J. Pharm. Pharmacol. 1999, 51: 1555–1560
Received May 19, 1999
Accepted July 30, 1999

Investigation of Plants used in Jamaican Folk Medicine for Anti-bacterial Activity

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Abstract

We have started a systematic scientific study of folklore medicinal plants currently used as alternative medicine in Jamaican society. In this initial study, extracts of plants widely used by the islanders are studied for antibacterial activity against five common pathogens; *Streptococcus* group A, *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Escherichia coli*.

These studies revealed that 25% (approx.) of the plant extracts had antimicrobial activity against at least one of the microbes used. Subsequent to these observations, extracts from *Mikania micrantha* were examined in detail. This led to the isolation of two sesquiterpenoids, mikanolide and dihydromikanolide, with activity against *S. aureus* and *C. albicans*.

The results suggest that traditional folk medicine could be used as a guide in our continuing search for new natural products with potential medicinal properties.

Chemical and biological investigation of folk-medicinal plants with the reputation of being curative has provided the world with many of the common clinical drugs of today. It has been reported that at least 119 compounds derived from 90 plant species can be regarded as important drugs currently in use, and that 77% of these are derived from traditional medicine (Cragg et al 1997). In rural Jamaican society and some urban settings traditional medicine is still widely practised and dramatic curative properties have been ascribed to several endemic plants.

The search for new antibacterial agents, in particular, has increased in the last decade, mainly because of the increase in bacterial infection, especially in countries with young populations and more so because of bacterial resistance to current antibiotics (Vaara 1996). Bacterial infection has also been implicated in complications of chronic conditions, specifically transplants, cancer and AIDS, because of weakened immunity (Stinson 1996).

Bacteria resist attack of antibiotics by three main mechanisms: modification of the target site or enzymes that the antibiotic normally attacks, prevention of access of the antibiotic and production of new antibiotic enzymes that destroy or inactivate the antibiotics (Mann 1995).

With these factors in mind we embarked upon widespread systematic screening of plants used in Jamaican traditional folk medicine. The organic extracts of plants (Table 1; Adams 1972; Ayensu 1981) were screened for antibacterial activity against five microorganisms by use of the disk-diffusion method (Bauer et al 1966; Ericsson & Sherris 1971; Boyd 1984; Atlas 1986). The plants were collected from random sites all over the island, if possible from plots cultivated specifically for medicinal use.

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Materials and Methods

Plant material

Plants were collected from different places throughout the island and identified by comparison with authentic samples present at the herbarium in the Botany Department of the University of the West Indies. Samples were deposited when necessary.

Extraction and preparation of test solutions

Aerial parts of each plant were collected and dried for 4 days. The material was then milled and a

mixture of acetone and ethyl acetate (1 : 1, 150 mL) was percolated through portions (20 g) of each plant. The resulting solutions were concentrated in-vacuo by rotary evaporation to yield dark green gums (average yield 2·8%). Test solutions were prepared from each extract by dissolving the gum (0·50 g) in a 1 : 1 mixture of acetone and ethyl acetate (10 mL) to give a 5% (w/v) solution. The negative control was a 1 : 1 mixture of acetone and ethyl acetate only.

Isolation of mikanolide and dihydromikanolide from Mikania micrantha

Dried milled leaves of the plant (254 g) were extracted with acetone–ethyl acetate (1 : 1, 2 L) and acetone (1 L), each for 4 days to give a dark green gum (11·7 g, yield 4·6%). Trituration of the gum with 1 : 1 chloroform–hexane (250 mL) and acetone (250 mL) resulted in the deposition of a light green solid (141 mg) which was isolated by decanting the supernatant. Repeated preparative TLC of this solid (silica gel, 70 : 30 hexane–ethyl acetate) yielded mikanolide (Figure 1A) (21 mg) and dihydromikanolide (Figure 1B) (9 mg) as the major components.

