BIO-POTENTIALS ACTIVITY OF *Sonneratia caseolaris* (MANGROVE) EXTRACT AS ANTIBACTERIAL COLLECTED FROM SOUTH SUMATERA

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Summary

Crude extracts of four mangrove species (leaf, fruit, bark and root), i.e. Avicennia alba, A. marina, Rhizophora mucronata, and Sonneratia caseolaris collected from Teluk Payo, Banyuasin, South Sumatera was extracted in methanol, ethyl acetate, hexane and tested for antibacterial (Escherichia coli and Staphylococcus aureus pathogen), and brine shrimp cytotoxic assay. The highest activity recorded was methanol extract of S. caseolaris in E. coli isolates (18 mm inhibition) and in S. aureus isolates (19 mm inhibition), exhibiting relatively high biopotency. Brine Shrimp Lethality Test showed that leaf of S. caseolaris methanol extract was not toxic to Artemia salina. The high bioactive mangrove extract evaluated further by HPLC showed that mangrove extracts likely contains flavonoid.

Keywords: Extract of *Sonneratia caseolaris*, antibacterial, BSLT, column chromatography, TLC, and HPLC

Introduction

Microorganisms have potential to cause human diseases. Most of the time viruses, bacteria and fungi act as major pathogenic organisms. The discovery of antibiotics in the early twentieth century provided an increasingly important tool to combat bacterial diseases. As antibiotics are increasingly used and misused, the bacterial strains become resistant to antibiotics rapidly. Therefore, antibacterial activity of medicinal plants is very important since vast number of medicinal plants have been used for centuries as remedies for human diseases. Among them extracts from different parts of mangroves and mangrove associates are widely used throughout the world. For instance, stem of *Avicennia marina* is used for ulcers and bark of *Bruguiera sexangula* is used for antitumors. Mangrove and mangrove associates contain biologically active antiviral, antibacterial and antifungal. They provide a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids and tannins [1]. Therefore, it is worth to screen mangrove plants for the presence of new antibacterial compounds to combat the normal pathogenic bacterial strains and hospital acquired antibiotic resistant bacterial strains.

Mangroves are one of the easiest tropical forest types to generate. They have the ability to grow where no other vascular plants can. The mangroves exist under stressful conditions such as violent environments, high concentration of moisture, high and low tides of water, and abundant living microorganisms and insects. They thrive in a very peculiar environment and serve as a bridging ecosystem between freshwater and marine systems. They possess an unusual morphology and physiognomy and the path of photosynthesis in mangroves is different from other glycophytes. They possess modifications to establish water and salt economy. There are modifications or alterations in other physiological processes such as carbohydrate metabolism or polyphenol synthesis and due to these reasons, they may have chemical compounds, which protect them from these destructive elements [2].

Mangroves from the coast of Teluk Payo, South Sumatra province were collected. Their extract of leaf, fruit, bark and root was scrutinized to determine their bioactive using *Escherichia coli* and *Staphylococcus aureus* and brine shrimp cytotoxic as target organism.

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Materials and Methods

Collection and Extraction of Mangrove Bioactive

Four species of mangroves i.e. *Avicenna marina*, *A. alba*, *Rhizophora mucronata* and *Sonneratia caseolaris* were collected and identified from the mangrove forest in Teluk Payo, South Sumatera during December 2009 (**Figure 1**). Prior to the extraction, leaves, fruits, barks and roots of respective species were cleaned, shade dried in order to prevent photolysis and thermal degradation, then chopped into small pieces and ground coarsely in a mechanical grinder.

