ANTIMICROBIAL ACTIVITIES AND BIOASSAY GUIDED IDENTIFICATION OF BIOACTIVE CONSTITUENTS OF SELECTED KENYAN MEDICINAL PLANTS

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DECLARATION

This thesis is my original work and has not been presented for award of a degree in any other university

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We confirm that the work reported in this thesis was carried out by candidate under our supervision as university supervisoras.

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ABSTRACT

The relationship between man and plants has been very close in almost all generations. Egyptians, around 1500 B.C. discovered that oil from beans of castor tree would bring relief when applied to burns and septic wounds. In this study the focus was to investigate the potential of *Trimeria grandifolia* (Hochst.) Warb (Flacourtiaceae) and *Capparis fascicularis* DC. (Cappaaraceae) used traditionally to manage microbial infections. This is due to the current infectious disease burden and the increasing rate of antimicrobial resistance to existing antibiotics. This creates the need to look for alternative antimicrobials in which pathogens have lower or no resistance. The study aimed to carry out antimicrobial activities of the constituents of different parts of *T. grandifolia* and *C. fascicularis* and characterize the most active constituent. Leaves, root bark and stem bark of the two plants were sampled from Nyandarua County in Kenya, dried, ground and sequentially extracted using solvents of increasing polarity (petroleum ether, ethyl acetate, methanol and water). Twenty-four extracts were obtained, screened against selected strains of bacteria and fungus (*Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, Escherichia coli and Candida albicans* respectively) using disc diffusion and tube dilution methods. The results were analysed by analysis of variance (ANOVA). The ethyl acetate extract of *C. fascicularis* root bark (CFR2) showed a significant inhibition against *S. aureus, B. subtilis* and *S. typh*i, compared to those extracted using other solvents ($F = 18.84$, $P = 0.0001$, $F= 18.84$, $P = 0.0001$ and $F = 9.27$, $P = 0.0001$ respectively). Minimum inhibitory concentration (MIC) of CFR2 against *S. tyhpi* and *B. subtilis* was at 500 µg/mL ($F = 14.38$, $P = 0.0001$) and 250 µg/mL ($F = 4.82$, $P = 0.0001$) respectively, which was significantly lower than other concentrations of plant extract. Therefore, CFR2 was considered the most active crude extract. Fractionation of CFR2 by column chromatography yielded seven fractions that were screened against the selected bacterial strains. Fraction 2 (F2), had the lowest MIC values and was considered the most active fraction. It inhibited *B. subtilis, E. coli* and *S. typhi* at 250 µg/mL ($F = 14.38$, $P = 0.0001$), 500 µg/mL ($F = 3.23$, $P = 0.003$) and 500 µg/mL ($F = 6.58$, $P = 0.0001$) respectively. F2 was further fractionated and four sub-fractions obtained. They were screened against the selected strains of bacteria. However, F2 (mean= 13.250 mm) was the most bioactive fraction compared to the four sub-fractions ($F = 66.79$, $P = 0.0001$). This suggested that, constituents of F2 worked in synergy. F2 was then screened for phytochemicals where phenols, terpenes and flavonoids were present. In addition, F2 was analysed by GC-MS. Two bioactive compounds were identified as, 2,4-Di-tert-butylphenol **(14)** and (*E,E*) -2,4-Decadienal **(18)** . F2 was also analysed by LC-ESI-MS and three reported bioactive compounds were identified as Tanshinone II A **(21),** Cryptotanshinone **(22)** and Danshensu **(23).** Better antimicrobial activities were displayed in *C. fascicularis* compared to *T. grandifolia.* CFR2 was the most active extract on bacteria and this suggested that most antimicrobial compounds are concentrated in the roots of *C. fascicularis*. Therefore, we recommend that further chromatographic separation be done to obtain all the chemical constituents and screen them against various strains of bacteria and fungi, toxicity studies of isolated compounds from *T. grandifolia* and *C. fascicularis* be carried out to determine their efficacy, blending and structure-activity relationships of isolated compounds with known antifungal and antibacterial drugs be carried out in order to determine if there is synergy or antagonism and derivitization of the isolated compounds be done so as to improve their bioactivity.