In Vitro Screening of Leishmanicidal Activity in Myanmar Timber Extracts

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Seventy-five Myanmar timber extracts belonging to 27 families were examined for their leishmanicidal activities. Some timber extracts had significant leishmanicidal activity, especially extracts of *Millettia pendula*, which exhibited the most potent activity (MLC 3.1 μg/ml, MIC 1.6 μg/ml). Other timber extracts showing potent activity included those from *Cedrela serrata*, *Cedrela toona*, *Cordia fragrantissima*, *Calophyllum kunstleri*, *Dalbergia cultrate*, *Grevillea robusta*, *Haplophragma adenophyllum*, *Michelia champaca*, and *Tectona grandis*. From a literature search for reports on the chemical constituents of these plants, most constituents were found to be quinone derivatives or other compounds with unsaturated carbonyl groups.

Key words leishmaniasis; timber; Myanmar; *Millettia pendula*; Leguminosae; forest resources

Myanmar is surrounded by several countries, and along the border between Thailand, Laos, and Myanmar (the "GOLDEN TRIANGLE") opium poppy is illegally cultivated. Because of tropical monsoons and its subtropical climate, Myanmar abounds in plant natural resources, especially timber such as teak. However, the medicinal properties of these plant materials have yet to be thoroughly investigated.

According to a report of the Japanese Ministry of Health, Labour and Welfare Quarantine Station, several infectious diseases, such as malaria, cholera, Japanese encephalitis, bubonic plague, filaria, and leishmania, are distributed throughout Myanmar (http://www.forth.go.jp). Malaria eradication is a priority. One of us (M. S.) established a nonprofit organization, the Myanmar Substitutionary Medicinal Plant (MSMP) Project to eradicate the illegal cultivation of opium poppy along the border by introducing medicinal plant cultivation. Timber samples in Myanmar were donated by the Ministry of Forestry of Myanmar. In general, woody plants biosynthesize defensive compounds within the heartwood to protect the tissue against attack by pathogens, such as fungi and bacteria, and from oxidants. Several pharmacologic properties of heartwood constituents (for example, antibacterial, antifungal, and antioxidant activities) that play a role in the defense of heartwood tissue have been reported.[1—3] Many quinone analogues have been isolated from the heartwood of woody plants[4—7] and they are thought to function as free radical scavengers of oxidants.

Leishmaniasis is endemic in tropical regions and currently affects 12 million people in 88 countries.[8] The disease is transmitted by small biting sandflies (*Phlebotomus* spp.). The first-line drugs for the treatment of leishmaniasis are pentavalent antimonials such as *N*-methylglucamine antimonate (Glucantime) and sodium stilbogluconate (Pentostam); however, these drugs are toxic and generally expensive. Cutaneous leishmaniasis is also found in Myanmar. Although some quinone derivatives are known to be antiprotozoal agents,[9,10] only a few with antiprotozoal activity have been extracted from heartwood (a rich source of quinone compounds). For these reasons, we focused our screening for antileishmanial activity on the heartwood constituents.

In this paper, we describe the leishmanicidal activity of Myanmar timber extracts to show that Myanmar has abundant forest resources, the cultivation of which might serve as alternatives to the illegally cultivated opium poppy.

MATERIALS AND METHODS

Materials All tested timbers used were from "Myanmar Timber Samples" produced by the Forest Research Institute, Forest Department, Yangon, and were kindly donated by the Ministry of Forestry of Myanmar.

Chemicals Tetra Color ONE was purchased from Seikagaku Kogyo Co. Ltd. Medium 199 was from Invitrogen Co. Ltd. ES Cell Qualified Fetal Bovine Serum was from Invitrogen Co. Ltd.

Extraction of Timbers All timbers (each 50 g) were pulverized and extracted with methanol (150 ml×3) at 40 °C. Extracts were evaporated *in vacuo* to obtain syrups.

