Bioactivity Screening of Crude Alkaloidal Extracts from Some Rubiaceae

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ABSTRACT

The crude alkaloidal extracts were isolated from the leaves of Morinda tomentosa, Nauclea cordifolia and Nauclea cadamba, belonging to the family Rubiaceae. These crude alkaloids were subjected to chemical test for alkaloid. The bioactivity screening of these plant extracts was also carried out, including anti-malarial, anti-bacterial, anti-cancer and anti-HSV1 activities and cytotoxicity. The crude alkaloidal extracts of M. tomentosa and N. cordifolia exhibited activities against Herpes simplex virus type 1 while that of N. cadamba showed activity against Plasmodium falciparum K 1 strain.

Key words: Bioactivity screening, Crude alkaloids, Rubiaceae

INTRODUCTION

The family Rubiaceae consists of 650 genera and 10,500 species under worldwide distribution. Most species are herbaceous. There are about 55 genera in the Northern part of Thailand (Kardner, 2000).

The most-popular member of this family is coffee, known as a major economic and the most important crop. Various species of Cinchona are the source of quinine used as a cure against malaria. This family is characterized by the opposite leaves or whorled and stipulate; epipetalous stamen and the inferior ovary; flower bisexual, regular; calyx tube adnate to ovary; fruit capsular, baccate or drupaceous; embryo large, in rich endosperm (Keng, 1969).

The genus Nauclea is known to produce many kinds of indole alkaloids, having some significant biological activities such as antiproliferative, antiparasitic and antimicrobial for use as seed molecules for drug development. The roots of *Morinda tomentosa*, crushed in alcohol, provide a medicine to stop vomiting in cholera (P/etelot, 1952). A decoction of the leaves and roots is prescribed to treat ague; a poultice of them is applied to the spleen (Ridley, 1906); the bark is astringent and febrifuge (Foxworthy, 1922). The roots of *Nauclea cordifolia* are used as an astringent in dysentery; the bark is acrid, bitter, pungent and is used to cure

inflammations, biliousness, disease of the blood and skin disease (Indian Medicinal Plants). The leaves of *Nauclea cadamba* are used as an ingredient of a gargle (Roger, 1939), an oiled-heated leaf is applied to the chest or abdomen to treat fever or malaria (Burkill and Haniff,1930). The bark is also tonic and bechic (Foucaud, 1953).

The aim of this research is to investigate the bioactivity of crude alkaloids from some Rubiaceae which may play an important role in drug development.

METERIALS AND METHODS

Plant Materials: Fresh leaves of *Morinda tomentosa*, *Nauclea cordifolia* and *Nauclea cadamba* were collected from Chiang Mai Province, North of Thailand in April, 2003 and identified by Maxwell at the Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand. Voucher specimens of these plants are deposited at the Herbarium of the Faculty of Pharmacy of the above University.

Extraction: The leaves of medicinal plants studied were dried at 50^{o}C and powdered. About 1–3 kg of plant materials were macerated with three successive portions of 3 litres of methanol and filtered. The combined filtrates were then concentrated under reduced pressure to dryness to give crude extracts. The extract of each plant material was then partitioned between ethyl acetate and 5% aqueous sulphuric acid. The water-soluble fraction was alkalinized with concentrated ammonium hydroxide (pH 10) and exhaustively extracted with chloroform. The organic layer was dried over anhydrous magnesium sulphate and evaporated to give crude alkaloidal fraction (Table 1). The crude alkaloidal fraction of each plant was subjected to chemical test for alkaloid and bioactivity screening test.

Chemical test for alkaloid:

Preparation of Dragendoff's test solution: Bismuth subnitrate 0.85 g was dissolved in 10 ml of glacial acetic acid and 40 ml of water (A). Eight grams of potassium iodide were dissolved in 20 ml of water (B). Both solutions (A) and (B) were stored and protected from light. Immediately before use, equal volumes of (A) and (B) were mixed with glacial acetic acid.

Preparation of Mayer's test solution: Mercury (II) chloride 1.358 g was dissolved in 60 ml of water. Five grams of potassium iodide were dissolved in 10 ml of water. Both solutions were mixed and adjusted to 100 ml with distilled water.

Primary Screening: Appropriate amounts of crude alkaloidal extract from each plant (Table 1) were dissolved in 0.5 ml of dilute hydrochloric acid. The solutions were tested with Dragendoff's test solution and Mayer's test solution.

Secondary Screening: Appropriate amounts of crude alkaloidal extract from each plant were dissolved in chloroform and applied on a silica gel GF_{254} TLC plate by means of a capillary tube. The separation was performed by using hexane:chloroform:methanol (3:5:2) as mobile phase.

Bioactivity Screening Test: Appropriate amounts of crude alkaloidal extract from each plant were subjected to bioactivity screening tests including: anti-malarial activity, anti-bacterial activity, anti-HSV-1 activity, anti-cancer activity and cytotoxicity.

