Original Article

Mahidol University Journal of Pharmaceutical Science 2012; 39 (1), 9-18

Biological Activities of Medicinal Plants from Mangrove and Beach Forests

N. Soonthornchareonnon[1], C. Wiwat[2], and W. Chuakul[3]*

[1] *Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand*
[2] *Department of Microbiology, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand*
[3] *Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand*

Abstract

Mangrove and beach forests 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 include antimicrobial activity and cytotoxicity as well as phytochemical screening and Thin Layer Chromatographic fingerprint of the samples are performed. Thirty one samples of 24 species from Welu wetland, Chanthaburi province were tested. The cytotoxicity was done by Brine Shrimps Lethality test (BST), plants possessed cytotoxicity are *Arcangelisia flava* Merr. (roots), *Melaleuca cajuputi* Powell. (leaves), and *Pluchea indica* Less. (leaves) with LD_{50} 31.7, 249.4 and 559.1 µg/ml, respectively. The majority of the 80% alcoholic extracts inhibited *Staphylococcus aureus,* and *Bacillus cereus*. *Bruguiera gymnorrhiza* (fruits) and *Lumnitzera littorea* (twig) exhibited the strong antimicrobial activity against *S. aureus,* and *B. cereus* with MIC 0.0312 mg/ml and 0.0625 mg/ml, respectively. Only *B. gymnorrhiza* (fruits) inhibited *Candida albicans* with MIC 0.0312 mg/ml. Phytochemical screening of the active plants was conducted by color test and TLC. The result revealed that the most chemical substances found in the plants were tannins, phenolic compounds and flavonoids.

Key words: Mangrove forest, Beach forest, Welu wetland, Chantaburi province, Antimicrobial activity, Cytotoxicity

INTRODUCTION

Mangrove and beach forests occur in most tropical and subtropical regions of the world. This group of plants that grows along the coastline is very important to the ecosystem diversity because they protect the coastline from destruction (maintain the ecosystem diversity) and provide many resources for utilization in the forestry, fisheries, food, agricultural and medicinal industries. Several plants are used for medical purposes e.g. the upper parts of *Acanthus ebracteatus* Vahl. for skin infection treatment[1], the leaf and flower of *Pluchea indica* for tuberculosis treatment[2], the bark of *Avicennia alba* Blume for wound[3], etc. The purpose of this study is to evaluate the biological potential of the plants in the mangrove and beach forests for antimicrobial activity and cytotoxicity, moreover the phytochemical screening will be tested for the medicinal plants which possess biological activities. The results from this study will serve as the basis for drug development.

***Corresponding author:** Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, 447 Sri-Ayuthaya Road, Rajthevi, Bangkok 10400, Thailand.
E-mail: pynsk@mahidol.ac.th Tel. +66 (0) 2644-8677-91 Ext. 5527 Fax +66 (0) 2644-8701

MATERIALS AND METHODS

Plant materials

Thirty one samples of 24 species from mangrove and beach forests in Welu wetland, Chanthaburi province were collected in March-May 2010. The taxonomic identity of the plants was confirmed by Professor Wongsatit Chuakul, Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University. Voucher herbarium specimens have been deposited at the herbarium of the Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University. The plant material was shade dried and then ground in a Wiley grinder with a 2 mm diameter mesh.

Brine shrimp lethality test (BST):

Brine shrimp lethality bioassay (BST) is a test which easily mastered, costs little, and utilizes small amount of test material[4,5]. It is predictive of cytotoxicity, antimalarial and pesticidal activities[6].

Hatching the brine shrimp

Brine shrimp eggs (*Artemia salina*, Sanders TM Great Salt Lake, Brine Shrimp Company L.C., U.S.A.) were hatched in artificial sea water prepared from commercial sea salt (Aqua Marine, Thailand) 40 g/l. The two unequal compartments plastic chamber with several holes on the divider was used for hatching. The eggs were sprinkled into the larger compartment which was dark, while the smaller compartment was illuminated. After 36 hours incubation at room temperature (25-29°C), nauplii (larvae) were collected by pipetting from the lighted side whereas their shells were left in another side.

