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Research Article

Antimicrobial Activity of 23 Endemic Plants in Madagascar

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Abstract

***Purpose**: To screen the crude methanol extracts obtained from 23 endemic plants in Madagascar for antimicrobial activity.*

***Method**s: In order to assess the antimicrobial properties of the extracts, their minimum inhibitory concentrations (MICs) were obtained using the broth microdilution method. The six test pathogenic species used were Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa and Candida albicans. Bioautography agar overlay test and phytochemical screening were also performed on the most active extracts.*

***Results**: From the 23 plants tested, 16 of which are used in traditional medicine, Poivrea phaneropetala (Combretaceae), Koehneria madagascariensis (Lythraceae) and Rhopalopilia perrieri (Opiliaceae) exhibited the broad spectrum of activity, being active against all the test organisms, while Monoporus clusiifolius (Myrsinaceae) showed the strongest antifungal activity against Candida albicans with a minimal inhibitory concentration of 0.250 mg/ml. Bioautography and phytochemical analysis of the five active extracts against bacterial strains and of one active extract against C. albicans indicate that the active compounds responsible for antimicrobial activity may be mainly flavonoids and/or terpenes.*

***Conclusion**: These preliminary results are the first antimicrobial studies on these plants and lend support for the use of some of them in traditional medicine.*

***Keywords:** Antimicrobial properties, Traditional medicine, Microdilution assay, Bioautography, Madagascar.*

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INTRODUCTION

Madagascar is host to approximately 12,000 vegetable species, more than 80 % of which are endemic to the island [1]. Owing to environmental degradation, increasing deforestation, slash and burn agriculture in primary forest, this unique patrimony is threatened by extinction. Only 9 % of the original areas are currently available and the country is among 25 most critical regions for plant life protection in the world [2].

In Madagascar, herbal medicines are often used as the first line of treatment of various diseases. The practice of traditional medicine is well-embedded in the lifestyle of the eighteen indigenous tribes of Madagascar. Previous investigations on Madagascan flora mainly dealt with ethnobotanical practices of plants in folk medicine [3-5]. Screening surveys have already shown some biological activities such as antiplasmodial [6], antiviral [7], and cytotoxic activities [8]. However, very few scientific studies have been carried out on the putative antimicrobial properties of endemic plants in the island although many of them have been claimed by local traditional healers to be effective in the treatment of infectious diseases.

The aim of this study was to investigate the antimicrobial properties of 23 endemic plants obtained from different parts of the country and to identify the phytochemical class of active components.

EXPERIMENTAL

Plant materials

The plants were collected from various locations in Madagascar and were authenticated by Dr Armand Rakotozafy, the curator of the Department of Botany at the Institut Malgache de Recherches Appliquées (IMRA), Antananarivo, Madagascar. Voucher specimens were deposited at the herbarium of the Parc Botanique et Zoologique de Tsimbazaza, Antananarivo, Madagascar. Plant names and their folkoric use are given in Table 1. Dried plant materials were ground into fine powders and preserved at the IMRA herbarium.

Table 1: List of 23 Madagascan endemic plants used in the study and their folkoric use

Family	Species name	Folkloric use
Annonaceae	*Xylopia buxifolia* Baill.	Tonic, jaundice
Apocynaceae	*Mascarenhasia lisianthiflora* A. DC.	n.i
Asteraceae	*Pluchea grevei* Humbert	Headache, tonic
Asteropeiaceae	*Asteropeia densiflora* Baker	n.i
Celastraceae	*Evonymopsis longipes* H. Perrier	Headache
Clusiaceae	*Symphonia clusioides* Baker	Hair ointment
Combretaceae	*Poivrea phaneropetala* (Baker) H. Perrier	Vermifuge
Combretaceae	*Poivrea grandidieri* (Baill.) H. Perrier	Vermifuge, icterus
Combretaceae	*Poivrea obscura* (Tul.) H. Perrier	Vermifuge, diuretic
Dilleniaceae	*Hibbertia coriacea* Baill.	Vaginitis, urethritis
Elaeocarpaceae	*Elaeocarpus sericeus* Baker	n.i
Fabaceae	*Piptadenia pervillei* Vatke	Antimalarial
Lythraceae	*Koehneria madagascariensis* (Baker) S.A.Graham, H.Tobe & P.Baas	n.i
Melastomaceae	*Dichaetanthera oblongifolia* Baker	Diarrhea, dysentery
Moraceae	*Pachytrophe dimepate* Bureau	Jaundice
Myrsinaceae	*Monoporus clusiifolius* H. Perrier	n.i
Opiliaceae	*Rhopalopilia perrieri* Cavaco & Keraudren	Antiseptic, wound healing
Rhamnaceae	Bathiorhamnus louvelii (H. Perrier) Capuron	Purgative
Sapindaceae	*Conchopetalum madagascariense* Radlk	n.i
Sarcolaenaceae	*Leptolaena pauciflora* Baker	Venereal disease
Sarcolaenaceae	*Leptolaena diospyroidea* (Baill.) Cavaco	Impotence to man
Sterculiaceae	*Rulingia madagascariensis* Baker	n.i
Thymelaeaceae	*Peddiea involucrata* Baker	Antimalarial

n.i = no previous ethnomedical indication reported

Preparation of plant extracts

Dried and powdered plants (10 g) were macerated with agitation in 50 ml methanol (MeOH) overnight at room temperature. After filtration, the solvent was eliminated by evaporation at reduced pressure and crude extracts were dried at 45 °C using a speed-vac concentrator (Savant), and stored at the IMRA bank at 4 °C.

Microorganisms

The bacterial strains used for the investigation were *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*, and were all obtained as clinical isolates from patients at the Microbiology Department, IMRA. The yeast strain, *Candida albicans* (MUCL 31360), was obtained from the Mycothèque de l'Université Catholique de Louvain (MUCL), Belgium. Stock cultures were maintained at 4 °C on slopes of nutrient agar (Difco) for bacteria and on Sabouraud dextrose agar (SDA, Difco) for the yeast, prior to their use.

Determination of minimum inhibitory concentration (MIC)

The broth microdilution method was used to assess the MIC of the different plant extracts in a 96-well microplate using a modified method [9]. Microbial suspensions were first prepared from an overnight culture of bacterial and fungal cells grown in flasks, each containing 10 ml of Mueller-Hinton Broth (Oxoid) for bacteria and Sabouraud dextrose broth (Difco) for yeast at 37 °C and 30 °C, respectively. The turbidity of the microbial suspensions was adjusted to 0.5 McFarland using Densicheck (BioMerieux). Stock solutions of the different extracts were prepared by re-suspending the crude methanol extracts in 10 % dimethyl sulfoxide (DMSO) to produce concentrations in the range of 1.25 – 160 mg/ml. These solutions were further diluted 10-fold with water and then sterilized by filtration through 0.22 µm membrane filters. A known volume (100 µl) of each solution was deposited in the wells. This was followed by the addition of 100 µl of the inoculum (approximately 10^6 CFU/ml for bacteria and 10^5 CFU/ml for *C. albicans*) was added to each well. The microplates were incubated overnight at 37 °C for bacteria and 30 °C for 48 h for *C. albicans*. After incubation, 40 µl of 0.2 mg/ml aqueous solution of methylthiazoyltetrazolium chloride (MTT) was added to each well and further incubated for 30 min at room temperature. MIC was defined as the lowest concentration in which no transformation of MTT was observed. Streptomycin sulfate and nystatin were used as positive controls and their MICs were determined using the same process. All samples were tested in triplicate and the tests were repeted twice.

