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IOP Conf. Series: Earth and Environmental Science **144** (2018) 012024 doi:10.1088/1755-1315/144/1/012024

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 *L.itsea 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 IC_{50} 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.

2.2Maceration

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% $AlCl_3 \cdot 6H_2O$ (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:

$$\%\, Scavenging\ Effect = \left(1 - \frac{Abs_{sample}}{Abs_{control}}\right) x100\% \quad (1)$$

2.7 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.

3. 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	+	+	-	+	+	-	+	+	+
	Leaf	+	+	-	-	+	-	+	+	-

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 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 indicates 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 $AlCl_3$ method test [10,11] and Folin-Ciocalteu method test [8,9].

Table 2. Total Phenol and Flavonoid content *L.garciae*

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	Branch	30±0.002	140±0.005
acetate?	Bark	40±0.004	160±0.005
	Leaf	40±0.004	210±0.004
	Branch	100±0.001	1010±0.002
Ethanol	Bark	90±0.009	800±0.001
	Leaf	100±0.001	240±0.001

Overall, the highest total phenol contained in *Litsea garciae* 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 garciae* 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. garciae* 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)				
		100	50	25	12.5	IC_{50}(ppm)
n-hexane	Branch	25±0.004	23±0.003	20±0.001	16±0.001	nd
	Bark	49±0.004	27±0.001	17±0.006	4±0.007	nd
	Leaf	19±0.004	15±0.033	4±0.005	3±0.004	nd
Ethyl	Branch	86±0.001	67±0.0010	34±0.003	27±0.008	41.54
acetate	Bark	61±0.0013	52±0.003	52±0.002	23±0.007	55.29
	Leaf	37±0.007	23±0.002	5±0.0012	0±0.007	nd
Ethanol	Branch	62±0.002	66±0.005	57±0.006	4±0.003	52.52
	Bark	60±0.001	58±0,002	52±0.001	46±0.002	19.26
	Leaf	72±0.005	56±0.002	33±0.003	24±0.003	53.76

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.garciae*.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. garciae* extract contains secondary metabolite alkaloids, flavonoids, tannins, carbohydrates, Coumarin which can inhibit free radicals and growth of *P. acnes*.bacteria.

Acknowledgment

The authors gratefully acknowledged to Ministry of Research, Technology and Higher Education, Republic of Indonesia, and also members of Laboratory of Forest Products Chemistry

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