

Initial Studies on Alkaloids from Lombok Medicinal Plants

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Abstract: Initial investigation of medicinal plants from Lombok has resulted in the collection of 100 plant species predicted to have antimicrobial, including antimalarial, properties according to local medicinal uses. These plants represent 49 families and 80 genera; 23% of the plants tested positively for alkaloids. Among the plants testing positive, five have been selected for further investigation involving structure elucidation and antimicrobial testing on the extracted alkaloids. Initial work on structural elucidation of some of the alkaloids is reported briefly.

Keywords: Alkaloids, Medicinal Plants, Lombok

Introduction

There is an urgent need to discover new antimicrobial agents for human and veterinary therapeutic uses, as resistance to current drugs increases in severity and extent. For instance, many *Plasmodium falciparum* strains, the parasite responsible for many fatalities from malaria, have become resistant to chloroquine [1]. This phenomenon has also occurred with some important pathogenic bacteria [2]. As a result, more than two billion people worldwide are at high risk of malarial/bacterial diseases. The identification of new and structurally novel natural products with antimicrobial activity, and hopefully new modes of action, is one of the ways of tackling this problem. While various approaches to locating such natural products have been undertaken, we are exploring a combined chemo- and bio-rational strategy based on alkaloids and medicinal plants respectively. By targeting alkaloid-containing medicinal plants, it is hoped that structural novelty with the required bioactivity will be achieved more efficiently. Alkaloids have diverse structures and many show a range of pharmacological activities

including antimicrobial activity [19]. They are also normally readily separable from the other plant metabolites as a result of their basicity.

Lombok, part of the tropical Indonesian archipelago, is a small island (4600 km²) with 2.4 million inhabitants which administratively is divided into three regions of West, Central, and East Lombok. It is situated between Bali and Sumbawa Islands, where transition from the western to the eastern Indonesian flora and fauna begins. The northern region of the island is mountainous and is dominated by tall trees and shrubs, while the south is a drier region with savanna vegetation. As a consequence, Lombok has a rich and varied flora. Local people have a long tradition of using plants for medicinal purposes and more than 70 percent are still utilising them. Aqueous plant extracts, which could extract alkaloid salts with naturally occurring acids, are made for internal medicinal use. For topical applications, plant material is normally crushed and applied directly or crushed in the presence of water before application. However, most of the plants have not been studied chemically and pharmacologically, and there is great potential for the isolation of novel, naturally-occurring bioactive compounds.

The overall aims of this current study were to locate, isolate, identify and evaluate novel bioactive alkaloids from plants traditionally used for medicinal purposes in Lombok, Indonesia. The plants investigated were those predicted to have antimicrobial and antimalarial properties based on the local uses of the plants. This study has involved plant specimen collection, field screening for alkaloids, extraction, purification, structure elucidation, and later will include bioactivity testing. The first three steps have been carried out at the University of Mataram, Lombok and further work is continuing at the University of Wollongong. Presently, we would like to report the results of the plants selected for alkaloid screening, as well as the results of initial work on molecular elucidation.

Results and Discussion

Plant Selection

A list of medicinal plants on Lombok was generated, consisting of 100 plant species based on the possibility of them having antimicrobial or antimalarial activities inferred from their local uses in medicine. The list was then reduced to 5 species for initial investigation on the basis of the following criteria: 1) the medicinal use of the plant and the possibility of it containing antibacterial, or antimalarial compounds, 2) the presence of alkaloids in the species, 3) the extent of chemical and biological work already undertaken on the species, and 4) the geographical distribution of the species. The plants thus selected were *Alstonia scholaris* R. Br. (Apocynaceae; antimalarial properties), *Voacanga foetida* (Bl.) Rolfe. (Apocynaceae; antimicrobial properties), *Psychotria malayana* Jack. (Rubiaceae; antimicrobial properties), *Clerodendron paniculatum* L. (Verbenaceae; antimicrobial properties), and *C. calamitosum* L. (Verbenaceae; antimalarial and antimicrobial properties). Although there are a number of reports on alkaloids from *A. scholaris* R. Br. [3,4], we were particularly interested in investigating alkaloids in the young plants, as local people have used the plant at this growth stage to treat malaria.