Bioassay

The procedure for BST was modified from the assay described by Solis et al.[5] and berberine sulfate was used as positive control. Two milligrams of the extracts were made up to 2 mg/ml in artificial sea water except for water insoluble compounds which were dissolved in DMSO 30 µl prior to adding sea water. Serial dilutions were made in the wells of 96-well microplates (Nunc, Denmark) in triplicate in 120 µl sea water. Control wells with DMSO were included in each experiment. A suspension of nauplii containing 4-5 organisms was added to each well. The plates were covered and incubated at room temperature (25-29°C) for 24 hours. Plates were then examined under the binocular stereomicroscope and the numbers of dead (non-motile) nauplii in each well were counted. One hundred microliters of methanol were then added to each well to immobilize the nauplii and after 15 minutes the total numbers of brine shrimp in each well were counted. Analysis of the data was performed by probit analysis on a Finney computer program to determine the lethal concentration to half of the test organisms (LC_{50})[7].

Antimicrobial test

Agar diffusion susceptibility test

Microorganisms tested

The microorganisms used to assess the antimicrobial property were four quality control isolates of bacteria; Gram positive: *Staphylococcus aureus* ATCC 25923, and *Bacillus cereus* ATCC 14579, Gram negative: *Escherichia coli* ATCC 25922 and *Salmonella typhimurium*, including a fungus; *Candida albicans* ATCC 10231. The microorganisms were grown in Mueller-Hinton broth for bacteria and Sabouraud Dextrose Broth for fungi and incubated at 37ºC for 16-18hr. The inoculum was prepared from direct colony suspension equivalent to 0.5 McFarland turbidity standard and standardized to yield 0.5 colony forming units (CFU)/ml. The suspension was swabbed on the agar surface (Mueller-Hinton agar for bacteria and Sabouraud-4% Glucose Agar for *C. albicans*) by using swab cotton.

Disc diffusion test:

Conventional disc diffusion method[8,9] was employed for the initial assessment of antimicrobial potential of the extracts. Sterile 6.0 mm diameter blank discs were impregnated

with test substances at the dose of 5 and 10 mg/disc. These discs, along with the positive control discs (ampicillin 10 µg/disc, sulphamethoxazole-trimethoprim 25 µg/disc, norfloxacin 10 µg/disc and amphotericin 100 µg/disc) were placed on petri dishes containing a suitable agar medium seeded with the test organisms using sterile transfer loop. The plates were kept in an incubator (37^0C) for 18-24 hr. to allow the growth of the microorganisms. The antimicrobial activity of the test extracts were determined by measuring the diameter of the zone of inhibition in terms of millimetre.

MIC and MBC determination using broth dilution methods

MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration**)** were determined by microdilution method[10] and using ampicillin and amphotericin B as antibiotic positive controls (Sigma Chemical Co., St. Louis, USA). Inoculates were prepared in the same medium at density adjusted to 0.5 McFarland turbidity standard and two fold dilution. The inoculated tubes were incubated at 37^0C and the MICs were recorded after 24 hr of incubation. The MIC was defined as the lowest concentration of plant extract or positive controls at which the microorganism tested did not showed visible growth, while MBC was defined as the minimum bactericidal concentration with negative subcultures on the agar medium. Values were means of three measurements.

Phytochemical screening

Phytochemical screening of 10 biological active plants was carried out according to Farnsworth (1966)[11].

Preparation of extract for phytochemical screening : Dried and powdered plant materials (100 g) were macerated with 80% ethanol (500 ml) for 5 days and then filtered to give an ethanolic extract.

Tests for alkaloids : The ethanolic extract (30 ml) was evaporated to dryness in an evaporating dish on a water bath. Five ml of 2N HCl were added and stirred while heating on the water bath for 10 min., cooled, filtered and the filtrate was treated with a few drops of Dragendorff's, Hager's, Marme's, Mayer's, Valser's, Wagner's and tannic acid reagents. The samples were then observed for the presence of turbidity or precipitation.

Tests for flavonoids : The ethanolic extract (30 ml) was evaporated to dryness on a water bath, cooled and the residue was defatted by washing several times with petroleum ether. The defatted residue was dissolved in 30 ml 80% ethanol and filtered. The filtrate was treated with 1 ml of concentrated HCl and magnesium ribbons (0.5 g). The presence of flavonoids was indicative if a pink or magenta-red color developed within 3 min.

Tests for tannins : The alcoholic extract (25 ml) was evaporated to dryness on a water bath. The residue was dissolved with saline solution, filtered and the volume of filtrate was adjusted to 10 ml with more saline solution. To 2 ml of this solution, few drops of gelatin solution were added, the samples were then observed for the presence of precipitation. Another 2 ml of the solution, few drops of ferric chloride test reagent were added. An intense green or blue color was taken as an evidence for the presence of hydrolysable or condensed tannins or polyphenolic compounds.

