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Research paper

Chemical composition, antimicrobial and antibiotic potentiating activity of essential oils from 10 tropical medicinal plants from Mauritius

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ABSTRACT

Infectious diseases and antibiotic resistance have become a public health issue of increasing magnitude. The discovery and development of new antimicrobial agents from herbal medicine to address this problem has attracted much attention and should be given high priority. This study was designed to evaluate the antimicrobial properties of essential oils (EOs) extracted from 10 common medicinal plants of Mauritius. Eighteen microorganisms (bacterial and fungal isolates) were used to evaluate the antimicrobial potential of the EOs as well as their ability to potentiate conventional antibiotics. The phytochemical profile was established using Gas chromatography–Mass spectrometry method. Antibacterial activities were recorded with low minimal inhibitory concentration for 4 of the EOs using the microbroth dilution assay. A synergistic effect of the EO of *Citrus hystrix* D.C., *Citrus reticulate* (Blanco) and *Melaleuca quinquenervia* S.T. Blake (Cav.) were observed against *Escherichia coli* (ATCC 25922) and *Staphylococcus epidermidis* (ATCC 12228) when combined with gentamicin. Fungicidal and fungistatic effects of the EOs were observed among all the fungi irrespective of the family except for *Trichophyton mentagrophytes*. Twenty eight major compounds were identified and predominantly composed of monoterpene hydrocarbons at a dose-content ranging from 0.68 to 88.58%. This study has provided key information on the antimicrobial property and phytochemical composition of some tropical medicinal plants. Hence, EOs studied in the present investigation may be considered as potential medicinal candidates that could be exploited as complementary and alternative therapies for the treatment and management of infectious diseases.

1. Introduction

Infectious diseases are nowadays considered as one of the leading causes of global morbidity and mortality, especially in developing countries. Additionally, antibiotic resistance has become a public health problem of increasing magnitude. Therefore, the discovery and development of novel antimicrobial agents from natural products to address this problem is of uttermost importance (Yala et al., 2001; Edward-Jones, 2013). The quest for novel effective antimicrobial agents from natural products has gained much momentum particularly in the health care sector, where microbial resistance is increasing at an alarming rate and offering new challenges. Phytochemicals with potential antimicrobial activities isolated from plants are thus being explored in view of potential therapeutic application to fight fatal opportunistic infections (Bakkali et al., 2008). Moreover, the problem of multidrug resistance has been observed over a wide range of pathogens and the most common example is Methicillin Resistant *Staphylococcus aureus* (MRSA). The appearance of multidrug resistance among the Gram-negative bacteria which have been correlated to the production of extended spectrum β-lactamase is also of growing concern worldwide. Thus, infectious diseases are becoming more challenging and difficult to treat. On the other hand, the establishment of new antibiotics is too expensive. Moreover, when the cost of such antimicrobials are affordable, the time for their development and implementation is very slow compared to the escalating rate of multidrug resistant pathologies (Edward-Jones, 2013).

In this respect, natural products derived from traditionally used medicinal plants like EOs have attracted much attention as

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alternative and complementary antimicrobials (Zu et al., 2010). EOs, which are complex natural mixtures of biologically active substances can be of therapeutic benefit in the treatment of a panoply of human diseases (Derwich et al., 2010).

The present study was thus designed to investigate the antimicrobial potential and antibiotic potentiating activity of some of the commonly used medicinal plants from Mauritius. The plants studied are *Citrus grandis* L. (CG), *Citrus hystrix* D.C. (CH), *Citrus reticulate* (Blanco) (CR), *Psidium guajava* L. (PG), *Lavandula* × *intermedia* var. Grosso L. (LI), *Cupressus macrocarpa* H. (CM), *Cymbopogon citratus* D.C. (Stapf) (CC), *Melaleuca quinquenervia* S.T. Blake (Cav.) (MQ), and *Triphasia trifolia* (Burm. f.) (P. Wilson) (TT). 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 10 EOs extracted from these selected plants.

2. Material and methods

2.1. Collection of plant materials

Traditionally used medicinal plants (Gurib-Fakim et al., 1996; Nunkoo and Mahomoodally, 2012) 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 *C. macrocarpa* H. (CM-2014-AE4), *C. grandis* L. (CGl-2014-AE5), *C. citratus* D.C. (CC-2014-AE10), *Lavandula* × *intermedia* var. Grosso L. (LI-2014-AE2), *M. quinquenervia* S.T. Blake (MQ-2014-AE11), *P. guajava* L. (PG-2014-AE14), *T. trifolia* P. Wilson (TT-2014-AE17) and fully ripened fruits of 3 citrus species namely *C. grandis* L. (CGp-2014-AE5), *C. hystrix* D.C. (CH-2014-AE8), and *C. reticulate* Blanco (CR-2014-AE18) were collected from the University farm (University of Mauritius, Réduit, Mauritius). A voucher specimen was kept in the Faculty of Science, (University of Mauritius, Réduit, Mauritius) with an identification code as specified in parentheses above. The Plant List (www.plantlist.org) and International Plant Name index (www.ipni.org), were used to validate plant scientific names as well as confirm author names. Our local repository database was updated for future reference and data mining.

2.2. Extraction of the EOs

The leaves of the plants were gently plucked, washed and finely cut into pieces. Fruits were carefully peeled with the use of a sharp knife to avoid any damage to 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 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 reference microorganisms from the American Type Culture Collection (ATCC) used in the present investigation were; *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), and *Trichophyton mentagrophytes* (ATCC 9533). Clinical Laboratory collection strains studied were *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. Powdered Mueller-Hinton agar (Sigma–Aldrich, Germany) was dissolved in distilled water and the mixture was autoclaved at 121 °C for 2 h. Cooled autoclaved agar was distributed evenly in petri dishes aseptically. 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 inoculum was set at 10^7 CFU/ml (Caraega et al., 2003). Three antibiotics were used as positive controls namely chloramphenicol, tetracycline and ampicillin pre-dosed at 30 μg/disc. Discs without samples were used as negative controls. 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 for 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 micro-dilution broth susceptibility assay as reported by Baker et al. (1980), Joshi et al. (2010) and Rapper et al. (2013). 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. Fifty microliters 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. Fifty microliters from the first wells were transferred, resulting in the samples of the first row to be serially diluted in descending concentrations. Muller Hinton broth was incorporated in the wells as negative controls.

Fifty microliters of inoculum (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 micro-organisms (when viable) with INT resulted in a color change from colorless to a reddish-pink. Wells with the lowest dilutions with no color change was 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 (no growth) in the MIC assay and corresponding to the MIC value, MICx2 (one dilution higher than MIC) and MICx4 (one dilution higher than MICx2) were inoculated on Muller Hinton Agar and incubated for 24 h at 37 ± 1 °C. The MBC was defined as the lowest EO concentration of the MIC wells in which bacteria failed to grow (Onawunmi, 1989). On the other hand, if growth occurred following inoculation, the concentration of the corresponding well used for its inoculation (MIC value, MICx2 and MICx4) was referred to as the bacteriostatic concentration (Smith-Palmer et al., 1998). For comparison, both negative and positive controls were included.