Alkaloid Screening

A hundred medicinal plants giving negative or positive tests for alkaloids (modified Culvenor and Fitzgerald procedure [5]) are listed in Table 1 and 2 respectively under family and species; the local uses of the plants are also included in these tables.

Table 1. Lombok medicinal plant species giving a negative test for alkaloids.

Family	Species	Locality*	Collection Code	Diseases/ Conditions treated**	Part Tested***
Acanthaceae	<i>Justicia gendarussa</i>	Narmada, WL	NWL08	Fever	Lf, bk, rt
Agavaceae	<i>Cordyline fruticosa L.</i>	Puyung, CL	PCL04	Diarrhoea	Lf, rh
	<i>C. rumphii Miq.</i>	Puyung, CL	PCL13	Dysentery	Lf, st, rt
Amaryllidaceae	<i>Crinum latifolium L.</i>	Kopang, EL	KEL01	Fever	Lf, bl
Anacardiaceae	<i>Bouea burmanica Griff.</i>	Masbagik, EL	MEL03	Ulcers, abscesses	Lf, bk, rt
	<i>Buchanania macrophylla Bl.</i>	Narmada, WL	NWL02	Fever, sore eyes	Lf, bk, rt
	<i>Dracontomelon celebicum Kds.</i>	Narmada, WL	NWL01	Fever, sore eyes	Lf, bk, rt
	<i>Gluta elegans Kurz.</i>	Puyung, CL	PCL08	Skin diseases	Lf, bk, rt
Annonaceae	<i>Xylopiya malayana</i>	Suranadi, WL	SWL04	Fever, malaria	Lf, bk, fl
Apiaceae	<i>Corriandrum sativum L.</i>	Mantang, CL	MCL05	Wounds	Lf, sd
	<i>Foeniculum vulgare Mill.</i>	Mantang, EL	MLT05	Cough, fever	Lf, st, rt, sd
Apocynaceae	<i>Alstonia macrophylla Wall.</i>	Puyung, CL	PCL07	Ulcers	Lf, bk, rt
	<i>Wrightia spp.</i>	Masbagik, EL	MEL11	Dysentery	Lf, bk, rt
Asclepiadaceae	<i>Cryptostegia madagascariensis Boj.</i>	Ampenan, WL	AWL04	Dysentery	Lf, bk, rt
Asteraceae	<i>Achillea millefolium L.</i>	Tetebatu, EL	TEL02	Wounds	Lf, st, rt
	<i>Bidens leuchantus Willd.</i>	Tetebatu, EL	TEL05	Swellings	Lf, bk, rt,
Capparidaceae	<i>Gynandropsis speciosa</i>	Narmada, WL	NWL06	Gonorrhoea, dysentery	Lf, st, rt
	<i>Polanisia icosandra</i>	Kotaraja, EL	KEL08	Skin diseases	Lf, bk, rt
Compositae	<i>Pluchea indica Less.</i>	Kotaraja, EL	KEL07	Fever, dysentery	Lf, bk, rt
Erythroxylaceae	<i>Phyllanthus acidus Skeels.</i>	Sepakek, CL	SCL02	Fever	Lf, bk, rt
Euphorbiaceae	<i>Aleurites moluccana Wild.</i>	Suranadi, WL	SWL05	Dysentery, itches	Lf, bk, fr
	<i>Antidesma cuspidatum Muell. Arg.</i>	Suranadi, WL	SWL09	Fever	Lf, bk, rt
	<i>A. montanum Bl.</i>	Pancor, EL	PEL02	Ulcers, wounds	Lf, bk, rt, sd