Tests for saponins: One gram of dried ethanolic extract was dissolved in 10 ml of distilled water in a test tube and shaked vigorously for 1-2 min. The presence of saponins was indicated by characteristic honeycomb froth at least 1 cm in height, which persisted for 30 min.

Tests for anthraquinone glycosides: One gram of the powdered plant material, 10 ml of 0.5 N potassium hydroxide containing 1 ml of 3% hydrogen peroxide solution was added. The suspension was boiled for 3-5 min. then cooled, filtered and the filtrate was acidified with 10 drops of glacial acetic acid. This acidified mixture was extracted by shaking with 10 ml of dichloromethane. A 5 ml aliquot of the dichloromethane solution was shaken with 3 ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. A pink to red coloration of the alkaline layer indicated the presence of anthraquinone.

RESULTS AND DISCUSSION

The plant species analyzed representing 31 samples of 24 species from mangrove and beach forests in Welu wetland, Chanthaburi province were shown in Table 1. The cytotoxicity was done by Brine Shrimps Lethality test (BST), crude extracts resulting in LC_{50} values of less than 250 µg/ml were considered significantly active and had the potential for further investigation[12]. They were the extracts of *Arcangelisia flava* Merr. (roots), and *Melaleuca cajuputi* Powell. (leaves) with LD_{50} 31.7 and 249.4 559.1 µg/ml, respectively (Table 2). The results presented in Table 3 demonstrate that most of the samles were effective towards Gram-positive bacteria than Gram-negative bacteria. It is therefore theorized that Gram-positive bacteria are more susceptible than Gram negative bacteria due to the differences in their cell wall structure. The crude extracts which showed remarkably potent antibacterial activity against *S. aureus* at the concentration of 5 mg/ml were *Wedelia biflora* (leaves), *Lumnitzera littorea* (twig), *Arcangelisia flava* (roots), *Ancistrocladus tectorius* (stem), *Phyllanthus emblica* (stem bark), *Thespesia populnea* (leaves), *Premna obtusifolia* (roots), *Uvaria rufa* (twig), *Heritiera littoralis* (twig), and *Avicennia alba* (leaves). The crude extracts which showed active towards Gram negative bacteria were *Wedelia biflora* (leaves), *Melaleuca quinquenervia* (leaves), *Ardisia helferiana* (roots), *Thespesia populnea* (leaves), *Salacia chinensis* (leaves), and *Phyllanthus emblica* (stem bark). Only *B. gymnorrhiza* (fruits) inhibited *C. albicans*. The MIC and MBC values of *B. gymnorrhiza* (fruits) towards *S. aureus*, *B. cereus*, and *C. albicans* were 0.312, 1.250; 0.625, and 0.625; 0.312, >5 mg/ml, respectively. The MIC and MBC values of *L. littorea* (twig) towards *S. aureus*, and *B. cereus* were 0.312, 0.625; and 0.625, 0.625 mg/ml, respectively. The MIC and MBC values of *W. biflora* (leaves) towards *S. aureus*, and *E. coli* were equal concentration of 0.625, and >5 mg/ml, respectively. The phytochemical screening of the 10 active crude extracts in the present study revealed the presence of flavonoids, tannins, and phenolic compounds (Table 5). From these findings, we can assume that flavonoids, tannins, and phenolic compounds in the extracts may show the antibacterial activities.

CONCLUSION

Our findings demonstrate for the first time that ethanolic extracts of *B. gymnorrhiza* (fruits), *L. littorea* (twig) and *W. biflora* (leaves) have potent antibacterial activity against Gram-positive bacteria and only *B. gymnorrhiza* (fruits) showed active towards yeast. The result concluded that *B. gymnorrhiza* (fruits) exhibited a broader spectrum of antibacterial activity. The demonstration of broad spectrum of *B. gymnorrhiza* (fruits) may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease. However, the effect of these plants on more pathogenic organisms, and toxicological investigations and further purification need to be carried out.

Based on the above results, the plants in the mangrove and beach forests showed the potential as a source of antimicrobial and cytotoxic agents and further studies may lead to drug development.

ACKNOWLEDGEMENTS

This research work was financially supported by Graduate School of Asian and African Area Studies, Kyoto University. The authors are grateful to Mr. Sommai Suppakun, Head of Welu wetland, Chanthaburi province for his kind providing the samples. The authors are thankful to Department of Pharmacognosy, and Department of Microbiology, Faculty of Pharmacy, Mahidol University for providing laboratory facilities.

