

Screening for anti-infective properties of several medicinal plants of the Mauritian 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 gloeosporioides*, *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 gloeosporioides*, *Sclerotinia sclerotium* and *Guignardia* sp.

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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 Silve, 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), *Erythroxyllum laurifolium* (Lam.) (Erythroxyllaceae), *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), *Erythroxyllum 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 Kunkel 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 gloeosporoides*, *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 aseptically 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 dishes 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 Hin-

ton 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

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

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
<i>Antidesma madagascariense</i>	–	–	–	–	–	–	+	–	–	–	–	+
<i>Aphloia theiformis</i>	–	–	–	–	–	–	+	–	–	+	–	+
<i>Erythroxylum laurifolium</i>	–	–	–	–	–	–	+	–	–	+	–	+
<i>Mangifera indica</i>	–	–	–	–	–	–	+	–	–	+	–	+
<i>Melia azedarach</i>	–	–	–	–	–	–	+	–	–	–	–	–
<i>Michelia champaca</i>	–	–	–	–	–	–	+	–	–	–	–	–
<i>Moringa oleifera</i>	–	–	–	–	–	–	+	–	–	+	–	+

(+) 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 gloeosporoides*.

^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
<i>Antidesma madagascariense</i>	–	–	+	–	+	+	++	–	–	+	–	–
<i>Aphloia theiformis</i>	–	–	–	–	++	+	–	–	+	–	–	–
<i>Erythroxyllum laurifolium</i>	–	–	+	–	++	–	–	–	+	–	–	++
<i>Mangifera indica</i>	–	–	+	–	–	–	–	–	–	–	–	–
<i>Melia azedarach</i>	–	–	+	–	++	+	–	–	–	–	–	–
<i>Michelia champaca</i>	–	–	–	–	++	+	++	–	–	+	–	–
<i>Moringa oleifera</i>	–	–	+	–	–	+	+	–	–	+	–	–

(+) 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 gloeosporoides*.

^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 *Erythroxyllum laurifolium* against *Candida albicans* (730 ml/g), methanol extract of *Melia azedarach* against *Enterobacter cloacae* (138 ml/g) and methanol extract of *Erythroxyllum 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
<i>Antidesma madagascariense</i>	+	+	++	+	++	+	–	–	–	–	–	+
<i>Aphloia theiformis</i>	+	–	++	+	+	+	–	–	+	–	–	+
<i>Erythroxyllum laurifolium</i>	–	–	++	+	+	+	–	–	+	–	–	++
<i>Mangifera indica</i>	–	–	++	–	+	+	–	–	+	–	–	++
<i>Melia azedarach</i>	–	+	++	–	++	+	–	–	+	–	–	++
<i>Michelia champaca</i>	–	+	++	+	+	+	–	–	–	+	–	+
<i>Moringa oleifera</i>	–	+	++	–	++	++	–	–	–	–	–	+

(+) 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 gloeosporoides*.

^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 ($\mu\text{g/ml}$)	Total activity (ml/g)
<i>Antidesma madagascariense</i>	<i>Salmonella enteritidis</i>	125	326
	<i>Staphylococcus aureus</i>	500	81
<i>Aphloia theiformis</i>	<i>Salmonella enteritidis</i>	500	74
	<i>Staphylococcus aureus</i>	500	74
<i>Erythroxylum laurifolium</i>	<i>Bacillus subtilis</i>	1000	91
	<i>Candida albicans</i>	125	730
	<i>Salmonella enteritidis</i>	500	182
<i>Mangifera indica</i>	<i>Candida albicans</i>	250	54
	<i>Enterobacter cloacae</i>	250	54
	<i>Salmonella enteritidis</i>	250	54
<i>Melia azedarach</i>	<i>Candida albicans</i>	250	277
	<i>Enterobacter cloacae</i>	500	138
	<i>Salmonella enteritidis</i>	500	138
<i>Michelia champaca</i>	<i>Salmonella enteritidis</i>	125	1043
<i>Moringa oleifera</i>	<i>Salmonella enteritidis</i>	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
<i>Bacillus subtilis</i>	7	100
<i>Enterobacter cloacae</i>	7	100
<i>Escherichia coli</i>	4	57.1
<i>Pseudomonas aeruginosa</i>	4	57.1
<i>Salmonella enteritidis</i>	7	100
<i>Staphylococcus aureus</i>	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 (%)
<i>Antidesma madagascariense</i>	6	100
<i>Aphloia theiformis</i>	5	83.3
<i>Erythroxylum laurifolium</i>	4	66.7
<i>Mangifera indica</i>	3	50
<i>Melia azedarach</i>	4	66.7
<i>Michelia champaca</i>	5	83.3
<i>Moringa oleifera</i>	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 gloeosporoides*, *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 gloeosporoides* 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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