Family	Species	Locality*	Collection Code	Diseases/ Conditions treated**	Part Tested***
Euphorbiaceae	<i>Aporosa frutescens</i> Bl.	Puyung, CL	PCL05	Fever	Lf, bk, rt
	<i>Baccaurea brevipes</i> Hook. f.	Puyung, CL	PCL06	Fever, dysentery	Lf, bk, rt
	<i>B. dulcis</i> Muell. Arg.	Puyung, CL	PCL11	Wounds	Lf, bk, rt
	<i>Croton argyratus</i> Bl.	Puyung, CL	PCL09	Dysentery	Lf, bk, rt
	<i>Euphorbia pulcherrima</i> Willd.	Puyung, CL	PCL10	Wounds	Lf, bk, rt
	<i>E. tirucalli</i> L.	Puyung, CL	PCL12	Ulcers	Lf, bk, rt
	<i>Sauropus androgynus</i> L.	Kotaraja, EL	KEL11	Fever	Lf, bk, rt
Gentianaceae	<i>Canscora ecussata</i> Schult.	Kopang, CL	KCL02	Ulcers, wounds	Lf, st, rt
Haloragaceae	<i>Gunnera macrophylla</i> Bl.	Suranadi, WL	SWL03	Dysentery, Diarrhoea	Lf, bk, fr
	<i>Myriophyllum brasiliense</i> Cambess.	Kotaraja, EL	KEL10	Diarrhoea	Lf, bk, rt
Hernandiaceae	<i>Artocarpus anisophylla</i> Miq.	Mantang, CL	MCL04	Dysentery	Lf, bk, rt, fr
	<i>A. dadah</i> Miq.	Pancor, EL	PEL05	Dysentery	Lf, bk, rt
	<i>A. champeden</i> Spreng.	Ampenan, WL	AWL02	Sore eyes, Diarrhoea	Lf, bk
	<i>A. gomeziana</i> Wall.	Kotaraja, EL	KEL09	Dysentery	Lf, bk, rt
	<i>Hernandia ovigera</i> L.	Ampenan, WL	AWL01	Sore eyes	Lf, st
Lamiaceae	<i>Coleus ambonicus</i> Lour.	Tetebatu, EL	TEL04	Diphtheria, tetanus	Lf, bk, rt, sd
	<i>Desmodium heterophyllum</i> DC.	Mantang, CL	MCL03	Scabies, itches	Lf, st, rt
	<i>Orthosiphon grandiflorus</i> Bold.	Sepakek, CL	SCL01	Syphilis	Lf, st, rt
Leguminosae	<i>Bauhinia variegata</i> L.	Mataram, WL	MWL05	Fever, cough	Lf, bk, rt
	<i>Derris elliptica</i> Benth.	Suranadi, WL	SWL07	Fever, scabies	Lf, bk, rt
Malvaceae	<i>Abelmoschus esculentus</i> Moench.	Pancor, EL	PEL03	Gonorr-hoea	Lf, bk, rt
	<i>Gossypium arboreum</i> L.	Pancor, EL	PEL06	Ulcers	Lf, bk, rt
Meliaceae	<i>Dysoxylum</i> sp.	Suranadi, WL	SWL08	Itches	Lf, bk, rt
	<i>Sweitenia macrophylla</i> King.	Masbagik, EL	MEL08	Malaria	Lf, bk, rt
Myristicaceae	<i>Horsfleidia glabra</i> Warb.	Masbagik, EL	MEL09	Itches	Lf, bk, rt
Myrtaceae	<i>Eugenia cumini</i> Merr.	Mataram, WL	MWL07	Sore eyes	Lf, bk, rt, sd
Oxalidaceae	<i>Averrhoa bilimbi</i> L.	Kotaraja, EL	KEL15	Fever, cough	Lf, bk, rt
	<i>A. carambola</i> L.	Kotaraja, EL	KEL06	Wounds, scabies	Lf, bk, rt
Palmae	<i>Cocos nucifera</i> L.	Narmada, WL	NWL05	Fever, dysentery	Lf, st, rt

