Isolation and identification of allelochemicals from methanolic extract of *Robinia pseudo-acacia* L. leaves

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Introduction: *Robinia pseudo-acacia* L. belongs to family Fabaceae and is commonly known as black locust. It is widely distributed in temperate and sub temperate zone. It is a nitrogen fixer and has rapid juvenile growth; it is widely planted as an ornamental, for shelterbelts, and for land reclamation. It was brought to Japan as an ornamental tree 100 years ago but now threatens to be an invader plant. Its widely distributed species have interesting biological properties and medicinal activities. It has been observed that places where these trees dominate others plants are lacking and the vegetation beneath the tree is poor. A bioactivity guided fractionation of the methanolic extract of leaves afforded robinetin as a potential allelochemical.

Method and material

The fresh leaves (1.5 kg) of *Robinia pseudo-acacia* were extracted with 80 % aqueous ethanol. The extract was concentrated to a crude dark brown gum (100 g). The crude fraction Fr-1 was bioassayed using lettuce as test plant and was found significantly inhibitory to the root and shoot growth. The EC_{50} of crude extract was found to be $0.2 \text{ mg g}^{-1} \text{ Fw}$ (Fig. 1).

Bioassay directed silica gel column chromatographic resolution of this crude fraction, gave 14 main fractions (Fig. 2), which were bioassays on with lettuce seeds and among these Fr-1-9, Fr-1-10 and Fr-1-6 were found to be the highly active as these fractions strongly inhibited the root and shoot growth of lettuce seedling (Fig. 3, 4 and 5). The Fr-1-6 fraction was accordingly purified on Sephadex column chromatography and reverse phase HPLC using MeOH-H₂O (9:1) as eluting solvents. It gave four main fractions, which were bioassayed (Fig. 6), and the most active one, Fr-1-6-2, that was high in amount too was further purified to yield the flavonoid 1 identified as robinetin (Fig. 8). Bioassay results (Fig. 7) reveal that this could be the possible allelochemical and play an important role in the allelopathy of *Robinia pseudo-acacia*.

Result and discussion

The ethyl acetate fraction being one of the highly active fractions was purified on repeated reverse phase HPLC using MeOH-H₂O as eluting solvent. The dark yellow fraction obtained was found highly inhibitory and inhibited the growth of lettuce root and shoot growth at all applied concentrations. The EC_{50} of this fraction was found to be as $10 \text{ } \mu\text{g g}^{-1} \text{ Fw}$. Purification of this fraction yielded a yellow powder, which was identified as 3,3',4',5'-pentahydroxy flavone robinetin by NMR spectroscopic analysis.

It has been observed that flavonoids with adjacent tri-hydroxyl or possessing catechol configuration exhibit high rate of plant growth inhibitory activities. There are still many active fractions on which work is in progress.