Bioautography agar-overlay assay with *B. subtilis* and *C. albicans*

Plant extracts showing significant antimicrobial activity with MICs values close to 1 mg/mL against *B. subtilis* or *C. albicans* were investigated by thin layer chroma-tography (TLC) bioautographic agar-overlay according to the method of Rahalison et al [10] with minor modifications. Twenty microlitres of different solutions of the methanol plant extract (400 µg) were applied to precoated Silica gel GF254 plates (Merck KGaA, Darmstadt, Germany). TLC plates were developed with ethyl acetate/methanol 1/1 (v/v) for *B. subtilis* and ethyl acetate/methanol/water 10/10/3 (v/v/v) for *C. albicans* and dried thoroughly overnight to achieve complete removal of the solvents. The developed TLC plates were thinly overlaid with molten malt extract agar and with SDA inoculated with an overnight culture of *B. subtilis* and *C. albicans*, respectively. The plates were incubated in a dark and humid chamber at 25 °C for 24 h for *B. subtilis* and 48 h for *C. albicans*. After incubation, cultures were sprayed with MTT and further incubated for 30 min at room temperature. Microbial growth inhibition appeared as clear zones around active

compounds against a purple background. The plates were in duplicate. One set was used for bioautography experiment and the other was intended for the reference chromatogram. The experiments were repeated twice.

Phytochemical screening

Plant extracts previously selected for TLC bioautographic assays were subjected to preliminary phytochemical screening to determine the major chemical groups corresponding to active compounds responsible for the observed inhibition zones. TLC was first developed with the same solvent systems as previously mentioned above. For the detection of alkaloid, coumarins, flavonoids and terpenes, phytochemical screening was performed using standard procedures [11]. Observations were made from two independent experiments.

RESULTS

A total of 23 methanol plant extracts of Madagascan endemic species belonging to 20 different botanical families, among which 16 are used in traditional medicine (Table 1), were tested for their antimicrobial activities against 5 bacterial species and one yeast. The results of these tests are summarized in Table 2. Eight extracts inhibited the growth of *B. subtilis* at MIC values lower or equal to 1 mg/ml. Among them, the extract of *Rhopalopilia perrieri* Cavaco & Keraudren (Opiliaceae) exhibited the lowest MIC value (0.13 mg/ml). Four of them were also found to be effective against *S. aureus* at 1 mg/ml. Five extracts were moderately active against *E. coli* and *S. typhi* with MIC values ranging from 2 to 4 mg/ml. None of the tested plant extracts displayed activity against *P. aeruginosa* (MIC > 8 mg/ml) except *Poivrea phaneropetala* (Baker) H. Perrier (Combretaceae), *Koehneria madagascariensis* (Baker) S.A.Graham, H.Tobe and P.Baas (Lythraceae) and *R. perrieri*. Of the 6 plant extracts that inhibited *C. albicans* at MIC lower or equal to 2 mg/ml, *Monoporus clusiifolius* H. Perrier (Myrsinaceae) showed the most interesting inhibitory activity against C. *albicans* with a MIC of 0.25 mg/ml

DISCUSSION

This study showed that all but two plant extracts possessed some degree of activity against at least one tested microorganism, at the highest tested dose of 8 mg/ml. Overall, Gram positive bacteria (*B.subtilis* and *S. aureus)* were more sensitive to plant extracts than Gram negative bacteria (*E. coli, S. typhi* and *P.aeruginosa*). Among them, *P. phaneropetala, K. madagascariensis* and *R. perrieri* demonstrated a broad spectrum of activity against all the tested organisms while *M. clusiifolius* was the most active against *C. albicans*. *R. perrieri* showed the most promising antibacterial activity, confirming its use in traditional medicine as antiseptic and wound healing. The present investigation also confirmed the anti-infective property of *H. coriacea* in the treatment of vaginitis and urethritis and that of *D. oblongifolia* which is used to treat diarrhoea and dysentery in folk medicine. In contrast, the use of *Leptolaena pauciflora* Baker (Sarcolaenaceae) as anti-infective agent for the treatment of venereal diseases was not supported by our investigation. Furthermore, the oral use of this plant containing several phenolic compounds could be toxic [12].

Based on this investigation, we are unable to say categorically that extracts having the same Rf values contained the same bioactive compounds. It is possible that the observed inhibition was likely due to one or more compounds sharing the same Rf in the solvent system used, particularly for low Rf spots. Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids and terpenes as the probable active compounds present in the crude extracts. It is well known that numerous members of these phytochemical groups have already demonstrated antimicrobial activity [13].

Table 2: Antimicrobial activity of 23 Madagascan endemic plants

Plant	Voucher no.	Microorganisms [a]/ MIC [b] (mg/ml)					
		B.s	**S.a**	**E.c**	**S.t**	**P.a**	**C.a**
Xylopia buxifolia	AML6	-	-	-	-	-	-
Mascarenhasia lisianthiflora	MOR23	2	-	-	-	-	-
Pluchea grevei	TUL16	4	-	-	-	-	4
Asteropeia densiflora	TUL8	0.5	2	-	-	-	2
Evonymopsis longipes	MOR33	2	8	-	-	-	8
Symphonia clusioides	AMB34	-	-	-	-	-	-
Poivrea phaneropetala	MOR12	0.5	1	4	4	8	2
Poivrea grandidieri	TUL10	4	8	8	8	-	8
Poivrea obscura	MOR26	1	1	4	4	-	-
Hibbertia coriacea	MKR44	1	2	4	2	-	4
Elaeocarpus sericeus	M447	1	4	-	-	-	4
Piptadenia pervillei	AA17	2	8	8	8	-	-
Koehneria madagascariensis	TUL5	0.5	1	4	4	8	2
Dichaetanthera oblongifolia	BP31	1	2	-	-	-	2
Pachytrophe dimepate	VAM282	2	-	-	-	-	-
Monoporus clusiifolius	AMB43	2	8	-	-	-	0.25
Rhopalopilia perrieri	TUL35	0.13	1	4	4	8	2
Bathiorhamnus louvelii	AA68	2	-	-	-	-	-
Conchopetalum madagascariense	VAT652	2	-	-	-	-	-
Leptolaena pauciflora	MKR692	4	8	8	8	-	8
Leptolaena diospyroidea	MOR56	4	-	-	-	-	-
Rulingia madagascariensis	BP14	4	8	-	-	-	8
Peddiea involucrata	AA48	4	8	-	-	-	-
References (µg/ml)							
Streptomycine		3.9	3.9	31.3	31.3	-	nd
Nystatine		nd	nd	nd	nd	nd	3.13

