

Isolation of Antibacterial Compounds from *Garcinia Cf Cymosa*

M. Dwi Wiwik Ernawati*, Yusnelti and Afrida
Faculty of Education, Department of Chemistry, Jambi University
Jl. Raya Jambi-Muarabulian Km 15 Mendalo Darat, Jambi, 36361 Indonesia
* Corresponding Author : wwkchem2@yahoo.co.id

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Abstract

Garcinia cf cymosa as part the family of *Guttiferae* was found out containing some active components of antibacterial *i.e.* xantone, flavonoid, terpenoid and phenolic derivatives. This study was intended to investigate the principal components as antibacterial through bioassay guided isolation using *n*-hexane, dichloromethane, and ethyl acetate as solvent. Based on this study have been isolated three xanthone derivatives *i.e.* α -mangostin(1), β -mangostin(2) and (-)- epicatechin(3). Compound 1 showed strong activity at concentrations of 100 ppm against *Shigella dysenteriae* where comp.3 demonstrated significant antibacterial activities against *Shigella dysenteriae* and *Pseudomonas auregynosa* at the same dose.

Keywords: *Garcinia cf cymosa*, (-)-epicatechin, *Shigelladysenteriae*, *Pseudomonas auregynosa*

1. Introduction

Plants produce a whole series of different compounds which are not of particular significance for primary metabolism, but represent an adaptive ability of a plant to adverse abiotic and biotic environmental conditions. They can have a remarkable effect to other plants, microorganisms and animals from their immediate or wider environment. All these organic compounds are defined as biologically active substances, and generally represent secondary metabolites, given the fact that they occur as an intermediate or end products of secondary plant metabolism. These secondary metabolites, apart from determining unique plant traits, such as: colour and scent of flowers and fruit, characteristic flavour of spices, vegetables, they also complete the functioning of plant organism, showing both biological and pharmacological activity of a plant. Therefore, medicinal properties of plants can be attributed to secondary metabolites (Hartmann, 2008).

Recently, the screening of plant extracts has been of great interest to scientists for the discovery of new compound effective in the treatment of bacterial infection. One of the important medicinal plant is *Garcinia cf cymosa*. It is the higher plants with a height of 30 meters including the family of *Guttiferae*, where widely distributed in Indonesia especially in Sumatera dan Kalimantan island, local name as "Manggis" (Heyne, K. 1987). This plant was reported potential as a source of bioactive chemical compounds which contained such as xanthenes, flavonoids, steroids, terpenoids and other phenolic derivatives (Josep, et al, 2005). These secondary metabolite were reported having diverse biological activities such as cytotoxic, anti-microbial, anti-oxidant, anti-inflammatory, anti-cancer, and anti-HIV(Lannang, et al, 2005).

Based on the previous research inspired us to investigate the principal components as antibacterial through bioassay guided isolation using *n*-hexane, dichloromethane, and ethyl acetate as solvent. As first step of this study was conducted screening the antibacterial potency from the bark of *Garcinia cf cymosa* indicated that dichloromethane and ethylacetate extract demonstrated strong antibacterial activities at the concentration of 500 ppm.

2. Experimental

2.1 General

The ¹H-NMR spectra was measured with a JEOL JNM-ECS 400, (DEPT 90°, 135°, HMBC, HMQC dan COSY). (¹H: 400 MHz, using Me₄Si (0 ppm) as internal reference. Chemical Shifts were reported in parts per million (ppm). FAB mass spectra was recorded on a JEOL SX-102. All solvents were distilled. Column chromatography was conducted using silica gel (E. Merck products). Thin-layer chromatography (TLC) analysis was performed on precoated Kieselgel 60F254 plates (0.25 mm, Merck). The spots were monitored under UV light (254 or 365 nm) and visualized by spraying agents cerium sulphate. Culture of *Staphylococcus aureus*, *Shigella dysenteriae*, *Pseudomonas auregynosa* and *Escherichia coli* were cultivated in a incubator (Hirayama) at 37°C with a gas-controlled environment of 5% CO₂. Detection of the bacteria were determined by light microscopy (Nikon Eclipse). Tetracycline was utilized as positive control for antibacterial assay.