Antibacterial assay

The widely used disk-diffusion method was used to investigate the antibacterial activity of each crude plant extract (50–60 μL, 2·5–3·0 mg). Isolates of *Streptococcus* group A, *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Escherichia coli* (pathogens of widespread occurrence in infections treated at the University Hospital of the West Indies, Kingston, Jamaica) were obtained and standardized culture suspensions of $1{\cdot}5 \times 10^8$ colony-forming units were used for each inoculum (National Committee for Clinical Laboratory Standards (NCCLS) 1993, 1994). After incubation the zone of inhibition of growth surrounding each disk was measured by use of a micrometer. If zonal inhibition of 14 mm or greater was observed, the organism was recorded as susceptible to the test extract and was assigned the code +++. If extracts produced zones of 12–14 mm the effect on the organism was considered to be intermediate, ++, and those extracts producing zones of 8–12 mm were considered to weakly active, +. If zones were less than 8 mm the organism was considered to be resistant. Gentamicin (10 μg) was used as standard.

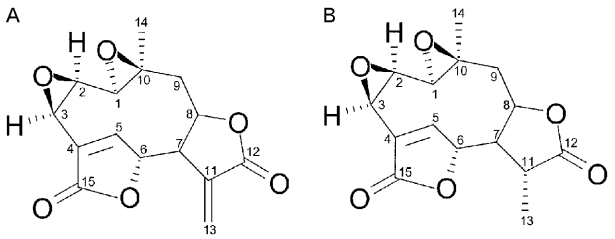

Figure 1. The structures of A. mikanolide and B. dihydromikanolide.

Results and Discussion

Of the plants screened for antibacterial activity, twenty-three (Table 1) were active, of these *Mikania micrantha* had the greatest activity; it was active against three of the five pathogens *Staphylococcus aureus*, *Streptococcus* group A and *Escherichia coli*.

The bacteria in the Gram-positive cocci group, *Staphylococcus aureus* and *Streptococcus* group A were more sensitive to the plant extracts than were the Gram-negative bacteria. However, those of the Gram-negative bacilli group, *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa*, were generally resistant to the extracts, with *P. mirabilis* being sensitive to only four plant extracts—*Cassia jamaicensis*, *Coccoloba krugii*, *Cordia brownei* and *Haematoxylum campechianum*, whereas *E. coli* was sensitive to the *Mikania micrantha* extract only.

P. aeruginosa was the most resistant of the five organisms tested. This organism has been reported to be naturally resistant to many widely used antibiotics and thus chemotherapy is a problem. This resistance is because of a resistant transfer plasmid factor carrying genes coding for the detoxification of a variety of antibiotics (Brock et al 1984)

The *Compositae* (*Asteraceae*) family furnished the largest number of active plant extracts; six of the ten plants screened in this family had activity against at least two bacterial species. The Gram-positive bacteria were more sensitive and thus the extract seemed to have a spectrum of activity similar to that of the cephalosporins, lincosamides, penicillins and macrolides. The results showed that four plants—*Cassia jamaicensis*, *Coccoloba krugii*, *Cordia brownei* and *Haematoxylum campechianum* were active against the Gram-negative bacterium *Proteus mirabilis* and against the Gram-positive bacteria. The extract of *Mikania micrantha* (Guaco) was the most active against the pathogens and was selected for activity-directed chemical isolation studies.

These studies revealed that the antibacterial activity of the extract could be attributed to the two known sesquiterpenes, mikanolide and dihydro-

Table 1. Plants, folk use and antibacterial activity.

