

ANTIBACTERIAL ACTIVITY OF SOME MEDICINAL PLANTS OF SRI LANKA

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Abstract: 101 Plant extracts from 55 plants were screened for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Mycobacterium fortuitum*. *Hortonia angustifolia* (root) showed very high activity against *Mycobacterium fortuitum*; *Artemisia dubia* (leaf), *Celtis cinnamomea* (stem), *Curcuma longa* (tuber), *Lobelia aromatica* (leaf), *Ocimum gratissimum* (aerial part), *Pimenta officinalis* (stem bark), *Thespesia populnea* (stem), *Ulex europaeus* (whole plant) showed significant activity against at least one of the above organisms.

Key words: Antibacterial activity, medicinal plants.

INTRODUCTION

The search for new antibiotics with hitherto unknown modes of action remains an urgent priority as microbial resistance to antimicrobial agents continues to unfold as one of the most difficult problems facing the physicians dealing with infectious diseases. There is virtually no drug that is free of this problem, as genes for resistance have emerged and spread at an alarming rate.^{1,2} So far the majority of clinically used antibiotics are obtained from microorganisms, lower plants such as fungi and algae, symbiotic lichens and mosses.³ On the other hand, higher plants contain a reservoir of antibiotics which have so far remained underutilized.³ Many plants which are resistant to microorganisms have been shown to produce antibiotics.³

The flora of Sri Lanka comprise about 3,300 flowering plants of which about 830 (25%) species are endemic to the island.⁴ The variations in soil conditions, temperature and humidity are reflected in a wide range of flora. Most of the plant species found in Sri Lanka are the same as those found in the Indian sub-continent. However, the high percentage of endemic plants in the island, compared to most countries in the region, makes it a fertile testing ground for the phytochemist. Attygalle⁵ has described a large number of plants of medicinal value in his compilation of 'Sinhalese Materia Medica'. The pioneering work by Chandrasena⁶ in 1935, has listed the earliest chemical studies and the pharmacological activity of Sri Lankan and Indian plants.

The present study describes the investigation of 101 plant extracts derived from 55 medicinal plants of Sri Lanka, for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Mycobacterium fortuitum*.

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S. aureus and *E. coli* were chosen because standard strains of these organisms are sensitive to almost all commonly used antibiotics. *S. aureus* is found in 10-20% of normal healthy adults and is one of the commonest pathogens in skin wounds.⁷ *M. fortuitum*, is an acid fast soil bacterium and was selected as representative of the genus *Mycobacterium* for the following reasons:⁸ It is a rapidly growing bacterium (results could be evaluated in 7-14 days) and grows in simple media with little enrichment. Furthermore, *M. fortuitum* is easier to handle being ordinarily non-pathogenic; it rarely causes pulmonary disease but usually causes wound infections, post infection abscesses, and contamination of the porcine valve used for heart valve replacement of humans. However, *M. fortuitum* shows full resistance to most antitubercular drugs.⁸

METHODS AND MATERIALS

Plant material

Plant material used in this study were mature (reproductive maturity) specimens (1-5 kg) collected from different localities of Sri Lanka, mostly from the Central Province of Sri Lanka. All plants collected were true species.^{9,4} Plant specimens were identified by comparison at the National Herbarium, Royal Botanic Gardens, Peradeniya.^{9,4} The specimens were immediately washed with running water to remove contaminated soil and other surface impurities. They were cut into small pieces about 3-6 cm in length. The specimens were immediately shade-dried and powdered in a laboratory mill.

Preparation of plant extracts

The air-dried and ground plant material (100 g) were extracted into 500 ml of hot methanol or successively with 500 ml each of hot hexane/light petroleum (40-60°C), dichloromethane, ethyl acetate, in a Soxhlet apparatus. Some were extracted directly with cold ethyl acetate and cold methanol in a bottle shaker. The solubles were concentrated to dryness separately using a rotavapor (below 45°C). The extracts were subjected to antibacterial assay as described below.