Family	Species	Locality*	Collection Code	Diseases/ Conditions treated**	Part Tested***
Pandanaceae	<i>Pandanus furcatus</i> Roxb.	Pancor, EL	PEL04	Dysentery	Lf, st, rt
Papilionaceae	<i>Abrus fruticulosus</i> Wall.	Puyung, CL	PCL01	Fever	Lf, fl, bk, rt
Pedaliaceae	<i>Sasamus indicum</i> L.	Masbagik, EL	MEL06	Diarrhoea	Lf, bk, rt
Pinaceae	<i>Pinus mercurii</i> <i>Jungh & De. Vr.</i>	Mataram, WL	MWL01	Ulcers	Lf, bk, rt
Piperaceae	<i>Piper baccatum</i> L.	Mataram, WL	MWL03	Fever, swelling	Lf, st
	<i>Peperomia pellucida</i> Kunth.	Pancor, EL	PEL01	Fever	Lf, bk, rt
Poaceae	<i>Dendrocalamus asper</i> Schult. F.	Kekait, WL	KWL03	Fever	Lf, st
Rafflesiaceae	<i>Brugmensia suaveolens</i>	Mataram, WL	MWL06	Wounds	Lf, bk, rt
Rhamnaceae	<i>Alphitonia moluccana</i> T&B.	Sepakek, CL	SCL03	Fever	Lf, bk, rt
Rubiaceae	<i>Borreria hispida</i> Schum.	Narmada, WL	NWL11	Wounds, dysentery	Lf, st, rt
	<i>B. setidens</i> (Miq.) Bold.	Masbagik, EL	MEL10	Fever	Lf, bk, rt
	<i>B. ocimoides</i> DC.	Ampenan, WL	AWL05	Ulcers	Lf, bk, rt
	<i>Catesbaea spinosa</i> L.	Mantang, CL	MCL01	Fever, swellings	Lf, bk, rt
Sapindaceae	<i>Schleichera oleaosa</i> L.	Masbagik, EL	MEL07	Malaria	Lf, bk, rt
Sapotaceae	<i>Manilka raachras Fosberg.</i>	Tetebatu, EL	TEL01	Fever, dysentery	Lf, bk, rt, fl
Sterculiaceae	<i>Melochia umbellata</i> Staff.	Narmada, WL	NWL09	Fever	Lf, bk, rt
Thymelaeaceae	<i>Aquilaria filaria</i>	Kekait, WL	KWL04	Malaria	Lf, bk, rt
Verbenaceae	<i>Callicarpa cuspidata</i> Roxb.	Tetebatu, EL	TEL03	Fever	Lf, bk, rt
	<i>Alpinia galanga</i> Sw.	Puyung, CL	PCL02	Cholera	Lf, rh
	<i>A. javanica</i> Bl.	Suranadi, WL	SWL01	Swelling, cholera	Lf, rh
Zingiberaceae	<i>Curcuma aerginosa</i> Roxb.	Tetebatu, EL	TEL 05	Fever	Lf, rh
	<i>C. zedoaria</i> Rosc.	Puyung, CL	PCL03	Malaria	Lf, rh
	<i>C. domestica</i> Val.	Sepakek, CL	SCL04	Diarrhoea, scabies	Lf, rh
	<i>Zingiber ffficinale</i> Rosc.	Suranadi, WL	SWL02	Itches, cholera, wounds	Lf, rh

*WL(West Lombok), CL(Central Lombok), EL(East Lombok); ** Information gathered by interviewing local people and confirmed by Perry (1980) [9]; ***bk(bark), st (stem), rt(root), bl(bulb), rh(rhizome), fl(flower), fr(fruit), sd(seed); plant parts printed in bold are those used medicinally

Table 2. Lombok medicinal plant species giving a positive test for alkaloids.