RESULTS AND DISCUSSION

The crude alkaloids of *Morinda tomentosa, Nauclea cordifolia* and *Nauclea cadamba* leaves were isolated and the percentage yield of each plant extract was determined. Results are presented in Table 1. which indicates that *Nauclea cadamba* gave higher percentage yield of crude alkaloid than *Nauclea cordifolia* and *Morinda tomentosa.*

Table 1. Percentage yields of crude alkaloid from Rubiaceae leaves.

Plant Name	Powdered leaves wt (kg)	Crude Extract wt (gm)	Crude Alkaloids	
			wt (gm)	% by wt.
Morinda tomentosa	1.4	321.01	1.04	0.01
Nauclea cordifolia	2.5	730.02	2.69	0.10
Nauclea cadamba	2.7	340.00	43.89	1.62

According to the primary chemical screening test, a few drops of Dragendoff's reagent were added into appropriate volumes of crude alkaloidal extract (in dilute hydrochloric acid) of each plant and yellow precipitates were observed. Another portion of the sample solution of each plant was tested with Mayer's reagent and white precipitates were observed. These indicated that there were alkaloids present in all samples. However heavy yellow and white precipitates were observed in the crude alkaloidal extract of *Nauclea cadamba.* Results are shown in Table 2.

Table 2. Primary chemical screening test of crude alkaloidal extracts.

Plant Name	Dragendoff's test	Mayer's test
Morinda tomentosa	+	+
Nauclea cordifolia	+	+
Nauclea cadamba	+++	+++

Note: + = slightly positive test, +++ = very strong positive test

In the case of secondary chemical screening test, thin-layer chromatographic technique was used to confirm the primary chemical screening test results. Appropriate volumes of crude alkaloidal extract from *Morinda tomentosa*, *Nauclea cordifolia* and *Nauclea cadamba* were applied on separate silica gel GF_{254} TLC plate, using hexane:chloroform:methanol (3:5:2) as mobile phase. After development, the chromatograms were sprayed with Dragendoff's reagent as shown in Fig 1. Positive test of alkaloids was observed on each chromatogram. However, for the chromatogram of *Nauclea cadamba*, very intense spots of alkaloid were present.

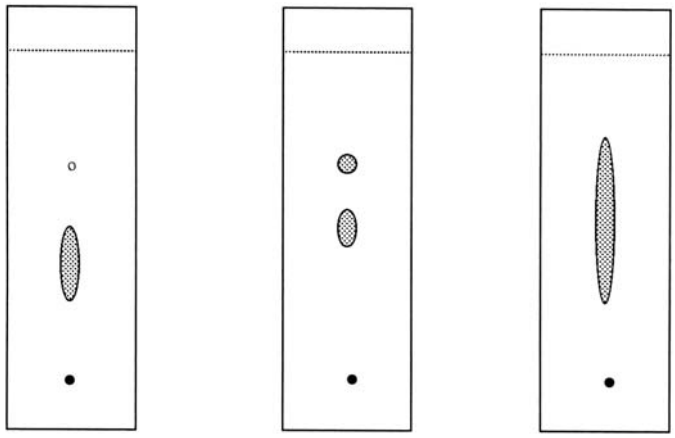

Figure 1. Thin-layer chromatogram of crude alkaloids.

Adsorbent: silica gel GF_{254}; Solvent: Hexane-chloroform- methanol (30+50+20); visualisation: spray with Dragendoff's reagent.

1. Nauclea cordifolia 2. Nauclea cadamba 3. Morinda tomentosa

Bioactivity Studies

Anti-malarial Activity: Appropriate amounts of *Morinda tomentosa, Nauclea cordifolia* and *Nauclea cadamba* were dissolved in dimethyl sulfoxide (DMSO) in three separate vials. The anti-malarial studies were carried out at the tested concentration of 10 μg/ml. The crude alkaloidal extract of *Nauclea cadamba* exhibited anti-malarial activity against *Plasmodium falciparum,* K1 strain with the activity EC_{50} = 3.7 μ/ml, using dihydroartimisinin as positive control with the activity EC^{50} = 4.4 nM. But the crude alkaloidal extracts of *Morinda tomentosa* and *Nauclea cordifolia* were inactive (Table 3).

Table 3. Anti-malarial activity of crude alkaloids against *Plasmodium falciparum,* K1 strain.

Crude Alkaloid of Plant	Dissolved Solvent	Activity EC_{50} (μg/ml)
Morinda tomentosa	DMSO	Inactive
Nauclea cordifolia	DMSO	Inactive
Nauclea cadamba	DMSO	3.7

Note: Tested concentration = 10 μg/ml

Dihydroartimisinin was used as positive control with EC_{50} = 4.4 nM

Anti-bacterial activity: The samples were dissolved in methanol. The anti-bacterial activity of each sample solution was investigated towards Gram-negative and Gram-positive bacteria. All samples inhibited the growth of bacteria in concentration of 10% weight by volume, using methanol as solvent (Table 4).