Antibacterial screening

Tests for antibacterial activity¹⁰ were carried out using type strains of *S. aureus* (NCTC 6571), *E. coli* (NCTC 10418) and *M. fortuitum* {3010/82 (4974) Brisbane}. The plant extracts (16 mg each) were weighed and dissolved in 4 ml of absolute alcohol in test tubes. From this solution 0.4 ml was transferred to a sterile nutrient broth solution (10 g peptone, 10 g beef extract and 5 g NaCl in 1000 ml of distilled water; the medium was autoclaved at 15 lb pressure for 15 min) to prepare tenfold dilutions. A series of doubling dilutions were then dispensed in 2 ml aliquots, in test tubes, with concentrations ranging from 1/10 to 1/5120 and inoculated with a fixed bacterial inoculum (0.1 ml of a 10⁻⁴ dilution of bacterial suspension with a density of a 2% aqueous suspension of barium sulphate). Different bacterial cultures were inoculated separately. Simultaneously, the

bacterial inoculum was also made into an identical set of tubes of dilutions made from ethanol alone, and a tube each of nutrient broth, these being used as controls. The tubes were incubated at 37°C for 24 h except for *M. fortuitum* which was incubated for 7 days. The tubes were examined for turbidity with the naked eye. The minimal inhibitory concentration (MIC), which is the lowest concentration which prevents the development of turbidity of the extracts was determined. To confirm the naked eye reading, a loopful of mixture from each of these tubes were then inoculated on nutrient agar and incubated as before. The subculture from the tube containing the MIC formed no more than a few colonies and the subcultures from the tubes containing bactericidal concentration formed no growth at all. The minimal bactericidal concentration (MBC) was considered to be that showing no growth or less than 20 colonies on a semisolid medium. In the interpretation of results, the test solutions were compared with those of alcohol dilutions. All experiments were carried out under sterile conditions.

RESULTS AND DISCUSSION

Most of the plants used in the study are used in ethnomedical preparations in Sri Lanka.⁶ Some plants have been selected on the basis that they are widely distributed and apparently free of pest/microbial attack. Some plants were chosen due to the presence of compounds that are useful and/or having interesting biological properties. For example *Curcuma longa* and *Artemisia dubia* are used ethnomedically. *C. longa* is used as both a colouring material and condiment.¹¹ This plant contains curcumin,¹² juvabione,¹³ and some sesquiterpenoids.¹⁴

Of the plants which are promising, most showed antibacterial activity against one organism only (Table 1). The hot methanol extract of *Hortonia angustifolia* (Rt) showed highest activity against *M. fortuitum*. Interestingly, the hot methanol extract of *H. angustifolia* has shown strong antifungal activity (against *Cladosporium cladosporioides*)¹⁵ while both its Rt and Lf has shown significant insecticidal activity against *Aphis craccivora*.^{16,17} The cold methanol extract of *Artemisia dubia* (Lf), hot methanol extract of *Celtis cinnamomea* (St), cold ethanol and methanol extracts of *Curcuma longa* (Tb), and hot methanol extracts of *Lobelia aromatica* (Lf), *Ocimum gratissimum* (Ap), *Pimenta officinalis* (St Bk), *Thespesia populnea* (St) and *Ulex europaeus* (Wp) showed significant activity. The hot methanol extracts of *Cestrum aurantiacum* (Lf and St), *Chrysopogon zeylanicus* (Wp), *Eupatorium odoratum* (Lf), *Gaultheria rudis* (Wp-Lf), *Glochidion montanum* (Lf), *Hortonia angustifolia* (St), *Hypericum mysurense* (St), *Leucas zeylanica* (Wp), *Lobelia nicotianifolia* (Lf), *Phyllanthus embilica* (Lf), *Phyllanthus reticulatus* (Lf) and *Psychotria nigra* (Lf) showed only moderate activity.

Table 1: Antibacterial activity of some medicinal plants of Sri Lanka against *Staphylococcus aureus*, *Escherichia coli* and *Mycobacterium fortuitum*.

