

ORIGINAL ARTICLE

Pharmacological studies of plants in the mangrove forest

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ABSTRACT

Mangrove areas are rich in medicinal and edible plants. Biological screening of the plants in this study may lead to drug and health product development. The biological tests includes antioxidation, antilipid peroxidation and cancer chemoprevention. Fifty seven samples of 32 species were tested. Calyces of *Sonneratia caseolaris* exhibited the strong antioxidant activity followed by stamens of *Sonneratia caseolaris*, calyces of *S. alba*, *Cynometra ramiflora* seeds, *Xylocarpus rumphii* fruit peel and branches. Some edible pods including *Bruguiera parviflora*, *Ceriops decandra*, *C. tagal*, *Rhizophora mucronata* etc., were also active. Some of these pods also exhibited antilipid peroxidation such as *Bruguiera parviflora* and *Rhizophora mucronata*. Besides, *Cynometra ramiflora* (seeds), *Lumnitzera racemosa* (leaves), *Nypa fruticans* (inflorescences) and *Sonneratia caseolaris*, exhibited strong antilipid peroxidation. Plants possessed cancer chemoprevention activities were *Bruguiera gymnorrhiza* (pods), *Acanthus ebracteatus* (leaves), *Avicennia marina* (leaves), *Flagellaria indica* (young shoot), *Phoenix paludosa* (young shoot) and *Trianthema decandra* (aerial part), of which *Bruguiera gymnorrhiza* pods exhibited strongest activity.

บทคัดย่อ

ป่าชายเลนอุดมไปด้วยพืชสมุนไพรและพืชอาหาร การศึกษาครั้งนี้เป็นการศึกษาฤทธิ์ทางเภสัชวิทยา เพื่อเป็นแนวทางในการพัฒนายาและผลิตภัณฑ์ โดยตรวจสอบฤทธิ์ต้านอนุมูลอิสระ ฤทธิ์ต้านการเกิด lipid

peroxide และฤทธิ์ป้องกันมะเร็ง ของพืช 32 ชนิด 57 ตัวอย่าง พบว่าพืชที่มีฤทธิ์ต้านอนุมูลอิสระที่ดีที่สุดคือ กลีบ-เลี้ยงดอกลำพู รองลงมาคือเกสรตัวผู้ของดอกลำพู กลีบเลี้ยงลำพูทะเล เมล็ดในมะคะ เปลือกลูกและกิ่งตะบัน และฝักของพืชในป่าชายเลนหลายชนิดก็มีฤทธิ์ดีเช่นกัน ได้แก่ ฝักอ่อนถั่วดำ ฝักอ่อนโปรงขาว ฝักอ่อนโปรงแดง ฝักอ่อนโกงกางใบใหญ่ ฝักแก่โกงกางใบใหญ่ ซึ่งพืชเหล่านี้ ยังมีฤทธิ์ต้านการเกิด lipid peroxidation ที่ดีด้วย เช่น ฝักอ่อนถั่วดำ ฝักอ่อนโกงกาง เป็นต้น พืชที่มีฤทธิ์ต้านการเกิด lipid peroxidation ยังได้แก่ เมล็ดในมะคะ ใบผาดดอกขาว จาก และผลลำพู ส่วนพืชที่มีฤทธิ์ต้านการเกิดมะเร็งที่ดีที่สุดได้แก่ พังกาหัวสุมดอกแดง นอกจากนี้ได้แก่ ใบเหวื่อปลาหมอ ใบแสมทะเล ยอดหวายลิง ยอดเป้ง ฝักเบ็ยทะเล

INTRODUCTION

Mangrove forest is economically and ecologically importance. The mangrove plants are useful for erosion protection and also for marine animals. Several plants are used for medical purposes e.g. *Acanthus ebracteatus* Vahl for chronic wound (Suchamuong, 1979), the bark of *Rhizophora apiculata* Blume for diarrhoea and wound (School of Thai traditional medicine, 1981; Traditional Medicine Association, 1980), the bark of *R. mucronata* for diarrhoea (Pongboonrod, 1976) and the bark of *Avicennia alba* Blume for wound (Yaadfon Association, 1981), etc. The purpose of this study is to evaluate the biological potential of the plants in the mangrove forest using antioxidant, lipid peroxidation inhibition and cancer chemoprevention tests. The results from this study will serve as the basis for drug development.

