IJPSR (2021), Volume 12, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 17 March 2020; received in revised form, 17 June 2020; accepted, 28 June 2020; published 01 March 2021

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF SECONDARY METABOLITE PRODUCED BY ENDOPHYTIC FUNGI ISOLATED FROM *LANNEA COROMANDELICA* (HOUTT.) MERR

Puteri Amelia [* 1], Putri Agni Kreativita Ivada [1], Narti Fitriana [2], Ismiarni Komala [1], Saiful Bahri [3] and Muhammad Hanafi [4]

Department of Pharmacy [1], Faculty of Health Sciences, Department of Biology [2], Faculty of Sciences and Technology, Universitas Islam Negeri (UIN) Syarif Hidayatullah Jakarta, Indonesia.
Faculty of Pharmacy [3], National Science Institute and Technology, Jakarta, Indonesia.
Indonesian Institute of Sciences (LIPI) [4], Research Centre for Chemistry, Indonesia.

Keywords:

Antioxidant, Antimicrobial, Citrinin, Endophytes, *Penicillium sp*, Spectroscopic

Correspondence to Author:
Puteri Amelia

M. Pharm,
Department of Pharmacy,
Faculty of Health Sciences,
Universitas Islam Negeri (UIN)
Syarif Hidayatullah Jakarta,
Tangerang, Banten - 15419,
Indonesia.

E-mail: puteri.amelia@uinjkt.ac.id

ABSTRACT: *Lannea coromandelica* (Houtt.) Merr. which is known as Kayu Jawa in Bugis community, Indonesia has been widely used empirically for the treatment of wounds. Ethanol extract from its stem bark is known to have antibacterial, anti-inflammation, and antioxidant activity. The purpose of this study were to isolate, characterize, and determine the antioxidant and antimicrobial activity of the endophytic fungi from the roots of this plant. In this paper, we reported antioxidant and antimicrobial activity of the isolated endophytic fungi from *Lannea coromandelica* (Houtt.) Merr. and the isolation of the bioactive compound known as citrinin. The structure of citrinin was determined by extensive analysis of the spectroscopic data (^{1}H-NMR, ^{13}C-NMR, 2D NMR, and LC-MS) and comparison to those of the previous report. The antioxidant activity showed that the isolated compound has moderate activity with AAI value of 0.671 and IC_{50} 145.9 ppm while the antimicrobial activity demonstrated that the isolated compound was not active against *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 14028, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853 (MIC: 1000 µg/ml).

INTRODUCTION: The bioactive compounds can be led from many sources; plants, animals, marine organisms, and endophytic microbes. Plants are known as a reservoir of endophytes, including fungi, bacteria, and actinomycetes [1, 2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(3).1588-92

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(3).1588-92

The ability of endophytic microbes to produce bioactive compounds has been widely investigated since the discovery of penicillin from *Penicillium notatum* by Alexander Fleming in 1928 [3].

Endophytic microbes are bacteria or fungi or actinomycetes that spend part or all of their life that inhabits internal tissues of plants without causing disease [4, 5]. Using endophytic microbes as a source of natural products and lead compounds for developing new drugs was very beneficial since it can be obtained without killing the host plant; hence the over-exploitation and over-cutting of the

plants can be reduced and the environmental protection can be preserved [6]. In our continuing search for bioactive natural products from tropical plants [7], herein we reported the antioxidant and antimicrobial activity of the endophytic fungi from *Lannea coromandelica* (Houtt.) Merr.

Both the leaves and the stem bark of this plant have astringent and painkilling properties and contain some secondary metabolites such as alkaloids, flavonoids, tannins, and saponins [8]. The new development of the isolation method and the advanced technology of screening biological activities have shortened the time required to isolate secondary metabolites compounds from natural sources. The combination of the instruments' uses, for example, high-performance liquid chromatography (HPLC), infrared (IR), mass spectroscopy (MS), and nuclear magnetic resonance (NMR) make the process of the isolation of bioactive compounds from a natural product is possible to do in a short time [9]. The aims of this study were to isolate and evaluate the antioxidant and antimicrobial activity of the endophytic fungi of *Lannea coromandelica* (Houtt.) Merr. and determined the structure of the isolated compound using spectroscopy analysis.

