

**Structures and properties of naphthoquinones produced by *Monacrosporium ambrosium*, an ectosymbiote of the shot hole borer beetle *Xyleborus fornicatus***

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The fungus, *Monacrosporium ambrosium* (syn. *Fusarium ambrosium*) has a symbiotic association with the shot-hole borer beetle, *Xyleborus fornicatus*, an insect pest that causes serious damage to the tea (*Camellia sinensis* var. *assamica*) plantations in Sri Lanka. Here we report the isolation of six naphthoquinones produced by the fungus *M. ambrosium* and the biological activities of the crude extracts of the fungal culture. *M. ambrosium* was fermented in potato dextrose broth and medium was extracted with ethyl acetate (EtOAc) and the mycelium with EtOAc and methanol (MeOH). Chromatographic separation of the combined two EtOAc extracts (Extract A) afforded six pigmented naphthoquinones, identified as anhydrojavanicin, dihydroanhydrojavanicin, 7-acetonyl-5, 8-dihydroxy-6-methyl-1, 4-naphthoquinone, javanicin, anhydrofusarubin and solaniol. Extracts were screened for antifungal activity, brine shrimp lethality, lettuce seed germination assay and  $\alpha$ -amylase inhibitory assay. Furthermore, the antifungal activity was examined against two endophytic fungi, *Pestalotiopsis camelliae* and *Phoma multirostrata* isolated from tea stems as well as three endophytic fungi, *Bipolaris sorokiniana*, *Daldinia eschscholizii* and *Glomerella magna* from *Costus speciosus*, *Phyllanthus acidus* and *Piper betel* respectively against *M. ambrosium*.

Extract A was found to possess several compounds with antifungal activity against *C. cladosporioides*. The shoot and root elongation of lettuce seeds were found to be completely inhibited by the EtOAc extract of culture broth and EtOAc extract of mycelium at 250 and 1000 ppm, respectively. The root elongation was completely inhibited at 2000 ppm of MeOH extract of mycelium while complete inhibition of shoot elongation was observed at 4000 ppm. The extracts were found to be positive in the brine shrimp lethality LD<sub>50</sub> -702, 1395 and 993 ppm for the EtOAc extract of culture broth, EtOAc extract of mycelium and MeOH extract of mycelium, respectively. None of the extracts showed good activity in the  $\alpha$ -amylase inhibitory activity assay. Extract A was found to inhibit the growth of two endophytic fungi *P. camelliae* (100% at 1000 ppm) and *P. multirostrata* (38.1% at 1000 ppm). *M. ambrosium* did not inhibit the growth of *B. sorokiniana*, *D. eschscholtzii* and *G. magna*. These results suggest that *M. ambrosium* produces antifungal naphthoquinones, which selectively inhibit the growth of two endophytic fungi living within tea stems.