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Screening of Some Extracts from *Garcinia parvifolia* Miq. (Guttiferae) for Antiplasmodial, Antioxidant, Cytotoxic and Antibacterial Activities

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Abstract: Crude extracts of some parts of *Garcinia parvifolia* Miq: root, stem bark, fruit and leaf, can be dissolved in various solvents, such as n-hexane, ethylacetate and methanol. The crude extracts of these plant parts were screened for antiplasmodial, antioxidant, cytotoxic and antibacterial activities. Most extracts show antiplasmodial activity but the extracts of roots and stem bark show strong antiplasmodial activities (IC_{50} 7.88 and 4.11 μg mL^{-1}). All of the extracts show antioxidant activities with IC_{50} <100 μg mL^{-1}. They all show cytotoxic activity with a value of LC_{50} <1000 μg mL^{-1}. N-hexane extract of leaf and fruit do not show antibacterial activity, while n-hexane and ethylacetae extracts of stem bark, root and fruit show strong antibacterial activity, especially against *Staphyloccocus aureus.*

Key words: *Garcinia parvifolia*, antiplasmodial, antioxidant, cytotoxic, antibacterial

INTRODUCTION

Garcinia is a large genus mainly occurring in the old world tropics. Some species have been used as traditional medicines and have been shown to contain of isoprenylated xanthones and biflavonoids (Waffo *et al*., 2006; Thoison *et al*., 2000).

Garcinia parvifolia's local name is asam kandis, the species of *Garcinia* are found mainly in Kalimantan, Indonesia. It is a rust colored fruit with orange likely rather acid and the young leaf is sometime eaten by residen of Kalimantan as a vegetable (Heyne, 1987). Extracts of the *Garcinia* genus, particularly *G. mangostana*, *G. kola*, *G. cowa* and *G. atroviridis* have been extensively reported to exhibit diverse biological activities such as antibacterial, antioxidant, anti-inflammatory, antitumor-promoting, cytotoxic and antiplasmodial, but only a few of these studies involved *G. parvifolia* (Grosvenor *et al*., 1995; Mackeen *et al*., 2000; Likhitwitayawuid *et al*., 1998). Although the occurrence of xanthones, benzophenones and biflavonoids is common in the *Garcinia* genus, to date, the isolation of only rubraxanthone, cowinin and parvixanthone have been isolated from this species (Xu *et al*., 2001; Jantan *et al*., 2002). In continuation of our search for bioactive natural products from Guttiferous plants, we have examined the extracts of some plant parts from *G. parvifolia* Miq. In this study we observed antiplasmodial, antioxidant, cytotoxic and antibacterial activities in leaves, steam barks, roots and fruits of *G. parvifolia* Miq.

MATERIALS AND METHODS

Plants materials: The fruits, roots, leaves and stem barks of *G. parvifolia* Miq. (Guttiferae) were collected in Nang Kalis, Kalimantan. The herbarium voucher specimen (GP-1) was identified and deposited at the Herbarium Bogoriense, Bogor.

Extraction: Dried and powdered parts of *G. parvifolia* Miq. were extracted under soxlet with n-hexane, ethylacetate and methanol. Evaporation in a vacuum concentrated the extracts into dried residues.

DPPH assay: The antioxidants assay was performed on scavenging effect of stable free radicals 1,1-diphenyl-2-picryl-hydrazyl (DPPH). An extract of 4 mg was dissolved in 4 mL DMSO to obtain 1000 μg mL^{-1} mother solution of test sample. This test sample was diluted with ethanol to concentrations of 10, 40, 200 and 1000 μg mL^{-1} extracts, respectively. These samples were mixed with an ethanol solution of 300 μM DPPH in a 90-well micro-titer plate and incubated at 37°C for 30 min. For each, extract three replicate trials were held. The absorption was measured at 517 nm. Inhibition percentage of the test samples was

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compared to that of the control (DMSO). The positive control test was a solution of hydroquinone. The IC_{50} value indicates the ability of the test sample to scavenge 50% of free radicals (Christov *et al*., 2005).

Brine shrimp lethality test: Brine shrimp (*Artemia saline* Leach) eggs (Premium Extra Brine Shrimp Eggs, USA) were placed in a hatching tank containing sea water for 48 h. Then 2 mg of plant extract were dissolved in 1 mL of chloroform (2 000 μg mL^{-1}). From this solution 500, 50 or 5 μL was transferred to vials corresponding to 1000, 100 or 10 μg mL^{-1}, respectively. Vials containing chloroform and extraction solvents (500 μL) were prepared as controls. For each extract, three were replicate trials. After incubation, 10 brine shrimp larvae (nauplii) were introduced into vials containing graded concentrations (ranging from 10 to 1000 ppm) of the test extracts. After 24 h, the number of surviving shrimp at each concentration of the extract were counted and data analyzed with the Finney computer program to determine the LC_{50} at a 95% confidence interval (Meyer *et al*., 1982).