MATERIAL & METHODS

1. Chemicals

50% glacial acetic acid (Merck), 35% perchloric acid (Mallinckrodt), potassium dihydrogen phosphate (Mayer+Baker htd), potassium chloride (Carlo erba), thiobarbituric acid, ethyl acetate (J.T.Baker), methanol

(Mallinckrodt), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma), Trolox[®](6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Aldrich), linoleic acid (Fluka)

2. Equipments

Shaking water bath, Centrifuge (Sorvall[®] RC 5C plus), Fluorometer (Shimadzu RF-10AXL), Spectrophotometer (Milton roy Spectronic 3000)

3. Methods

3.1 Scavenging effect on DPPH radicals

The free radical scavenging effect was determined by DPPH method (Hatono et al., 1988; Duh and Yen, 1997; Ancerwiz et al., 1998, Buyapraphatsara et al., 2002) and TLC method (Sanchez-Medina et al., 2001). Ethyl acetate fraction of methanol extract were submitted to TLC analysis using silica gel G60 as an adsorbent and 2 mobile phases, CHCl₃ – MeOH 9:1 and CHCl₃ – MeOH – H₂O (62:35:8). The developed plates were sprayed with 0.2 % DPPH, the yellow spots appeared after 8 hours were considered to be active.

3.2 *In vitro* antilipid peroxidation

The inhibition of lipid peroxidation in mice brain homogenate was determined using thiobarbituric acid method (Kamasud, 2001,

Bunyapraphatsara et al., 2002)

3.3 Assay of quinone reductase induction

Cancer chemopreventive activity was screened by using quinone reductase induction activity (Prochaska and Santamaria, 1988; Prodask et al., 1992 ; Bunyapraphatsara et al., 2002)

RESULTS

Eighteen Plants in the mangrove forest which showed antioxidant activity with ED₅₀

less than 10 µg/ml, were flower of *Avicennia alba* Blume (7.67 µg/ml), *A. marina* (Forsk.) Vierh (8.67), *Barringtonia asiatica* (L.) Kurz twig (8.93), *Bruguiera parviflora* Wight & Arn.ex Griff.young pods (5), *B. decandra* Ding Hua young pods (6.38), *Ceriops tagal* (Perr.) C.B. Robinson young pods (6.67), *Cynometra ramiflora* L. seeds (3.33), *Rhizophora mucronata* Poir. young pods (3.83), *Sonneratia caseolaris* (L.) Engl. (2.93), etc. (Table 1).

Table 1. Antioxidant activity of plants in the mangrove forest

	Plants	Part Used	EC ₅₀ (µg/ml)	Number of active spot (TLC)
1	<i>Acanthus ebracteatus</i> Vahl	leaves	49.33	7
2	<i>Acanthus illicifolius</i> L.	seeds	162.00	9
		bark	79.67	6
3	<i>Acrostichum aureum</i> L.	twig	103.00	3
4	<i>Avicennia alba</i> Blume	fruit	20.67	10
		leaves	102.67	3
		flowers	7.67	8
5	<i>Avicennia marina</i> (Forsk.) Vierh	branches	89.00	3
		leaves	66.00	3
		flowers	8.67	8
6	<i>Barringtonia asiatica</i> (L.) Kurz.	branches	28.00	6
		twig	8.93	3
		leaves	66.00	3
7	<i>Bruguiera cylindrica</i> Blume.	pod	47.00	7
8	<i>Bruguiera gymnorrhiza</i> (L.) Savigny	young pods	11.67	8
		flowers	11.67	8
		leaves	18.00	8
		branches	16.00	10
9	<i>Bruguiera parviflora</i> Wight & Arn.ex Griff.	young pods	5.00	9
		leaves	105.00	10
10	<i>Bruguiera sexangular</i> Poir	young pods	16.67	12
11	<i>Ceriops decandra</i> Ding Hou	young pods	6.38	10

Table 1. Antioxidant activity of plants in the mangrove forest (cont.)