[a] *MIC: minimal inhibitory concentration (mg/ml)*
[b] *Microorganisms: B.s = Bacillus subtilis; S.a = Staphylococcus aureus; E.c = Escherichia coli; S.t = Salmonella typhi; P.s = Pseudomonas aeruginosa; C.a = Candida albicans (-): MIC > 8 mg/ml for plant extracts and MIC > 64 µg/ml for streptomycin; nd = not determined*

Overall, five plant extracts with MICs lower than 1 mg/ml against either B. subtilis or C. albicans were subjected to TLC bioautographic agar overlay test. Rf value of the inhibition zones and the probable chemical group of compound responsible for the inhibition are listed in Table 3. The tests based on colour development suggested that the active compounds belong mainly to flavonoid or terpene groups. Furthermore, a positive test with Dragendorf reagent suggests that Asteropeia densiflora Baker (Asteropeiaceae) and R. perrieri may contain alkaloids at Rf of 0.02 (at base line).

Table 3: Phytochemical screening of active compounds responsible for microbial inhibition based on bioautography assays

Plant	Rf value	Phytocompound [a]			
		A	**C**	**F**	**Te**
Monoporus clusiifolius [b]	0.68	-	-	+	+
Poivrea phaneropetala [c]	0.1	-	-	+	+
Koehneria madagascariensis [c]	0.1	-	-	+	+
Asteropeia densiflora [c]	0.02	+	-	+	+
	0.75	-	-	+	+
Rhopalopilia perrieri [c]	0.02	+	-	+	+

[a] *A= Alkaloids; C= Coumarins; F= Flavonoids; Te= Terpenes*
[b] *Test performed on TLC bioautographic agar-overlay with solvent system EtOAc/MeOH/H_2O (10/10/3) using C. albicans as organism test*
[c] *Test performed on TLC biautographic agar-overlay with solvent system EtOAc/MeOH (1/1) using B. subtilis as organism test*

In many cases, these substances serve as plant defense mechanisms against aggression by microorganisms, insects, and herbivores either synthesized during the plant normal development (constitutive resistance factors) or induced only after contact with the pathogen (induced resistance factor) [14].

To the best of our knowledge, no previous antimicrobial survey has been reported on these plants. However, some of them were previously reported for other biological activities. Literature searches indicate that *Evonymopsis longipes* (H. Perrier) H. Perrier (Celastraceae) was particularly potent and could completely inactivate the Herpes Simplex Virus at a concentration lower than 25 µg/ml [7]. *Peddiea involucrata* Baker (Thymelaeaceae) and *Piptadenia pervillei* Vatke (Fabaceae) were said to possess antimalarial activity [15-16]. From this latter was isolated (+)-catechin 5-gallate and (+)-catechin 3-gallate which demonstrated, *in vitro*, high activity against the chloroquine-resistant strain FcB1 of *Plasmodium falciparum* as well as (+)-catechinant ethylgallate which were less effective [16]. With regard to antibacterial activity, howerver, these plants were less effective, being active only against *B. subtilis* with MIC ranging from 2 to 4 mg/ml.

While no ethnomedicinal practices were reported to date for 7 of the 23 plants, the study revealed that *A. densiflora* and *K. madagascariensis* were found to possess effective antibacterial activity; in addition, the extract from *M. clusiifolius* may be a good candidate in the search for potential antifungal compounds. Further stuydies on these plants are being undertaken in our laboratory to isolate and elucidate the compounds responsible for the observed anti-candidiasis and antibacterial activities.

CONCLUSION

This survey contributes new data to the existing knowledge of antimicrobial activity of the endemic flora of Madagascar and may serve as a basis for further pharmaceutical investigation. The findings also lend support for the use of some of these plants in traditional medicine in Madagascar.

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REFERENCES

1. *Robinson JG. An island of evolutionary exuberance. Science, 2004; 304 (5667): 53.*
2. *Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GAB, Kent J. Biodiversity hotspots for conservation priorities. Nature, 2000; 403: 853-858.*
3. *Beaujard P. Plants and traditional medicine in Southeast Madagascar. J Ethnopharmacol, 1988; 23: 165-265.*
4. *Novy JW. Medicinal plants of the eastern region of Madagascar. J Ethnopharmacol, 1997; 55: 119-226.*
5. *Norscia I, Borgognini-Tarli SM. Ethnobotanical reputation of plant species from two forests of Madagascar: a preliminary investigation. S Afr J Bot, 2006; 72 (4): 656-660.*
6. *Rasoanaivo P, Ramanitrahasimbola D, Rafatro H, Rakotondramanana D, Robijaona B, Rakotozafy A, Ratsimamanga-Urveg S, Labaied M, Grellier P, Allorge L et al. Screening extracts of Madagascan plants in search of antiplasmodial compounds. Phytother Res, 2004; 18(9): 742-747.*
7. *Hudson JB, Lee MK, Rasoanaivo P. Antiviral activities in plants endemic to Madagascar. Pharmaceut Biol, 2000; 38 (1): 36-39.*
8. *Williams RB, Norris A, Miller JS, Razafitsalama LJ, Andriantsiferana R, Rasamison VE, Kingston DG. Two new cytotoxic naphthoquinones from Mendoncia cowanii from the rainforest of Madagascar. Planta Med, 2006; 72: 564-566.*
9. *Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med, 1998; 64: 711-713.*
10. *Rahalison L, Hamburger M, Hostettmann K, Monod M, Frenk E. A bioautographic agar overlay method for the detection of*

antifungal compounds from higher plants. Phytochem Anal, 1991; 2:199-203.