Table 1. Plant species analyzed from mangrove and beach forests

Family	Plant species	Trivial name	Part used
Acanthaceae	*Acanthus ebracteatus* Vahl.	Ngueak Plamo	Twig
Ancistrocladaceae	*Ancistrocladus tectorius* (Lour.) Merr.	Lin kwang, Khan song	Stem
Annonaceae	*Polyalthia evecta* (Pierre) Finet&Gagnep.	Nom noi	Leaves
Annonaceae	*Uvaria rufa* Blume	Phi phuannoi	Twig
Avicenniaceae	*Avicennia alba* Blume	Samaekhao	Twig, Leaves
Celastraceae	*Salacia chinensis* L.	Kamphaengchetchan	Leaves
Combretaceae	*Lumnitzera littorea* (Jack) Voigt	Fat daeng	Twig
Asteraceae (Compositae)	*Pluchea indica* (L.) Less.	Khlu	Leaves
Asteraceae (Compositae)	*Wedelia biflora* (L.) DC.	Phakkhratthale, Ben chamatnamkem	Leaves
Dilleniaceae	*Tetracera loureiri* (Finet&Gagnep) Pierre ex Craib	Rot sukhon	Leaves, Vines
Euphorbiaceae	*Phyllanthus emblica* L.	Ma khampom	Stem bark
Lamiaceae (Labiatae)	*Premna obtusifolia* R.Br.	Cha lueat	Roots
Fabaceae	*Intsia bijuga* (Colebr.) Kuntze	Lumphothale,	Twig, Leaves
Fabaceae	*Peltophorum dasyrachis* (Miq.) Kurz	A rang, In si	Stem bark, Twig
Fabaceae	*Derris trifoliate* Lour.	Thopthaepnam	Twig
Malvaceae	*Hibiscus tiliaceus* L.	Po thale	Leaves
Malvaceae	*Thespesia populnea* (L.) Soland.ex Corr.	Pho thale	Twig, Leaves
Melastomataceae	*Melastoma saigonense* (Kuntze) Merr.	Khlongkhlengyuan	Leaves
Menispermaceae	*Arcangelisia flava* (L.) Merr.	Khaminkhruea, Khaminpra	Roots
Myrsinaceae	*Ardisia helferiana* Kurz	Som kung khon, Ta pla	Roots
Myrtaceae	*Melaleuca quinquenervia* (Cav.) S.T. Blake	Sametkhao	Leaves
Rhizophoraceae	*Bruguiera gymnorrhiza* (L.) Savigny	Kongkanghua sum, Prasak	Leaves, Fruit
Rutaceae	*Glycos mispentaphylla* (Retz.) DC.	Khoei tai	Leaves
Sterculiaceae	*Heritiera littoralis* Dryand.	Ngonkaithale	Leaves, Twig

Table 2. Brine shrimp bioassay of plant extracts

Plant species	Part used	% yield	LC_{50} (µg/ml)
Acanthus ebracteatus Vahl	Twig	2.6	>1000
Ancistrocladus tectorius (Lour.) Merr.	Stem	2.8	>1000
Polyalthia evecta (Pierre) Finet&Gagnep.	Leaves	0.9	>1000
Uvaria rufa Blume	Twig	2.8	>1000
Avicennia alba Blume	Leaves	6.2	>1000
	Twig	2.1	>1000
Salacia chinensis L.	Leaves	2.6	>1000
Lumnitzera littorea (Jack) Voigt	Twig	2.4	>1000
Pluchea indica (L.) Less.	Leaves	2.7	559.1
Wedelia biflora (L.) DC.	Leaves	4.3	>1000
Tetracera loureiri (Finet&Gagnep) Pierre ex Craib	Leaves	4.6	>1000
	Vines	2.9	>1000
Phyllanthus emblica L.	Stem bark	4.3	>1000
Premna obtusifolia R.Br.	Roots	3.7	>1000
Intsia bijuga (Colebr.) Kuntze	Leaves	11.4	>1000
	Twig	3.1	>1000
Peltophorum dasyrachis (Miq.) Kurz	Stem bark	11.7	>1000
	Twig	1.4	>1000
Derris trifoliate Lour.	Twig	2.0	>1000
Hibiscus tiliaceus L.	Leaves	3.8	>1000
Thespesia populnea (L.) Soland.ex Corr.	Leaves	13.0	>1000
	Twig	1.5	>1000
Melastoma saigonense (Kuntze) Merr.	Leaves	5.5	>1000
Arcangelisia flava (L.) Merr.	Roots	2.7	31.7
Ardisia helferiana Kurz	Roots	2.9	>1000
Melaleuca quinquenervia (Cav.) S.T. Blake	Leaves	7.2	249.4
Bruguiera gymnorrhiza (L.) Savigny	Leaves	5.2	>1000
	Fruit	6.1	>1000
Glycosmis pentaphylla (Retz.) DC.	Leaves	4.4	>1000
Heritiera littoralis Dryand.	Leaves	3.2	>1000
	Twig	2.7	>1000
Berberine sulfate			22.3

