

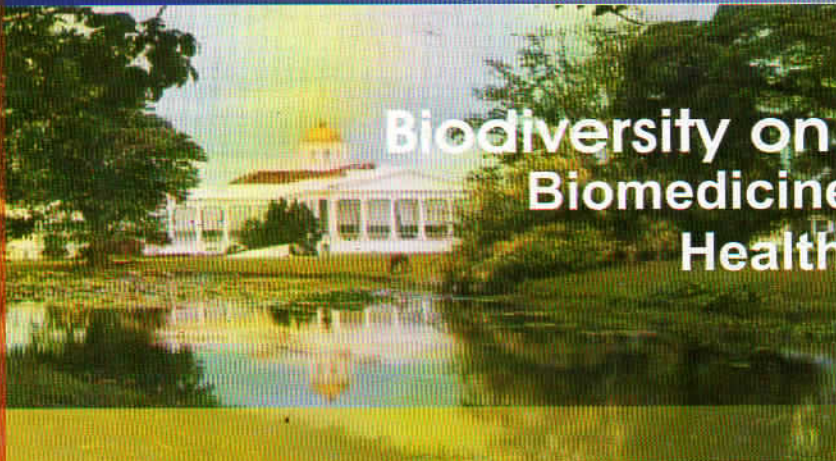
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PROCEEDINGS OF
INTERNATIONAL SYMPOSIUM
on BIOMEDICINES

18th and 19th September 2003

Theme :

**Biodiversity on Traditional
Biomedicines for Human
Health and Welfare**



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**Biopharmaca Research Center
Bogor Agricultural University
Bogor - Indonesia**

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INTERNATIONAL SYMPOSIUM ON BIOMEDICINES

Bogor, 18th – 19th September 2003

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**Biodiversity on Traditional Biomedicines for Human
Health and Welfare**

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Prospect of Seeds of Kolowe (*Chydenanthus excelsus*) as Source of Saponins Bactericide

Laode Rijai'), *Ratu Safitri*'), and *Henny Hilmi*)'

Chemistry Department, Mulawarman University, Samarinda

Biology Department, Padjadjaran University, Bandung

Biology Department, Padjadjaran University, Bandung

Abstracts

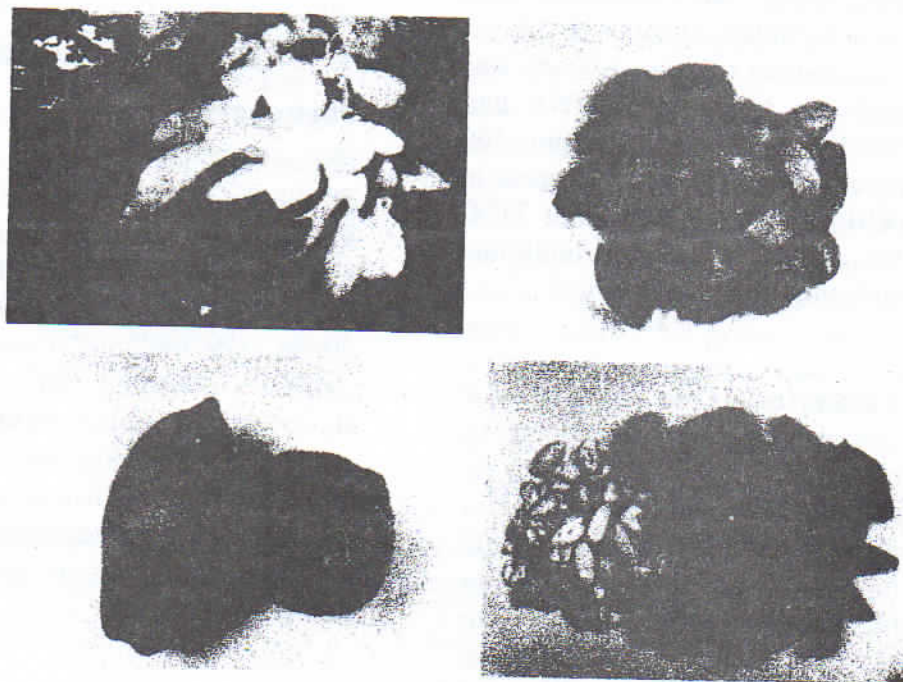
Traditionally, seeds of kolowe used to poison of fishes. In Kamaru, Buton island, Indonesia their seeds are used as captor tool for fishing. This purpose is supported by geographical site of the area that is in coastal area. For phytochemically seeds of kolowe contain saponins that present in methanol, n-butanol, and ethylacetate fractions. Third of the extracts are active against plant bacteria pathogen. The antibacterial is presumably from saponins since saponins are known to have antibacterial activity. So seeds of kolowe prospect as source antibacterial saponins.

