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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 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), *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.