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**PROCEEDING OF
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in occasion of

the 38th Meeting of National Working Group on Indonesian Medicinal Plant

**21-21 July 2010
Surabaya, Indonesia**

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BIOLOGICAL ACTIVITIES AND DEVELOPMENT OF HERBAL PRODUCTS FROM *SONNERATIA* *CASEOLARIS* L. ENGL.

Enih Rosamah^{1*}, IrawanWijaya Kusuma¹, and Farida Aryani¹

¹Forestry Faculty, Mulawarman University, Samarinda, East-Kalimantan

*Corresponding author, e-mail: enihros@yahoo.com

Abstract: Since the phytochemical analysis of the ethanolic extracts showed the presence of alkaloids, flavonoids, triterpenoids, steroids, saponins, and tannins, that further investigation were carried out covering antibacterial- and antifungal assay, antioxidant and toxicity of the extracts of fruits, leaves and twigs of Rambai sungai (*Sonneratia caseolaris*). Antioxidant activity was determined by using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging method, antibacterial and antifungal assays were conducted by agar diffusion method against several strains of pathogenic bacteria and fungi. Toxicity of the extracts was evaluated by using *brine shrimp lethality test* against *Artemia salina* Leach. Rambai sungai water extracts showed neither antifungal activity nor antibacterial activity. On the other hand, Rambai sungai water extracts possessed antioxidant potency and comparable to the antioxidant of ascorbic acid with 96% activity. Based on the toxicity test, *S. caseolaris* has possibility to be developed as a safe product for human; and the higher antioxidant potency lead the plant to be developed as herbal products sources.

Keywords : *Sonneratia caseolaris*, antioxidant, toxicity

INTRODUCTION

Indonesian tropical forest area include of 110 mill. Ha, are riched on medicinal plants. In so far, there are about 1.000 species have been used world-wide as traditional medicines (Pramono 2002).

In the last two decades, the utilization of natural resources medicine are tremendous grown up, which is indicated by higher variety in herbal medicine and higher number of herbal medicine producers. In contras, there are changed in deseases pattern, donated by degenerative and cronical deseases, i.e chollesterol, hypertency, diabetic, cancer, and new infection deseases, more resistance against deseases caused by microbial agents. Those cases lead to the people tendency not to choose chemical medicine, but the more safely natural medicine. Increasing of herbal products, supported by more investigation of natural medicines. Based on traditional wisdom of indigenous people, plants were determined as herbal raw materials. Rambai sungai (*Sonneratia caseolaris*) is one of potential mangrove plants as herbal medicine based on traditional utilization information by indigenous people. Mangrove plants potency as medicinal plant was reported by Purnobasuki, (2004).

In Bangladesh, Shaddu *et al.*, (2006) isolated flavonoid as antioxidant from *S. caseolaris*. Leaf and fruits of this plant traditionally used as herbal medicine for stomach, bleeding, antiseptic. Based on this information, it is very urgent to investigate the potency of Rambai sungai for herbal products. This research aimed to study the potency for antimicrobial agent from Rambai sungai plant.

MATERIALS AND METHODS

Materials

Sonneratia caseolaris plant from the Mahakam river side Samarinda East-Kalimantan. Tested fungi: *Aspergillus niger*, *Candida albicans*, and *Trichopyton mentagrophytes*.

Tested bacteria culture: *Salmonella thypi*, *Bacillus cereus* from BPOM Samarinda and *Staphylococcus aureus* from Healthy Laboratory Samarinda. *Artemia salina* Leach for Brine Shrimp Lethality Test.

Extraction

Samples were extracted twice by diluted powdered samples with hot water (ratio of 1:10) put in a waterbath at 100 °C for 3 h. After filtration, the aqueous solution was evaporated by rotary vacuum evaporator at 40°C to yield gummy water extracts.

Toxicity test

Toxicity of extracts were tested against brine shrimp (*Artemia Salina* Leach). Extracts were decided as toxic, when the $LC_{50} < 1000$ mg/ml.