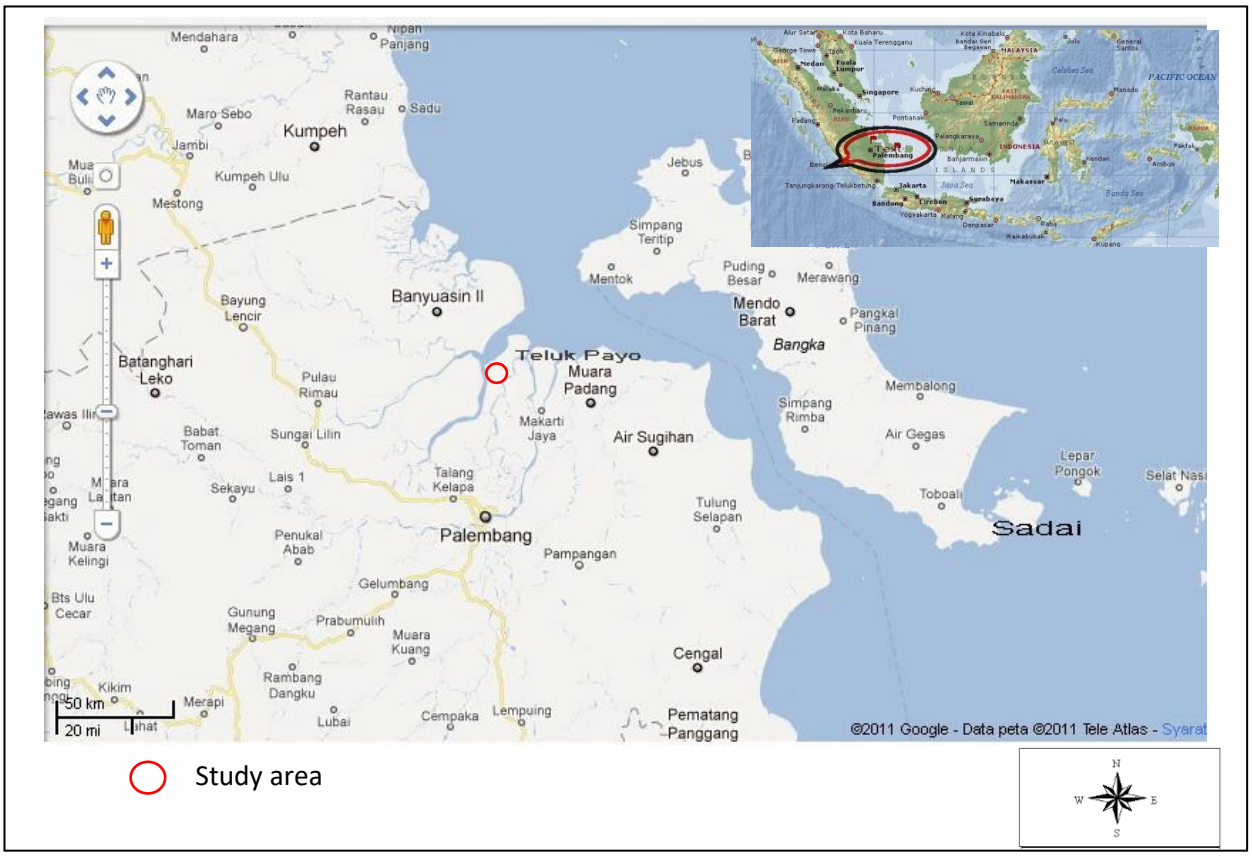

Figure 1. Map showing the study area, Teluk Payo, South Sumatera (Indonesia)

Extraction of Bioactive

Around 100 g of powdered mangrove material was extracted for 3 x 24 hour using methanol 80%, ethyl acetate 80% and hexane 80% as much 250 ml. The extracts were filtered using Whatman no. 1 filter paper. The fraction was evaporated at rotary evaporator at 40 - 50°C, then collected in air–tight plastic vials and stored in the refrigerator for further studies.

Bioassays

Antimicrobial assay was carried out as described by Abesinghe and Wanigatunge [3] against *Escherichia coli* NBRC 13276 and *Staphylococcus aureus* NBRC 14237 pathogen. The cytotoxic activity of mangrove extracts was tested against freshly hatched free-swimming nauplii of *Artemia salina* Leach. The assay system was prepared with 3 ml of filtered seawater containing chosen concentration of mangroves extract in cavity blocks (embryo cup) and 10 nauplii each was transferred in experimental, vehicle control and negative control wells. Invariably the concentration of the experimental systems was determined on the basis of exploratory experiments. The percentage of mortality was determined by comparing the mean surviving larvae of the test and control tubes. The LC_{50} value was determined using probit scale [4].

Column Chromatography and Thin Layer Chromatography (TLC)

The mangroves extract (2 ml) was loaded on a silica gel 60 Fe_{254} (Merck) column packed with chloroform and eluted with chloroform and methanol (9:1 to 1:9) to yield fractions. The potential fractions were then examined by TLC on 25 TLC alluminium silica gel 60 Fe_{254} (Merck) using same solvent with column chromatography. Individual fractions were collected and tested for bioactivity.

High Performance Liquid Chromatography (HPLC)

Mangrove extracts derived from column chromatography were injected to HPLC KNAUER, column Eurospher100-5C_{18} (4.6 x 150 mm, 5 µm), injection volume 20 µl, PDA detector with 200-400 nm, flow rate of gradient 1 ml/minute, methanol:water (v/v) (at 0 minute 0% water, at 22 minute 100% methanol, at 30 minute 100% methanol, at 33 minute 100% water, at 40 minute 100% water).

Results and Discussion

Extraction of Component Bioactive

Methanol solvent produced more weight extract percentage than that of ethyl acetate and hexane solvent (**Table 1**).