11. *Wagner H, Bladt S. Plant drug analysis. A thin layer chromatography atlas. Springer-Verlag, Berlin Heidelberg; 1996.384p.*
12. *Paris R, Jacquemin H, Linard A. Madagascar plants. XV. Chlaenaceae from Malagasy. Leptolaena pauciflora, L. diospyroidea cavaco var tampoketsensis, and Sarcolaena multiflora. Presence of myricetol heterosides. Plantes Méd et Phyto, 1975; 9: 230-237.*
13. *Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev, 1999; 12 (4): 564-582.*
14. *Castro MS, Fontes W. Plant defense and antimicrobial peptides. Protein Pept Lett, 2005; 12: 11-16.*
15. *Randrianarivelojosia M, Rasidimanana VT, Rabarison H, Cheplogoi PK, Ratsimbason M, Mulholland DA, Mauclere P.. Plants traditionally prescribed to treat tazo (malaria) in the eastern region of Madagascar. Malar J, 2003; 2: 25.*
16. *Ramanandraibe V, Grellier P, Martin MT, Deville A, Joyeau R, Ramanitrahasimbola D, Mouray E, Rasoanaivo P, Mambu L. Antiplasmodial phenolic compounds from Piptadenia pervillei. Planta Medica, 2008; 74: 417-421.*

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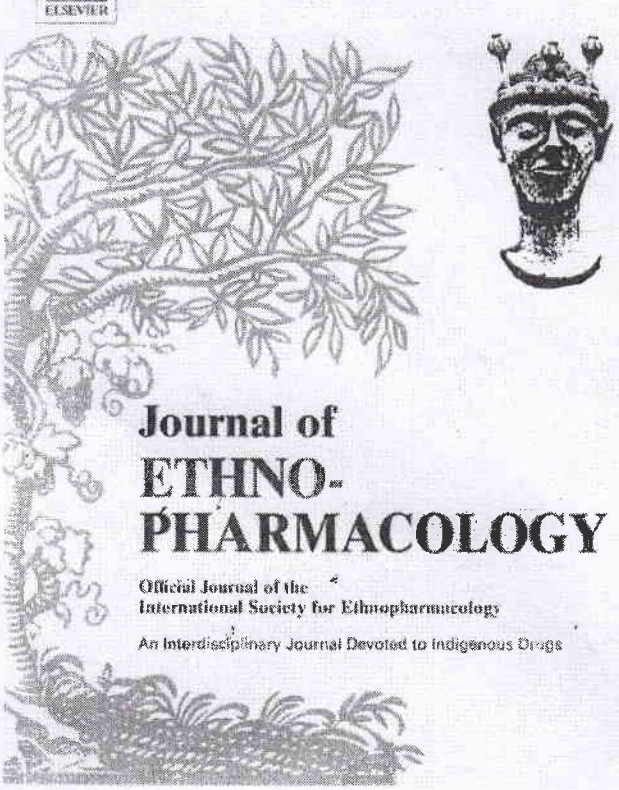

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Screening for anti-infective properties of several medicinal plants of the Mauritians flora

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Abstract

Several plants of the Mauritian flora alleged to possess anti-infective properties were studied against different strains of pathogenic bacteria and fungi. The grounded dried plant materials were extracted with different extractants and screened for anti-microbial activity using the disk diffusion and the micro-dilution techniques. Preliminary screening revealed that the methanol extracts were most active. *Salmonella enteritidis*, *Enterobacter cloacae* and *Bacillus subtilis* were the three test organisms, which were found to be susceptible to all the crude methanolic extracts of the different plants investigated (100% susceptibility), followed by *Escherichia coli* (57.1%) and *Pseudomonas aeruginosa* (57.1%), and *Staphylococcus aureus* (28.6%). The lowest minimum inhibitory concentration recorded for the different crude methanol extracts against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*, *Enterobacter cloacae*, *Bacillus subtilis* and the mould fungus *Candida albicans* were 500, 1000, 125, 250, 1000 and 125 μg/ml, respectively. Bioautography using *Cladosporium cucumerinum* revealed that dichloromethane (DCM) extracts had the highest activity against the phytopathogenic fungus. It was also noted that the DCM extracts of *Michelia champaca* and *Antidesma madagascariense* yielded the maximum number of growth inhibiting compounds against *Cladosporium cucumerinum*. Activity of the different crude extracts was also investigated against several phytopathogenic filamentous fungi, *Colletotrichum glocosporoides*, *Rhizoctonia solani*, *Sclerotinia sclerotium*, *Guignardia* sp. and *Fusarium oxysporum*. It was found that crude hexane extracts as well as crude DCM extracts exhibited marked activity against several strains of fungi, especially *Colletotrichum glocosporoides*, *Sclerotinia sclerotium* and *Guignardia* sp.

Keywords: Medicinal plants; Mauritius; Infections; Bioautography; Anti-microbial activity; Micro-dilution assay

1. Introduction

Anti-microbial agents are undeniably one of the most important therapeutic discoveries of the 20th century. However, with the 'antibiotic era' barely five decades old, mankind is now faced with the global problem of emerging resistance in virtually all pathogens (Peterson and Dalhoff, 2004). Surveys have revealed that almost no group of antibiotics has been introduced to which resistance had not been observed (Eloff, 2000). This is indeed quite alarming when considering that in 1990, out of the 39.5 million of death in the developing world, 9.2 million were estimated to have been caused by infectious and parasitic diseases, and that 98% of death in children in developing countries resulted mostly from infectious diseases (Murray and Lopez, 1997). Bacterial resistance is beyond doubt the consequence of years of widespread indiscriminate use, incessant misuse and abuse of antibiotics (Peterson and Dalhoff, 2004). In human medicine alone, the US Centre for Disease Control and Prevention estimates that approximately one-third of the 150 million prescriptions for antibiotics written each year were not needed.

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Because of the limited life span of antibiotics, it is of utmost importance to find appropriate solutions to impede, or perhaps even reduce, the development of drug resistance associated with many microbial species (Martini and Eloff, 1998).

Since time immemorial, medicinal plants had been a dependable source of therapeutics for the treatment of various ailments (Hoareau and Da Silva, 1999) but since the advent of the use of fermentation-based antibiotics work on anti-microbial agents from plants sources has been greatly overshadowed (Mitscher et al., 1987). The rapid propagation in antibiotic resistance and the increasing interest in natural products, however, have placed medicinal plants back in the front lights as a reliable source for the discovery of active anti-microbial agents and possibly even novel classes of antibiotics (Shultes, 1992).

Plants are complex chemical storehouses of undiscovered biodynamic compounds with unrealized potential for use in modern medicine (Plotkin, 1988). It has long been established that naturally occurring substances in plants have anti-bacterial and anti-fungal activities. In Mauritius, medicinal plants, for centuries, have been used for the treatment of a wide range of ailments, many of which are still in use today and hold favored positions among local tradi-practitioners. Situated between the southern latitude of 19°50′ and 20°32′ and longitude 57°18′ and 57°46′, Mauritius is a tropical island, which has emerged some 8 million years ago from the Indian Ocean. Certain conditions such as the topography of the land and the rain distribution have ensured the island a diverse microclimatic regime, which has had a direct consequence on both the endemic and exotic vegetation. Its old age and geographic isolation has provided the Mauritian flora a high degree of endemism. The island possesses seven phanerogams all of which are endemic (Gurib-Fakim, 2002). For that reason, Mauritius is a rich source of natural products of great therapeutic value that wait to be uncovered. Which is why emphasis in this work was laid on several plants of the Mauritian flora.