Table 3. Antimicrobial activity of crude extracts against standard microorganisms.

Plants	Part used	Clear zone (mm.)									
		S. aureus		***B. cereus***		***E. coli***		***S. typhimurium***		***C. albicans***	
		5 mg	**10 mg**	**5 mg**	**10 mg**	**5 mg**	**10 mg**	**5 mg**	**10 mg**	**5 mg**	**10 mg**
Acanthus ebracteatus	Twig	-	9.2	-	-	-	-	-	-	-	-
Ancistrocladus tectorius	Stem	15.0	15.1	9.9	11.0	-	-	-	-	-	-
Polyalthia evecta	Leaves	9.8	11.1	10.0	10.8	-	-	-	-	-	-
Uvaria rufa	Twig	12.1	12.9	9.8	10.2	-	-	-	-	-	-
Avicennia alba	Twig	10.5	12.8	-	7.9	-	-	-	-	-	-
	Leaves	11.5	14.5	9.0	10.4	-	-	-	-	-	-
Salacia chinensis	Leaves	8.0	9.4	7.0	7.8	7.5	8.0	-	-	-	-
Lumnitzera littorea	Twig	18.5	19.0	11.8	12.8	-	-	-	-	-	-
Pluchea indica	Leaves	9.5	11.5	7.9	10.0	-	-	-	-	-	-
Wedelia biflora	Leaves	22.5	32.5	-	-	13.4	21.5	-	-	-	-
Tetracera loureiri	Leaves	11.1	12.2	9.2	10.4	-	-	-	-	-	-
	Vines	12.6	14.1	9.8	10.9	-	-	-	-	-	-
Phyllanthus emblica	Stem bark	14.9	16.4	10.6	12	-	7.5	-	-	-	-
Premna obtusifolia	Roots	13.0	14.1	-	-	-	-	-	-	-	-
Intsia bijuga	Twig	9.6	11.3	6.9	8.6	-	-	-	-	-	-
	Leaves	-	7.2	-	-	-	-	-	-	-	-
Peltophorum dasyrachis	Stem bark	11.0	13.1	9.5	10.1	-	-	-	-	-	-
Derris trifoliata	Twig	-	-	9.5	11.1	-	-	-	-	-	-

Table 3. (cont.) Antimicrobial activity of crude extracts against standard microorganisms.

Plants	Part used	Clear Zone (mm.)									
		S. aureus		*B. cereus*		*E. coli*		*S. typhimurium*		*C. albicans*	
		5 mg	10 mg	5 mg	10 mg	5 mg	10 mg	5 mg	10 mg	5 mg	10 mg
Hibiscus tiliaceus	Leaves	6.1	6.6	7.2	8.1	-	-	-	-	-	-
Thespesia populnea	Twig	14.5	15.1	-	-	-	-	-	-	-	-
	Leaves	10.6	12.1	10.2	11.2	-	7.4	-	-	-	-
Melastoma saigonense	Leaves	-	8.1	7.8	9.5	-	-	-	-	-	-
Arcangelisia flava	Roots	17.2	17.1	9.6	8.0	-	-	-	-	-	-
Ardisia helferiana	Roots	9.5	11.2	8.0	10.4	-	8.2	-	6.9	-	-
Melaleuca quinquenervia	Leaves	10.9	13.4	10.2	11.5	9.0	14.1	-	-	-	-
Bruguiera gymnorrhiza	Leaves	6.8	9.1	7.2	8.5	-	-	-	-	-	-
	Fruit	11.1	13.4	10.6	12.5	-	-	-	-	38.1	44.1
Glycosmis pentaphylla	Leaves	-	-	-	-	-	-	-	-	-	-
Heritiera littoralis	Twig	11.6	13.6	11.4	12.4	-	-	-	-	-	-
	Leaves	9.9	11.5	10.2	11.0	-	-	-	-	-	-
Ampicillin (10 µg)		47.0		13.86		38.7		26.7		-	
SXT (25 µg)		31.8		14.2		30.7		30.4		-	
Norfloxacin (10 µg)		29.6		26.8		38.3		37.1		-	
Amphotericin B (100 µg)		-		-		-		-		18.9	