Cultivation of *Leishmania promastigotes* Medium 199 was used for the cultivation of promastigotes of *Leishmania major* (MHOM/SU/73/5ASKH). Promastigotes were cultured in medium [supplemented with heat-inactivated (56 °C for 30 min) fetal bovine serum (10%)] at 27 °C, in an atmosphere of 5% CO_2 in an incubator.

Leishmanicidal Activity Assay The leishmanicidal effects of timber extracts were assessed by the improved 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrasodium bromide (MTT) method as follows. Cultured promastigotes were seeded at 4×10^5/50 μl of medium/well in 96-well microplates, and then 50 μl of different concentrations of test compounds dissolved in a mixture of DMSO and medium were added to each well. Each concentration was tested in triplicate. The microplate was incubated at 27 °C in 5% CO_2 for 48 h. Tetra Color ONE (10 μl) [a mixture of WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt) and 1-methoxy PMS (1-methoxy-5-methylphenazinium methosulfate)] was added to each well and the plates were incubated at 27 °C for 6 h. Optical density values (test wavelength 450 nm; refer-

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ence wavelength 630 nm) were measured using a microplate reader (Thermo BioAnalysis Japan Co., Ltd., Kanagawa, Japan). The leishmanicidal activity was expressed as the minimum lethal concentration (MLC) and minimum inhibitory concentration (MIC). The inhibitory concentration of 50% (IC_{50}) for pendulone was estimated from the graph.

Isolation of Pendulone Powdered *Millettia pendula* Benth timber was extracted with hot methanol (2 l×2) and then filtered. The filtrate was concentrated *in vacuo* and the residue (4.8 g) was purified by column chromatography on silica gel using chloroform–methanol to obtain 20 fractions. Fractions eluted with 10—30% methanol/chloroform were combined and then rechromatographed on silica gel using chloroform–ethyl acetate to give 100 fractions. Fractions eluted with 10—20% ethyl acetate/chloroform were combined and rechromatographed on Sephadex LH-20 using methanol as an eluent to give pendulone (85 mg). Its physical properties were identical to the reported data.[11]

RESULTS AND DISCUSSION

Seventy-five timbers belonging to 27 families were examined for their leishmanicidal activities. Local names in Myanmar and their scientific names for tested timbers are listed in Table 1. Some timber extracts had significant leishmanicidal activity, especially extracts of *Millettia pendula* Benth. (syn. *M. leucantha* Kurz.) (Leguminosae) (no. 61; local name, *thinwin*), which exhibited the most potent activity (MLC 3.1 μg/ml, MIC 1.6 μg/ml). Other timber extracts showing potent activity included those from *Cedrela serrata* Royle. (Meliaceae) (no. 51; local name, *taung-tama*), *Cedrela toona* Roxb. (syn. *Toona ciliata* M. Roem.) (Meliaceae) (no. 63; local name, *thitkado*), *Cordia fragrantissima* Kurz. (Boraginaceae) (no. 44; local name, *sandawa*), *Calophyllum kunstleri* King. (Guttiferae) (no. 57; local name, *tharapi*), *Dalbergia cultrate* Grah. (Leguminosae) (no. 70; Local name, *yindaik*), *Grevillea robusta* A-Cun. (Proteaceae) (no. 18; Local name, *kha daw-hmi*), *Haplophragma adenophyllum* (Wall.) Dep. (Bignoniaceae) (no. 37; local name, *petthan*), *Michelia champaca* Linn. (Magnoliaceae) (no. 43; local name, *sagawa*), and *Tectona grandis* Linn. f. (Verbenaceae) (no. 22; local name, *kyun*).