Plant (Family) [#] [Sinhala/Tamil]	Collection	Part ^a	Solvent ^b	Minimal Bactericidal Concentration (MBC) µg/ml ^c		
				<i>Stap.</i>	<i>Myc.</i>	<i>E. coli</i>
+ <i>Actinodaphne speciosa</i> Nees. (Lauraceae) [S-Alikan]	Horton Plains	Lf	MeOH ^h	-	-	-
<i>Allaeophania decipiens</i> Thw. (Rubiaceae)	Horton Plains	Ap	MeOH ^h	-	-	-
		St	MeOH ^h	-	-	-
<i>Alpinia abundiflora</i> Burt & Smith (Zingiberaceae)	Horton Plains	Rh	*Pet ^h	-	-	-
			CH ₂ Cl ₂ ^h	-	-	-
			*MeOH ^h	-	-	-
+ <i>Alpinia fax</i> Burt & Smith (Zingiberaceae)	Rakwana	Tb	MeOH ^h	-	-	-
<i>Alpinia nigra</i> (Gaertn.) Burt (Zingiberaceae) [S-Alugas, T-Shitai rattai]	Hakgala	Ft	MeOH ^h	-	-	-
		Tb	MeOH ^h	-	-	-
<i>Anotis richardiana</i> (Arn.) Hook.f. (Rubiaceae)	Pattipola	Wp	MeOH ^h	-	-	-
<i>Artemisia dubia</i> var. <i>grata</i> Wall (Compositae) [S-Walkolundu, T-Marukolundu]	Ambawela	Lf	MeOH ^c	25	-	-
		Lf	MeOH ^h	-	-	-
		St	MeOH ^c	-	-	-
		St	MeOH ^h	-	-	-
		Rt	MeOH ^c	-	-	-
<i>Bridelia retusa</i> (L.) Spreng (Euphorbiaceae) [S-Ketakela, T-Adamarudu]	Kandy	Lf	MeOH ^h	-	-	-
<i>Butea monosperma</i> (Lam.) Taub. (Leguminosae) [S-Gaskela, T-Parasu]	Mahiyangana	St Bk	EtOH ^c	-	-	-
<i>Celtis cinnamomea</i> Lindl. ex Planch. (Ulmaceae) [S-Gurenda, T-Pinari]	Hakgala	Lf	MeOH ^h	-	-	-
		St	MeOH ^h	25	-	50
<i>Cestrum aurantiacum</i> Lindl. (Solanaceae)	Hakgala	Lf	MeOH ^h	50	-	-
		St	MeOH ^h	-	-	-
<i>Chrysopogon zeylanicus</i> (Steud.) Thw. (Gramaceae) [S-Gawara]	Hakgala	Wp	MeOH ^h	100	-	50
<i>Costus speciosus</i> (Koen.) Sm. (Zingiberaceae) [S-Tebu, T-Khoshadam]	Kalutara	Rh	MeOH ^h	-	-	-
<i>Curcuma longa</i> L. (Zingiberaceae) [S-Kaha, T-Manchal]	Kandy	Tb	EtOH ^c	-	25	-
		Tb	MeOH ^c	-	25	-
<i>Curcuma zedoaria</i> (Berg.) Roscoe. (Zingiberaceae) [S-Harankaha, T-Kasturimangal]	Kandy	Tb	MeOH ^h	-	-	-
<i>Eupatorium inulifolium</i> HBK. (Compositae)	Hakgala	St	MeOH ^h	-	-	-
		StBk	MeOH ^h	-	-	-
<i>Eupatorium odoratum</i> L. (Compositae) [S-Podisinghomaran]	Kandy	Lf	MeOH ^h	50	-	-

Table 1 Contd.