Phytochemical analysis

Extract was subjected to phytochemical analysis (Harborne, 1987). Phytochemical analysis were conducted by coloring test, included testing for alkaloids, steroids and triterpenoids, saponin, flavonoids, carbohydrates, and tannin (Kokate, 2001).

Antimicrobial activities assay

Extracts were tested for antifungal activities assay against *Aspergillus niger*, *Candida albicans*, and *Trichopyton mentagrophytes* in a *paper disk diffusion* agar method. For anti bacterial activities assay, extracts were subjected to *Salmonella thypi*, *Bacillus cereus* and *Staphylococcus aureus*. Antimicrobial assay by diffusion method with extracts concentrations of 40 µg, 60 µg, 80 µg. Antimicrobial activity was determined based on the *Minimum Inhibitory Concentration* (MIC). Myconazole and terramycine were used as positive control for antifungal and antibacterial assay respectively, Acetone was used as negative control. The mean values of inhibition were obtained from triplicate experiments.

Testing for Antioxidant activity against DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical

Testing for antioxidant activity of extracts against DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical solution were conducted by spectrophotometer at ambient temperature (25°C) with wave length of 514 nm. For this test, ascorbic acid was used as positive control.

Making of herbal products

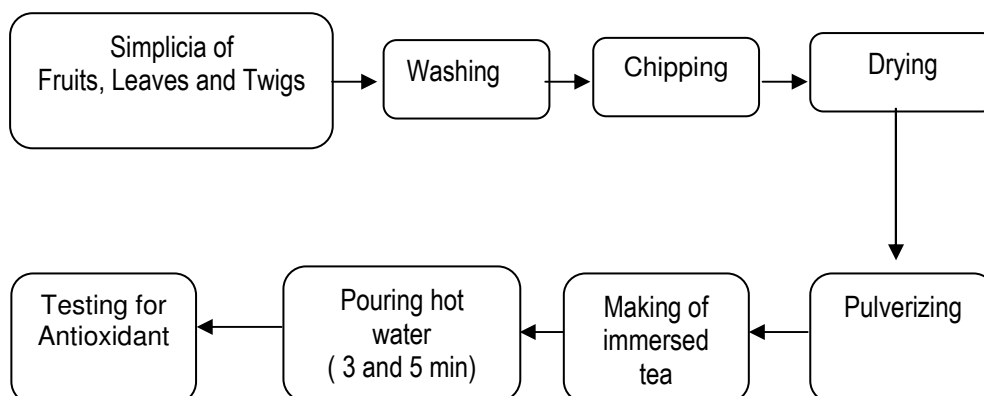


Figure 1. Scheme of Herbal Production from *S. caseolaris*

RESULTS AND DISCUSSION

Extracts Yield

The total extracts from the *S. caseolaris* by aqueous solution at 100°C for 3 h, after filtering and drying (based on oven-dried) are given in Table 1.

Table 1. Water Extracts yields of *S. caseolaris*

Extracts	Part of Plants	Sample (g)	Extracts (g)	Yield (%)
Water extracts	Fruit	44.08	20.71	46.98
	Leafs	43.12	4.96	11.50
	Twig	44.50	6.95	15.62

Pulverizing the samples before extraction has a large influence on the extractable amount of extractives. Grinding can increase the leaching of water soluble organic compounds ten times more than that without grinding. The higher extracts yield may also be caused by higher polarity of aqueous solution, which dissolved some chemical compounds.

Phytochemical Analysis

Phytochemical analysis of *S. caseolaris* ethanolic extracts showed the main active compounds as presented in Table 2.