Family	Botanical name (Common name)	Folk uses	Pathogens				
			E. coli	*S. aureus*	*P. mirabilis*	*P. aeruginosa*	*Streptococcus A*
Acanthaceae	*Asystasia gangetica* (L.) T. Andres		–	+ + +	–	–	–
Acanthaceae	*Justicia pectoralis* Jacq. (Fresh cut)	Wounds, colds	–	–	–	–	–
Amaranthaceae	*Achyranthes indica* L. (Devil's horse whip)	Flu, marasmus, venereal disease	–	–	–	–	–
Anacardiaceae	*Mangifera indica* L. (Julie)	Fever, diarrhoea	–	+	–	–	–
Annonceae	*Annona muricata* L. (Soursop)	Tranquillizer	–	–	–	–	–
Annonceae	*Annona squamosa* L. (Sweetsop)	Fever, painful spleen, labour pains	–	–	–	–	–
Apocynaceae	*Catharanthus roseus* (L.) G. Don (Periwinkle)	Leukaemia, astringent	–	–	–	–	–
Asclepiadaceae	*Asclepias curassavica* L. (Red top)	Vermifuge, sores	–	–	–	–	–
Bignoniaceae	*Catalpa longissima* (Jacq.) Dum–Cours (French oak)	Haemorrhoid, angina, astringent	–	–	–	–	–
Bignoniaceae	*Tecoma stans* (L.) Kunth (Yellow cedar)	Fever	–	–	–	–	–
Boraginaceae	*Cordia brownei* (Friesen) I. M. Johnston. (Black sage)	Colds, fever, insomnia	–	+ +	+	–	–
Boraginaceae	*Cordia sebestena* L. (Geiger tree)	Sharpening appetite	–	–	–	–	–
Boraginaceae	*Symphytum officinale* L. (Europ.) (Comfrey)	Anaemia, fatigue, pains, colds	–	–	–	–	–
Boraginaceae	*Tournefortia volubilis* L. (Chigger nut)	Sores, baths, ''restore manhood''	–	–	–	–	–
Brassicaceae	*Lepidium virginicum* L. (Wild pepper grass)	Relieve gas	–	–	–	–	–
Caesalpincaceae	*Cassia alata* L. (King of the forest)	Eczema, purgative, hypertension, vermifuge	–	–	–	–	–
Caesalpincaceae	*Cassia jamaicensis*[a] (Britton) Adams		–	+ + +	+	–	–
Caesalpincaceae	*Cassia occidentalis* L. (Dandelion)	Liver and bladder dysfunction	–	–	–	–	–
Caesalpiniaceae	*Haematoxylum campechianum* L. (Logwood leaves)	Wound dressing	–	+ +	+	–	–
Caesalpiniaceae	*Tamarindus indica L. (Tamarind leaves)*	Colds, measles, chicken pox	–	–	–	–	–
Campanulaceae	*Hippobroma longiflora* (L.) G. Don (Madam fate)	Poultice used for injury	–	+	–	–	–
Campanulaceae	*Lobelia accuminata*[a] Sw.		–	–	–	–	–
Campanulaceae	*Lobelia viridiflora*[a] McVaugh		–	–	–	–	–
Cannabaceae	*Cannibis sativa* L. (Ganja)	Asthma, vision improvement	–	–	–	–	–
Capparaceae	*Cleome rutidosperma* L.		–	–	–	–	–
Capparaceae	*Cleome viscosa* L. (Wild caia)		–	–	–	–	+
Commelinaceae	*Commelina benghalensis* L. (Water grass)	Thrush, burns, sore throats, strangury	–	–	–	–	–
Compositae (Asteraceae)	*Bidens cynapiifolia* Kunth (Spanish needle)	Fever, Stomach problems	–	–	–	–	–

Continued overleaf

Table 1. (*continued*)

