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The antimicrobial activity and essential oil composition of medicinal aromatic plants used in African traditional healing

Van Vuuren, Sandra Freda

2008-02-29T12:14:13Z
http://hdl.handle.net/10539/4505

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Sandy van Vuuren (0202739E)

PhD

Abstract

A study on the essential oil chemistry and microbiological activity of South African indigenous medicinal plants with the aim of establishing a scientific rationale for their anti-infective properties was undertaken. For the purpose of this study, nine medicinal aromatic plants were selected. The hydrodistilled essential oil was analyzed by gas chromatography combined with mass spectrometry (GC-MS) and various techniques were used to document the antimicrobial activity.

Disc diffusion studies on *Myrothamnus flabellifolius* indicated highest activities against the fungal test organisms *Cryptococcus neoformans* and *Aspergillus niger* with radial inhibition zones of 8 mm and 10 mm respectively. The highest antimicrobial activity noted in the minimum inhibitory concentration (MIC) assay was for *C. neoformans* (2 mg/mL). Time-kill studies demonstrated the death kinetic progression on *M. flabellifolius* essential oils where the killing rate was greatest for *Candida albicans*. *Osmitopsis asteriscoides*, a plant used traditionally for cuts and swellings showed highest disc diffusion antimicrobial efficacy against *Staphylococcus aureus* (3 mm) and *C. neoformans* (3 mm). The MIC study indicated highest susceptibilities (4 mg/mL) for *Escherichia coli* and *Staphylococcus epidermidis*. Death kinetics for the three organisms studied demonstrated that the killing rate was greatest for *C. albicans*. The role of the two major constituents (1,8-cineole and (-)-camphor) act synergistically to enhance antimicrobial activity. Disc diffusion assays undertaken on *Artemisia afra* showed highest activity against *Candida tropicalis* (5 mm). In the MIC assay the highest susceptibility was against *Serratia odorifera* (4 mg/mL). Time-kill assays on *Artemisia afra* showed a concentration dependent bactericidal activity, with evidence that the major constituents independently and in combination were not responsible for the overall activity of the plant. *Lippia javanica*, a plant used to treat coughs, colds and bronchitis, indicated highest susceptibility against the respiratory pathogen *Klebsiella pneumoniae* (5 mm) with the disc diffusion assay. The MIC assay indicated highest susceptibilities (4

mg/mL) against *C. neoformans* and *E. coli*. Death kinetic assays for three test organisms showed that the killing rate was the greatest for *K. pneumoniae.* The time-kill study for *L. javanica* in combination with *A. afra* demonstrated that the oils in combination act synergistically against *K. pneumoniae*. The antimicrobial activity of the essential oils and extracts were determined for *Helichrysum cymosum* subsp. *cymosum* where the extracts demonstrated at least a six times greater MIC efficacy than the essential oils. Using column chromatography, the antimicrobially active compound was isolated from *H. cymosum* subsp. *cymosum* and identified as helihumulone. The traditional use of plants as a treatment for infectious diseases is not always restricted to a single part of the plant as was noted in the study on *Croton gratissimus* var. *subgratissimus*, where the leaf, bark and root extracts were investigated singularly and combined in various ratios to establish possible interaction. The MIC and fractional inhibitory concentration (FIC) results indicated variable efficacies for the plant combinations. The greatest synergistic profile was noted for *C. neoformans* in the leaf and root combination (MIC 0.4 mg/mL and FIC of 0.4). Further isobologram combination studies were thereafter conducted on varying ratios of leaf and root extracts, indicating greatest synergy for *Bacillus cereus, Enterococcus faecalis, C. albicans* and *C. neoformans*. While seasonal variation had very little impact on the MIC results obtained from *Heteropyxis natalensis*, the ratio of the two major compounds (1,8-cineol and limonene) fluctuated on a monthly basis. Moderate antimicrobial activity (3.0-16.0 mg/mL) was found for most pathogens with higher sensitivities for *C. neoformans*. The geographical variation of *H. natalensis* essential oil indicated similar profiles for Gauteng, Nelspruit and Waterberg samples. The Lagalametse sample, however, showed distinct variation both chemically and microbiologically where efficacy was higher than in all other samples. The impact of the enantiomeric configuration was investigated for limonene in combination with 1,8-cineole with (+/-)-limonene in combination with 1,8-cineole having the most significant synergistic ratios against *Pseudomonas aeruginosa.* The antimicrobial activities of the non-volatile and volatile fractions of *Tarchonanthus camphoratus* and *Plectranthus grandidentatus,* singularly and in combination demonstrated that the volatile constituents contribute to the total efficacy of the plant. Isobologram representation of the combination of various ratios of *T. camphoratus* and *P. grandidentatus* essential oil and

non-volatile extracts devoid of essential oils present a predominant synergistic profile for all pathogens studied. A comparative study on five indigenous oils (*M. flabellifolius, O. asteriscoides, H. natalensis, A. afra* and *L. javanica*) was undertaken with five popular commercial oils (*Lavendula angustifolia*, *Thymus vulgaris*, *Melaleuca alternifolia*, *Mentha piperita* and *Rosmarinus officinalis*). The highest antimicrobial activity was noted for *Thymus vulgaris* in the MIC assay, followed by *M. flabellifolius, O. asteriscoides* and *M. alternifolia*. With the time-kill assay, *M. flabellifolius* showed the most rapid cidal effect against all three pathogens tested. The comparative evaluation of commercial essential oils with indigenous oils validated the use of South African aromatic plants for their anti-infective properties.