(-) = no inhibitory effects observed; S. aureus = Staphylococcus aureus ATCC 25923, B. cereus = Bacillus cereus ATCC 14579, E. coli = Escherichia coli ATCC 25922, S. typhimurium = Salmonella typhimurium, C. albican = Candida albicans ATCC 10231.

Table 4. The MIC and MBC values of active plant extracts.

Plants	Part used	MIC and MBC (mg/ml)			
		S. aureus	***B. cereus***	***E. coli***	***C. albicans***
Bruguiera gymnorrhiza	Fruits	MIC = 0.312 MBC = 1.250	MIC = 0.625 MBC = 0.625	-	MIC = 0.312 MBC >5
Lumnitzera littorea	Twig	MIC = 0.312 MBC = 0.625	MIC = 0.625 MBC = 0.625	-	-
Wedelia biflora	Leaves	MIC = 0.625 MBC >5	-	MIC = 0.625 MBC >5	
Ampicillin		0.5 µg/ml	2.0 µg/ml	0.5 µg/ml	-
Amphotericin B		-	-	-	1.0 µg/ml

Table 5. Phytochemical screening of 10 active plant extracts.

Plants	**Part used**	**Alkaloids**	**Anthraquinones**	**Flavonoids**	**Saponins**	**Tannins**	**Phenolic compounds**
Ancistrocladus tectorius	Stem	-	-	+	-	+	+
Avicennia alba	Leaves	-	-	+	-	-	+
Lumnitzera littorea	Twig	-	-	+	-	+	+
Wedelia biflora	Leaves	-	-	+	-	-	+
Phyllanthus emblica	Stem bark	-	-	+	-	+	+
Premna obtusifolia	Roots	-	-	+	-	+	+
Thespesia populnea	Leaves	-	-	+	-	+	+
Bruguiera gymnorrhiza	Leaves	-	-	+	-	+	+
	Fruit	-	-	+	-	+	+
Heritiera littoralis	Twig	-	-	+	-	+	+

REFERENCES

1. Sittiwet C, Niamsa N, Puangpronpitag D. Antimicrobial activity of *Acanthus ebracteatus* Vahl. aqueous extract: The potential for skin infection treatment. *Int J Biol Chem* 2009; 3:95-8.
2. Mohamad S, Zin NM, Wahab HA, *et al*. Antituberculosis potential of some ethnobotanically selected Malaysian plants. *J Ethnopharmacol* 2011; 133 (3): 1021-6.
3. Yaadfon Association. Use of medicinal plants in mangrove forest. Trang: Green Group, 1981.
4. Meyer BN, Ferrighi NR, Putnam JE, *et al*. Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica* 1982; 45:31-4.
5. Solis PN, Wright CW, Anderson MM, *et al*. A microwell cytotoxicity assay using *Artemia salina* (brine shrimp). *Planta Medica* 1993; 59:250-2.
6. Ghisalberti EL. Detection and isolation of bioactive natural products. In Colegate SM, Molyneux RJ (Eds). Bioactive Natural Products: Detection, Isolation and Structure Elucidation. Boca Raton: CRC Press, 1993. pp. 15-8.
7. Finney DJ. Probit Analysis. 3thed. Cambridge: Cambridge University Press, 1971. pp. 76-80.
8. Bauer AW, Kirby WMM, Sherris JC, *et al*. Antimicrobial susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1966; 45:493-6.
9. Cruickshank R. Medical microbiology: A guide to diagnosis and control of infection. London: E. and S. Livingstone Ltd., 1968. p. 888.
10. NCCLS. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically: Approved Standard, 5th ed. NCCLS documents M7-A5. NCCLS: Wayne, PA, USA, 2000.
11. Farnswortth NR. Biological and phytochemical screening of plants. *J Pharm Sci* 1966; 55(3):245-76.
12. Rieser MJ, Gu ZM, Fang XP, *et al*. Five novel mono-tetrahydrofuran ring acetogenins from the seeds of *Annona muricata. J Nat Prod* 1996; 59:100-8.