MATERIALS AND METHODS:

Materials: *Lannea coromandelica* (Houtt.) Merr which was collected from South Sulawesi, Indonesia, and determined in Center for Plant Conservation – Bogor Botanical Gardens, Indonesia, media NA (nutrient agar), PDA (potato dextrose agar), PDY (potato dextrose yeast) DMSO (dimethyl sulfoxide), DPPH (2, 2-difenil-1-pikrilhidrazil), NaCl 0,85% (b/v), *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 14028, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, organic solvents (ethanol, ethyl acetate, acetone, methanol), bleach solution, distilled water, silica for column chromatography and TLC (thin layer chromatography). All other chemicals used in this study were analytical grade.

Isolation of Endophytic Fungi [5]: The roots of *Lannea coromandelica* (Houtt.) Mer. plant materials were surface-sterilized respectively in sterile water, 10% bleach solution, 70% ethanol, and distilled water. The sterilized samples incubated for 2 weeks on PDA (potato dextrose agar) supplemented with chloramphenicol (50 µg/ml) and streptomycin sulphate (250 µg/ml) to suppress bacterial growth. The developed mycelia as pure cultures were transferred onto fresh PDA plates free of antibiotics and cultivated for 14 days on PDA plates at 28 °C plates and used as stock and working culture.

Fermentation [5]: Five pieces (0.5 × 0.5 cm^2) of mycelia agar plugs were selected and inoculated into liquid fermentation media PDY and incubated at room temperature for four weeks under stationary conditions. The broth culture was filtered to separate the filtrate and mycelia. The filtrate was extracted three times by shaking with an equal volume of ethyl acetate and evaporated until dry solid ethyl acetate extract was obtained and its activities were evaluated.

Antimicrobial Activity Test [10]: The antimicrobial activity of the crude ethyl acetate extracts was tested against *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 14028, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 using agar diffusion method according to the Clinical Laboratory Standards Institutes 11 with minor modification. Pre-warmed nutrient agar plates were seeded with 106 CFU suspension of test bacteria. Paper disc saturated with Ethyl acetate extract which dissolved in Ethyl acetate (1 mg/ml) and pipetted 20 µl onto sterile paper discs and placed onto the surface of inoculated agar plates. Plates were incubated at 37 °C for 24 h.

The clear zone around the disc demonstrated a microbe's inhibition zone (mm) produced by the extracts. Chloramphenicol was used as positive control while ethyl acetate was used as the negative control.

Antioxidant Activity Test: The ability of crude ethyl acetate extracts to scavenge DPPH free radicals was assessed by using the method described by Takao *et al.* with some modifications [12]. Briefly, various concentration of Ethyl acetate extract (200; 100; 50; 25; 12,5; 6,25 µg/ml) were mixed with 3 ml of methanolic solution of DPPH (0.1 m). An equal amount of methanol and DPPH without sample was served as control, while

ascorbic acid was used as the positive control. After 30 min of reaction at room temperature in the dark, the absorbance was measured at 517 nm against methanol as a blank using a UV-VIS spectrophotometer. Each sample was analyzed in triplo, and the average values were plotted to obtain the IC_{50} against DPPH by linear regression. The radical scavenging activity was evaluated as the percentage of inhibition according to the following equation:

% Inhibition = [(absorbance of control – absorbance of sample) / absorbance of control)] × 100

The IC_{50} value is the effective concentration at which 50% of DPPH radicals were scavenged. It was obtained from the graph of scavenging activity (%) versus the concentration of samples. The low IC_{50} value indicates the strong ability of the extract to act DPPH scavenger [13].