Family	Botanical name (Common name)	Folk uses	Pathogens				
			E. coli	*S. aureus*	*P. mirabilis*	*P. aeruginosa*	*Streptococcus A*
Compositae (Asteraceae)	*Eupatorium odoratum* L. (Jack in the bush)	Wound dressing	–	+ +	–	–	–
Compositae (Asteraceae)	*Eupatorium villosium* Sw. (Bitter bush)	Lotion for skin rash	–	+ +	–	–	–
Compositae (Asteraceae)	*Mikania micrantha* Kunth (Guaco)	Skin itch, athlete's foot, wound dressing	+	+ + +	–	–	+
Compositae (Asteraceae)	*Parthenium hysterophorus* L. (Dog flea weed)	Sores and wound dressing	–	+	–	–	–
Compositae (Asteraceae)	*Pseudelephantopus spicatus* (B. Juss. ex Aubl.) C. F. Baker (Dog tongue)	Fever, ophthalmic, sprains	–	–	–	–	+
Compositae (Asteraceae)	*Synedrella nodiflora* (L.) Gaertn. (Fatten barrow)	Colds	–	–	–	–	–
Compositae (Asteraceae)	*Tridax procumbens* L. (Bakenbox)	Fever, catarrh	–	–	–	–	–
Compositae (Asteraceae)	*Veronia pluvialis*[a] Gleason	Tea for colic	–	+ +	–	–	+
Compositae (Asteraceae)	*Wedelia trilobata* L. Hitchc. (Running marigold)	Abortion, fever, sores, colds	–	–	–	–	–
Convoluvlaceae	*Evolvulus arbuscula* Poir. (Sea thyme)	Bellyache	–	–	–	–	–
Crassulaceae	*Bryophyllum pinnatum* (Lam.) Oken. (Leaf of life)	Cold, pain, dysmonorrhoea	–	–	–	–	–
Cucurbitaceae	*Momordica charantia* L. (Cerasee)	Sores, infusion for menstrual disorder	–	+	–	–	+ +
Euphorbiaceae	*Croton eluteria* (L.) Sw (Cascarilla)	Stimulant, tonic	–	–	–	–	–
Euphorbiaceae	*Croton flavens* L. (Yellow balsam)	Earache, sore throat, pneumonia	–	–	–	–	–
Euphorbiaceae	*Croton linearis* Jacq. (Rosemary)	Tranquillizer, circulation problems	–	–	–	–	–
Euphorbiaceae	*Euphorbia cyathophora* L.	Latex used for wound, skin ulcers	–	+	–	–	–
Euphorbiaceae	*Ricinus communis* L. (Oil nut bush)	Flu, pains, gonorrhoea	–	–	–	–	–
Gesneriaceae	*Rytidophyllum tomentosum*[a]. (L.) Mart ex G. Don (Search me heart)	General drink for colds	–	+ +	–	–	+
Labiatae (Lamiaceae)	*Hyptis pectinata* (L.) Poit. (Piaba)	Sores, colds, headache, tonsillitis	–	–	–	–	–
Labiatae (Lamiaceae)	*Hyptis verticillata* Jscq. (John Charles)	Colds, gout	–	–	–	–	–
Labiatae (Lamiaceae)	*Leonotis nepetiflora* (L.) Ait. F. (Christmas candle–stick)	Fever, abortifacient	–	–	–	–	–
Labiatae (Lamiaceae)	*Ocimum basilicum* L. (Sweet parsley)	Colds, liver ailments	–	–	–	–	–
Lythraceae	*Cuphea parsonsia* (L.) R. Br. ex Steud. (Strong back)	Menstrual pains	–	+ +	–	–	–
Malvaceae	*Hibiscus rosa–sinensis* L. (Double hibiscus)	Colds, hernia	–	–	–	–	–
Malvaceae	*Sida acuta* Burm. f. (Broom weed)	Fever, colic	–	–	–	–	–

Table 1. (*continued*)