Table 4. Anti-bacterial activity of crude alkaloids against Gram-negative and Gram-positive bacteria.

Description	Inhibition zone (MM.)		
	Staphylococcus aureus **ATCC 25923**	***Escherichia coli*** **ATCC 25922**	***Pseudomonas aeruginosa*** **ATCC 27853**
Control (MeOH)	0	0	0
Crude Alkaloids of:			
Morinda tomentosa	13	0	12
Nauclea cordifolia	12	0	10
Nauclea cadamba	27	0	20

Note: The alkaloids inhibited the growth of bacterial in the concentration of 10% weight by volume, using methanol as solvent.

Anti-HSV-1 Test and Cytotoxicity Test: The crude alkaloidal extracts of *Morinda tomentosa, Nauclea cordifolia* and *Nauclea cadamba* were tested against *Herpes simplex* virus type 1, using acyclovir as positive control with IC_{50} = 1.0±0.2 μg/ml. (Table 5). Only the crude alkaloids of *Morinda tomentosa* and *Nauclea cordifolia* showed activities against *Herpes simplex* virus type 1. Cytotoxicity of these sample solutions were also tested against vero cell at the tested concentration of 50 μg /ml, using ellipticine as positive control with IC_{50} = 0.5±0.1 μg/ml. Each sample solution exhibited no effect on vero cell (IC_{50} > 50 μg/ml) as shown in Table 5.

Table 5. Anti-HSV-1 test and cytotoxicity test for crude alkaloids.

Crude Alkaloid of Plant	Cytotoxicity (IC50 : μg/ml)	Anti-HSV-1 (IC50 : μg/ml)
Morinda tomentosa	> 50	16.9
Nauclea cordifolia	> 50	48.2
Nauclea cadamba	> 50	inactive

Note: Tested concentration 50 μg/ml
For cytotoxicity : Ellipticine was used as positive control : IC_{50} = 0.5±0.1 μg/ml
For anti-HSV-1 test : Acyclovir was used as positive control : IC_{50} = 1.0±0.2 μg/ml

Anti-cancer Test: The anti-cancer tests of these sample solutions were also carried out against NC1-H187 (Human small cell lung cancer), KB (Human mouth carcinoma) and BC (Breast cancer), using Ellipticine and Doxurubicin as positive control. No activity was found for all sample solutions.

CONCLUSION

Maceration of dried leaves of *Morinda tomentosa*, *Nauclea cordifolia* and *Nauclea cadamba* with methanol was carried out. The crude alkaloids of these plants were subjected to bioactivity screening. The results showed that crude alkaloids of *Morinda tomentosa* and *Nauclea cordifolia* exhibited activities against *Herpes simplex* virus type 1 while the crude alkaloid of *Nauclea cadamba* showed activity against *Plasmodium falciparum* K1 strain. This indicated that the crude alkaloidal extracts of these three medicinal plants may have different bioactive compounds which can be used as seed molecules for drug development. Further extraction, purification and structure elucidation will be investigated and presented in the subsequent publication.

ACKNOWLEDGEMENTS

The authors would like to thank the then Ministry of University Affairs; Graduate School, Chiang Mai University and Faculty of Pharmacy, Chiang Mai University for partial support of this research work.

REFERENCES

Burkill, I. H., and M. Haniff. 1930. Malay village medicine. Gard. Bull. Straits Settle. 6: 165–321.

Foucaud, R. 1953. Contributions a letude des plants Medicinales du Nord-vietnam Trav. Lab. Mat. Med. Pharm. Gal. Fac. Pharm. Paris 38(1): 1–206.

Foxworthy, F. W. 1922. Minor forest products of the Malay peninsulia. Malayan Forest Record 151–217.

Keng, H. 1969. Order and families of malayan seed plants. Cathay Press, Hong Kong. p. 244–247.

Kardner, S. 2000. Filed guide to forest trees of Northern Thailand. Kobfai Publishing Project, Thailand. p. 217–218.

Lenette, E. H. 1985. Manual of clinical microbiology. 3rd ed. American Society for microbiology, Washington DC. p. 649–651.

Pételot, A. 1952. Les plantes médicinales du Cambodge, du Laos et eu Viet-nam. 2: 31.

Ridley, N. N. 1906. Malay drugs, ed. 2Agr. Bull. Straits Fed. Malay States. 5: 246.

Roger, A. 1939. A handbook of the forest products of Burma. Office of Conservator of forests. p. 104–105.

Thomas, O. O. 1989. Fitoterapia 60: 135.

Washington, J. A. 1981. Laboratory procedures in clinical microbiology, Springer-Verlag, New York. p. 286, 447, 457.