Plant (Family) ^a [Sinhala/Tamil]	Collection	Part ^a	Solvent ^b	Minimal Bactericidal Concentration (MBC) µg/ml ^c		
				<i>Stap.</i>	<i>Myco</i>	<i>E. coli</i>
<i>Eupatorium riparium</i> Regel. (Compositae)	Hakgala	Ap	MeOH ^h	-	-	-
		Rt	*Hex ^h *CH ₂ Cl ₂ ^h	-	-	-
<i>Gaultheria rudis</i> Stapf. (Ericaceae) [S-Wal kapuru]	Horton Plains	Wp-Lf	MeOH ^h	100	-	-
		Lf	MeOH ^h	-	-	-
<i>Glochidion montanum</i> Thw. (Euphorbiaceae)	Hakgala	Lf	MeOH ^h	-	100	-
<i>Gynostemma laxum</i> (Wall.) Cong. (Cucurbitaceae)	Hakgala	Ap	MeOH ^h	-	-	-
+ <i>Hortonia angustifolia</i> (Thw.) Trim. (Monimiaceae)	Sinharaja	Lf	MeOH ^h	-	-	-
		St	MeOH ^h	-	100	-
+ <i>Hortonia floribunda</i> Wight ex Arn. (Monimiaceae) [S-Gawara]	Hakgala	Lf	MeOH ^h	-	-	-
		St	MeOH ^h	-	-	-
<i>Hypericum mysurense</i> Wight & Arn. (Gutiferae)	Pattipola	Lf	MeOH ^h	-	-	-
		St	MeOH ^h	100	-	-
<i>Justicia betonia</i> L. (Acanthaceae) [S-Sudu puruk]	Kandy	Ap	MeOH ^h	-	-	-
<i>Leucas biflora</i> (Vahl.) Benth. (Labiatae) [S-Geta tumba, T-Peyt tumpi]	Horton Plains	Wp	MeOH ^h	-	-	-
<i>Leucas zeylanica</i> (L.) Benth. (Labiatae) [S-Geta tumba, T-Mudi tumpi]	Hakgala	Wp	MeOH ^h	-	-	100
<i>Lobelia aromatica</i> Moon ex Wight (Lobeliaceae)	Horton Plains	Lf	MeOH ^h	12.5	-	-
		St Bk	MeOH ^h	-	-	-
<i>Lobelia nicotianifolia</i> Roth ex R. & Schult (Lobeliaceae) [S-Waldunkola, T-Kattupoillai]	Hakgala	Lf	MeOH ^h	-	-	-
		St Bk	MeOH ^h	-	-	-
+ <i>Mastixia tetrandra</i> (Wight ex Thwaites) C.B. Clarke (Cornaceae) [S-Matawara]	Hakgala	Lf	MeOH ^h	-	-	-
<i>Ocimum gratissimum</i> L. (Labiatae) [S-Gastala, T-Elumichantulasi]	Kandy	Ap	MeOH ^h	25	-	25
		Rt	MeOH ^h	-	-	-
<i>Osbeckia cupularis</i> D. Don ex Wight & Arn. (Melastomaceae) [S-Bowitia]	Horton Plains	Wp	MeOH ^h	-	-	-
<i>Pedaliium murex</i> L. (Pedaliaceae) [S-Ethnerenchi, T-Perunerrenchi]	Puttalam	Lf	MeOH ^h	-	-	-
<i>Phyllanthus embilica</i> L. (Euphorbiaceae) [S-Nelli, T-Nelli]	Hakgala	Lf	MeOH ^h	-	50	-
<i>Phyllanthus reticulatus</i> Poir. (Euphorbiaceae) [S-Welkayila, T-Pulla]	Sigiriya	Lf	MeOH ^h	-	100	-
<i>Pimenta officinalis</i> Lindl. (Myrtaceae)	Kandy	Lf	MeOH ^h	-	-	-
		St Bk	MeOH ^h	25	-	-

Table 1 Contd.