Family	Species	Locality*	Collection Code	Diseases/ Conditions treated**	Part tested(Result)
Amaryllidaceae	<i>Crinum asiaticum L.</i>	Masbagik, EL	MEL03	Wounds, abscesses	Lf(++), bl(++)
Annonaceae	<i>Annona squamosa L.</i>	Kotaraja, EL	KEL13	Fever	Lf(+), bk(++), rt(++)
Apocynaceae	<i>Alstonia cholaris R.Br.</i>	Kotaraja,EL	KEL3	Malaria	Lf(+++), bk(+++), rt(+++)
	<i>Voacanga foetida (Bl.) Rolfe</i>	Kekait, WL	KWL01	Almost all skin diseases	Lf(+++), bk(+++), fr(+++), sd(+++)
Caesalpiniaceae	<i>Cassia siamea</i>	Kotaraja,EL	KEL02	Malaria	Lf(+++), bk(++), rt(++)
Caricaceae	<i>Carica papaya L.</i>	Narmada, WL	NWL04	Malaria, Ulcers	Lf(++), st(-), rt(-), fr(-)
Convolvulaceae	<i>Ipomoea batatas Polr.</i>	Narmada, WL	NWL03	Wounds	Lf(+), rh(-)
Cucurbitaceae	<i>Momordica charantia L.</i>	Pancor, EL	PEL07	Malaria	Lf(++), st(++), fr(+)
	<i>M. bicolor Bl.</i>	Narmada, WL	NWL10	Malaria	Lf(++), st(++), rt (+)
Euphorbiaceae	<i>Jatropha multifida L.</i>	Ampenan, WL	AWL03	Swellings, wounds	Lf (-), bk(-), sd(+)
Lamiaceae	<i>Dryophylla auricularia (L.) Bl.</i>	Masbagik, EL	MEL05	Fever	Lf(++), St(++), rt(+)
Loganiaceae	<i>Strychnos ligustrina Bl.</i>	Masbagik, MEL	MEL12	Malaria	Lf(-), bk(+++), rt(++)
Magnoliaceae	<i>Michelia champaca L.</i>	Mataram, WL	MWL06	Fever, wounds	Lf(+++), bk(+++), rt(++)
	<i>M. alba DC.</i>	Narmada, WL	NWL07	Malaria	Lf(++), bk(++), rt(++)
Meliaceae	<i>Azadirachta indica Juss.</i>	Kopang, EL	KCL02	Dysentery malaria	Lf(++), bk(++), rt(+)
Mimosaceae	<i>Crotalaria retusa L.</i>	Kotaraja, EL	KEL14	Fever, wounds	Lf(++), st(+), rt(++), fr(-)
Moraceae	<i>Ficus septica</i>	Mataram, WL	MWL05	Wounds	Lf(+++), bk(+++), rt(+++)
Moringaceae	<i>Moringa oleifera Lamk.</i>	Mataram, WL	MWL08	Fever, Wounds	Lf (++) , bk (++) , rt (++)
Rubiaceae	<i>Psychotria malayana Jack.</i>	Suranadi, WL	SWL04	Wounds, skin diseases	Lf(++), bk(++), rt(-), fr(-)
Sterculiaceae	<i>Sterculia foetida Linn.</i>	Kotaraja,EL	KEL01	Fever, malaria	Lf(++), k(+++), rt(+++)

Family	Species	Locality*	Collection Code	Diseases/ Conditions treated**	Part tested(Result)
Verbenaceae	<i>Clerodendron alamosum</i> L.	Kotaraja, EL	KEL12	Malaria, wounds	Lf (++)
	<i>C. paniculatum</i> L.	Narmada, WL	NWL06	Sore eyes	Lf(-), fl(++), rt(-)
Zingiberaceae	<i>Curcuma xanthorrhiza</i> Roxb.	Mataram, WL	MWL02	Diarrhoea, malaria	Lf (+), rh (+)

*WL(West Lombok), CL(Central Lombok), EL(East Lombok); ** Information gathered by interviewing local people and confirmed by Perry (1980) [7]; ***bk(bark), st (stem), rt(root), bl(bulb), rh(rhizome), fl(flower), fr(fruit), sd(seed); plant parts printed in bold are those used medicinally

The plants collected were widely distributed in 49 families and 80 genera, and this is an indication of the variety of Lombok medicinal plants. Of these plants, twenty-three species (23%) contained alkaloids. In a survey of plants of Tasmania, Australia, which focused mainly on endemic species in this cool temperate environment [6], 15 % of the plant species gave positive alkaloid tests. However, in a similar alkaloid survey in Queensland, Australia, with many tropical and sub-tropical species, 20% of species were positive [10]. Further details on the five selected plants are as follows:

Alstonia scholaris R.Br. This is the tallest tree belonging to the family Apocynaceae growing to a height of 20-25 m and 40-80 cm in diameter. In Lombok, this tree, known as 'nita', is common in areas up to 900 m above sea level. The concentrated aqueous extract of leaves or bark of the young tree has been used to treat malaria in Lombok. Antimalarial testing with *Plasmodium falciparum*, revealed that the alkaloids obtained from leaves of *A. scholaris* from Lombok were active [8], but no specific mention was made on the age of the trees from which the alkaloids were isolated. We have found that alkaloids are distributed through the whole plant including leaves, bark, roots, and fruit.

Clerodendron calamitosum L. The leaves of this plant in the Verbenaceae, under the local name 'keji beling', have been used commonly to treat malaria and wounds, as well as to destroy kidney stones. It is an erect scrub, which grows in dry, shady areas in villages, coconut groves, and at village borders and roadsides.