Table 2. Water Extracts yields of *S. caseolaris*

Extracts Compound	Part of Plant		
	Fruit	Leaf	Twig
Alkaloid	+	+	+
Flavonoid	+	+	+
Saponin	-	+	+
Tannin	+	+	+
Triterpenoid and Steroid	(-/-)	(-/ +)	(-/-)

(+) = active, (-) = not active

Antimicrobial Activity Assay

In particular, aqueous solution are able to dissolve simple sugars, i.e. carbohydrates and starch, which are foodstuffs for microorganism. The heating while the extraction processing could be able to damage the thermolabile-active compounds of extracts and may degrade the chemical structures of active compounds (Fengel dan Wegener, 1984; Harborne, 1987). Those should be the reason why, antimicrobial activity assay of *S. caseolaris* extracts shown neither antifungal- nor antibacterial activities. Antimicrobial secondary metabolites-compounds of *S. caseolaris* supposedly did not dissolve in aqueous solution.

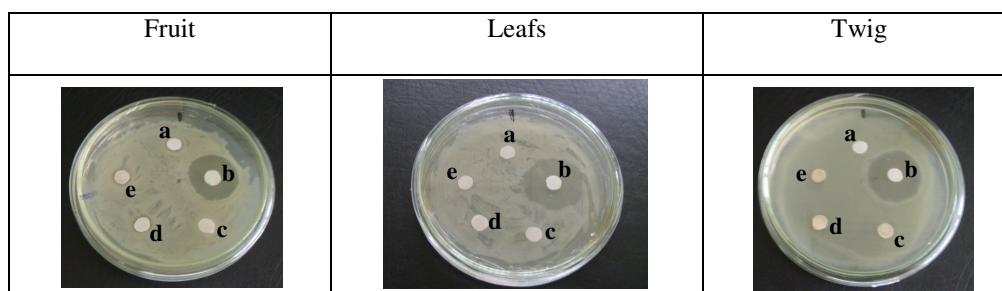


Figure 2. Antibacterial activity assay against *Salmonella thypi* of rambai sungai water extract

(a)= Negative control, (b)= Terramycine, (c)= Extracts 40µg, (d)= Extracts 60µg, (e)= Extracts 80µg

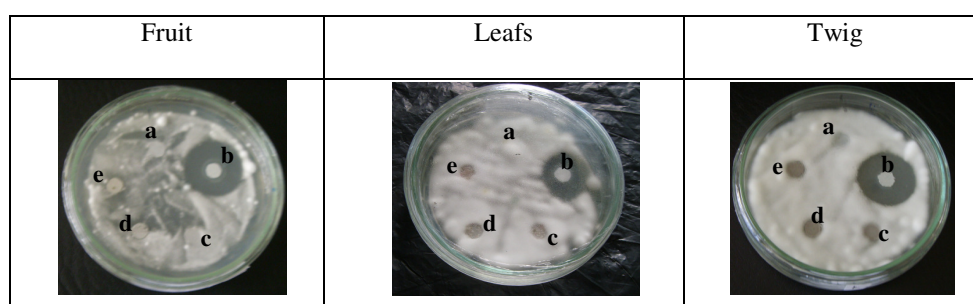


Figure 3. Antifungal activity assay against *Tricophyton mentagrophytes* of rambai sungai water extract

(a)= negative control, (b)= Myconazole, (c)= Extracts 40µg, (d)= Extracts 60µg, (e)= Extracts 80µg

Testing of antioxidant Activity

In order to explore the mechanism of the free radical scavenging effect of *S. caseolaris* extracts, the antioxidant activity was examined. It was hypothesized that the antioxidant activity of *S. caseolaris* extracts may protect from oxygen free radicals. The study was designed to measured how well *S. caseolaris* extracts scavenged DPPH free radicals.

The data from this study indicate that *S. caseolaris* extracts is strong antioxidant which can scavenge DPPH free radicals. The higher inhibition are in water extracts of leaves and twigs (94%), nearly that of ascorbic acid activity (96%) as shown in Fig. 4.