Table 1. Mangrove Extracted with MeOH, EtOAc, and Hexane

Species of Mangrove	Part	Original weight (g)	Weight Extract (g)			Crude Extract (% b/v)		
			MeOH	EtOAc	Hexane	MeOH	EtOAc	Hexane
A. marina	Leaf	100	7.71	6.80	3.34	7.71	6.80	3.34
	Bark	100	4.22	4.08	2.21	4.22	4.08	2.21
	Fruit	100	11.8	10.89	2.23	11.80	10.89	2.23
	Root	100	5.22	4.72	2.56	5.22	4.72	2.56
A. alba	Leaf	100	7.93	7.23	4.23	7.93	7.23	4.23
	Bark	100	9.34	8.67	4.21	9.34	8.67	4.21
	Fruit	100	10.31	9.98	4.22	10.31	9.98	4.22
	Root	100	8.30	7.56	3.67	8.30	7.56	3.67
R. mucronata	Leaf	100	10.89	9.79	2.58	10.89	9.79	2.58
	Bark	100	6.73	5.56	2.78	6.73	5.56	2.78
	Root	100	6.75	6.34	3.53	6.75	6.34	3.53
S. caseolaris	Leaf	100	8.67	6.65	3.45	8.67	6.65	3.45
	Bark	100	6.89	6.34	3.12	6.89	6.34	3.12
	Fruit	100	8.90	7.54	3.45	8.90	7.54	3.45
	Root	100	9.78	8.67	4.34	9.78	8.67	4.34

Antibacterial Activity

Crude extracts of mangrove (leaf, fruit, bark and root) had the ability to inhibit bacterial growth of *E. coli* and *S. aureus*. The invitro antibacterial activity revealed that methanol extract of mangroves had remarkable antibacterial activity. Among four species tested, methanol extract of the leaf *S. caseolaris* exhibited wide spectrum of activity which suppress the growth of all tested bacteria, produced a mean zones of inhibition in *E.* coli culture (18 mm) and in *S. aureus* culture (19 mm) (**Table 2** and **Table 3**).

Table 2. Antibacterial activity screening of mangrove extracts towards *E. coli*

Species of Mangroves	Part	Diameter of inhibition (mm)*							
		MeOH (20µl/5µg)	EtOAc (20µl/5µg)	Hexane (20µl/5µg)	A (-)	B (-)	C (-)	D (+)	E (+)
A.marina	Leaf	10	8	0	0	0	0	10	15
	Bark	12	0	0	0	0	0	10	15
	Fruit	16	12	0	0	0	0	10	15
	Root	9	9	0	0	0	0	10	15
A.alba	Leaf	9	7	0	0	0	0	10	15
	Bark	10	8	0	0	0	0	10	15
	Fruit	13	9	0	0	0	0	10	15
	Root	9	8	0	0	0	0	10	15
R.mucronata	Leaf	11	9	0	0	0	0	10	15
	Bark	11	9	0	0	0	0	10	15
	Root	9	8	0	0	0	0	10	15
S.caseolaris	Leaf	**18**	17	0	0	0	0	10	15
	Bark	12	11	0	0	0	0	10	15
	Fruit	14	11	0	0	0	0	10	15
	Root	9	8	0	0	0	0	10	15

info: * optimization hour observation at the 16th, A (-) MeOH, B (-) EtOAc, C (-) Hexane, D (+) Penicillin 10 µg, E (+) Chloramphenicol 30 µg

Table 3. Antibacterial activity screening of mangrove extract towards *S. aureus*

Species of Mangroves	Part	Diameter of inhibition (mm)*							
		MeOH (20µl/5µg)	EtOAc (20µl/5µg)	Hexane (20µl/5µg)	A (-)	B (-)	C (-)	D (+)	E (+)
A.marina	Leaf	10	7	0	0	0	0	10	15
	Bark	12	11	0	0	0	0	10	15
	Fruit	12	9	0	0	0	0	10	15
	Root	7	10	0	0	0	0	10	15
A.alba	Leaf	9	7	0	0	0	0	10	15
	Bark	8	7	0	0	0	0	10	15
	Fruit	10	8	0	0	0	0	10	15
	Root	9	8	0	0	0	0	10	15
R.mucronata	Leaf	10	8	0	0	0	0	10	15
	Bark	10	8	0	0	0	0	10	15
	Root	9	8	0	0	0	0	10	15
S.caseolaris	Leaf	**19**	16	0	0	0	0	10	15
	Bark	16	11	0	0	0	0	10	15
	Fruit	11	11	0	0	0	0	10	15
	Root	10	8	0	0	0	0	10	15

info: * optimization hour observation at the 16th, A (-) MeOH, B (-) EtOAc, C (-) Hexane, D (+) Penicillin 10 µg, E (+) Chloramphenicol 30 µg

The result of bioactivity tests showed that mangrove extract bioactive compounds have strong power. According to Davidstout [5], that the power of antibacterial is pointed out by growth inhibition zone. The growth inhibition of >20 mm means very strong, inhibition zone of 10-20 mm means strong, inhibition zone of 5-10 mm means medium, and inhibition zone of <5 mm means weak.