No alkaloid studies appear to have been reported on this plant. A Mayer's alkaloid test on whole plants produced a weak positive result (+). Further testing on separated parts indicated that the alkaloid accumulated mainly in the leaves (++) , therefore, isolation of the alkaloid was focused on this part. At least five major alkaloids have been shown to be present in the crude alkaloidal extract (0.04% based on fresh material, 250 g) and some of their characteristics are noted in Table 3.

Table 3. Some characteristics of components in the crude alkaloidal extract of *C. calamitosum* L.

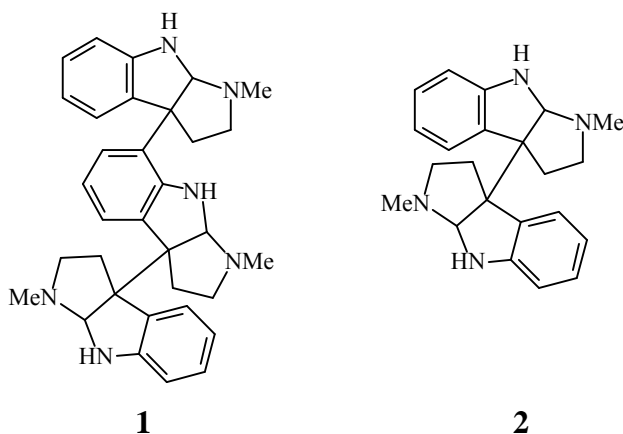
Component No.	Rf*	UV light (333 nm)**	Iodine Test***	Dragendorff Test****	M ⁺ (CIMS)*****
1	0.34	abs.	brown	yellow	285 & 309
2	0.41	abs.	brown	yellow	285
3	0.56	abs.	brown	yellow	308
4	0.81	blue fl.	-	-	270
5	0.91	blue fl.	-	-	277

* TLC (silica gel, ethyl acetate : isopropanol : NH₄OH = 95 : 10 : 5 by vol.); ** Abs (absorbance), fl (fluorescence); *** By using iodine vapour [9], - no colouration; **** Reference [9], - no colouration; ***** CIMS (Chemical Ionisation Mass Spectrometry)

By comparing molecular weights of the few known indole-based alkaloids isolated previously from other species in the genus [11-13], all the components appear to be new alkaloids from the genus *Clerodendron*. Molecular elucidation of these components is currently in progress.

Clerodendron paniculatum L. Having a beautiful red flower, the plant is commonly cultivated in gardens for ornamental value. Like other species in the Verbenaceae, the plant, known locally as 'kembang aik terjun', grows as an erect shrub, with a height up to 1.5 m. The plant is reported [14] as a native in South East Asia, and has been used to treat sore eyes by extracting the leaves with sterile water and applying the extract as eye drops. The concentrated water extract of the leaves also has been utilised to treat wounds. Only the mature flowers tested positive for alkaloids, and young flowers (less than two months old) gave a negative alkaloid test. It is possible that the leaves contain alkaloids at very low concentration, but in this study it was decided to extract mature flowers only.

Psychotria malayana Jack. This small tree, locally known as 'lolon jarum', and which grows to a height of 1-4 m, is largely distributed in the west Indonesia archipelago. In Java, there have been no reports indicating local uses [14], however, people in Lombok have utilised this plant (aqueous extracts of either leaves or bark) for protecting skin from infection from open wounds and for other skin diseases. The alkaloids are concentrated in the leaves and bark. Further separation of the crude alkaloid extract from the leaves (0.9% based on air dried material, 100 g) by the use of preparative TLC (silica gel; solvent system CH₂Cl₂: CH₃OH: NH₄OH/ 90 : 15 : 1) showed at least four alkaloids to be present; hodgkinsine **1** (Rf 0.56), a trimeric N_b-methyltryptamine alkaloid, was the major constituent. It was readily identified by electron impact mass spectrometry (EIMS) with three principal peaks at m/z 172, 344, and 518 for the trimeric structure (Chemical Ionisation HRMS m/z 519.3200, calc 519.3236 for C₃₃H₃₈N₆+H). The other minor alkaloid found was chimonanthine **2** (Rf 0.63), a dimeric N_b-methyltryptamine, having two main fragments at m/z 172 and 130 (Chemical Ionisation HRMS m/z 347.2224, calc 347.2235 for C₂₂H₂₆N₄+H) in the EI mass spectrum. Polymeric tryptamines are commonly found in *Psychotria* species [15-18]. Two other constituents with molecular weights of 186 (Chemical Ionisation HRMS m/z 187.1230, calc 187.1235 for C₁₂H₁₄N₂+H) and 574 are currently under investigation and will be reported separately. Alkaloids with these molecular weights have not been reported from *Psychotria* previously.