Aiming for new compounds responsible for anti-infective properties, a thorough literature search was undertaken through ethnobotanical published data looking for plants used in Mauritius to combat fever and diseases caused by bacteria and fungi. The plants selected were as follows: *Antidesma madagascariense* (Lam.) (Euphorbiaceae), *Aphloia theiformis* (Vahl.) (Aphloiaceae), *Erythroxylum laurifolium* (Lam.) (Erythroxylaceae), *Mangifera indica* (L.) (Anacardiaceae), *Melia azedarach* (Meliaceae), *Michelia champaca* (L.) (Magnoliaceae) and *Moringa oleifera* (Lam.) (Moringaceae).

The use of medicinal plants towards certain types of illnesses has roots in the Mauritian traditional pharmacopoeia. This study was undertaken to determine possible inhibitory effects of some plants that are used against common infectious diseases in Mauritius.

2. Material and methods

2.1. Plant material

Fresh leaves of all the plants except leaves of *Moringa oleifera*, *Mangifera indica* and *Melia azedarach* were collected from trees grown in the local Botanical Garden while leaves of *Moringa oleifera*, *Mangifera indica* and *Melia azedarach* were collected from trees growing in the hot northwestern part of the island, mainly, the capital, Port Louis. The Curator of the Botanical Garden identified the different plants and voucher specimen were collected for all the plants, i.e., *Antidesma madagascariense* (23,376), *Aphloia theiformis* (24,121), *Erythroxylum laurifolium* (24,045), *Mangifera indica* (19,368), *Melia azedarach* (15,549), *Michelia champaca* (11,664) and *Moringa oleifera* (10,122) and deposited at the Department of Chemistry, then transferred to join the vast collection of the National Herbarium of the Mauritius Sugar Industry Research Institute, MSIRI, after confirmation of their identities from comparison with botanical descriptions and the collaboration of the botanist in charge of the herbarium.

2.2. Preparation of plant material and extraction

All the plant materials used were in the form of finely grounded dried powder. The different plants collected were processed similarly. After their authentication, the plants were collected in large quantity, thoroughly washed with water and dried in a drying cabinet at about 40 °C for several days till complete removal of water then processed to a fine powder using a Jankel and Künkel Model A10 mill. The dried powdered plant materials were then extracted via maceration in a serial manner using hexane, dichloromethane (DCM) and methanol (10:1 solvent to dry weight ratio) for two successive 24-h periods. The extracts were filtered, combined and dried under reduced pressure.

2.3. TLC analysis

Thin layer chromatography (5 μl of a 100 mg extract/ml solution) was on Silica Gel 60 coated on glass plates (Merck TLC F254) with hexane/ethyl acetate 1/1 (v/v) and DCM/methanol/water 65/35/0.5 (v/v/v) as eluants. The separated components were visualised under visible and ultraviolet light (254 and 360 nm, Camag Universal UV lamp TL-600) or using spray reagents such as 5% anisaldehyde in a 5% sulphuric acid in ethanol solution, vanillin and Dragendorff (Martini and Eloff, 1998).

2.4. Microorganisms

The test organisms used were *Bacillus subtilis*, *Enterobacter cloacae*, *Escherichia coli*, *Salmonella enteritidis*, *Staphylococcus aureus*, the yeast mould *Candida albicans* and filamentous phytopathogenic fungi; *Colletotrichum glocosporoides*, *Cladosporium cucumerinum*, *Fusarium oxysporum*, *Guignardia* sp., *Rhizoctonia solani* and *Sclerotinia sclerotium*. The different bacteria were obtained as clinical isolates from patients of the Department of Microbiology, Institut Malgache des Recherches Appliquées (IMRA), Antananarivo, Madagascar, as well as the yeast mould *Candida albicans* while the filamentous fungi and *Enterobacter cloacae* were isolated from different plant species.

2.5. Bioassay

2.5.1. Preliminary screening using the disk diffusion technique

2.5.1.1. Procedure used for the different bacteria and Candida albicans. The disk diffusion method was used as a preliminary test to find out if the plant extracts were active (Matsen, 1979). Stock solutions of the different extracts at a known concentration (8 mg/ml) were prepared in the solvents used for extraction and suitably stored. Loops full of the different bacteria and *Candida albicans* were transferred aseptically into test tubes containing peptone water (10 ml) and incubated at 37 °C for 24 h. After the incubation period, the turbidity of the solution was adjusted to 0.5 McFarland. Hundred microlitres of this inoculum (5×10^5 CFU/ml) was then transferred onto the surface of solidified Muller Hinton agar and spread evenly across the whole surface of the agar in the petri dish (85 mm). In the case of *Candida albicans* sabouraud dextrose agar media was used (NCCLS, 1992). Sterile paper disks (6 mm diameter, prepared from Whatman number 1 filter paper) were then dipped into the stock solution of the extract (concentration 8 mg/ml) and transferred ascetically onto the surface of the agar bearing the bacteria. The tests were run in duplicate. Similarly, paper disks containing standard concentration (8 mg/ml) of Ampicillin were used as positive control. The petri dishes were then incubated at 37 °C for 24 h. The results recorded were the average of the duplicated test.

2.5.1.2. Procedure used for the filamentous fungi. The different fungi were sub-cultured on potato dextrose agar (PDA) and incubated in a humid atmosphere at 26 °C for 48 h or until the petri dishes were completely invaded by the fungi. Cubes (0.5 cm × 0.5 cm) were cut aseptically from the mother petri dish and deposited in the centre of fresh petri dishes containing sterile PDA and these were then incubated in a humid atmosphere at a temperature of 26 °C until a growth diameter of about 2 cm was observed. The sterile disks impregnated with the plant extracts were then deposited onto the PDA in circle about 10 mm from the growing fungi. The Petri dished were incubated for 48 h and zone of inhibition around the disks if any were recorded. The tests were run in duplicate and the results recorded were the average of the duplicated test. Anti-microbial activities of the plant extracts were expressed in terms of: + (positive with corrected value for diameter of inhibition with respect to blank less than 4 mm); ++ (positive with corrected value for diameter of inhibition with respect to blank more than 4 mm); − (negative, when no distinct zone of inhibition is observed). Blanks were prepared by dipping the filter paper disk in the different solvents used for extraction.

2.5.2. Determination of minimum inhibitory concentration (MIC) using the micro-dilution technique on the different strains of bacteria and Candida albicans

MIC analyses were conducted via broth micro-dilution techniques according to the National Committee for Clinical Laboratory Standards procedures for aerobic testing (NCCLS, 1990). Each of the bacteria were sub-cultured twice on Muller Hinton agar, colonies (5–7) were then transferred aseptically from the second transfer plate into individual tubes containing sterile nutrient broth (10 ml). The tubes were incubated for a period of 8–12 h at 37 °C to ensure that the bacteria were in the log phase. Subsequently, the bacterial suspensions were visually adjusted to 0.5 McFarland and then further diluted 1:100 with fresh sterile broth to yield starting inoculums of approximately 10^6 CFU/ml. Stock solutions of the different extracts at a concentration of 16 mg/ml were prepared in the solvent used for extraction. A known volume (100 μl) of each solution was placed in the first well of a 96-well microplate and two-fold serially diluted with sterile distilled water (Klepser et al., 1996). A known volume of the inoculum (100 μl) was then added to each well. The plates were then incubated at 37 °C for 24 h. After incubation, 40 μl of MTT (0.2 mg/ml) was added to each well and incubated for a further 10–15 m. Bacterial growth is denoted by a blue coloration of the wells. The well of lowest concentration in which no blue coloration is observed is taken as the MIC. Streptomycin sulphate and Gentamicin sulphate were used to compare the susceptibility of the different microorganisms. The procedure used was as described above, only instead of doing serial dilution of the extracts; serial dilution of the standard antibiotics was done (Bonjar, 2004).