Millettia pendula Benth. (local name, *thinwin*): The isolation of several prenylated isoflavanones from the root bark of the genus *Millettia* have been reported.[12] From the heartwood of *M. pendula*, two isoflavane-quinone derivatives have been reported to date.[11] Their chemical structures include a quinone moiety, and many leishmanicidal constituents from natural sources have quinone moieties in their molecules. For this reason, it was assumed that this moiety was responsible for the leishmanicidal activity of this timber extracts. To support this assumption, an active component was isolated from this plant extract (see experimental). It was found to be pendulone, which has already been reported as a constituent of *M. pendula*. It showed potent activity (IC_{50} 0.066 μg/ml).

Cedrela serrata Royle. (local name, *taung-tama*) and *C. toona* Roxb. (local name, *thitkado*): From the heartwood of *Cedrela* spp., some triterpenoids and essential oils have been extracted from *C. odorata*.[13] Two limonoids were isolated from *C. toona* seeds.[14] However, whether these limonoids have antiprotozoal properties was not mentioned in the paper, although several limonoids (2,6-dihydroxyfissinolide, fissinolide, and methyl 3-acetoxy-6-hydroxy-1-oxomeliac-14-enoate isolated from plants of another genus) are reported to have leishmanicidal activities.[15] Other constituents of this plant are some cadinane-type sesquiterpenes and a coumarin derivative.[13,16] Moderate leishmanicidal activity of coumarin isolated from a Peruvian folk medicine was noted in our previous study,[17] although the activity of its derivatives has not been investigated. Therefore the identity of the active principle in this timber extract is not obvious.

Cordia fragrantissima Kurz. (local name, *sandawa*): Several benzoquinone and hydroquinone derivatives called cordiaquinones, cordiaquinols, cordiachromes, alliodorols, and allioquinols have been isolated from other *Cordia* spp. These constituents possess a *p*-benzoquinone or *p*-hydroquinone moiety.[18—21] Despite many reports of the isolation of *Cordia* species constituents, none have reported the isolation of chemical components from *C. fragrantissima*. However, that such quinone derivatives exist in *C. fragrantissima* is easily accepted because of the many isolated from other species of this genus.

Dalbergia cultrate Grah. (local name, *yindaik*): This plant genus is mainly distributed in tropical, subtropical, and south African regions. Timbers of this genus (known as rosewood) are used to make upscale furniture. *Dalbergia latifolia* is called "Indian rosewood" and *Dalbergia nigra* is called "Brazilian rosewood." This genus generally contains neoflavonoids [named generically for dalbergions, 4-arylchromene derivatives (=neoflavans) and 4-arylcoumarin derivatives (=dalbergins)]. The heartwood constituents of this plant include 4-arylcoumarin derivatives [(*S*)-4-methoxydalbergione and stevenin], a *p*-hydroquinone derivative (dalbergin), and diphenylpropene derivatives (3,3-diphenylprop-1-ene and 3′-hydroxydalbergiphenol).[22] However, their pharmacologic properties are ambiguous.

Calophyllum kunstleri King. (local name, *tharapi*): Calanolide A, isolated from *Calophyllum* species, is known as a potent anti-HIV coumarin and is a strong candidate anti-HIV drug.[23] To our knowledge, no constituents of *C. kunstleri* have been reported.

Grevillea robusta A-Cun. (local name *kha daw-hmi*): Timber of this woody plant called "silk oak" is commercially valuable for making furniture. Arubtin derivatives, macrocyclic phenols, and cinnamic acid derivatives have so far been isolated from the leaves of *G. robusta*.[24] Our previous research found that some cinnamic acid derivatives had leishmanicidal activity.[17] Macrocyclic phenols with the resorcinol moiety are characteristic compounds of this species[25,26] and much attention has been focused on the bioactivity of these compounds, although none has been focused on their existence in heartwood.