2.2. Plant material

The bark part of *Garcinia cf cymosa* was collected from *Botanical Garden of Bukit Sari*, Tebo Regency Jambi Province in 2012. Identification and classification of the plant material was performed at the Biology Department, University of Andalas. The voucher specimens of the plants are deposited in the Herbarium of the Faculty of Mathematics and Science, University of Andalas. The collected plant materials were air-dried under

shade at room temperature and then ground into dried powder.

2.3 Isolation and Purification of antibacterial compounds

Dried, ground plant material (5 Kg) was extracted three times by direct maceration with methanol at rt for 6 h and subsequently three times under reflux for 4 h gave 652 g extract. The MeOH extract (250 g) was fractionated by vacuum liquid chromatography on silica gel using combination of *n*-hexane, dichloromethane, ethyl acetate and methanol with increasing polarity as eluent to give 5 fractions. All of collected fractions were examined the anti-bacterial activity so that the most promising fractions (fr. 6 to fr. 10) in which have large obtained and exhibited strong antibacterial activity at a concentration of 500 ppm. Briefly, 21.4 g of dichloromethane fraction was subjected to silica gel (SiO₂) column chromatography with mobile phase (*n*-hexane:AcOEt) = 3 : 1 afforded three major component *i.e.* α -mangostine(1), β -mangostin(2) and epicatechine(3). The structure of isolated compounds 1-3 were elucidated by spectroscopy techniques; UV, IR, NMR-1D (¹H-NMR, ¹³C-NMR), and NMR-2D (HMBC and HMQC) then compared the date with published data led to determine the structure.

2.4. Antibacterial Assay

The following bacteria were used: *Staphylococcus aureus* ATCC, *Shigella dysenteriae* ATCC and *Escherichia coli* ATCC. Bacteria are stored in the Laboratory of Bioprocess Research Center for Biology -LIPI Cibinong, West Java. Bacterial suspension were prepared from overnight cultures by the direct colony method(Haine et al, 2008). Basically a thin agar plate is poured which has the bacteria *Staphylococcus aureus*, *Shigella dysenteriae*, *Pseudomonas auregynosa* and *Escherichia coli*. Holes are punched in the agar and a sample of (0.5 – 1 μ l) is placed in each of these wells. The sample will diffuse through the agar and will become more dilute the further from the well it is. At a certain distance from the well the antibacterial activity of the sample will be at the minimum concentration to inhibit the growth of *Staphylococcus aureus*, *Shigella dysenteriae*, *Pseudomonas auregynosa* and *Escherichia coli*. After a few nights of incubation could be seen where this minimum concentration has been reached, because there will a clear circle around the well. Beyond this clear zone the agar is cloudy because the bacteria have grown. The higher the concentration of antibacterial substances in the sample, the larger will be the clear zone around the well.

3. Results and Discussion

Antibacterial activity of all the five crude plant extracts were carried out against four bacterial strains. Our results showed that the most promising fractions (fr. 6 to fr. 10) in which have large obtained and effective against all the bacterial strains tested at a concentration of 500 ppm. Therefore, in order to isolate the major components which represent the antibacterial efficacy. It was purified by chromatography technique and eluted by using *n*-hexane:AcOEt (3 : 1) led to isolation of three compounds. Purified active subfractions were elucidated the structure on the basis of spectroscopy data(NMR, IR, UV and MS spectrum) then compared with spectroscopy data of known compounds previously isolated from the same genus to show that two compounds as xanthone derivatives *i.e.* α -mangostine(1) and β -mangostin(2) then a flavan-3 ol known as epicatechine(3). Structure of isolated compounds were seen in the **Figure 1**.

