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Antimicrobial, antibiotic potentiating activity and phytochemical profile of essential oils from exotic and endemic medicinal plants of Mauritius

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ABSTRACT

The aim of this study was to evaluate the antimicrobial properties of essential oils (EOs) isolated from 7 exotic and 2 endemic medicinal plants of Mauritius. Eighteen microorganisms (bacterial and fungal isolates) have been used to evaluate the antimicrobial potential of the EOs as well as their ability to potentiate conventional antibiotics. Significant antibacterial activities were recorded with low minimal inhibitory concentration for 8 of the EOs using the microbroth dilution assay except for *Salvia officinalis*, where the activity recorded was comparable to that of the antibiotics. The synergistic effect of the EOs of *Pimenta dioica*, *Psiadia arguta* and *Piper betle* were observed against *Escherichia coli* and *Staphylococcus epidermidis* when combined with gentamicin. The fungicidal and fungistatic effect of the EOs were observed among all the fungi irrespective of the family except for *Trichophyton mentagrophytes*. Forty three major compounds were identified using the gas chromatography–mass spectrometry method and predominantly composed of oxygenated monoterpenes at a dose ranging from 0.45% to 69.76%, while, in the case of *P. dioica*, the EO was predominantly composed of aromatic compounds at a dose of 89.22%. This study has provided key information on the antimicrobial property and phytochemical composition of some tropical medicinal plants from Mauritius.

1. Introduction

The emergence of multidrug-resistant pathogens and their impact on healthcare, along with the inappropriate use of antibiotics has become an urgent issue for patient safety (Edward-Jones, 2013). Indeed, infectious diseases including multidrug resistant related diseases are considered as one of the leading causes of global morbidity and mortality, especially in developing countries. Additionally, antibiotic resistance has become a major public health problem of increasing magnitude, and the discovery and development of novel antimicrobial agents from natural products to address this problem is of uttermost importance (Edward-Jones, 2013; Yala et al., 2001). The quest for novel effective antimicrobial agents from natural products has attracted much attention particularly in the health care sector, where microbial resistance is increasing at an alarming rate and offering new challenges. Antimicrobial phytochemicals isolated from medicinal plants are thus being explored and their components probed in view of medical application to fight fatal opportunistic infections (Bakkali et al., 2008). Moreover, the problem of multidrug resistance has been observed from a wide range of pathogens and the most common example is methicillin resistant *Staphylococcus aureus* (MRSA). The prevalence of MRSA infection has experienced a breakthrough in acute care and chronic care, thereby increasing the number of patients at risk of potential complications from contact precautions. Also, the appearance of multidrug resistance among the Gram-negative bacteria which have been correlated to the production of extended spectrum β-lactamase is of growing concerns worldwide and hence such infectious diseases are becoming more challenging and difficult to manage and/or treat. On the other hand, the establishment of new antibiotics are too expensive and when the cost are affordable, the time for its development and implementation are very slow compared to the rate of increase of multidrug resistance pathologies (Edward-Jones, 2013).

For this purpose, natural products like EOs have attracted much attention and are coming more within the scope of phytomedicine

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and attracted much attention as complementary and alternative therapy (Zu et al., 2010). Additionally, they offer potential novel template molecules and mixtures of bioactive compounds that can be exploited industrially as bio-products for both the wellness, pharmaceutical and food industry. EOs, which are complex mixtures of biologically active substances, are classified as natural products having pharmacological potential that can be of therapeutic benefit in the management of human diseases (Derwich et al., 2010).

In our continuous exploration of antimicrobial agents from local resources that could be subsequently developed as novel bioproducts industrially, we have thus designed the present work to investigate the antimicrobial potential of some of commonly used medicinal plants from Mauritius. Nine plants have been included among which 2 are endemic (*Psiadia arguta* Pers. (Voigt) and *Psiadia terebinthina* A.J. Scott), and the remaining 7 plants (*Pimenta dioica* L., *Salvia officinalis* L., *Laurus nobilis* L., *Piper betle* L., *Rosmarinus officinalis* L., *Cinnamomum zeylanicum* Nees and *Schinus terebinthifolius* R.) are exotic to Mauritius. These medicinal plants were assessed for their antimicrobial potential *in vitro* against key infective bacterial and fungal strains among which 4 of the microorganisms (MRSA, *Pseudomonas aeruginosa, Escherichia coli* and *Enterococcus faecalis* have been highlighted by the National Committee for Clinical Laboratory Standards (NCCLS) as major causative agents of nosocomial infections. Additionally, we have evaluated the phytochemical composition and yield of the EOs extracted from the 9 selected plants.

2. Material and methods

2.1. Collection of plant materials

Plants included in the present study are those used traditionally by the local people as medicinal plants to treat and manage several infectious diseases (Gurib-Fakim et al., 1996; Nunkoo and Mahomoodally, 2012). Plants were collected from the central region of Mauritius which is 151 m above sea level and benefitting from a mild tropical maritime climate throughout the year. The leaves of *P. dioica* L. (PD), *S. officinalis* L. (SO), *L. nobilis* L. (LN), *P. betle* L. (PB), *R. officinalis* L. (RO), *C. zeylanicum* Nees (CZ), *S. terebinthifolius* R. (ST), *P. arguta* Pers. (PA) and *P. terebinthina* A.J. Scott (PT) were collected at the University farm. Each plant was identified by a local botanist.