Plant (Family)* [Sinhala/Tamil]	Collection	Part ^a	Solvent ^b	Minimal Bactericidal Concentration (MBC) $\mu\text{g/ml}^c$		
				<i>Stap.</i>	<i>Myco</i>	<i>E. coli</i>
<i>Pleiospermium alatum</i> (Wight & Arn.) Swingle (Rutaceae) [S-Tumpathkurundu, T-Mailadikurundu]	Sigiriya	St Bk	Hex ^h	-	-	-
		Rt Bk	[*] EtOAc ^b	-	-	-
<i>Psychotria bisulcata</i> (Wight & Arn.) (Rubiaceae)	Horton Plains	Lf	[*] MeOH ^h	-	100	-
		St	MeOH ^h	-	-	-
<i>Psychotria nigra</i> var. <i>coronata</i> Hk.f. (Rubiaceae)	Horton Plains	Lf	MeOH ^h	-	-	-
		St	MeOH ^h	-	-	-
<i>Ricinus communis</i> L. (Euphorbiaceae) [S-Ernadu, T-Chittamanakku]	Sigiriya	St Bk	MeOH ^h	-	-	-
<i>Saprosma foetens</i> (Wight) K.schum. (Rubiaceae)	Kandy	Lf	MeOH ^h	-	-	-
		St Bk	MeOH ^h	-	-	-
<i>Sarcococca prunifoliosa</i> Lindl. (Buxaceae)	Hakgala	Lf	MeOH ^h	-	-	-
		St	MeOH ^h	-	-	-
+ <i>Semecarpus coriacea</i> Thw. (Anacardiaceae) [S-Badulla]	Hakgala	Lf	MeOH ^h	-	-	-
		St	MeOH ^h	-	-	-
<i>Semecarpus nigro-viridis</i> Thw. (Anacardiaceae) [S-Gatabadulla]	Hakgala	Lf	MeOH ^h	-	-	-
		St	MeOH ^h	-	-	-
		St Bk	MeOH ^h	-	-	-
+ <i>Semecarpus obscura</i> Thw. (Anacardiaceae) [S-Badulla]	Hakgala	Lf	MeOH ^h	-	-	-
		St	MeOH ^h	-	-	-
		St Bk	MeOH ^h	-	-	-
<i>Sesamum indicum</i> L. (Pedaliaceae) [S-Walthala, T-Ellu]	Rambukkana	Lf	EtOH ^c	-	-	-
<i>Solanum giganteum</i> Jacq. (Solanaceae)	Hakgala	Rt	MeOH ^h	-	-	-
		Rt	MeOH ^h	-	-	-
<i>Strobilanthes hookeri</i> Nees. (Acanthaceae)	Horton Plains	Lf	MeOH ^h	-	-	-
		St	MeOH ^h	-	-	-
<i>Strobilanthes viscosa</i> (Nees) T. Anders (Acanthaceae)	Horton Plains	Lf	MeOH ^h	-	-	-
		St	MeOH ^h	-	-	-
<i>Strobilanthes auriculatus</i> Nees. (Acanthaceae)	Horton Plains	Lf	MeOH ^h	-	-	-
		St	MeOH ^h	-	-	-
<i>Theobroma cacao</i> L. (Sterculiaceae) [S-Cocoa, T-Coco]	Kalutara	Rt	EtOAc ^c	-	-	-
<i>Thespesia populnea</i> (L.) Soland ex Corr. (Malvaceae) [S-Gansuriya, T-Kavarachu]	Kandy	Lf	MeOH ^h	-	-	-
		St	MeOH ^h	25	-	-
<i>Ulex europaeus</i> L. (Leguminosae)	Horton Plains	Wp	MeOH ^h	100	12.5	-
<i>Wendlandia bicuspidata</i> Wight & Arn. (Rubiaceae) [S-Balalhulla]	Hakgala	Lf	MeOH ^h	-	-	-
		St	MeOH ^h	-	-	-
		St Bk	MeOH ^h	-	-	-
<i>Zingiber zerumbet</i> (L.) Sm. (Zingiberaceae) [S-Walinguru]	Hakgala	Tb	MeOH ^h	-	-	-

: Nomenclature follows W.M. Bandaranayake et al.⁴, + : Species endemic to Sri Lanka⁴,
* : Successive extraction, a : Ap - aerial part; Lf - leaf, Rh - rhizome; Rt - root; St Bk - stem
bark; Tb - tuber; Wp-whole plant, b : h - hot extraction; Hex - n-hexane, c: (-) - no activity.

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