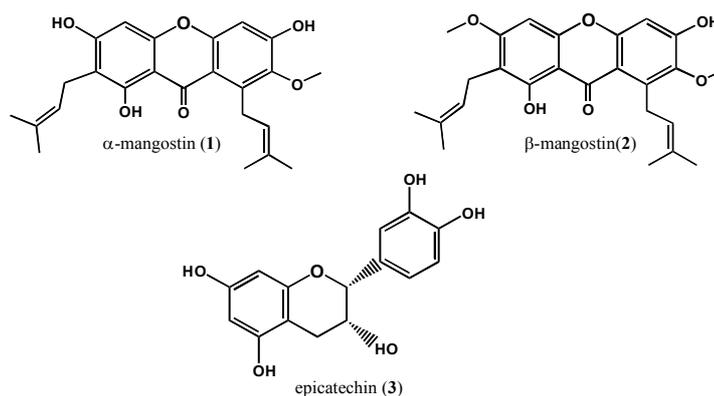


Figure 1. Structure of Isolated compounds from *Garcinia cf cymosa*

The results of antibacterial activity of compound 1-3 from *Garcinia cf cymosa* are presented on the **Table 1**. Isolated compounds showed different activity. Compound 1 showed strong activity at concentrations of 100 ppm against *Shigella dysenteriae*(2) and at the same dose, compound 3 acted against *Pseudomonas auregynosa*(4) while Compound 1 showed moderate activity against *Staphylococcus aureus*(1), and comp.2 exhibited very low activity against *Staphylococcus aureus*(1) and *Escherichia coli* (3). Antibacterial activity of comp.3 is more pronounced than comp.1 and 2. Similar results were obtained by Masika et al, 2004, among tested isolated

compounds, comp.2 was one of the low antibacterial activity. It may caused by mehtylation at C-7 position could reduce the antibacterial potency.

Table 1: The Antibacterial Activities of Comp.1-3 against *Staphylococcus aureus*(1), *Shigella dysentriae*(2), *Escherichia coli* (3) and *Pseudomonas auregynosa*(4)

Run	Conc. (ppm)	Inhibition Zone (cm)												
		1			2			3			Positive control (Tetracycline)			
		(1)	(2)	(3)	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)		
1	100	0.16	0.02	0.35	0.47	0.02	0.47	0.60	0.61	0.84	0.63			
2	200	0.16	0.06	0.43	0.47	0.06	0.47	0.61	0.62	0.96	0.64			
3	300	0.18	0.06	0.55	0.47	0.11	0.50	0.65	0.68	1.10	0.72			
4	400	0.20	0.07	1.00	0.57	0.11	0.54	0.74	0.71	1.17	0.78			
5	500	0.23	0.08	1.05	0.82	0.11	0.58	0.82	0.78	1.24	0.85			
6	750	0.23	0.15	1.33	0.87	0.26	0.72	0.85	0.82	1.47	0.99			
7	1000	0.25	0.17	1.34	0.87	0.26	0.72	0.87	0.88	1.58	1.03			
8	2500	0.26	0.23	1.44	0.87	0.32	0.80	0.87	0.98	1.68	1.34			
9	5000	0.26	0.32	1.44	0.88	0.32	0.90	1.00	1.03	2.33	1.61			
10	10000	0.26	0.44	1.56	1.05	0.32	1.12	1.13	1.72	2.60	1.84			

Note : Comp. 1 : α -mangostine, Comp.2 : β -mangostine, Comp. 3 : epicatechin

4. Conclusion

The Bioassay-guided isolation was used to separate the antibacterial compounds from the MeOH plant extract. Three antibacterial compounds were successfully isolated from MeOH extract of the *Garcinia cf cymosa*. The isolated epicatechin (3) demonstrated significant antibacterial activities against *Shigella dysentriae*(2) and *Pseudomonas auregynosa*(4). TLC analysis confirmed that these isolated compounds are predominant components in whole plant MeOH extract, indicating their significant contribution to the overall antibacterial activity. Furthermore some related publication reported their potential use in the treatment of bacterial diseases.

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