The highest activity recorded was methanol extract of the leaf *S. caseolaris* inhibiting the growth of E. *coli* isolates (18 mm inhibition) and the growth of *S. aureus* isolates (19 mm inhibition).

The difference between the antibacterial activities of mangrove extract could be due to the type and quantity of antimicrobial substances present in each form.

Brine Shrimp Assay

The brine shrimp assay is considered as a reliable indicator for the preliminary assessment of toxicity [6]. This assay is widely employed in the screening process of botanical for the isolation of bioactive metabolites.

The extract of four mangrove species showed different mortality rate at different concentrations (**Table 4**). The mortality rate increased with the increase of concentration of each sample. The crude extracts of the leaf *S. caseolaris* indicated the highest LC_{50} value of 34914,03 µg/ml meaning not toxic.

Table 4. *Artemia* cytotoxicity profile of mangrove extracts

Concentration µg/ml	Percentage of Mortality			
	Fruit of *A. marina*	Fruit of A.*alba*	Leaf of *R. mucronata*	Leaf of *S.caseolaris*
0 (Control)	0.00	0.00	0.00	0.00
10	3.33	3.33	3.33	13.33
100	3.33	6.67	10.00	16.67
200	3.33	30.00	13.33	23.33
500	6.67	30.00	23.33	33.33
1000	10.00	36.67	33.33	30.00
LC_{50} (µg/ml)	532108.08	6714.288	1043.019	34914.03
Category of toxicity [7]	Not toxic	Not toxic	Not toxic	Not toxic

Purification with Column Chromatography

First step of identification of bioactive compound of mangroves plant is by usage of column chromatography. Solvent applied is chloroform:methanol (9:1 to 1:9). For extract of the leaf *S. caseolaris* as much 20 fractions (yield extract) are produced. Then all fractions were antibacterial tested (*S. aureus* and *E. coli*).

Purification with TLC

Fraction showing bioactivity then underwent thin layer chromatography TLC). Its Rf point (*Retardation fraction*) was measured. Extract of the leaf *S. caseolaris* eluted by chloroform:methanol (9:1 to 1:9) showed Rf points 0,78. (**Figure 2**). Spot on TLC is taken further and tested by antibacterial (*S. aureus* and *E. coli*). The growth inhibition was 7 mm.

Figure 2. Profile of bioactive fraction of *S. caseolaris* leaf on TLC

HPLC Characteristic of Bioactive Substance

Analysis of HPLC with detector photodiode array (PDA) (λ = 200 - 400 nm) showed that the dominant peak with its retention time and wavelength characteristic was similar with those of the standard compound characteristic stored in HPLC data base.

HPLC analysis of the leaf *S. caseolaris* showed have 4 peak dominant at retention time (Rt) 14,50 (λ = 234 nm), Rt 15,92 (λ = 258 nm), Rt 19,68 (λ = 230 nm), and Rt 23,13 (λ = 223 nm and 277

nm) (**Figure 3**).

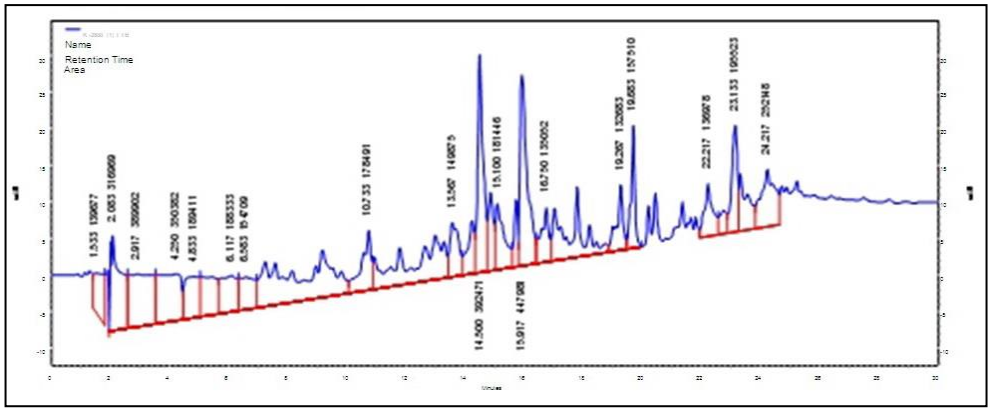

Figure 3. HPLC Analysis of leaf *S. caseolaris* extract

Conclusions

Among four mangrove species screened, the broadest activity was showed by *S. caseolaris* extract, therefore this mangrove plant might posses potential source for further study of its bioactive compounds for the purpose of biopharmaceuticals.

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