Haplophragma adenophyllum (Wall.) Dep (local name, *petthan*): The genus *Haplophragma* is a rare species in the Bignoniaceae family. Three naphthoquinones (dehydro-α-lapachone, α-lapachone, and β-lapachone) have been isolated from heartwood and roots, and one (β-lapachone) has antitrypanosomal activity.[27]

Michelia champaca Linn. (local name *sagawa*): Several sesquiterpene lactones were isolated from *M. champaca* along with some aliphatic chain compounds.[28,29] Among sesquiterpene lactones, parthenolide and michampanolide

Table 1. Tested Timber Samples and Their Leishmanicidal Activity against *L. major*

Entry no.	Voucher no.	Local name	Scientific name	Family name	Leishmanicidal activity (μg/ml)	
					MLC	MIC
1	M-0001	*Aukchinsa*	*Diospyros ehretioides* Wall.	Ebenaceae	>400	400
2	M-0002	*Baing*	*Tetrameles nudiflora* R. Br.	Datiscaceae	400	400
3	M-0003	*Binga*	*Mitragyna rotundifolia* O. Ktze.	Rubiaceae	>400	>400
4	M-0004	*Bonmeza*	*Albizzia chinensis* (Osbeck.) Merr.	Mimosaceae	400	400
5	M-0005	*Chinyok*	*Garuga pinnata* Roxb.	Burseraceae	400	400
6	M-0006	*Didu*	*Salmalia insignis* Schott. & Endl.	Malvaceae	>400	>400
7	M-0007	*Gwe*	*Spondias pinnata* (Linn.) Kurz.	Anacardiaceae	>400	>400
8	M-0008	*Gyo*	*Schleichera oleosa* (Lour.) Merr.	Sapindaceae	>400	>400
9	M-0009	*Gyok*	*Diospyros montana* Roxb.	Ebenaceae	>400	400
10	M-0010	*Hnaw*	*Adina cordifolia* Hook. f.	Rubiaceae	>400	>400
11	M-0011	*In*	*Dipterocarpus tuberculatus* Roxb.	Dipterocarpaceae	400	200
12	M-0012	*Ingyin*	*Pentacme siamensis* (Miq.) Kurz.	Dipterocarpaceae	>400	>400
13	M-0013	*Kambala*	*Sonneratia apetala* Ham.	Lythraceae	>400	>400
14	M-0014	*Kanyaung*	*Shorea thorelli* Pierre.	Dipterocarpaceae	>400	>400
15	M-0015	*Kanyin*	*Dipterocarpus alatus* Roxb.	Dipterocarpaceae	>400	400
16	M-0016	*Kathit*	*Erythrina Suberosa* Roxb.	Leguminosae	>400	400
17	M-0017	*Kaunghmu*	*Anisoptera scaphula* (Roxb.) Pierre.	Dipterocarpaceae	>400	400
18	M-0018	*Kha Daw-Hmi*	*Grevillea robusta* A-Cun.	Proteaceae	100	50
19	M-0019	*Kokko*	*Albizzia Lebbek* Benth.	Mimosaceae	400	400
20	M-0020	*Kuthan*	*Hymenodictyon excelsum* Wall.	Rubiaceae	>400	>400
21	M-0021	*Kyana*	*Xylocarpus moluccensis* (Lam.) Roem.	Meliaceae	>400	50
22	M-0022	*Kyun*	*Tectona grandis* Linn. f.	Verbenaceae	100	50
23	M-0023	*Lein*	*Terminalia pyrifolia* Kurz.	Combretaceae	>400	400
24	M-0024	*Letpan* (Cotton wood)	*Salmalia malabarica* (Dc.) Schott. & Endl.	Malvaceae	>400	>400
25	M-0025	*Leza*	*Lagerstroemia tomentosa* Presl.	Lythraceae	>400	200
26	M-0026	*Magyi-Pway*	*Diospyros pendula* Hasselt. ex. Hassk.	Ebenaceae	>400	>400
27	M-0027	*Maniauga*	*Carallia brachiata* (Lour.) Merr.	Rhizophoraceae	>400	>400
28	M-0028	*Ma-U-Kadon*	*Nauclea orientalis* Linn.	Rubiaceae	>400	400
