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Original Article

TOTAL FLAVONOID CONTENT AND ANTIMICROBIAL PROPERTIES OF FOUR SPECIES OF ZINGIBERACEAE

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ABSRACT

Objectives: The objectives of this research were to study flavonoid content and antimicrobial activity of four species of Zingiberaceae plants that were collected randomly in West Java (Indonesia).

Methods: Preparation of extract were perfomed by reflux using ethanol as a solvent. The extracts were vaporated using rotavapor. Total flavonoid content was measured using modified method adapted from Chang. For antimicrobial activity microdilution broth method was used.

Results: Antimicrobial activity of four Zingiberaceae plants showed various potency. *Hornstedtia pininga*, *Amomum hochreutineri* and *Hedychium roxburghii* showed strong activity against *Bacilus subtilis* (MIC at 500 µg/mL). The strongest activity against *E. coli* were showed by *H. pininga* and *H. roxburghii* the MIC were 31.25 µg/mL. Whereas, the strongest antimicrobial activity against *P. aeruginosa* was *H. roxburghii* (MIC was 62.5 µg/mL). In line with total flavonoid content results, the highest yield was obtained by *H. roxburghii* rhizome extract (0.162%), followed by *A. hochreutineri* (0.76%), *Nicolaia hemisphaerica* (0.071%) and *H. pininga* (0.04%).

Conclusion: In the present study, ethanolic extract of four Zingiberaceae showed an antimicrobial activity with various potency. *Hornstedtia pininga*, *Amomum hochreutineri* and *Hedychium roxburghii* showed strong activity against *Bacilus subtilis* (MIC at 500 µg/mL).

Keywords: Zingiberaceae, Flavonoid, Antimicrobial, Hedychium, Hornstedtia.

INTRODUCTION

Zingiberaceae is one of the common plants in Indonesia which used as traditional medicine, whereas subfamily Zingiberoidae distributed in Indo-Malaysia [1]. The essential oil of Zingiberaceae have been studied as an antimicrobial, larvisidal, and repelan [2], [3]. In addition, previous studies have been conducted, and reported that sesquiterpenoid diarylheptanoid were compounds commonly found in Zingiberaceae plants [4], [5], [6]. The Zingiberaceae known as a source of flavonoids and often contain tannins. Matsuda et al (2002) isolated the flavonoid compound from *Hedychium coronarium* [7] and the rhizome of *H. spicatum* [8]. Previous research assumed that were correlation between antimicrobial activity and flavonoid content. The antimicrobial activity of the alcoholic and aqueous extract of ginger (Zingiberaceae) with the presence of (6)-gingerol that argue as active composition, were showed optimum activity against *Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* [9] The objective of this research were to study antimicrobial properties and flavonoid content of four Zingiberaceae plants which obtained randomly at some place on West Java – Indonesia. The species were *Nicolaia hemisphaerica* (Blume) Horan*, Hornstedtia pininga* (Blume) Valet*., Amomum hochreutineri* Val. and *Hedychium roxburghii* Blume.

MATERIALS AND METHODS

Chemicals

Muller Hinton Broth (MHB) (Flucka ®), Muler Hinton Agar (MHA) (Sigma-Aldrich®), quercetine (Sigma-Aldrich®), tetracycline, nystatine, methanol, n-hexane, ethyl acetate, ethanol 95%, chloroform, aquadest, toluene, hydrochloride acid, sulfuric acid, magnesium powder, amyl alcohol, Fe(III)chloride, sodium sulfat anhydride, sodium acetate, gelatine, sodium hydroxide, kalium iodide, alumunium chloride. All other reagents were analytical grade.

Rhizome samples

The rhizomes of *Nicolaia hemisphaerica* (Blume) Horan, *Hornstedtia pininga* (Blume) Valet., *Amomum hochreutineri* Val. and *Hedychium roxburghii* Blume were collected from some of area in West Java – Indonesia. The rhizomes were thoroughly washed with water, wet sortation, sliced, and grinded into powder.

Extract preparation

Extraction was conducted by reflux method using ethanol as a solvent then evaporated with rotavapor (Buchi ®).

Determination of total flavonoid content

Total flavonoid content was measured using modified method adapted from Chang [10]. Preparation of standard solution: 1000 µg/mL of quercetin (in methanol) diluted to 20, 40, 60, 80, 100, 120, dan 140 µg/mL. Crude drugs (0.8 g) were extracted by maceration method with 10 mL ethanol 24 hours. Extract was vaporated then diluted volumetric with 5 mL of methanol. Extract or standar solution (0.5 mL), 1,5 mL of methanol, 0.1 mL of AlCl3 10% (in aquadest), 0.1 mL of sodium acetate 1 M and 2.8 mL aquadest were incubated at room temperature in 30 minutes. The absorbance was fixed at λ 415 nm. The total flavonoid content was measured as percentage of total g quercetin equivalens per 100 g extract (g QE/100 g).

Microorgamism

The microorganism were Staphylococcus aureus ATCC 6538, Bacillus subtilis ATCC 6633, Pseudomonas aeruginosa ATCC 9027, Escherichia coli ATCC 8939, Candida albicans ATCC 10231 were used for determined antimicrobial activity. Each microorganism stock cultures were incubated overnight at 37oC on Nutrient Agar for bacteria and 25oC on Saburoud Dextrose Agar for fungal.