Phytochemical Screening, Isolation and Structure Elucidation: Phytochemical screening of the ethyl acetate extract was evaluated based on the methodology of Harborne [14]. The ethyl acetate extract was purified by subjected to column chromatography over silica gel and eluted with hexane/ethyl acetate (49:1 to 1:1, v/v), followed by $CHCl_3$/methanol (49:1 to 100%) to give some fractions. Concentrated fractions were spotted on the TLC plate and eluted with several eluent mixtures. The pure compound was analyzed by TLC, LC-MS, and NMR spectroscopy.

Identification of Endophytes Fungi: The characterization of fungal endophytes (AP21C) was performed by microscopic identification and macroscopic morphological, including the color of the colonies, the exudate drops, zonation, and also types of hyphae and micelium [15].

RESULTS AND DISCUSSION: *Lannea coromandelica* (Houtt.) Merr was collected from South Sulawesi, Indonesia. In this paper, we reported the antioxidant and antimicrobial activity from ethyl acetate extract of isolate (AP21C) endophytic fungi from roots of *Lannea coromandelica* (Houtt.) Merr. and the structure elucidation of the isolated compound (compound 1).

The antioxidant activity assay of ethyl acetate extract of isolate AP21C was determined by the use of the methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) reagent with several variations of sample concentration and quercetin as the standard. DPPH method is used in the study of antioxidant effects of complex biological compounds and of their ability to reduce the free radical's activity. The reduction of DPPH (purple) to the corresponding hydrazine (yellow) is a fast and simple method for evaluating radical scavenging activity [13].

The reaction can be monitored spectro-photometrically by following the decrease in absorbance at $\lambda = 515$ nm. The antioxidant activity assay showed that the ethyl acetate extract of isolate AP21C has moderate activity with AAI value of 0.671 and IC_{50} 145.9 ppm. The antimicrobial activity of ethyl acetate extract of isolate AP21C was tested by the agar diffusion method with chloramphenicol as positive control and ethyl acetate as the negative control. Chloramphenicol was chosen as a positive control because of its wide spectrum against Gram-positive and Gram-negative bacteria, and it is also known as a bacteriostatic agent. The result of the anti-microbial activity test is summarized in **Table 1**.

TABLE 1: THE INHIBITION ZONE OF THE ETHYL ACETATE EXTRACTS OF ISOLATE (AP21C) ENDOPHYTIC FUNGI FROM *LANNEA COROMANDELICA* (HOUTT.) MERR

S. no.	Test Organism	Diameter of Inhibition Zone (mm)*		
		Positive Control (Chloramphenicol)	Negative Control (Ethyl Acetate)	Ethyl Acetate Extract of Isolate AP21C
1	*E. coli*	25.5 ± 0.2	-	-
2	*S. typhi*	33.0 ± 0.4	-	9.0 ± 0.2
3	*S. aureus*	22.5 ± 0.5	-	13.9 ± 0.3
4	*P. aeruginosa*	19.5 ± 0.3	-	-

*Values are means of three replicates ± standard deviation.

Determination of the MIC of ethyl acetate extract of isolate AP21C that showed inhibitory activity was assayed by dilution methods. The MIC values against *Staphylococcus aureus* ATCC 25923 and

Salmonella typhi ATCC 14028 is 1000 µg/ml. Hence, based on the MIC values, the ethyl acetate extract of isolate AP21C was categorized has weak antibacterial activity. Isolation and identification of chemical compounds in the ethyl acetate extract of isolate AP21C was conducted using column chromatography monitored by TLC. Compound (1) was obtained as a yellow powder. Identification by using GC-MS and analysis of the ^{1}H and ^{13}C NMR data compared with the literature suggested that compound (1) is citrinin. Citrinin was first isolated from *Penicillium citrinum* and was first recognized as a potential antibiotic but later found as nephrotoxin [16]. Compound (1) 1H NMR-δ (multiplicity, J in Hz):): 15.89 (1H,s), 15.10 (1H, s), 9.54 (H-1,1H, s), 4.76 (H-3, 1H, dq, j 0.7 and 6.7 Hz), 2.98 (H-4, 1H, q, 7.2 Hz), 2.0 (H-11, 3H, s), 1.32 (H-9, 3H, d, 6.7 Hz) and 1.20 (H-10, 3H, d, 7.2 Hz). 13C NMR-δ: 183.8, 177.3, 163.1, 163.1, 139.3, 123.1, 107.5, 100.3, 81.8, 34.6, 18.3, 18.6, 9.5 ppm.

