Potential of Endophytes and Rhizosphere Bacteria from Selected Indigenous Kenyan

Plants around Juja as Sources of Antimicrobial Compounds

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ABSTRACT

An increase in the number of people in the world having health problems caused by certain cancers, drug-resistant bacteria, parasitic protozoans, and fungi has caused alarm. Endophytes are a potential source of novel chemistry and biology products/compounds to assist in helping solve not only human health, but plant and animal health problems also. Rhizosphere microorganisms may produce a myriad of substances of potential use in modern medicine. By isolating the endophytes and rhizosphere bacteria and growing them in culture media, it is possible to harvest the bioactive compounds that they produce and these may contain potential novel compounds that may be effective candidates for treating emerging and re-emerging infectious human diseases.

The study was conducted with the aim of isolating endophytes and rhizosphere bacteria with antagonistic activity against pathogenic microbes. They were obtained from indigenous Kenyan plants around Juja that included; *Cleodendrum myricoides, Dombeya rotundifolia, Dalbergia menaloxylon, Lannea flavus, Dichrostachys cinerea, Gomphocarpus fruticosus, Balanites aegyptica, Schrebera alata, Jasminium floribundum* and *Hibiscus fuscus.* Characterization of the bacteria was done using morphological, physiological and molecular techniques while characterization of bioactive substances from culture filtrates was done using bioassay guided fractionation and spectroscopic methods. A total of fourty eight isolates (48) were obtained from both the endosphere and the rhizosphere regions. They were subjected to a cross streak antimicrobial screening against bacteria (*Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli*) and fungi (*Candida albicans*), to determine their range of *in vitro* activity before extraction of the crude products. They showed a range of antagonistic activity against the test organism. Ten isolates (26%) were selected and investigated

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depending on their broad range of *in vitro* antimicrobial activity against the test bacteria and fungi and were further characterized. One isolate DM30 was subjected to molecular characterization. Phylogenetic analysis of amplified 16S rDNA sequence revealed that isolate DM30 belonged to the genus *Bacillus* and species *subtilis*. The crude products of the ten isolates were extracted (using ethyl acetate), yields determined and tested against the test bacteria and fungi. The products had different levels of activity and this was dependent on the test organism. There were significant differences ($P \le 0.05$) in the antagonistic activity of the different crude products against the test organisms. Bioassay guided separation was carried out on the crude products after preparative thin layer chromatography. The products separated in different bands/fractions and the most active fraction (s) with an inhibition diameter of \geq 7mm from each sample was further characterized to detect the secondary metabolites and the active components present with the aid of GC-MS machine. The identified compounds ranged from amines, acids, quinines, indole, steroids, azoles and many more. Compounds such as azoles have been known to be good antifungal agents. Toluene which was frequently detected in different fractions has been known to inhibit pathogenic microorganisms. The study demonstrated that a large number and range of secondary metabolites were present in the products. Further work might show whether these metabolites can be used to develop antimicrobial agents to replace the existing ones, once resistance builds up or for emerging pathogenic microorganisms.