Introduction

Kolowe is a scarce plants the plants are grown in Kamaru, Buton island, Indonesia and Andaman island, Birma (Harriman, 1999). The plants belong to *Chydenanthus* genera and there are two only species that is *Chydenanthus excelsus* and *Chydenanthus dentate-serrtatus*. Kolowe continuously bears many fruits. Traditionally the seeds are used to poison fish while the bark used to poison wild pig. Peoples of Kamaru, has used seeds of kolowe to poison fish. Therefore, the kolowe has been

everlasting by community in Kamaru. Phytochemically, seeds of kolowe contain saponins that are in methanol, n-butanol, and ethylacetate fraction. There were some evidence as antibacterial for saponins isolated from roots of *Bongardia chrysogonum* (Rahman dkk., 2000); and leaves of *Trevesia pulmata* (De Tomasi dkk., 2000). So poison properties of kolowe seeds have prospect as antibacterial. This research examined antibacterial effect of kolowe seeds against some plants bacterial pathogen. Antibacterial substances is presumably from saponins.

Figure 1. Fruits and Seeds of Kolowe (*C. excelsus*)



Research Method

Plant Materials. Plant materials were collected from Kamaru, Buton island, Indonesia. Identification of plant was done in Herbarium Bogoriense laboratory, Balitbang Botani Puslitbang Biologi-LIPI, Bogor on 2001.

Extraction and Partition. Fresh seeds of kolowe (500 g) were crash in methanol then maserated for 5 x 500 ml with methanol. Evaporation was done with low pressure to obtaine 285 g of methanol extracts. 100 g of the extract was suspended with water then partitioned with petroleum eter, ethylacetate, n-butanol successively. Result of partition was obtained from

20 g of ethylacetate and 55 g of n-butanol fractions.

Phytochemistry. Saponins test of methanol, n-butanol, and ethylacetate extract was done based on Froth and Liebermann-Burchard method.

Bacterial Target. The tested bacteria were *Ralstonia solanacearum* Y., *Xanthomonas campestris* pv *vesicatoria* D., *Erwinia cartovora*, and *Clavibacter nichigenense*. All of these are plant bacterial pathogens.

- **Minimum Inhibition Concentration (MIC) Test.** The media consist of a mixture of Nutrien Agar (NA) and

physiological NaCl sterilized at 121°C for 15 minute. The bacteria were made into suspension with their turbidity equal 9×10^9 *E. coli*. Extract of kolowe seeds was add into liquid media (NA and NaCl) up to 1 ppm; 10 ppm; 100 ppm; 1 %; 2 % concentration in petri dish then incubated at 33°C for 24 hour. Inhibition concentration was observed.

- **Antibacteria Test.** Preparation of extracts solution was done in methanol at 3 %; 2.5 %; 1.5 %; and 1 % along with NA media in water then sterilized at 121°C for 15 minute respectively. Strain of bacteria were turned into suspension using physiological NaCl up to muddy. Bacterial suspension 0.1 ml was put into petri dish followed by add are NA come closer together. A hole up to 8 mm were made on to the media mixed bacteria then 0.1 ml extract of various concentrations were put in the hole succesively then incubated at 33°C for 24

hour. Diameter inhibition area were noted.

Result and Discussion

Phytochemistry

Phytochemically, their major compounds of seeds of kolowe were saponins based on Froth and Liebermann-Burchard methods that is in methanol, n-butanol, and ethylacetate fractions. Third of these extracts consisted of alkaloids, flavanoids, polyphenols, and tannins. So saponins are the major compounds on kolowe seeds. Result of analysis of total saponins are 42.8 %. This indicates its prospect as source of saponins.