Voacanga foetida (Bl.) Rolfe. Locally called 'kumbi', this plant in the Apocynaceae is distributed throughout Indonesia but Lombok is the main region. It grows in areas about 400 m above sea level and reaches 10-15 m in height. The aqueous extract of the leaves or bark is used commonly to treat a wide range of skin conditions such as wounds, itches, and swellings. Initial alkaloid screening showed that all parts of the plant contained high concentrations of alkaloids, although a previous report indicated that only small amounts of alkaloids occurred in the bark [7].

Conclusions

Initial work on Lombok medicinal plants has resulted in a hundred species being collected and screened for the presence of alkaloids and the five selected plants are being investigated for determination of the alkaloid structures and antimicrobial activity. At least five novel alkaloids appear to be present in *C. calamitosum* L. It has also been found that hodgkinsine is the major alkaloid in *Psychotria malayana* Jack. Further work on the isolation and structural elucidation of alkaloids from the selected plants, and testing of the alkaloids for antibacterial and antimalarial properties, will be reported separately.

Experimental

General

Chemical Ionisation and Electron Impact (at 70 eV) mass spectra were obtained on a Shimadzu QP-5000 by the direct insertion technique. High resolution CI mass spectra were obtained on a Fisons/VG Autospec-TOF-oa Mass Spectrometer. Preparative TLC was performed on Merck silica gel 60 PF₂₅₄, 0.3 mm thick plates, and observed under UV light (333 nm). All solvents were distilled before use.

Plant Collection

The plants were collected throughout Lombok with permission of the Local Government and in collaboration with the University of Mataram. All plants were collected between April and June 1999, and were identified with the assistance of Mr. Made Sudana, a botanist at the University of Mataram, Lombok. In addition, plant specimens were sent to the Research and Development Centre for Biology, Bogor, Indonesia, for confirmatory identification. A local herbarium collection was created and stored in a pest-proof wooden container at the Laboratory of Biology, the University of Mataram. The collection codes noted in Tables 1 and 2 are also the voucher specimen codes in this herbarium.

Alkaloid Testing

The method of alkaloid testing followed the procedure of Culvenor and Fitzgerald [5]. In the procedure, finely ground plant material is rapidly extracted with ammoniacal chloroform (CHCl_3) or dichloromethane (CH_2Cl_2). After filtration, the solution is extracted in turn with aqueous sulphuric acid (0.1M, H_2SO_4). The presence of most alkaloids in the aqueous phase can be detected by the formation of a precipitate on addition of Mayer's reagent (K_2HgI_4). The semi-quantitative results of the method have been rated from + for faint turbidity to ++++ for a heavy curdy precipitate. Modifications to the procedure introduced by Bick *et. al.*[6], notably the use of a domestic coffee grinder to grind the plant material, were also used in this study.

In general, the procedure is rapid and produces consistent results. Water soluble alkaloids, however, are not detected, since they are not extracted by the ammoniacal CHCl_3 or CH_2Cl_2 . Separate water extractions of plant material were not undertaken in this investigation.

Isolation of Alkaloids

Before extraction, parts of the plants were prepared by air drying at room temperature (*ca.* 27°C) followed by grinding separately in a coffee grinder. In the case of plants giving a negative alkaloid result after drying, although giving a positive test when fresh (such as *Clerodendron calamitosum* L.), fresh material was ground and extracted to reduce the loss of alkaloid.

The procedure for extraction of the alkaloid from the five plants selected is outlined in Figure 1. Ground plant material was extracted with cold distilled methanol (CH_3OH) with occasional swirling. Methanol extraction was continued until the plant material gave a negative test for alkaloids (Mayer's test). After filtration, the solvent was removed under reduced pressure at 40°C, to minimise any thermal degradation of the alkaloids.

