Bioassay Guided Isolation of *Cassia alata* Stem Bark Extract

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Abstract: Plants have the ability to synthesize chemical compounds that help them to defend against attack from a wide variety of predators such as insects, fungi and herbivorous mammals. Although some of these compounds, whilst being toxic to plant predators, turn out to be effective drugs for human diseases. Most medicinal plants are used as biological alternatives to synthetic fungicides. For example, we found that *Cassia alata* is an important medicinal plant in the subfamily Caesalpinioideae. In the preliminary work on this project, extracts of leaves and stem bark of the *Cassia alata* we obtained through sequential extraction method using hexane, ethylacetate and methanol. Antifungal assay and preliminary phytochemical analysis carried out. On these extract revealed higher antifungal activity by the ethylacetate extract of stem bark of *C.alata* against *Pythium* sp and *Alternaria* sp. Objective of the present study is identifying the antifungal active compounds present in the ethylacetate crude of stem bark of *C.alata*. In this study, we carried out bioassay guided isolation of ethyl acetate extract of *C.alata* stem bark using column chromatography. The ethyl acetate extract was fractionated into five fractions by VLC method. Based on the TLC analysis, these five fractions were combined into two fractions (A and B) and the antifungal bioassay was performed with different concentrations (50, 25 and 12.5 ppm) of the fractions A and B against the fungi *Alternaria* sp, *Aspergillus* sp, *Collectotrichum* sp, *Fusarium* sp and *Trichoderma* sp. The fraction B showed higher antifungal activity against all tested fungi. Subsequently column chromatographic analysis was carried out on the fraction B to identify the active antifungal compound/s. We were able to successfully isolate three pure compounds from fraction B of ethyl acetate extract of stem bark of *C.alata*, which is believed to possess antifungal activity. These three compounds have to be characterized through NMR studies and mass spectrometry. Further analysis of these three compounds can be carried out to reconfirm their antifungal potentials.