L. garciae

| Solvent     | Part   | Alk | Flav | Sap | Tan | Triter | Ste | Car | Cou | Caro |
|-------------|--------|-----|------|-----|-----|--------|-----|-----|-----|------|
| n-hexane    | Branch | +   | -    | +   | -   | +      | +   | +   | -   |       |
|             | Bark   | +   | -    | +   | -   | +      | +   | +   | -   |       |
|             | Leaf   | +   | -    | +   | -   | +      | +   | +   | -   |       |
| Ethilasetat | Branch | +   | -    | -   | +   | +      | +   | -   | +   | -     |
|             | Bark   | +   | -    | +   | -   | +      | +   | +   | -   |       |
|             | Leaf   | +   | -    | +   | -   | +      | +   | +   | -   |       |
| Ethanol     | Branch | +   | -    | +   | -   | +      | +   | -   | +   | -     |
|             | Bark   | +   | -    | +   | -   | +      | +   | -   | +   | -     |

Remarks : (+) positive contains, (-) negative contains
Alk: alkaloids, Flav: flavonoids, Sap: saponins, Tan: tannins, Triter: triterpenoid, Ste: steroid, Car: carbohydrate, Cou: Coumarin, Caro: Carotenoids.

Test results show that all extracts formed by using different solvents from branch, bark, Leaf from L. garciae extract contain phytochemicals such as alkaloids, flavonoids, terpenoids, steroids as well as carotenoid. The test results showed that all the extracts formed by using different solvents from L. garciae plants contain phytochemicals such as alkaloids, flavonoids, alkaloids, steroids, and tannins. However, saponins were found to be absent in all of the extract samples from the three solvents.
These results indicate that the different solvents, as the differences in polarity, could selectively extract different secondary metabolites [12]. Containing flavonoid have an effect on arachidonic acid metabolism, thus could have anti-inflammatory, anti-allergic, antithrombotic or vasoprotective effects. The presence of alkaloids in this plant has further confirmed its medicinal use as antibacterial agent [13].

3.2 Phenol and Flavonoid content
Below is a table of test results of total phenol and flavonoid by method Colorimetric AlCl₃ method test [10,11] and Folin-Ciocalteu method test [8,9].

| Solvent   | Part     | Phenolic µg GAE/mg extract | Flavonoid µg GAE/mg extract |
|-----------|----------|----------------------------|-----------------------------|
| n-hexane  | Branch   | 30±0.002                   | 190±0.004                   |
|           | Bark     | 40±0.002                   | 170±0.003                   |
|           | Leaf     | 30±0.001                   | 110±0.002                   |
| Ethyl acetate | Branch | 30±0.002                   | 140±0.005                   |
|           | Bark     | 40±0.004                   | 160±0.005                   |
|           | Leaf     | 40±0.004                   | 210±0.004                   |
| Ethanol   | Branch   | 100±0.001                  | 1010±0.002                  |
|           | Bark     | 90±0.009                   | 800±0.001                   |
|           | Leaf     | 100±0.001                  | 240±0.001                   |

Overall, the highest total phenol contained in Litsea gareicae was obtained from ethanol-soluble extract, averaging 100 µgGAE/ mg extract. The soluble extract of n-hexane and ethyl acetate has a total phenol content of almost the same that ranges between 300-400 µg GAE/ mg extract. In the total flavonoid test, the stem sample part of Litsea gareicae extract has the total content of Flavonoid reaches 1010 µgGA / mg extract. In previous research, methanol, ethanol, acetone, ethyl acetate have been used to extract phenols [14].The recovery of phenols from plant materials is influenced by solubility of the phenolic compounds in the solvent used for the extraction process. Otherwise, solvent polarity will play a key role in increasing phenolic solubility [15]. Total flavonoid, as one of the most diverse and widespread groups of natural compounds, are probably the most natural phenolics [16]. Both flavonoid and phenolic compounds are known to have multiple biological effects, including antioxidant and anti-inflammatory properties [17].

3.3 Antioxidants
In simple terms, antioxidants are expressed as compounds capable of inhibiting or preventing oxidation. The results of the antioxidant testing of L. gareicae plant parts of the three solvents and concentrations can be seen in the table.
Table 3. Antioxidant activity of *L. garciae*

| Solvent    | Part   | Antioxidant (ppm) | IC<sub>50</sub>(ppm) |
|------------|--------|-------------------|-----------------------|
| n-hexane   | Branch | 25±0.004          | 16±0.001             |
|            | Bark   | 49±0.004          | 4±0.007              |
|            | Leaf   | 19±0.004          | 3±0.004              |
| Ethyl acetate | Branch | 86±0.001          | 27±0.008             |
|            | Bark   | 61±0.0013         | 23±0.007             |
|            | Leaf   | 37±0.007          | 0±0.007              |
| Ethanol    | Branch | 62±0.002          | 57±0.006             |
|            | Bark   | 60±0.001          | 52±0.001             |
|            | Leaf   | 72±0.005          | 33±0.003             |

nd = not detected

The antioxidant activity test was performed by capture free radical of DPPH (1,1-diphenyl diphenyl-2-picrylhydrazyl) by using UV / VIS 1200 spectrophotometer. This method was chosen because it is a simple, quick, and easy method for screening the radical trapping activity of some compounds, but this method proves to be accurate and practical [18]. The antioxidant inhibitory value resulting from the extract sample testing has decreased value at each concentration from 100ppm-12.5ppm. The strong active of *L. garciae* were obtained in bark ethanol extract with IC50 at 19.26 ppm. Differences in the polarity may explain differences in extraction yield and antioxidant activity [19].