Antiplasmodial *in vitro* assay: The malarial parasite, *Plasmodium falciparum* (FCR-3, chloroquine resistant strain), was cultured according to the method of Trager (1976). To determine the antimalarial activity of each extract, the parasite was placed in a 24-well culture plate in the presence of a wide concentration range of each extract. For each extract there were three replicate trials. Total parasitemia was calculated as the number of parasites-observed, divided by 2000 erythrocyte multiplied by 100%. The parasitemia of each parasite stage was counted.

The concentration response parasite growth data was analyzed by a linear regression function (SYSTAT Sigma Plot Software) using the 50% inhibitory concentration (IC_{50}).

Antibacterial activity: Antibacterial activity of the organic extracts from the plant samples were evaluated by the paper disc diffusion method. For the determination of antibacterial activity, bacterial cultures were adjusted to 0.5 McFarland turbidity standard and inoculated onto nutrient agar plates. Sterile filter paper discs impregnated with 100 μL of extracts dilutions reconstituted in a minimum amount of solvent at concentrations of 2 and 20% were applied over each of the culture plates previously seeded with the 0.5 McFarland and 10^6 cfu mL^{-1} cultures of bacteria. Bacterial cultures were then incubated at 35-37°C for 18-24 h. Antibacterial activity was determined by measuring the zone of inhibition around each paper disc. For each extract three replicate trials were conducted against each organism (Acar *et al*., 1991; Doughari, 2006).

RESULTS AND DISCUSSION

Plants of the Guttiferae family are used worldwide in traditional medicine for the treatment of diseases and are well known to be rich sources of xanthones, biflavonoids and benzophenones. Particularly for trees belonging to the genus *Garcinia*, it has been found that approximately half of the species studied contain xanthones which are of chemotaxonomic interest. The plant used in our study is used as a traditional medicinal plant.

The results of the *in vitro* tests showed the methanol extract of the root and the n-hexane extract of the root and the stem bark have antiplasmodial activity that is stronger than the extracts of the other parts of the plant with IC_{50} <10 μg mL^{-1} (Table 1). A value of IC_{50} <10 μg mL^{-1} is classified as having strong antiplasmodial activity (Gessler *et al*., 1994). The different strengths of antiplasmodial extracts from different plant parts maybe due to the different amount of xanthone in those parts of the plant. *G. parvifolia* bark has been shown to contain nine minor xanthones (Xu *et al*., 2001).

Five xanthones have also been isolated from *G. cowa* and show moderate antimalarial activity (Likhitwitayawuid *et al*., 1998). One of the four xanthones isolated from the roots of *Andrographis paniculata* exhibited antimalarial activity during *in vitro* and *in vivo* study (Dua *et al*., 2004). Recently, xanthones have been identified as novel antimalarial agents.

Table 1: Antiplasmodial, antioxidant, activity of some extracts of different part of *G. parvifolia* Miq.

Solvent	Used part	Antiplasmodial IC_{50} (μg mL^{-1})	Antioxidant IC_{50} (μg mL^{-1})	Yield (%)
n-hexane	Leaf	19.29±1.21	47.38±4.53	1.20
	Roots	7.88±0.91	31.89±1.28	0.34
	Fruits	>100.00±2.34	118.26±8.98	0.92
	Stem barks	4.11±1.23	85.47±9.89	0.88
Ethylacetate	Leaf	16.87±2.34	71.82±8.34	6.97
	Roots	81.80±5.67	23.00±3.43	2.42
	Fruits	>100.00±12.34	87.61±2.35	23.01
	Stem barks	40.33±7.81	67.63±1.89	2.89
Methanol	Leaf	10.00±1.98	85.47±4.57	5.04
	Roots	3.27±8.97	93.83±3.45	1.52
	Fruits	>100.00±7.89	105.98±6.78	24.56
	Stem barks	10.36±3.45	35.97±5.78	4.19
	Standard	0.47±0.98	73.47±2.31	

Standard, chloroquine for antiplasmodial, hydroquinone for antioxidant activity and for each extract three replicate trials; [a]: Values are means±standard deviations of the means

The antioxidant activity was evaluated with the 2.2 diphenyl-1-picrylhydrazyl (DPPH) assay. The assay used a free radical which shows a characteristic absorption at 517 nm (purple) (Velaskez *et al*., 2003). The scavenging reaction between (DPPH•) and an antioxidant (H-A) can be written as:

$$(DPPH^{\bullet}) + (H\text{----}A) \rightarrow DPPH\text{--}H + (A^{\bullet})$$
purple yellow

Antioxidants react with DPPH•, which is a stable free radical and is reduced to the DPPH--H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability (Benabadji *et al*., 2004). Compared to extracts of one plant parts, fruit extracts had a lower IC_{50} >100 μg mL^{-1}. These difference is probably due to the variance of the major components in this part of the plant. The antioxidant activity of the extracts was expected, because xanthones isolated from other species of *Garcinia* have shown this quality (Minami *et al*., 1998). In fact, it has been proven that the plasmodium infected erythrocyte is under constant oxidative stress. This is caused by exogenous reactive oxidant species and reactive nitrogen species produced by the immune system of the host and by endogenous production of reactive oxidant species generated during the digestion of host cell haemoglobin and concomitant biochemical reactions (Ngouela *et al*., 2006).