Antibacterial

- **Minimum Inhibition Concentration Test (MIC).** The aim of the test was to determine the minimum concentration of extracts which still inhibit growth of the test bacteria. This test also can describe the sensitivity and resistant of bacteria.

Table 1. Result of MIC antibacterial test using extracts of kolowe seeds.

Specieses	Concentrations (%)		
	Methanol extracts	n-butanol extracts	Ethylacetate extracts
<i>C. michigenense.</i>	1	>2	1
<i>R. soanacearum</i> Y.,	2	>2	2
<i>X. capmestris</i> pv <i>vesicatoria</i> D.,	2	>2	2
<i>E. cartovora,</i>	>2	>2	>2

Species of *C. michigenense* very sensitive to methanol and n-butanol extracts and *E. cartovora* resistant to all extracts.

- **Antibacterial Activity.** Result indicated a significance difference in antibacterial activity of the third extracts. There are three factors in these experiment that are A (bacteria species), B (extracts), and C (various concentration). Effect of these factors based on varian analysis is able to know response between of factors. Difference response of among factors was analysed using Duncan double distance analysis (Tabel 2).

All of the bacterial test have different sensitivity *C. michigenense* was very sensitive and *E. cartovora* resistant. *C. michigenense* was sensitive since this gram positive bacteria posses one layer cell wall composed peptidoglican that susceptible to antimicrobial substances (Agrous, 1977 and Tortora, 1986). *R. soanacearum* Y., *X. camestris py vesicatoria* D., and *E. cartovora* are gram negative that posses three layers cell wall which may cause resistantancy (Agrios, 1997 and Pelzcar, 1988).

Table 2. Result of Duncan Double Distance Analysis (D3A) with diameter inhibition of growth bacteria as parameter

Species	Diameter inhibiting growth bacteria by extracts of kolowe seeds
<i>C. michigenense</i>	10,09
<i>R. soanacearum</i> Y.,	9,49
<i>X. capmestris py vesicatoria</i> D.,	9,33
<i>E. cartovora</i>	8,27
Standard (methanol)	8

Table 3. Average inhibition of extracts of kolowe seeds on growth of bacteria

Extracts	Diameter inhibition activity of extracts kolowe seeds on growth of bacterias (mm)
Methanol	10,07
n-butanol	9,52
Ethylacetate	8,30
Standard (methanol)	8

Table 4. Result of D3A on the correlation between extract concentrations and bacteria growth inhibition

Concentration in %	Diameter Inhibition of bacterial growth (mm)
5	10,19
4,5	9,44
4	9
3,5	8,55
3	8,04
0	8

Antibacterial compounds in seeds of kolowe is synergic which methanol fraction more active than other extracts. For pesticides application crude extracts (methanol extracts) is more effective, therefore activity of pure compounds has still no information.

There is correlation between increase of extract concentration with bacteria growth inhibition. This phenomenon reflects the prospect on using seeds of kolowe source of bactericide. To increase antibacterial activity of extracts, their concentration also has to increase. Therefore, research on maximum concentration of extracts which save to environment in needed.

Conclusion

There are difference sensitivity of bacteria tested against extracts of kolowe seeds. Most of the bacteria are sensitive to the extract. For the three extracts there are also different in their activity against bacterial target, therefore most of them have antibacterial effect. Phytochemically,

all extract fractions contain saponins that is 42,8 % on their crude extracts. So seeds of kolowe potential as source of saponins bactericide.

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Figure 2. Inhibition area by methanol extracts of kolowe seeds to *C. michiganense* bacteria at 3% (a); 2.5 % (b); 2 % (c); 1.5% (d), 1% (e) concentrations.

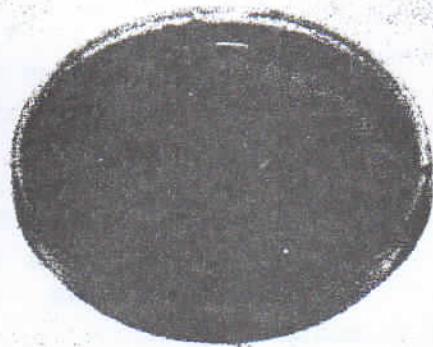


Figure 3. Inhibition area of methanol extracts of kolowe seeds to *R. solanacearum* bacteria at a); 2.5 % (b); 2 % (c); 1.5% (d), 1% (e) concentrations.