29	M-0029	*Mau-Lettan-She*	*Anthocephalus cadamba* Miq.	Rubiaceae	>400	>400
30	M-0030	*Myaukchaw*	*Homalium tomentosum* Benth.	Samydaceae	>400	400
31	M-0031	*Myauklok*	*Artocarpus lakoocha* Rxb.	Urticaceae	>400	>400
32	M-0032	*Myaukngo*	*Duabanga grandiflora* (Roxb.) Walp.	Lythraceae	>400	400
33	M-0033	*Nabe*	*Lannea grandis* Engler.	Anacardiaceae	>400	>400
34	M-0034	*Padauk*	*Pterocarpus macrocarpus* Kurz.	Leguminosae	>400	400
35	M-0035	*Panga*	*Terminalia chebula* Retz.	Combretaceae	>400	100
36	M-0036	*Pantama*	*Melia burmanica* Kurz.	Meliaceae	>400	>400
37	M-0037	*Petthan*	*Haplophragma adenophyllum* (Wall.) Dep	Bignoniaceae	100	50
38	M-0038	*Pinle-Kanazo*	*Heritiera fomes* (Buch.)	Sterculiaceae	>400	400
39	M-0039	*Pyaukseik*	*Holoptelea integrifolia* Planch.	Urticaceae	>400	400
40	M-0040	*Pyinkado*	*Xylia dolabriformis* Benth.	Leguminosae	>400	50
41	M-0041	*Pyinma*	*Lagerstroemia speciosa* Pers.	Lythraceae	>400	400
42	M-0042	*Sagasein*	*Cananga odorata* Hook. f. & T.	Annonaceae	200	100
43	M-0043	*Sagawa*	*Michelia champaca* Linn.	Magnoliaceae	100	50
44	M-0044	*Sandawa*	*Cordia fragrantissima* Kurz.	Boraginaceae	25	12.5
45	M-0045	*Sawbya*	*Pterocymbium tinetorium* (Blanco.) Merr.	Sterculiaceae	>400	>400
46	M-0046	*Sha*	*Acacia catechu* Willd.	Leguminosae	>400	100
47	M-0047	*Sit*	*Albizzia procera* Benth.	Mimosaceae	>400	100
48	M-0048	*Tamalan*	*Dalbergia oliveri* Gamble.	Leguminosae	>400	200
49	M-0049	*Taukkyan*	*Terminalia tomentosa* W&A.	Combretaceae	400	100
50	M-0050	*Taung-Peinne*	*Artocarpus calophylla* Kurz.	Urticaceae	>400	400
51	M-0051	*Taung-Tama*	*Cedrela serrata* Royle.	Meliaceae	50	12.5
52	M-0052	*Taung-Thayet*	*Swintonia floribunda* Griff.	Anacardiaceae	>400	>400
53	M-0053	*Thabye*	*Syzygium cumini* (Linn.) Skeels.	Myrtaceae	>400	400
54	M-0054	*Thadi*	*Protium serrata* Engler.	Burseraceae	>400	>400
55	M-0055	*Thame*	*Avicennia officinalis* Linn.	Verbenaceae	>400	400
56	M-0056	*Thande*	*Stereospermum personatum* Chatt.	Bignoniaceae	>400	400
57	M-0057	*Tharapi*	*Calophyllum kunstleri* King.	Guttiferae	50	25
58	M-0058	*Thayet*	*Mangifera indica* Linn.	Anacardiaceae	>400	400
59	M-0059	*Thingadu*	*Parashorea stellata* Kurz.	Dipterocarpaceae	>400	>400
60	M-0060	*Thingan*	*Hopea odorata* Roxb.	Dipterocarpaceae	>400	>400
61	M-0061	*Thinwin*	*Millettia pendula* Benth.	Leguminosae	3.1	1.6
62	M-0062	*Thitka*	*Pentace burmanica* Kurz.	Tiliaceae	>400	>400
63	M-0063	*Thitkado*	*Cedrela toona* Roxb.	Meliaceae	100	12.5
64	M-0064	*Thit-Magyi*	*Albizzia odoratissima* Benth.	Mimosaceae	>400	200
65	M-0065	*Thit-Payaung*	*Neonauclea excelsa* Bi.	Rubiaceae	>400	>400
66	M-0066	*Thitsein*	*Terminalia belerica* Roxb.	Combretaceae	>400	400