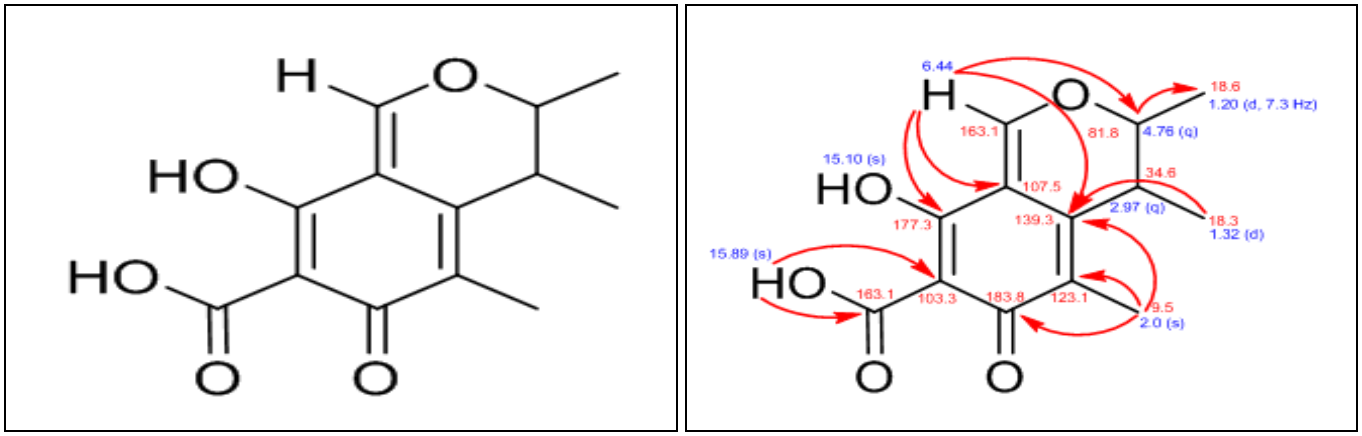

FIG. 1: STRUCTURE OF COMPOUND (1) AND ITS HMBC CORRELATIONS

The identification of endophytes fungi (AP21C) was determined as Penicillium sp. by the morphological characteristics of the colony and microscopic examinations [16]. The colonies of isolate are rapidly growing, flat, filamentous, and velvety.

The colors of the colonies are initially white and become blue-green, gray-green, olive-gray, while plate reverse is usually pale to yellowish. The microscopic examinations showed septate hyaline hyphae (1.5 to 5 µm in diameter), simple or branched conidiophores, metulae, phialides, and conidia. The conidia (2.5-5 µm in diameter) are round, unicellular, and visualized as unbranching chains at the tips of the phialides.

CONCLUSION: One bioactive compound known as citrinin had been isolated from the ethyl acetate extract of endophytic fungi from *Lannea coromandelica* (Houtt.) Merr. The antioxidant activity showed that the isolated compound has moderate activity with AAI value of 0.671 and IC_{50} 145.9 ppm while the antimicrobial activity demonstrated that the isolated compound was not active against the bacterial test.

ACKNOWLEDGEMENT: We gratefully acknowledge the financial support by the Research and Community Outreach (LP2M) UIN Syarif Hidayatullah Jakarta through "Conference Travel Grants 2016" and "Grant of Natural Science Development 2017".

CONFLICTS OF INTEREST: Authors have no conflict of interest regarding this article.