Preparation of inoculum

A loopful of inoculum inoculated into 10 mL Muller Hinton Broth. The broth suspension was incubated overnight at 37oC for bacteria and 25oC for fungal.

Antimicrobial activity test

Antimicrobial activity was measured using microbroth dilution method adapted from CLSI [11]. The suspension for inoculation was

prepared by diluted the broth culture with medium solution to obtained 0.5 McFarland (5 x 105 CFU/mL) suspension then diluted 1:20 with medium. When 0.01 mL of this suspension was inoculated into 96 well microtiter plate. The final concentration of extracts were 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, 15.6 µg/mL, 7.8 µg/mL. Tetracycline, ciprofloxacine, nystatine and ketoconazole were used as positive controls.

RESULTS

The total flavonoid content of four Zingiberaceae plants were measured by extrapolation to the standard curve calibration of quercetin. The equation of standard curve was y = 0,007x + 0,021; R^2= 0,995. The results showed different flavonoid content of each species in range of 0.020-0.162 % (w/w extract). The highest yield was shown by *Hedychium roxburghii* rhizome extract (0.162%), followed by *Amomum hochreutineri* (0.76%), *Nicolaia hemisphaerica* (0.071%) and *Hornstedtia pininga* (0.04%).

Table 1: Total flavonoid content of four Zingiberaceae plants

Species	Total Flavonoid (%)
N. hemisphaerica	0.071 ± 0.006
H. pininga	0.040 ± 0.003
A. hochreutineri	0,076 ± 0,007
H. roxburghii	0.162 ± 0.004

The antimicrobial activity of four Zingiberaceae plants showed various minimum inhibitory concentration and broad activity against test-microorganism. The results shown on the Table 2.

Table 2: Minimum Inhibitory Concentration of Four Zingiberaceae plants

Species	Minimum Inhibitory Concentration [MIC] (µg/mL)				
	B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***	***C. albicans***
N. hemisphaerica	1000	>1000	250	500	>1000
H. pininga	500	>1000	31.25	125	>1000
A. hochreutineri	500	>1000	125	125	>1000
H. roxburghii	500	>1000	31.25	62.5	>1000
Tetracycline	0.2	0.2	12.5	>25	-
Nystatine	-	-	-	-	0.78

DISCUSSION

Flavonoids are the secondary metabolite which increasingly becoming the subject of medical research. They have been showed many pharmacologycal properties, including antioxidant activity, antiallergic activity [12], anti-inflammatory activity, oestrogenic activity, antimicrobial activity [13],[14], vascular activity and cytotoxic activity [15]. Many folk medicine was reported containing flavonoids as the principal physiologically active constituent. For example, the plant *Tagetes minuta* was used traditionally to treat infectious diseases in Argentine, it was known contain quercetagentin-7-arabinosyl-galactose [16].

Actually, when reports of the antibacterial activity of flavonoids were compared, the results appeared widely conflicting. Recently, a set of guidelines was published for standard broth microdilution methods [16]. Among all the tested rhizome of four zingiberaceae, *H. roxburghii* showed the highest content of total flavonoid (0,162 ± 0,004%) followed by *A. hochreutineri* (0.76%), *N. hemisphaerica* (0.071%) and *H. pininga* (0.04%). The flavonoid compound of those plants have not identified yet, but other plants with the same genera showed the flavonoid presence. The flavonoid compound wich were known are quercitrin, quercitroside and isoquercitroside and they were identified *Amomum villosum* Lour. from the rhizomes [17], alpinetin from the seed of *A. subulatum* Roxb. [18]. The flavonoid compound which was characterized as 5-hydroxy-3,7,4'-trimethoxyflavone was isolated from *H. coronarium* (7), whereas the flavonoids from the rhizome of *H. spicatum* were identified as chrysin and teptochrysin (8).

In this study, antimicrobial test results of four Zingiberaceae by broth microdilution method showed in line correlation with total flavonoid content whereas *H. roxburghii* Bl. showed a broad antibacterial spectrum and the strongest activity especially against *P. aeruginosa* (MIC 62.5 µg/mL).

CONCLUSION

In the present study, ethanolic extract of four Zingiberaceae showed an antimicrobial activity with various potency. *Hornstedtia pininga*, *Amomum hochreutineri* and *Hedychium roxburghii* showed strong activity against *Bacilus subtilis* (MIC at 500 µg/mL). The strongest activity against *E. coli* were showed by *H. pininga* and *H. roxburghii* at MIC 31.25 µg/mL. Whereas, the strongest antimicrobial activity against *P. aeruginosa* was *H. roxburghii* (MIC 62.5 µg/mL). In line with total flavonoid content, the highest yield was obtained by *H. roxburghii* rhizome extract (0.162%), followed by *A. hochreutineri* (0.76%), *Nicolaia hemisphaerica* (0.071%) and *H. pininga* (0.04%).

CONFLICT OF INTERESTS

Declared None

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