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Phythochemical Screening and Antimicrobial Activity of Sub Fractions Asam Kandis (*Garcinia diocia* Blume)

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ABSTRACT

Fruit flesh of asam kandis *Garcinia diocia* Blume has been used people in Kalimantan Barat to preserve fresh fish. One of compounds which is probably responsible as a preservative agent is antimicrobial compounds. In this research, flesh fruit of asam kandis was extracted using methanol as a solvent. Furthermore, the crude extract was partitioned using various solvent such as n–hexane and ethyl acetate. On the basis of antimicrobial activity, methanolic and ethyl acetate fractions can inhibit growth of tested microorganisms. The best antimicrobial fraction was showed by ethyl acetate fraction due to inhibit all of tested microorganism but sub fraction E4 can only inhibit *Candida albicans* which are possibly responsible for antimicrobial activity were categorized as flavonoid.

INTRODUCTION

Garcinia is one of genus from Clusiaceae family which its spread in Asia, Africa, New Caledonia and Polynesia(Merza *et al*., 2004). Based on review of literature and observation of herbarium specimen, Indonesia has 64 species of *Garcinia* (*Garcinia* spp.) and 25 species of *Garcinia* found in Kalimantan Island (Uji, 2007). Extract ethyl acetate, acetone, and methanolic from seed of *Garcinia cola* exhibit antibacterial against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Plesiomonas shigelloides* and *Salmonella typhimurium* (Seanego and Ndip, 2012). Extract of feel, leaves, and bark *Garcinia mangostana* L. showing strong antibacterial against *Listeria monocytogenes* and *S.aureus* (Palakawong *et al*., 2010). Asam kandis (*Garcinia diocia* Blume) has used Dayak and Malay communities in the District of West Kalimantan province for agent preservation. Dry powder pulp of fruit flesh asam kandis combined with salt can preserve fresh fish. This treatment will be extend the shelf life of fish at room temperature for several days.

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Preservative agents have various mechanisms to preserve foodstuffs. One of mechanisms is antimicrobial activity. The first step to find out mechanism of asam kandis fruis as preservative agent, we evaluated antimicrobial activity from its extract, fraction and sub fraction. In addition, compound class of the extract and the sub fraction is evaluated by phytochemical screening with various specific reagents.

MATERIALS AND METHODS

Sampling of asam kandis fruit

Asam kandis fruit was collected from Mengkiang Village, Kecamatan Sanggau Kapuas Provinsi Kalimantan Barat. The sample was determined at Herbarium Bogoriense, Research Center for Biology Indonesian Institute Science, Indonesia**.**

Preparation and extraction of sample

Fruit flesh of asam kandis was sliced and dried gently. The dried sample was ground to be powder with size 60 mesh. The powder (2.5 kg) was macerated at room temperature using methanol as solvent. The maceration was carried out many times until colorless solvent. Furthermore, it was filtered using paper filter, Whatman No 1. The filtrate was concentrated using a vacuum rotary evaporator to get crude extract.

Partition of the crude extract

The crude extract was partitioned by various solvent, n-hexane and ethyl acetate which is different the polarity. The fractions were concentrated using a rotary evaporator. All of the fractions were performed antimicrobial assay.

Partial purification of the crude extracts

The fraction showing antimicrobial activity was purify continuously using various chromatography technique such as vacuum column chromatography (Si gel Merck 60, 70-230mesh) and gravity column chromatography (Si gel Merck 60, 70-230mesh).

Phytochemical test

The extract was put on a white plate and added some drops of various reagents, which is Dragendorff, Mayer, Wagner, Mg-HCl and $FeCl_3$. The color which is formed was recorded and used to predict class-compounds in the extract/fraction.

Antimicrobial assay of the extract

Antimicrobial activity of bacteria extract was determined based on modified well-difusion agar methods (Valgas *et al*., 2007). Well with 6 diameter mm was made using a punch in plate containing nutrient agar medium then its medium was spreaded with inoculum of bacterial test. Furthermore, the well was filled with 200 µg/well of the bacterial extract and incubated at 37 ^oC for 24 h. The extract having antimicrobial activity was signed with formation of inhibition zone around isolate. Diameter of inhibition zone was measured from the edge of the colony to the edge of the clear zone and recorded.

RESULTS AND DISCUSSION

Antimicrobial activity of crude extract and 3 fractions from flesh fruit of asam kandis

In this study, the crude extract of asam kandis fruit can inhibit 11 of 12 microorganism tests (Table 1). The antimicrobial activity was categorized as broad spectrum antimicrobial activity. On the basis of the result, preservative activity of asam kandis fruit is probably caused by antimicrobial activity.

When it was partitioned, the fraction showing antimicrobial activity is ethyl acetate fraction and methanol dissolved fraction. The crude extract and methanolic and ethyl acetate fractions showed similarity in inhibition of the number and species of tested microorganisms inhibited even though it differed in average diameter of clear zone.