2.5.3. Quantitative evaluation of anti-microbial activity

There exist different ways of expressing the biological activity of plant extracts based on the technique used. The agar diffusion method led to results being given in terms of width of the inhibition zone (mm or cm) while the micro-dilution method yield MIC values, the minimum concentration at which inhibition is observed (mg/ml). In this work new ways of expressing anti-bacterial efficiency as comparative numerical values are used. Beside results being recorded in terms of MIC (mg/ml), total activity values as described by Eloff (2000) was employed, as well as percent activity values which demonstrates the total anti-microbial potency of particular extracts and bacterial susceptibility index (BSI) as described by Bonjar (2004), which is used to compare the relative susceptibility among the bacterial strains:

- Total activity

$$\text{total activity} = \frac{\text{quantity of material extracted from 1 g of plant material}}{\text{MIC}}$$

These values would indicate the largest volume to which biologically active compounds in 1 g of plant material can be diluted and still inhibits the growth of bacteria (Eloff, 2004).

- Percent activity:

$$\text{activity (\%)} = \frac{100 \times \text{no. of susceptible stains to a specific extract}}{\text{total no. of tested bacterial strains}}$$

The percent activity demonstrates the total anti-microbial potency of particular extracts. It shows number of bacteria found susceptible to one particular extract.

- Bacterial susceptible index, BSI:

$$\text{BSI} = \frac{100 \times \text{no. of extracts effective against each bacterial strain}}{\text{no. of total samples}}$$

BSI is used to compare the relative susceptibility among the bacterial strains. BSI values ranges from '0' (resistant to all samples) to '100' (susceptible to all sample).

2.5.4. Bioautography using Cladosporium cucumerinum

The protocol was as described by Homans and Fuchs (1970). Bioautography involved the development of chromatogram of the different crude extracts under investigation as described above (Section 2.3). After separation, the TLC plates were thoroughly dried to remove all traces of solvents. The bioautography tests were done using *Cladosporium cucumerinum*, a parasitical fungus, which attacks different plants most particularly those of the Cucurbitaceae family. A suspension of the fungus was made half an hour before the bioautography was to be set. Loops full of the spore were transferred aseptically to sabouraud malt broth (20 ml) to which was then added 8 mg of chloramphenicol, which is a standard anti-bacterial agent.

The inoculum was thoroughly shaken every 10 min for 30 min to make a homogenised suspension of the *Cladosporium cucumerinum*, which was then sprayed thinly over the developed TLC plates and then incubated for 48 h at room temperature in a humid atmosphere. Nystatine (10 μl of a 1 mg/ml aqueous solution) and chloramphenicol (10 μl of a 1 mg/ml ethanol solution) spotted separately were used as control. Growth of the fungus was denoted by a greyish green coloration of the TLC plates with zones of inhibition, which appears as clear spots (Begue and Kline, 1972).

3. Results and discussion

Results clearly demonstrated that the plants investigated exhibited significant anti-microbial activity (Tables 1–3). The results showed that out of all the three solvents used for extraction, the methanol extracts displayed a broader spectrum of anti-bacterial activity (Table 3). The DCM extracts also showed relatively good anti-bacterial activities, most particularly against *Salmonella enteritidis*, *Enterobacter cloacae* and *Bacillus subtilis* but very little to no activity was noted against *Escherichia coli* and *Pseudomonas aeruginosa* (Table 2). The crude hexane extracts were of no interest as none showed marked activity (Table 1).

In the present investigation, the extracts were prepared serially with the same dried and grounded plant material, the first extractant being hexane followed by the other solvents of increasing polarity. This serial extraction led to some fractionation of the anti-microbial compounds of the plants studied. It was observed that in most of the medicinal plants analysed, the anti-bacterial potency resided in the most polar extractant used, i.e., methanol. This corroborate well with the ethnobotanical claims on the different plants, as in the local folklore, the beneficial medicinal properties of these plants are derived in most cases from decoctions and infusions in aqueous medium. The results revealed that the methanol extracts hold the most promise for further work.

In vitro anti-microbial screening via the micro-dilution technique provided the required preliminary observation to select among the crude methanolic plant extracts those with potentially useful properties for further chemical and pharmaceutical investigations. According to NCCLS standards, a breakpoint for a pure antibiotic susceptibility is 8 mg/l. The lowest minimum inhibitory concentration recorded for the different crude methanolic extracts against *Staphylococcus aureus*

Table 1
Results of preliminary anti-microbial screening of crude hexane extract using the disk diffusion method

Scientific name	SA[a]	EC[b]	SE[c]	PA[d]	EC[e]	BS[f]	CG[g]	RS[h]	SS[i]	GS[j]	OF[k]	CA[l]
Antidesma madagascariense	–	–	–	–	–	–	+	–	–	–	–	+
Aphloia theiformis	–	–	–	–	–	–	+	–	–	+	–	+
Erythroxylum laurifolium	–	–	–	–	–	–	+	–	–	+	–	+
Mangifera indica	–	–	–	–	–	–	+	–	–	+	–	+
Melia azedarach	–	–	–	–	–	–	+	–	–	–	–	–
Michelia champaca	–	–	–	–	–	–	+	–	–	–	–	–
Moringa oleifera	–	–	–	–	–	–	+	–	–	+	–	+

(+) Positive result with corrected diameter of inhibition with respect to blank less than 4 mm; (++) positive result with corrected diameter of inhibition with respect to blank more than 4 mm; (–) negative, no distinct zone of inhibition.