REFERENCES:

1. Lini IF, Afroz F, Begum N, Rony SR, Sharmin S, Moni F and Sohrab MH: Identification and bioactive potential of endophytic fungi from marine weeds available in the coastal area of Bangladesh. Int J Pharm Sci & Res 2020; 11(3): 1249-57.
2. Mane RS and Vedamurthy AB: Structure elucidation and therapeutic applications of endophytic fungi derived bioactive compounds obtained from *Ximenia americana* Western Ghats of Karnataka India. Int J Pharm Sci & Res 2020; 11(1): 212-25.
3. Bhardwaj A and Agrawal P: A Review Fungal Endophytes: As a Store House of Bioactive Compound. Word Journal of Pharmacy and Pharmaceutical Sciences 2014; 3: 228-237.
4. Deepthi VC, Sumathi S, Faisal M and Elyas KK: Isolation and identification of endophytic fungi with antimicrobial activities from the leaves of *Elaeocarpus sphaericus* (gaertn.) K. Schum. and *Myristica fragrans* houtt. Int J Pharm Sci & Res 2018; 9(7): 2783-91.

5. Kumala S, Yuliani DK and Simanjuntak P: Antimicrobial activity of secondary metabolites produced by endophytic fungi isolated from stems of Jati Tree (*Tectona grandis* L.F). Int J Pharm Sci Res 2015; 6(6): 2349-53.
6. Khare E, Mishra J and Arora NK: Multifaceted interactions between endophytes and plant developments and prospects. Front. Microbiol 2018; 9: 2732.
7. Amelia P, Nugroho AE and Hirasawa Y: Two new sarpagine-type indole alkaloids and antimalarial activity of 16-demethoxycarbonylvoacamine from *Tabernaemontana macrocarpa* Jack. J Nat Med 2019; 73(4): 820-25.
8. Rahman M, Khatun A, Uddin SJ and Shilpi J: Comparative effect of *Lannea coromandelica* (Houtt.) merr. leaves and stem barks on acetic acid induced pain model in mice and chromogenic reagents: exploring the analgesic potential and phytochemical groups. Pharmacologyonline 2016; 1: 146-52.
9. Deepika K, Mahesh Kumar MVS, Radhika TSD and Rajagopal SV: Studies on isolation, purification and structure elucidation of chemical constituents from methanolic flower extract of *Blepharis molluginifolia* pers. and their biological activities. Int J Pharm Sci Res 2016; 7(12): 4893-04.
10. Sultana C, Kundo NK, Islam S, Ahmed R, Afrin S, Saqueeb N and Wahed MII: Antioxidant, analgesic and antimicrobial activities of different fractions from methanolic extract of *Psidium guajava* L. leaves. Int J Pharm Sci & Res 2020; 11(6): 2733-38.
11. CLSI: Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition. CLSI document M02-A11. Wayne, PA: Clinical and Laboratory Standards Institute 2012.
12. Takao T, Kitatani F, Watanabe N, Yagi A and Sakata K: A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Biosci Biotechnol Bioche 1994; 58: 1780-3.
13. Amelia P, Elya B, Hanafi M: Antioxidant activity of xanthone from *Garcinia benthami* pierre leaves. Int.J. PharmTech Res 2014-2015; 7(2): 254-57.
14. Laldingngheta J and Vabeiryureilai LM: Determination of phytochemical contents and anti-oxidant activities of ethanol extract of the leaves of *Scurrula parasitica* L. Int J Pharm Sci & Res 2020; 11(5): 2211-16.
15. Mushimiyimana I, Kimonyo A and Nsabimana P: Colonial and morphological characteristics of various fungi species isolated from soil in Bangalore City. Bull Env Pharmacol Life Sci 2016; 6(1): 17-21.
16. Wang W, Liao Y, Zhang B, Gao M, Ke W, Li F, Shao Z: Citrinin monomer and dimer derivatives with antibacterial and cytotoxic activities isolated from the deep sea-derived fungus *Penicillium citrinum* NLG-S01-P1. Mar Drugs 2019; 17: 46.

How to cite this article:
Amelia P, Ivada PAK, Fitriana N, Komala I, Bahri S and Hanafi M: Antioxidant and antimicrobial activity of secondary metabolite produced by endophytic fungi isolated from *Lannea coromandelica* (Houtt.) Merr. Int J Pharm Sci & Res 2021; 12(3): 1588-92. doi: 10.13040/IJPSR.0975-8232.12(3).1588-92.