Fractionation of crude extract by n-hexane and ethyl acetate increased antimicrobial activity of ethyl acetate fraction especially on C*itrobacter freundii*. This suggest that the fractionation removed others chemical compound which have antagonism of various compounds on each other. The antagonistic interactions are showed that the combine activity of two or more chemical compounds is lower than their individual activities (Nelson and Kursar,1999).

The active compound of antimicrobial from flesh fruit of asam kandis might be semipolar compound which dissolved in ethyl acetate solvent. On the basis phytochemical screening and antimicrobial activity, ethyl acetate fraction will be used to further purification.

Generally, the larger the inhibition zone that forms the greater the antimicrobial activity. However, polarity of compounds shows an important role in this case. The polar compound will diffuse easily into medium so that the clear zone is bigger. Non polar antimicrobial compounds are difficult to spread into medium so that it will give lower clear zone.

Phytochemical Screening.

The phytochemical screening showed each fraction containing various class of compound. However, the ethyl acetate fraction exhibited interested compound class namely: alkaloid, flavonoid, phenolic, and saponin (Table 2). Phytochemical screening exhibits different distribution of compound groups in each fraction (Table 2).

Table. 1: Antimicrobial activity of various fractions of asam kandis (500 ug/well)

Microorganism Test	Gram	Average diameter of clear zones (mm)			
		The crude extract	*n*-Heksana fraction	Ethyl acetate fraction	Methanolic fraction
B. subtilis	positive	13.60	-	13.86	10.34
S. aureus	positive	9.40	-	8.93	8.08
B. cereus	positive	11.26	-	12.26	9.65
L. monositogenes	positive	9.57	-	11.40	9.10
Bacillus sp.	positive	13.25	-	13.40	10.40
Enterobacter sp.	negative	9.79	-	9.58	8.98
K. pneumoniae	negative	13.37	-	12.14	9.58
Salmonella sp.	negative	11.42	-	10.20	12.89
E. coli	negative	8.76	-	9.63	9.63
V. cholerae	negative	10.48	-	13.90	16.01
C. freundii	negative	-	-	9.92	-
C. albicans	Fungi	11.15	-	12.02	10.39

- = no clear zone

Table. 2: Phytochemical screening of various asam kandis fractions.

Class of compound	Methanolic fraction	Ethyl acetate fraction	n-hexane fraction
Alkaloid	-	+	-
Flavonoid	-	+	-
Phenolic	+++	++	-
Saponin	-	+	-

Note. (+) present; (-) absent

Phenolic group seems to distribute into 2 fractions namely n-methanolic and ethyl acetate which are responsible for antimicrobial activity. It proved that n-hexane fraction do not contain phenolic group so that it is not active against tested microorganisms. The presence of phenolic group in plants is to protect them from microbial, insect, and herbivores damage (Cowan, 1999).

Antimicrobial activity of sub fractions fractionated by vacuum column chromatography and gravity column chromatography

Ethyl acetate fraction was separated by vacuum column chromatography so that was produced 6 sub fractions (Table 3). Sub fraction E and F are active toward *Escherichia coli*, *Bacillus subtilis* and *Candida albicans*. The best antimicrobial activity between sub fraction E and F is E if it compared to diameter of clear zone.

Table. 3: Antimicrobial activity of sub fractions resulted by vacuum column chromatography (300 ug/well).

Sub Fraction	Average Diameter of Clear Zone (mm) for Each Tested Microorganisms		
	E. coli	***B. subtilis***	***C. albicans***
A	-	-	-
B	-	-	-
C	-	-	-
D	-	-	-
E	10.28	10.51	3.96
F	8.58	7.20	1.98

- = no clear zone

Table. 4: Antimicrobial activity of sub fractions resulted by gravity column chromatography (20 ug/well)

Tested microorganism	Average Diameter of Clear Zone (mm)					
	E_1	**E_2**	**E_3**	**E_4***	**E_5**	**E_6**
E. coli	-	-	-	-	-	-
B. subtilis	-	-	-	-	-	-
C. albicans	-	-	-	2.54	-	-

- = no clear zone.

Sub fraction E re-fractionated by gravity column chromatography was obtained E_1, E_2, E3, E_4, E_5, and E_6 (Table 4). E_4 only inhibits growth *C. albicans*. On the basis of this result, the crude extract and fraction contain more than one antimicrobial substance, which each the substance has responsible at certain tested microorganisms. After the second fractionation, the fraction contains fewer the number of substances but the concentration each substances is increase.

Therefore, lower the concentration E_4 put on the well still active against *C. albicans* which is categorized as narrow spectrum. The substances contained E4 are possibly categorized as flavonoid.

CONCLUSION

Asam kandis fruit is potential as preservative agent. One of mechanism to preserve food due to antimicrobial activity. Antimicrobial activity of the extract and fraction is categorized as a broad spectrum but sub fraction E_4 can only active against *C. albicans* and categorized as narrow spectrum. The compounds which are responsible for antimicrobial activity were categorized as flavonoid.

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