2.2. Extraction of the EOs

The leaves of the plants were gently plucked, washed and finely cut into pieces, while the fruits were peeled off carefully with the use of a sharp knife to avoid any damage of the oil glands and finely reduced to uniform size. The plant materials were then subjected to the hydrodistillation process for a period of 3 h using a Clevenger type apparatus (Kulisic et al., 2004; Soković and Van Griensven, 2006). The distillates of the EOs thus yielded were then dried over anhydrous sodium sulfate, filtered and stored at −4 °C until further analysis (Hussain et al., 2008).

2.3. Antimicrobial activity

2.3.1. Microbial strains

The microorganisms used in the present investigation included reference strains from the American Type Culture Collection (ATCC) (*E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853), *Staphylococcus epidermidis* (ATCC 12228), *Propionibacterium acnes* (ATCC 6919), *Candida albicans* (ATCC 10231), *Candida tropicalis* (ATCC 750), *Aspergillus niger* (ATCC 16404), *Trichophyton mentagrophytes* (ATCC 9533)) and and clinical – laboratory collection strains (*Streptococcus peroris*, *Klebsiella pneumonia*, MRSA, *E. coli*, *E. faecalis*, *P. aeruginosa*, *Acinetobacter baumannii*, *Proteus vulgaris*, and *C. albicans)*. The clinical isolates were obtained from Victoria Hospital, Candos, Mauritius.

2.3.2. Antibacterial screening

2.3.2.1. Disc diffusion method. The disc diffusion method was adapted from Lesueur et al. (2007) for the determination of the antibacterial activities of the EOs. For this purpose, paper discs (6 mm) were impregnated with 10 μl of EO. The discs were then placed on the inoculated Petri dishes containing 0.1 ml of bacterial suspension adjusted to 10^6 CFU/ml except for *S. aureus* where the innoculum is set at 10^7 CFU/ml (Caraega et al., 2003). Three antibiotics were used as positive control namely chloramphenicol, tetracycline and ampicillin pre-dosed at 30 μg/disc. Discs without samples were used as a negative control. The zones of inhibition including the diameter of the EO impregnated discs were compared with those of the controls after incubation at 37 ± 1 °C after 24 h. The presence or absence of an absolute inhibition zone was used as criteria for the definition of active or inactive EOs. The tests were carried out in triplicate for each EO (Rodriguez Vaquero et al., 2007; Sacchetti et al., 2005)

2.3.2.2. Micro-dilution broth susceptibility assay. The minimum inhibitory concentration (MIC) was calculated from the microdilution broth susceptibility assay as reported by Baker et al. (1980); Rapper et al. (2013) and Joshi et al. (2010). Briefly, 100 μl of a mixture of 1% (v/v) of DMSO and Muller Hinton broth was distributed in all the wells of the 96 well microplates. 50 μl of EOs (or antibiotics for the positive controls) were transferred into the wells of the first row of the 96 well microplates. Serial dilutions of the samples (EOs or antibiotics) were carried out such that 50 μl from the first wells are transferred, resulting in the samples of the first row to be serially distributed in descending concentrations. Negative controls were included whereby instead of the samples, Muller Hinton broth was incorporated.

50 μl of innoculum (at 0.5 McFarland) were then added to each well and the plates allowed to incubate for 24 h at 37 ± 1 °C. Following incubation, 40 μl of INT (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride) at 0.2 mg/ml was added to the reaction mixture. The interaction of the microorganisms (when viable) with INT give rise to a color change from colorless to a redish-pink color. The wells with the lowest dilutions whereby no color change were observed were considered as the MIC for these tested samples.

2.3.2.3. Minimum bactericidal concentration. For the determination of the minimum bactericidal concentration (MBC), growth inhibitory assays were performed as described by Celiktas et al. (2007) with slight modification. Briefly, 10 μL of broth from the uncolored wells (whereby no growth were observed in the previous MIC assay), corresponding to the MIC value, MICx2 (one dilution higher than MIC) and MICx4 (one dilution higher than MICx2) were inoculated on Muller Hinton Agar (MHA) and incubated for 24 h at 37 °C. The MBC was defined as the lowest recorded EO concentration of the MIC wells in which bacteria failed to grow on the MHA (Onawunmi, 1989). On the other hand, if growth occurred following inoculation on MHA, the concentration of the corresponding well used for inoculation (MIC value, MIC × 2 and MIC × 4) was referred to be as the bacteriostatic concentration (Smith-Palmer et al., 2001). For comparison, both negative and positive controls were set.

2.3.2.4. Total antimicrobial activity of selected EOs. The total antimicrobial activity of the EOs was calculated as described by Eloff (2004). This method gives an indication on the efficacy at which