[a] *Staphylococcus aureus.*
[b] *Escherichia coli.*
[c] *Salmonella enteritidis.*
[d] *Pseudomonas aeruginosa.*
[e] *Enterobacter cloacae.*
[f] *Bacillus subtilis.*
[g] *Colletotrichum glocosporoides.*
[h] *Rhizoctonia solani.*
[i] *Sclerotinia sclerotium.*
[j] *Guignardia* sp.
[k] *Fusarium oxysporum.*
[l] *Candida albicans.*

Table 2
Results of preliminary anti-microbial screening of crude DCM extract using the disk diffusion method

Scientific name	SA[a]	EC[b]	SE[c]	PA[d]	EC[e]	BS[f]	CG[g]	RS[h]	SS[i]	GS[j]	OF[k]	CA[l]
Antidesma madagascariense	−	−	+	−	+	+	++	−	−	+	−	−
Aphloia theiformis	−	−	−	−	++	+	−	−	+	−	−	−
Erythroxylum laurifolium	−	−	+	−	++	−	−	−	+	−	−	++
Mangifera indica	−	−	+	−	−	−	−	−	−	−	−	−
Melia azedarach	−	−	+	−	++	+	−	−	−	−	−	−
Michelia champaca	−	−	−	−	++	+	++	−	−	+	−	−
Moringa oleifera	−	−	+	−	−	+	+	−	−	+	−	−

(+) Positive result with corrected diameter of inhibition with respect to blank less than 4 mm; (++) positive result with corrected diameter of inhibition with respect to blank more than 4 mm; (−) negative, no distinct zone of inhibition.
[a] *Staphylococcus aureus*.
[b] *Escherichia coli*.
[c] *Salmonella enteritidis*.
[d] *Pseudomonas aeruginosa*.
[e] *Enterobacter cloacae*.
[f] *Bacillus subtilis*.
[g] *Colletotrichum glocosporoides*.
[h] *Rhizoctonia solani*.
[i] *Sclerotinia sclerotium*.
[j] *Guignardia* sp.
[k] *Fusarium oxysporum*.
[l] *Candida albicans*.

(500 μg/ml), *Escherichia coli* (1000 μg/ml), *Salmonella enteritidis* (125 μg/ml), *Enterobacter cloacae* (250 μg/ml), *Bacillus subtilis* (1000 μg/ml) and the mould fungus *Candida albicans* (125 μg/ml) (Table 4) were all above the breakpoint recommended. Nevertheless, these MIC values, according to Fabry et al. (1998) are demonstrative of the potential clinical use and interest of these extracts as they are crude extracts of uncertain composition and with components that can have synergistic or antagonistic effects.

Total activity values (Table 4) revealed that methanol extract of *Michelia champaca* has a high magnitude of anti-bacterial activity, as the anti-bacterial component(s) from this plant can be diluted in 1043 ml of solvent and still inhibits growth of *Salmonella enteritidis* (total activity = 1043 ml/g), followed by methanol extract of *Erythroxylum laurifolium* against *Candida albicans* (730 ml/g), methanol extract of *Melia azedarach* against *Enterobacter cloacae* (138 ml/g) and methanol extract of *Erythroxylum laurifolium* against *Bacillus subtilis* (91 ml/g).

BSI values (Table 5) were useful in evaluating the susceptibility of the different strains of bacteria towards the plant extracts investigated. *Bacillus subtilis*, *Enterobacter cloacae* and *Salmonella enteritidis* were the three test organisms found to be

Table 3
Results of preliminary anti-microbial screening of crude methanol extract using the disk diffusion method

Scientific name	SA[a]	EC[b]	SE[c]	PA[d]	EC[e]	BS[f]	CG[g]	RS[h]	SS[i]	GS[j]	OF[k]	CA[l]
Antidesma madagascariense	+	+	++	+	++	+	−	−	−	−	−	+
Aphloia theiformis	+	−	++	+	+	+	−	−	+	−	−	+
Erythroxylum laurifolium	−	−	++	+	+	+	−	−	+	−	−	++
Mangifera indica	−	−	++	−	+	+	−	−	+	−	−	++
Melia azedarach	−	+	++	−	++	+	−	−	+	−	−	++
Michelia champaca	−	+	++	+	+	+	−	−	−	+	−	+
Moringa oleifera	−	+	++	−	++	++	−	−	−	−	−	+

(+) Positive result with corrected diameter of inhibition with respect to blank less than 4 mm; (++) positive result with corrected diameter of inhibition with respect to blank more than 4 mm; (−) negative, no distinct zone of inhibition.
[a] *Staphylococcus aureus*.
[b] *Escherichia coli*.
[c] *Salmonella enteritidis*.
[d] *Pseudomonas aeruginosa*.
[e] *Enterobacter cloacae*.
[f] *Bacillus subtilis*.
[g] *Colletotrichum glocosporoides*.
[h] *Rhizoctonia solani*.
[i] *Sclerotinia sclerotium*.
[j] *Guignardia* sp.
[k] *Fusarium oxysporum*.
[l] *Candida albicans*.

Table 4
Minimum inhibitory concentration values of the most active methanol extracts

Plants	Test organisms	MIC (μg/ml)	Total activity (ml/g)
Antidesma madagascariense	*Salmonella enteritidis*	125	326
	Staphylococcus aureus	500	81
Aphloia theiformis	*Salmonella enteritidis*	500	74
	Staphylococcus aureus	500	74
Erythroxylum laurifolium	*Bacillus subtilis*	1000	91
	Candida albicans	125	730
	Salmonella enteritidis	500	182
Mangifera indica	*Candida albicans*	250	54
	Enterobacter cloacae	250	54
	Salmonella enteritidis	250	54
Melia azedarach	*Candida albicans*	250	277
	Enterobacter cloacae	500	138
	Salmonella enteritidis	500	138
Michelia champaca	*Salmonella enteritidis*	125	1043
Moringa oleifera	*Salmonella enteritidis*	500	169

Table 5
Bacterial susceptibility index, BSI, calculated for the different strain of bacteria used for screening of the seven different methanol[a] extracts

Test organisms	No. of active extracts	BSI values
Bacillus subtilis	7	100
Enterobacter cloacae	7	100
Escherichia coli	4	57.1
Pseudomonas aeruginosa	4	57.1
Salmonella enteritidis	7	100
Staphylococcus aureus	2	28.6

[a] BSI values were sought only for the methanolic extracts, on which emphasis was laid due to their high activity.

susceptible to the crude methanol extracts of the different plants investigated (100% susceptibility), followed by *Escherichia coli* (57.1%) and *Pseudomonas aeruginosa* (57.1%), and *Staphylococcus aureus* (28.6%).

Percent activity values recorded, further rationalized the folkloric use of these plants in the treatment of infectious diseases as the values in general were found to be above 50% (Table 6). Methanol extract of *Antidesma madagascariense* in particular showed noticeable efficiency (100% activity) against the different bacterial strain used (Table 6).