Family	Botanical name (Common name)	Folk uses	Pathogens				
			E. coli	*S. aureus*	*P. mirabilis*	*P. aeruginosa*	*Streptococcus A*
Malvaceae	*Sida rhombiflora* L.	Indigestion, haemorrhoids	–	–	–	–	–
Oleaceae	*Jasminiun fluminense* Vell. (Azores jasmine)	Snake bite antidote	–	–	–	–	–
Papaveraceae	*Bocconia frutescens* L. (John crow bush)	Purgative, vermifuge, skin ulcer	–	–	–	–	–
Papilionaceae (Fabaceae)	*Alysicarpus vaginalis* (L.) DC. (Medina)	Aphrodisiac	–	+ + +	–	–	–
Papilionaceae (Fabaceae)	*Crotalaria juncea* L. Sp. (Sunnhemp)	Heamoptysis	–	–	–	–	–
Papilionaceae (Fabaceae)	*Gliricida sepium* (Jacq.) Kunth ex Griseb (Quick stick)	Fever, colds, pain, gonorrhoea	–	–	–	–	–
Papilionaceae (Fabaceae)	*Lonchocarpus latifolius* (Willd.) DC. (Dog wood)	Vermifuge	–	–	–	–	–
Passifloraceae	*Passiflora maliformis* L. (Sweet cup)	Tonic	–	–	–	–	–
Phytolacaceae	*Petiveria alliacea* L. (Guinea hen weed)	Fever, antidote to poisoning	–	–	–	–	–
Phytolacaceae	*Rivina humilis* L. (Dog blood)	Colds, diarrhoea, marasmus	–	–	–	–	–
Piperaceae	*Peperomia pellucida* (L.) Kunth (Rat ears)	Flu, diuretic, hypertension, diarrhoea	–	–	–	–	–
Piperaceae	*Piper amalago* L. (Pepper elder)	Flatulence, constipation, cough, colds	–	–	–	–	–
Polygonaceae	*Antigonon leptipus* Hook. & Arn. (Corallita)	Cough, throat constriction	–	–	–	–	+ +
Polygonaceae	*Coccoloba krugii* Lindau		–	+ +	+	–	–
Rubiaceae	*Bauhinia divaricata* L. (Bull hoof)	Bronchitis, sores, liver & kidney ailments	–	–	–	–	–
Rubiaceae	*Borreria laevis* (Lam.) Griseb. (Button weed)	Colds, constipation	–	–	–	–	–
Rubiaceae	*Morinda royoc* L. (Strong back)	Tonic, aphrodisiac	–	–	–	–	–
Rutaceae	*Citrus aurantifolia* (Christm.) Swingle (Lime)	Stomach aches, insomnia	–	+	–	–	–
Rutaceae	*Murraya paniculata* (L.) Jack (Sweet neem)	Diarrhoea, wound dressing	–	+	–	–	–
Sapindaceae	*Blighia sapida* Konig (Ackee)	Colds, pains	–	–	–	–	–
Sapindaceae	*Melicoccus bijugatus* Jacq. (Guinep)	Hypertension, fever, cough	–	–	–	–	–
Simaroubaceae	*Simarouba glauca* DC (Bitter damson)	Malaria, diarrhoea	–	–	–	–	–
Solanaceae	*Solanum torvum* SW. (Susumber)	Flu, colds, vermifuge	–	–	–	–	–
Turneraceae	*Turnera ulmifolia* L. (Ram goat dash-a-long)	Colds, debility	–	–	–	–	–
Urticaceae	*Pilea microphylla* var *microphylla* Liebm (Baby puzzle)	Diarrhoea, labour	–	+	–	–	–
Urticaceae	*Pilea microphylla* var *trianthemoide* (Sw.) Griseb (Artillery plant)	Diarrhoea, asthma	–	–	–	–	–
Verbenaceae	*Clerodendrum philippinum* Schauer (Lady Nugent rose)	Headache	–	–	–	–	–

Continued overleaf

Table 1. (*continued*)

Family	Botanical name (Common name)	Folk uses	Pathogens				
			E. coli	*S. aureus*	*P. mirabilis*	*P. aeruginosa*	*Streptococcus A*
Verbenaceae	*Lantana camara* L. (White sage)	Gonorrhoea, measles, chicken pox	–	++	–	–	–
Verbenaceae	*Petrea volubilis* L. (Purple wreath)	Abortifacient	–	–	–	–	–
Verbenaceae	*Priva lappulacea* (L.) Pers (Clammy bur)	Abortifacient	–	–	–	–	–
Verbenaceae	*Stachytarpheta jamaicensis* (L.) Vahl (Vervine)	Eczema, colds, worms	–	–	–	–	–

[a]Endemic. +++, organism susceptible (zone > 14 mm); ++, intermediate activity (zone 12–14 mm); +, plant weakly active (zone 10–12 mm); –, organism resistant (zone < 10 mm).

Table 2. Antimicrobial activity of mikanolide and dihydromikanolide against *Staphylococcus aureus* and *Candida albicans* (Fermin et al 1975).

Compound	Minimum inhibitory concentration (mg/disc)	
	S. aureus	*C. albicans*
Mikanolide	45	42
Dihydromikanolide	48	6

mikanolide (Figures 1A, B). The identities of these compounds were confirmed by comparison of spectral data (Bardon et al 1988).

Although structural modification at carbon 11 did not alter the efficacy of the compounds against *Staphylococcus aureus*, the dihydro derivative was seven times more toxic to the fungus *Candida albicans* (Table 2).

Conclusion

These studies support the view that systematic scientific investigation can confirm the validity of the use of plants in traditional folklore medicine. For example, *Mikania micrantha* ('guaco'), which had the greatest anti-bacterial activity, is used in the form of decoctions for skin infections and ulcers (Ayensu 1981). Our separation chemistry revealed that mikanolide and dihydromikanolide were two of the active compounds in the organic extract of the plant.

Acknowledgements

The authors are grateful to the University of the West Indies, Mona, Jamaica for financial support of this work. Thanks also to Mr Patrick Lewis, Herbarium Curator, Department of Botany, University of the West Indies, Mona, and all other persons who assisted with the collection of plant material.

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