	Plants	Part Used	EC ₅₀ (µg/ml)	Number of active spot (TLC)
12	<i>Ceriops tagal</i> (Perr.) C.B. Robinson.	young pods	6.67	9
13	<i>Cissus carnosus</i> Roxb.	fruit	Negative	-
14	<i>Cordia cochinchinensis</i> Pierre	fruit	93.67	4
15	<i>Cynometra ramiflora</i> L.	rind	31.33	6
		seeds	3.33	10
16	<i>Derris trifoliata</i> Lour.	leaves	13.33	7
17	<i>Flagellaria indica</i> L.	twig(dried)	123.33	4
		twig(fresh)	384.00	4
18	<i>Lumnitzera racemosa</i> Willd.	leaves	5.87	10
		fruit	11.33	6
19	<i>Nypa fruticans</i> Wurmb.	young inflorescence	50.67	7
		pollen	42.00	8
20	<i>Phoenix paludosa</i> Roxb.	twig(dried)	38.00	6
		twig(fresh)	12.28	6
21	<i>Rhizophora apiculata</i> Blume	leaves	28.67	9
		young pods	36.80	4
		branches	10.10	5
22	<i>Rhizophora mucronata</i> Poir.	mature pods	4.33	8
		young pods	3.83	12
		leaves	7.67	11
23	<i>Sarcolobus globosus</i> Wall.	rind	Negative	-
		seeds	Negative	-
24	<i>Sonneratia alba</i> J. Smith	sepals	2.57	8
25	<i>Sonneratia caseolaris</i> (L.) Engl.	stamen	2.93	8
		sepals	6.10	7
		fruit	4.17	10
26	<i>Sonneratia ovata</i> Back	fruit	28.67	3
27	<i>Stenochlaena palustris</i> (Burm.f.) Bedd.	twig (dried)	215.70	2
		leaves(dried)	398.00	8

Table 1. Antioxidant activity of plants in the mangrove forest (cont.)

	Plants	Part Used	EC ₅₀ (µg/ml)	Number of active spot (TLC)
28	<i>Suaeda maritima</i> Dum. (Nakornsrithamarat)	twig	121.33	8
	<i>Suaeda maritima</i> Dum. (Samut songkram)	twig	189.33	8
	<i>Suaeda maritima</i> Dum. (Samut songkram)	twig	193.67	5
29	<i>Trianthema decandra</i> L.	twig (dried)	77.67	8
30	<i>Weddellia biflora</i> (L.) DC.	flowers	32.00	6
31	<i>Xylocarpus granatum</i> Koen.	seeds	5.60	8
		leaves	29.00	7
		rind	4.67	10
32	<i>Xylocarpus rumphii</i> (Kostel.) Mabblerley	rind	3.67	10
		seeds	114.00	5
		branches	3.67	12
		leaves	38.67	12
	Trolox ^R		3.70	

Among the plants tested, 6 plants showed strong lipid peroxide formation inhibition activity (IC₅₀ <1 µg/ml), 26 plants showed IC₅₀ <10 µg/ml. *Sonneratia casseolaris* (L.) Engl. fruit showed the strongest activity (0.083 µg/ml). (Table 2).

Table 2 Lipid peroxidation inhibition of plants in the mangrove forest

	Plant names	Part Used	IC ₅₀ (µg/ml)
1	<i>Acanthus ebracteatus</i> Vahl	leaves	28.998
2	<i>Acanthus illicifolius</i> L.	seeds	19.186
		rind	38.373
		leaves	12.093
3	<i>Acrostichum aureum</i> L.	twig	28.090
4	<i>Avicennia alba</i> Blume	fruit	7.909

Table 2 Lipid peroxidation inhibition of plants in the mangrove forest (cont.)

	Plant names	Part Used	IC ₅₀ (µg/ml)
		leaves	38.298
		flowers	6.712
		branches	12.524
5	<i>Avicennia marina</i> (Forsk.) Vierh	leaves	23.466
		flowers	6.774
		branches	5.677
6	<i>Barringtonia asiatica</i> (L.) Kurz.	twig	2.920
7	<i>Bruguiera cylindrica</i> Blume.	Pods	23.100
8	<i>Bruguiera gymnorrhiza</i> (L.) Savigny	young pods	4.425
		flowers	4.550
		leaves	3.625
		branches	2.825
9	<i>Bruguiera parviflora</i> Wight & Arn.ex Griff.	young pods	0.375
		leaves	42.600
10	<i>Bruguiera sexangular</i> Poir	young pods	4.661
11	<i>Ceriops decandra</i> Ding Hou	young pods	2.635
12	<i>Ceriops tagal</i> (Perr.) C.B. Robinson.	young pods	2.646
13	<i>Cissus carnosa</i> Roxb.	fruit	weak act.
14	<i>Cordia cochinchinensis</i> Pierre	fruit	54.385
15	<i>Cynometra ramiflora</i> L.	rind	10.259
		seeds	0.8992
16	<i>Derris trifoliata</i> Lour.	leaves	11.250
17	<i>Flagellaria indica</i> L.	twig (fresh)	weak act.
18	<i>Lumnitzera racemosa</i> Willd.	leaves	0.199
		fruit	3.838
19	<i>Nypa fruticans</i> Wurmb.	inflorescence	0.950
		stamen	16.670
20	<i>Phoenix paludosa</i> Roxb.	twig (fresh)	16.915
21	<i>Rhizophora apiculata</i> Blume	leaves	9.896
		young pods	3.850
		branches	2.359

Table 2 Lipid peroxidation inhibition of plants in the mangrove forest (cont.)