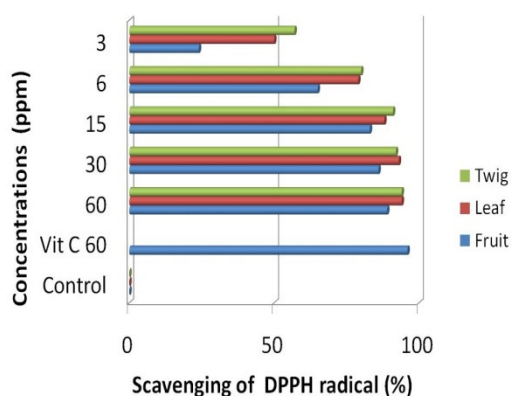


Figure 4. DPPH free radical scavenging activities of water extracts

The testing of Rambai sungai extracts against DPPH free radical showed a positive reaction. These results are supported by the presence of flavonoid in overall part of the plant. The flavonoid compounds are able to be scavenging of free radicals. The higher activity of fruit part was caused not only by presence of flavonoid compounds, but also by presence of glycosides from carbohydrates and supposing of vitamine C containing.

Testing of antioxidant Activity of Rambai Sungai Herbals

Figure 5 showed the scavenging of DPPH free radical of herbal samples higher than 50% . The higher scavenging activities are given by fruits herbal, 85% and 89% for pouring time of 3 and 5 min respectively.

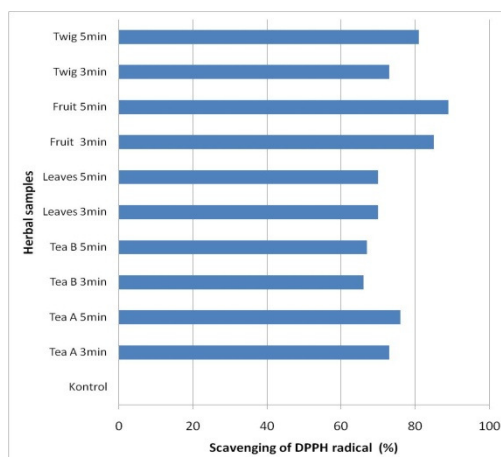


Figure 5. DPPH free radical scavenging activities of herbal products from Rambai sungai

Those scavenging values are higher than that of commercial tea A and tea B as positive control. The higher DPPH free radicals scavenging of fruits herbals in hot water caused by dissolved flavonoid, tannin, and other secondary metabolite compounds, which have antioxidant properties. The sour fruits-tasted are supposed containing of ascorbic acid, well-known as antioxidant.

Toxicity Test (*Brine Shrimp Lethality Test*)

Toxicity test was conducted based on the *brine shrimp lethality test* by using *Artemia salina* Leach (Meyer et al., 1982). The testing results are given in Tabel 3.

Tabel 3. Toxicity test of Rambai Sungai (*S. caseolaris*) extracts

Extracts	Part of Plant	Mortality (%)				
		25 ppm	50 ppm	100 ppm	250 ppm	500 ppm
Water extracts	Fruits	0	0	0	0	2
	Leaves	0	0	0	0	0
	Twigs	0	0	0	0	0
Gallic acid		0	20	50	90	90
Negative control		0	0	0	0	0

Based on the results of investigation with different extracts concentrations, Rambai sungai water extracts showed the mortality of 2 %. The tested extracts overall possessed $LC_{50} > 1000$ ppm. This showed that Rambai sungai extracts have the quality of non toxic. Meyer *et al.*, (1982) stated that a compound identified as a toxic, if it possesses $LC_{50} < 1000$ ppm.

The toxicity of a plant extracts affected to the utilisation of plants as oral medicine. Rambai sungai showed the lower mortality against *Artemia salina*, that the plant is safe to be produced as herbal products. Preparation and processing of this plant lead to the utilisation of Rambai sungai, either as medicines or as foods products.

CONCLUSION

Rambai sungai (*S. caseolaris*) aqueous extracts plays role as antioxidant and can be developed as herbal products. The aqueous extracts showed neither antifungal activities nor antibacterial activities.

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