Table 6
Percent activity values of the different methanolic extracts, demonstrating the total anti-microbial potency of the extracts

Plants	Number of susceptible bacterial strains	Percent activity values (%)
Antidesma madagascariense	6	100
Aphloia theiformis	5	83.3
Erythroxylum laurifolium	4	66.7
Mangifera indica	3	50
Melia azedarach	4	66.7
Michelia champaca	5	83.3
Moringa oleifera	4	66.7

Cladosporium cucumerinum is often used to detect the presence of anti-fungal compounds in plant extracts. Rahalison et al. (1993) investigated the activity of several plant extracts against the phytopathogenic fungus *Cladosporium cucumerinum* and the yeast mould *Candida albicans*. Out of 20 plant-derived compounds they analysed, they found that 15 gave a positive response with *Cladosporium cucumerinum*, out of which 13 were found active against *Candida albicans*. In the present work, results of bioautography with *Cladosporium cucumerinum* revealed that the DCM extracts exhibited by far the most appreciable activity, followed by the hexane extracts while the methanol extracts demonstrated no activity. The results on the bioautograms supported those obtained via the disk diffusion assay, as in both case, the DCM extracts illustrated the most outstanding activity. The DCM extracts of the different plants possessed broader spectrum of activity than extracts of methanol, ranging from *Colletotrichum glocosporoides*, *Sclerotinia sclerotium*, *Guignardia* sp. and *Fusarium oxysporum* (Table 2). The serial extraction method used here can account for the low anti-fungal activity of the methanol extracts, as the previous solvent, DCM, had most probably removed the active components from the plant materials. Manifest activity against *Colletotrichum glocosporoides* was noted for the DCM extract of *Antidesma madagascariense* and *Michelia champaca* in particular (Table 2).

The thorough literature search that was achieved prior to the beginning of the experiments had yielded a wide choice of protocols that aimed at quantifying the activity of plant extracts against filamentous fungi. However, as our ambition in the present study was only to demonstrate the broad spectrum of activity of the different plants chosen, the disk diffusion assay employed was found suitable and easily manageable for such preliminary screening. The anti-fungal activity recorded (Table 3) for some of the extracts were at levels that hint at a probable therapeutic worth. We intent to pursue the *in vitro* anti-fungal experiments with a broader collection of yeast, mould and filamentous fungi using established protocols to determine MIC values.

After a careful examination of the results of bioautography, the DCM extract of *Michelia champaca*, which possessed a maximum of five clearly distinguishable *Cladosporium cucumerinum* growth inhibiting compounds (R_f = 0.13, 0.32, 0.43, 0.61 and 0.97) was further fractionated on a Sephadex column, using DCM/methanol 1/1 as eluant. This yielded six different fractions, all of which exhibited growth of *Cladosporium cucumerinum*. The fraction eluted last being predominantly more active with at least two compounds inhibiting the growth of *Cladosporium cucumerinum* (R_f = 0.54, 0.74).

The results clearly indicate that the different plants screened possess substantial anti-microbial activity, which agrees with the use of these plants in the traditional Mauritian pharmacopoeia as plants having therapeutic anti-infective potential. This preliminary investigation of the activity of these crude extracts shows that it is important to continue screening medicinal plants as an alternative for finding new or better anti-microbials.

As far as we know, this study is the first in Mauritius to demonstrate the anti-infective properties of medicinal plants using the referred micro-dilution assays. Moreover, the wide

difference in polarity of the anti-microbial components detected may suggest possible clinical application. Active components are being isolated for certain plant extracts for chemical characterisation.

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References

Begue, W.J., Kline, R.M., 1972. The use of tetrazolium salts in bioautographic procedures. Journal of Chromatography 64, 182–184.

Bonjar, G.H.S., 2004. New approaches in screening for antibacterials in plants. Asian Journal of Plant Sciences 3, 55–60.

Eloff, J.N., 2000. On expressing the antibacterial activity of plant extracts—a small first step in applying scientific knowledge to rural primary health care. South African Journal of Science 96, 16–118.

Eloff, J.N., 2004. Quantifying the bioactivity of plant extracts during screening and bioassay-guided fractionation. Phytomedicine 11, 370–371.

Fabry, W., Okema, P.O., Ansorg, R., 1998. Antibacterial activity of plant extracts of East African medicinal plants. Journal of Ethnopharmacology 60, 79–84.

Gurib-Fakim, A., 2002. Mauritius through its medicinal plantstowards a better understanding of medicinal plants of the Indian Ocean Islands. Edition Le Printemps, Mauritius.

Hoareau, L., Da Silve, E.J., 1999. Medicinal plants: a re-emerging health aid. Journal of Biotechnology 2.

Homans, A.L., Fuchs, A., 1970. Direct bioautography on thin layer chromatograms as a method for detecting fungitoxic substances. Journal of Chromatography 51, 327–329.

Klepser, M.E., Banevicius, M.A., Qintiliani, R., Nightingale, C.H., 1996. Characterization of bactericidal activity of clindamycin against *Bacteroides fragilis* via kill curve methods. Antimicrobial Agents and Chemotherapy, 1941–1944.

Martini, N., Eloff, J.N., 1998. The preliminary isolation of several antibacterial compounds from *Combretum erythrophyllum* (Combretaceae). Journal of Ethnopharmacology 62, 255–263.

Matsen, J.M., 1979. Bacterial susceptibility testing and assays. In: Henry, J.B. (Ed.), Clinical Diagnosis and Laboratory Methods. WB Saunders, Philadelphia, pp. 1900–1938.

Mitscher, L.A., Drake, S., Gollapudi, A.R., Okwute, S.K., 1987. A modern look at folklore use of anti-infective agents. Journal of Natural Products 50, 1025–1040.

Murray, C.J.L., Lopez, A.D., 1997. Mortality by cause for eight regions of the world: global burden of disease study. Lancet 349, 1269–1276.

National Committee for Clinical Laboratory Standards (NCCLS), 1990. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grows Aerobically. Approved Standard (M7-A2). NCCLS, Villanova, PA.

National Committee for Clinical Laboratory Standards (NCCLS), 1992. Approved Standard M100-S4 (M2-A4). Performance Standards for Antimicrobial Disk Susceptibility Testing. NCCLS, Villanova, PA (Fourth Informational Supplement).

Peterson, L.R., Dalhoff, A., 2004. Towards targeted prescribing: will the cure for antimicrobial resistance be specific, directed therapy through improved diagnostic testing? Journal of Antimicrobial Chemotherapy 53, 902–905.

Plotkin, M.J., 1988. Conservation, ethnobotany and the search for new jungle medicines: pharmacognosy comes of ageagain. Pharmacotherapy 8, 257–262.

Rahalison, L., Hamburger, M., Monod, M., Frenk, E., Hostettmann, K., 1993. Antifungal tests in phytochemical investigations: comparison of bioautographic methods using phytopathogenic and human pathogenic fungi. Planta Medica 60, 41–44.

Shultes, R.E., Hofman, A., 1992. Plants of Gods. Healing Art Press, Rochester, Vermont.

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Y. Yvon, **EG. Raoelison**, R. Razafindrazaka, A. Randriantsoa, M. Romdhane, N. Chabir, M.G. Mkaddem, J. Bouajila. Relation between chemical composition or antioxidant activity and antihypertensive activity of six essential oils. *Journal of Food Science* 2012, **77**(8), H184-H191.