	Plant names	Part Used	IC₅₀ (µg/ml)
22	<i>Rhizophora mucronata</i> Poir.	mature pods young pods leaves	1.125 0.2918 1.975
23	<i>Sonneratia alba</i> J. Smith	sepals	0.840
24	<i>Sonneratia caseolaris</i> (L.) Engl.	leaves stamens sepals fruit	1.228 1.105 2.213 0.083
25	<i>Sonneratia ovata</i> Back	fruit	15.485
26	<i>Stenochlaena palustris</i> (Burm.f.) Bedd.	twig (dried) leaves (dried)	87.931 30.052
27	<i>Suaeda maritima</i> Dum. (Nakornsrihamarat) <i>Suaeda maritima</i> Dum. (Samut songkram) <i>Suaeda maritima</i> Dum. (Samut songkram)	twig twig twig	28.950 weak act. weak act.
28	<i>Trianthema decandra</i> L.	twig (dried)	8.900
29	<i>Weddelia biflora</i> (L.) DC.	flowers	8.165
30	<i>Xylocarpus granatum</i> Koen.	seeds leaves rind	3.250 6.150 1.425
31	<i>Xylocarpus rumphii</i> (Kostel.) Mabblerley	rind seeds branches leaves	1.250 38.875 1.700 1.725
	Trolox ^R		9.162

Bruguiera gymnorrhiza (L.) Savigny young pods showed strongest quinone reductase induction with CD 2.9 µg/ml. *Acanthus ebracteatus* Vahl leaves, *Cissus carnosa* Roxb. fruit, *Flagellaria indica* L. twig, *Phoenix paludosa* Roxb. inflorescence, *Trianthema decandra* L. twig, *Avicennia marina* (Forsk.) Vierh leaves showed the activities with CD 9.5, 3.6, 9.9, 8.8, 9.2 and 5 µg/ml, respectively. (Table 3).

Table 3 Quinone reductase induction activity of plants in the mangrove forest

	Plant Name	Part Used	QR	
			CD ($\mu\text{g/ml}$)	IC ₅₀ ($\mu\text{g/ml}$)
1	<i>Acanthus ebracteatus</i> Vahl	leaves	9.5	17.6
2	<i>Acanthus illicifolius</i> L.	seeds	>10	>20
		bark	>10	>20
3	<i>Acrostichum aureum</i> L.	twig	>10	>20
4	<i>Avicennia alba</i> Blume	fruit	>10	>20
		leaves	>10	>20
		flowers	>10	>20
		branches	>10	>20
5	<i>Avicennia marina</i> (Forsk.) Vierh	leaves	5	>20
		flowers	>10	>20
		branches	>10	>20
6	<i>Barringtonia asiatica</i> (L.) Kurz.	twig	>10	>20
		bark	>10	>20
7	<i>Bruguiera cylindrica</i> Blume.	young pods	>10	>20
8	<i>Bruguiera gymnorrhiza</i> (L.) Savigny	young pods	2.9	>20
		flowers	>20	>20
		leaves	>20	>20
		branches	>20	>20
9	<i>Bruguiera parviflora</i> Wight & Arn.ex Griff.	young pods	>10	>20
		leaves	>20	>20
10	<i>Bruguiera sexangular</i> Poir	young pods	10.2	>20
11	<i>Ceriops decandra</i> Ding Hou	young pods	>10	>20
12	<i>Ceriops tagal</i> (Perr.) C.B. Robinson.	young pods	>10	>20
13	<i>Cissus carnos</i> a Roxb.	fruit	3.6	>20
14	<i>Cordia cochinchinensis</i> Pierre	fruit	>10	>20
15	<i>Cynometra ramiflora</i> L.	rind	>10	>20
		seeds	>10	>20
16	<i>Derris trifoliata</i> Lour.	leaves	>10	>20
17	<i>Flagellaria indica</i> L.	twig (dried)	>10	>20
		twig (fresh)	9.9	>20

Table 3 Quinone reductase induction activity of plants in the mangrove forest (cont.)