The crude alkaloid mixture was then separated from neutral and acidic materials, and water solubles, by initial extraction with aqueous acetic acid ($\text{CH}_3\text{CO}_2\text{H}$) followed by dichloromethane extraction and then basification of the aqueous solution and further dichloromethane extraction.

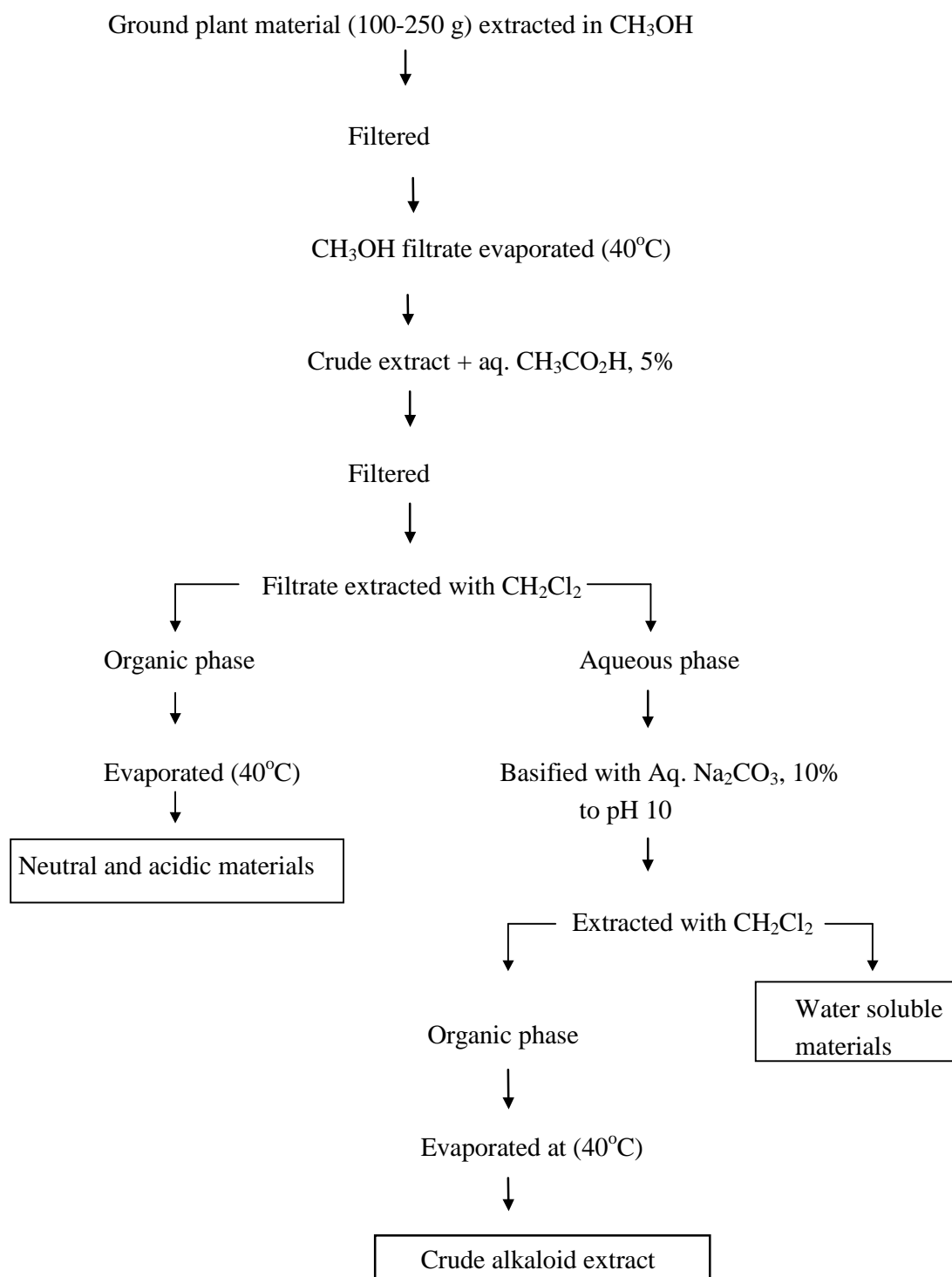


Figure 1. Outline of the extraction procedure for alkaloids carried out for selected medicinal plants of Lombok.

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Sample availability: Samples available from the authors.

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