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Comparative Production of Secondary Metabolites by Prunus africana (Hook. F.) Kalkman Provenances in Cameroon and Some Associated Endophytic Fungi

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Abstract : Prunus africana (Hook. F.) Kalkman, commonly known as Pygeum or African cherry belongs to the Rosaceae family. It is a medium to large, evergreen tree with a spreading crown of 10 to 20 m. It is used by the traditional medical practitioners for the treatment of over 45ailments in Cameroon and sub-Sahara Africa. In modern medicine, it is used in the treatment of benign prostrate hyperplasia (BPH), prostate gland hypertrophy (enlarged prostate glands). This is possible because of its ability to produce some secondary metabolites which are believed to have bioactivity against these ailments. The ready international market for the sale of Prunus bark, uncontrolled exploitation, illegal harvesting using inappropriate techniques and poor timing of harvesting have contributed enormously to making the plant endangered. It is known to harbor a large number of endophytic fungi with the potential to produce similar secondary metabolites as the parent plant. Alternative sourcing of medicinal principles through endophytic fungi requires succinct knowledge of the endophytic fungi. This will serve as a conservation measure for Prunus africana by reducing dependence on Prunus bark for such metabolites. This work thus sought to compare the production of some major secondary metabolites produced by P. africana and some of its associated endophytic fungi. The leaves and stem bark of the plant from different provenances were soaked in methanol for 72 hrs to yield the methanolic crude extract. The phytochemical screening of the methanolic crude extracts using different standard procedures revealed the presence of tannins, flavonoids, terpenoids, saponins, phenolics and steroids. Pure cultures of some predominantly isolated endophyte species from the difference Prunus provenances such as Curvularia sp, and Morphospecies P001 were also grown in Potato Dextrose Broth (PDB) for 21 days and later extracted with Methylene dichloride (MDC) solvent after 24hrs to produce crude culture extracts. Qualitative assessment of crude culture extracts showed the presence of tannins, terpenoids, phenolics and steroids particularly β-Sitosterol, (a major bioactive metabolite) as did the plant tissues. Qualitative analysis by thin layer chromatography (TLC) was done to confirm and compare the production of β-Sitosterol (as marker compounds) in the crude extracts of the plant and endophyte. Samples were loaded on TLC silica gel aluminium barked plate (Kieselgel 60 F254, 0.2 mm, Merck) using acetone/hexane, (3.0:7.0) solvent system. They were visualized under an ultra violet lamp (UV254 and UV360). TLC revealed that leaves had a higher concentration of β-sitosterol in terms of band intensity than stem barks from the different provenances. The intensity of β-sitosterol bands in the culture extracts of endophytes was comparable to the plant extracts except for Curvularia sp (very minute) whose band was very faint. The ability of these fungi to make β-sitosterol was confirmed by TLC analysis with the compound having chromatographic properties (retention factor) similar to those of β-sitosterol standard. The ability of these major endophytes to produce secondary metabolites similar to the host has therefore been demonstrated. There is, therefore, the potential of developing the in vitro production system of Prunus secondary metabolites thereby enhancing its conservation.

Keywords : Caneroon, endophytic fungi, Prunus africana, secondary metabolite
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