A COST-EFFECTIVE MODIFICATION OF MICROTITER PLATE ASSAY FOR DETERMINING MINIMUM INHIBITORY CONCENTRATION (MIC) OF PLANT FRACTIONS AND OILS

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Testing for antimicrobial activity using MIC determination and activity-guided fractionation of plant material is currently very popular. The agar diffusion method is a qualitative test used to screen antimicrobial activity while the agar dilution and micro broth dilution tests are quantitative methods used to determine the MIC. Large volumes of extract/media are needed for the agar dilution method whereas the micro broth dilution tests make it more suitable for the low volumes of fractions/oils available. However, plant fractions are usually dark in colour, preventing accurate reading of end points using turbidity, normally used to assess bacterial growth in the wells. The use of indicators such as tetrazolium salts and resazurin dyes or spectrophotometry using the principle of colorimetry have been attempted, which requires specific equipment or costly reagents. This study describes a less costly method for determining the MIC of plant fractions and oils. Serial dilutions of the oil was made in a 96 well microtitre in either Brain Heart Infusion (BHI) broth or Sugar Basal Glucose (SBG). The plates were incubated at 37 °C for 24 h after inoculation with a panel of microorganisms including *S aureus*NCTC6571, *E coli* NCTC10418, *P aeruginosa* NCTC10662, 5 clinical isolates of Methicillin Resistant *Staphylococcus aureus*, multiresistant *Klebsiella pneumoniae* and *Acinetobacter* spp, ESBL producing *K pneumoniae*, *Proteus* spp., *Enterobacter cloacae* and 5 *Candida* spp. including *C tropicalis*ATCC13803, *C glabrata* ATCC 90030, *C krusei* ATCC6258, *C albicans* ATCC90028 and *C parapsillosis* ATCC22019 and 3 clinical isolates of *C albicans*. After overnight incubation, growth (lack of inhibition) was noted by change of colour to pink with SBG and turbidity with BHI. The lowest concentration of oil at which no colour change (SBG)/turbidity (BHI) was recorded as the MIC of the oil for the organisms. MIC of tested organisms was similar with both media except vancomycin resistant *Enterococcus* (VRE) and *Enterobacter cloacae*. As *Pseudomonas aeruginosa* is a non-lactose fermenting bacteria, the lactose containing substrate is not suitable for use. Reading the end points with SBG was very easy as there were sharp demarcation between colourless wells and the pink coloured well. This novel method of SBG was correlated with BHI broth method. This SBG method could therefore be used to determine the MIC against an acid positive organism. This method is easy to determine the MIC of plant fractions or oils which are in small amount and also could determine the MIC against eight organisms using one plate. Hence, time could be saved. SBG is cheaper than commercial indicators (Tetrazolium) and allows effective reading of the MIC visually without any equipment.

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