	Plant Name	Part Used	QR	
			CD ($\mu\text{g/ml}$)	IC ₅₀ ($\mu\text{g/ml}$)
18	<i>Lumnitzera racemosa</i> Willd.	leaves	>10	>20
		fruit	>20	>20
19	<i>Nypa fruticans</i> Wurmb.	young inflorescence	>10	>20
20	<i>Phoenix paludosa</i> Roxb.	twig (dried)	8.8	>20
		twig (fresh)	>20	>20
21	<i>Rhizophora apiculata</i> Blume	leaves	>20	>20
		young pods	>20	>20
		branches	>20	>20
22	<i>Rhizophora mucronata</i> Poir.	mature pods	>10	>20
		young pods	>20	>20
		leaves	>10	>20
23	<i>Sarcolobus globosus</i> Wall.	rind	>10	>20
		seeds	>10	>20
24	<i>Sonneratia alba</i> J. Smith	sepals	>10	>20
25	<i>Sonneratia caseolaris</i> (L.) Engl.	stamen	>10	>20
		sepals	>10	>20
		fruit	>20	>20
26	<i>Sonneratia ovata</i> Back	fruit	>10	>20
27	<i>Stenochlaena palustris</i> (Burm.f.) Bedd.	twig (dried)	>20	>20
		leaves (dried)	>10	>20
		twig	>10	>20
28	<i>Suaeda maritima</i> Dum. (Nakornsrihamarat)	twig	>10	>20
	<i>Suaeda maritima</i> Dum. (Samut songkram)	twig	>20	>20
	<i>Suaeda maritima</i> Dum. (Samut songkram)	twig	>20	>20
29	<i>Trianthema decandra</i> L.	twig (dried)	9.2	>20
30	<i>Weddelia biflora</i> (L.) DC.	flowers	>20	>20

Table 3 Quinone reductase induction activity of plants in the mangrove forest (cont.)

	Plant Name	Part Used	QR	
			CD ($\mu\text{g/ml}$)	IC ₅₀ ($\mu\text{g/ml}$)
31	<i>Xylocarpus granatum</i> Koen.	seeds	>10	>20
		rind	>20	>20
32	<i>Xylocarpus rumphii</i> (Kostel.) Mabblerley	rind	>20	>20
		seeds	>20	>20

QR = quinone reductase induction, CD = double specific activity, IC₅₀ = 50% inhibition of cell growth

DISCUSSION

The oxidation process attributes to several degenerative diseases and contribute significantly to the risk of human aging, cancer and atherosclerosis. The attempt to search for new antioxidant may lead to development of antiatherosclerotic or anticarcinogenic drugs.

Among the plants tested, *Sonneratia caseolaris* (L.) Engl. sepals showed the strongest activity with EC₅₀ 2.57 mg/ml and *S. caseolaris* (L.) Engl. stamens, *Cynometra ramiflora* L. seeds, *Xylocarpus rumphii* rind also showed strong activity. It has been reported that phenolic compounds including tannin possess antioxidant activity (Hatano et al., 1989; Satoshi & Hara, 1990; Wang et al., 1999). β -Carotene, the compound commonly found in the green vegetable is also active (Young, 1991). It is possible that all these compounds may be responsible for the free radical scavenging activity of the plants from the mangrove forest. The antioxidant compounds were detected by TLC and found 2-12 active compounds. Further studies on isolation of the active compounds are

recommended.

Lipid peroxidation plays an important role in aging process and some chronic diseases including diabetes, nervous disorder, cardiovascular diseases and cancer (Takahashi et al., 1992, Iwatsuki et al., 1995, Wang et al., 1999). Some of the plants tested also showed strong activity e.g. *Bruguiera parviflora* Wight & Arn.ex Griff. young pods, *Cynometra ramiflora* L.seeds, *Lumnitzera racemosa* Willd. leaves, *Nypa fruticans* Wurmb. inflorescence, *Rhizophora mucronata* Poir. young pods and *Sonneratia caseolaris* (L.) Engl. fruit. Some of these plants are edible, further studies will lead to finding other sources of antioxidant compounds.

Many antioxidant compounds exhibited carcinogenesis inhibition, therefore in this study, quinone reductase induction test was used. Quinone reductase is Phase II enzyme which is a detoxifying enzyme. The stimulation of phase II enzyme is important in cancer chemoprevention process (Van Bladeren, 1993; Pezzuto,

1995). Among the samples tested, *Bruguiera gymnorrhiza* (L.) Savigny flowers exhibited the strongest activity with CD 3.6 mg/ml. Further isolation of active components is in progress.

Based on the above results, the plants in the mangrove forest showed the potential as a source of antioxidant and cancer chemoprevention agents and further studies may lead to drug development.

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