Table 1. (Continued)

Entry no.	Voucher no.	Local name	Scientific name	Family name	Leishmanicidal activity (μg/ml)	
					MLC	MIC
67	M-0067	*Thitsi*	*Melanorrhoea usitata* WALL.	Anacardiaceae	>400	400
68	M-0068	*Thitya*	*Shorea oblongifolia* THW.	Dipterocarpaceae	>400	>400
69	M-0069	*Yemane*	*Gmelina arborea* LINN.	Verbenaceae	>400	>400
70	M-0070	*Yindaik*	*Dalbergia cultrata* GRAH.	Leguminosae	50	25
71	M-0071	*Yinma*	*Chukrasia tabularis* A. JUSS.	Meliaceae	>400	100
72	M-0072	*Yinzat*	*Dalbergia fusca* PIERRE.	Leguminosae	>400	400
73	M-0073	*Yon*	*Anogeissus acuminata* WALL.	Combretaceae	>400	100
74	M-0074	*Zaungbale*	*Lagerstroemia villosa* WALL.	Lythraceae	>400	100
75	M-0075	*Zinbyun*	*Dillenia pentagyna* ROXB.	Dilleniaceae	400	100

contain α-methylene-γ-butylolactone moieties. Previously, we reported isolating seven leishmanicidal sesquiterpene lactones from the Peruvian folk medicine, *Elephantopus mollis* H.B.K. (Compositae), and that the α-methylene-γ-butylolactone moiety was essential for the expression of leishmanicidal activity.[30] A literature search for reports on *M. champaca* constituents found one on the root bark constituent liriodenine.[31] Liriodenine has also been obtained from the trunk bark and roots of *Annona spinescens* (Annonaceae) and found to be active against promastigotes of *Leishmania braziliensis*, *Leishmania amazonensis*, and *Leishmania donovani*.[32] Many isoquinoline alkaloids have been isolated from plants of the genus *Michelia*.[33,34] Isoquinoline alkaloids form a large group of natural leishmanicidal compounds. Although liriodenine or its analogues have not yet been isolated from *M. champaca* heartwood, it would be reasonable to assume that leishmanicidal activity in heartwood is due to such compounds.

Tectona grandis LINN. f. (local name, *kyun*): Teakwood is well known as a material for furniture and is the most important export next to rice from Myanmar. *T. grandis* has been reported to contain several naphthoquinones, lapachol, trichione, 5-hydroxylapachol, *etc.*[35] Among them, lapachol is widely distributed in the woody plant kingdom (especially in the Bignoniaceae family) and is mainly found in heartwood. Its leishmanicidal activity against amastigotes of *L. donovani* in mouse peritoneal macrophages has been reported.[36] The activity of timber extracts is probably due to lapachol or its derivatives.

In conclusion, we found that some timber extracts, especially extracts of *M. pendula* BENTH., had the most leishmanicidal activity. From a literature search for reports on chemical constituents of these plants, most constituents were found to be quinone derivatives or some other compounds with unsaturated carbonyl groups (*e.g.*, coumarin derivatives). Such compounds are probably part of the defense mechanism of wood. Moreover, a number of quinone derivatives have been found to be leishmanicidal components of plants in their natural environment; therefore these natural products might be used for antileishmanial treatment. However, in some species, chemical components have not been investigated, and thus new compounds might be found. This would improve utilization of forest resources and furnish an alternative to the illegal cultivation of opium poppy in Myanmar.

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