Brine shrimp nauplii have been used previously in a number of bioassay systems, which use natural product extracts, fractions, or pure compounds. The brine shrimp assay has advantages of being rapid (24 h), inexpensive and simple. It easily utilizes a large number of organisms for statistical validation and requires no special equipment and a relatively small amount of sample (McLaughlin and Rogers, 1998). It is a convenient preliminary toxicity test, since brine shrimp are highly sensitive to a variety of chemical substances (Franssen *et al*., 1997).

Plant extracts show toxicity to brine shrimp except extract of ethylacetate leaf (LC_{50} >1000 μg mL^{-1}). The stem bark and root extracts of ethylacetate show a strong toxicity to brine shrimp (LC_{50} 1.56±0.35 μg mL^{-1}) and (2.97±0.98 μg mL^{-1}), respectively. The results of the brine shrimp lethality bioassay experiments indicate the presence of active cytotoxic compounds in the extracts of the root and stem bark. Inhibitory effects on the growth of bacteria was determined using the disk diffusion method as outlined in Tortora *et al.* (2004). The zones of bacterial inhibition were measured to the nearest whole millimeter (mm). Diameter of zones of inhibition >10 mm were considered active (Dosumu *et al*., 2006). This effect may be a result of the pattern of radial diffusion penetration troughs of uneven growth distribution within the bacterial lawn, giving rise to microchannels of less dense or sparse bacterial accumulation (Srinivasan *et al*., 2001). Out of the three solvents used for extraction, the ethylacetate extracts showed the highest activity against the test organisms, followed by the methanol extracts and n-hexane extracts. Different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent (Marjorie, 1999). The ethylacetate extracts in this study might have had higher solubility for more phytoconstituents, consequently the highest antibacterial activity.

On the Table 3 below, the obtained inhibition-zone data varies, perhaps because the extracts used in this research are from different parts of the plant. Acar and Goldstein (1991) states that inhibition zone can be influenced by the degree of compound concentration, extract concentration and the antibacterial compound. The diameter of inhibition zone is proportional to the extract concentrations. The greater the concentration, the bigger inhibition zone. The results showed that the stem bark and root extracts are more effective than the leaf

Table 2: LC_{50} of some extracts of different part of *G. parvifolia* against *A. salina*

Solvent	Used part	LC_{50} (μg mL^{-1})[a]
n-hexane	Leaf	97.32±10.32
	Roots	23.85±9.89
	Fruits	55.60±11.54
	Stem barks	6.12±1.02
Ethylacetae	Leaf	>1000.00
	Roots	2.97±0.98
	Fruits	71.09±12.40
	Stem barks	1.56±0.35
Methanol	Leaf	78.25±12.51
	Roots	14.68±3.41
	Fruits	92.53±10.41
	Stem barks	24.62±7.46

[a]: Values are means±standard deviations of the means

Table 3: Zone inhibition diameter (mm) of some extracts of different part of *G. parvifolia*

		E. coli (%)		*S. aureus* (%)	
Solvent	Used part	2	20	2	20
n-hexane	Leaf	0.0	0.0	0.0	0.0
	Roots	0.0	0.0	8.0	15.0
	Fruits	0.0	0.0	0.0	0.0
	Stem barks	0.0	0.0	6.0	12.0
Ethylacetate	Leaf	0.0	0.0	7.5	10.0
	Roots	6.0	12.0	7.0	14.0
	Fruits	7.0	14.0	8.0	14.0
	Stem barks	7.5	15.0	8.0	19.0
Methanol	Leaf	0.0	0.0	7.0	19.0
	Roots	5.0	11.0	6.0	12.0
	Fruits	0.0	12.5	8.0	20.0
	Stem barks	8.0	14.0	9.0	19.0

extract. This may be due to the fact that the stem bark and root were more developed and mature than the leaves and may contain fewer pigments and other phenolics which have been reported to interfere with the antimicrobial activity of the extracts (Doughari, 2006). Other research (Mackeen *et al*., 2000) has shown that the extracts of all part of *G. atroviridis* show strong antibacterial activity. The strong antibacterial activity of extracts maybe due to the presence of xanthones and related metabolites that have been implicated for potent antibacterial activity in other species of *Garcinia* (Linuma *et al*., 1996).

CONCLUSION

Therefore, at a preliminary stage it may be suggested that the *G. parvifolia* showed antiplasmodial, antioxidant, cytotoxic and antibacterial activities and have potential to be developed as medicine. The range of biological activities of the extracts dissolved in various solvents shows the differences of active compounds in the roots, stem bark, leaf and fruits. Other work will be done to continue the biologically guided fractionation in order to isolate and identify the active compounds, as well as to understand the mechanism of inhibition.

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