3.4 Antibacterial assay

Antibacterial testing using bacteria *Propionibacterium acnes*. Antibacterial activity was performed using Elkhair et al [9] method that has been modified. The concentrations used were 1250, 625 and 312.5 ppm.

Table 4. Minimal Inhibitory Concentration (MIC) of *L. garciae*

| Solvent    | Part   | MIC (ppm) |
|------------|--------|-----------|
| n-hexane   | Branch | 625       |
|            | Bark   | 312.5     |
|            | Leaf   | 625       |
| Ethyl acetate | Branch | 625       |
|            | Bark   | 1250      |
|            | Leaf   | 312.5     |
| Ethanol    | Branch | 625       |
|            | Bark   | 312.5     |
|            | Leaf   | 625       |

In the bark sample using n-hexane and ethanol solvent, inhibition occurred at the smallest concentration 312.5ppm. The antimicrobial agents are expressed its potency as minimum inhibitory concentration (MIC) in this method. The method was carried out in a broth dilution test, in which a specific amount of bacteria was added to the serial dilution of antimicrobial agents in broth wells.
After incubation, bacterial growth was indicated by turbidity and its lack was indicated as growth inhibited by the antimicrobial agent. Among the extractions assayed, the n-hexane and ethanol bark and ethyl acetate leaves extracts of L. garciæ showed least activity. The have potency as bacterial growth inhibitor. As seen in table 4, all the sample could inhibit the bacteria growth at small concentration range (312.5-625 ppm). Except the stem part ethyl acetate extracts from stem. The investigated plants that did not show strong antibacterial activity; however, do not mean absence of bioactive constituents nor is that the plant inactive. Active compound(s) may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed [20]. The results can be due to the method of plant extraction, the type of microbes used in study, and variation of plant part [21].

4. Conclusion
Based on the results of research conducted, it is known that L. garciæ extract contains secondary metabolite alkaloids, flavonoids, tannins, carbohydrates, Coumarin which can inhibit free radicals and growth of P. acnes bacteria.

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References
[1] Guenther E 2006 Essential Oils 1st Edition translate by S Ketaren (Jakarta: UI Press) [Indonesia]
[2] Tanaka H, Yatsuhashi S, Yasuda T, Sato M, Sakai E and Xiao C 2009 A new amide from the leaves and twigs of Litseaauriculata J. Nat. Med. 63: p 331-334
[3] Duke SO 1990 Natural Pesticides From Plants Edunewcrop Proceedings VI p 511
[4] Cannell JPR 1998 Natural Products Isolation (Humana Press)
[5] Harborne JB 1987 Phytochemical Method: Modern method Guidelines of plants analysis (Bandung: ITB) [Indonesia]
[6] Kokate CK 2001 Pharmacognosy 16th Edn (Mumbai India: NialiPrakasham)
[7] Senthilmurugan GB, Vasanthe and Suresh K 2013 Screening and Antibacterial Activity Analysis of some Important Medicinal Plants International Journal of Innovation and Applied Studies 2(2) p 146-152
[8] Velioglu YS, Mazza G, Gao L, and Oomah BD 1998 Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J Agr. Food Chem. 46: p 4113-4117
[9] Dewanto V, Wu X, Adom KK and Liu RH 2002 processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agr. Food Chem. 50: p 3010-3014.
[10] Shimizu K, Kondo R, Sakai K, Takeda N, Nagahata T and Oniki T 2001 novel vitamin E Devirate with 4-substituted Resorcinol Moiety has both antioxidant and Tyrosinase Inhibitory Properties Lipids 36: p 21 – 25
[11] Elkhair E, Fadda H and Mohsen UA 2010 Antibacterial Activity and Phytochemical Analysis of Some Medicinal Plants from Gaza Strip–Palestine Journal of Al Azhar University
[12] Rebey IB, Bouroug S, Debez IBS, Karoui IJ, Sellami IH and Msaada K 2012 Effects of extraction solvents and provenances on phenolic contents and antioxidant activities of cumin (Cuminumcyminum L.) seeds. Food and Bioprocess Technology 5: p 2827–2836.
[13] Evans WE 2002 Treas and Evans Pharmacognosy. 15th ed. WB Saunders Co. Ltd, Philadelphia, Freidewald WT, RI Levy and DS Frederickson 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin.
[14] Tan ES, A Aminah H M Khalid, M Y Maskat and M A Ghani 2012 Antioxidant properties of three banana cultivars (Musa acuminata ‘Berangan’, ‘Mas’ and ‘Raja’) extracts Sains Malays 41 : p 319-324.

[15] Naczk M and Shahidi F J 2006 Pham Biomed Anal 41: p 1523-1542.

[16] Shimoi K, Masuda S, Shen B and Kinae N 1996 Mutat. Res 350 : p 153-161.

[17] Amarowicz R 2007 Eur. Lipid Sci. Technol 109: p 549–551

[18] Febriani K 2012 Antioxidant Activity of extract and fraction of Cocculusorbiculatus(L) DC using DPPH method and Identification of Chemical components group from active fraction (Depok: FMIPA Universitas Indonesia) [Indonesia]

[19] Naczk M, F Shahidi 2006 Phenolics in cereals fruits and vegetables: occurrence, extraction, and analysis. Pharm. Biomed. Anal 41 : p 1523-1542.

[20] Parekh J, and Chanda S 2007 Antibacterial and phytochemical studies on twelve species of Indian medicinal plants J African of Biomedical Research 10: p 175 – 181.

[21] Ncube N S, Afolayan A J, and Okoh AI 2008: Assessment techniques of antimicrobial properties of natural compounds of plant origin current methods and future trends J. African of Biotechnology 12 : p 1797-1806.