Isolation of allelochemicals from comfrey (*Symphytum officinale* L.): A candidate for allelopathic ground cover crop

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Introduction: *Symphytum officinale* L., commonly known as comfrey, is an evergreen herb belonging to the borage family (Boraginaceae). Comfrey is a native herb of Eurasia, and often invades other places. *S. officinale* is also being widely consumed as a medicinal herb for the treatment of various illnesses. Its leaf extract and root powder have been used since long as anti inflammatory, analgesic and tissue regenerating herbal medicine in America, Asia, and in different countries of Europe. Bioactivity guided extraction and chromatographic techniques were used to isolate and purify the allelochemicals from the aqueous ethanolic extract of comfrey leaves.

Methods and Materials

Fresh leaves of comfrey (1.5 kg) were extracted with 80 % aqueous ethanol. The extract was evaporated to dryness under vacuum and suspended in ca. 100 ml of water. The water suspension was partitioned three times with hexane (ca. 100 ml). The residual water fraction was then acidified to pH 2.5 with HCl and partitioned three times with EtOAc (ca. 100 ml). The residual water fraction was then neutralized with NaOH and used for bioassays and further purification (Figure 2). The crude extract along with its organic solvent fractions was subjected to bioassays (Figure 3). The bioassay results revealed that most of the inhibitory activity of the crude extract was passed to ethyl acetate soluble fraction. The bioactive EtOAc fraction was subjected to reversed-phase silica gel chromatography and eluted successively with water, water-methanol (1:1), methanol and finally with methanol-chloroform (1:1). All of these fractions were tested for their activity and among these, water-methanol (1:1) was found to be the most active followed by the aqueous fraction (Figure 4). Purification of this fraction on lobar column using chloroform-methanol as eluting solvent gave compounds **A**, **B** and **C**, which are being, characterized using extensive mass and ¹H and ¹³C NMR spectroscopic techniques.

Results and Discussion

S. officinale showed strong plant growth inhibitory activity on lettuce by plant box method (Figure 1). The results revealed that it releases some chemicals, which are deterrent to other plants in its vicinity. The crude ethanolic extract and its ethyl acetate soluble fraction inhibited the radicle growth of lettuce seedling by 65 % relative to control (Figure 3). After chromatographic resolution of EtOAc the inhibitory activity was mainly found into water and 50 % water- methanol eluted fractions (Figure 4). So far, the most active water-methanol (1:1) has been further purified by HPLC. Three major bioactive fractions obtained by 60% MeOH-elution, which were further purified and designated as compound **A**, **B** and **C**. The inhibitory activity of compound **B** on the elongation of lettuce roots was higher than that of the compounds **A** and **C** (Figure 5) at higher concentration however, EC₅₀ values of all these three compounds seem to be the same. Preliminary examination of the chromatographic fractions has revealed that these are alkaloid in nature. Characterization and identification of structures of these compounds is in process.

It is well known that plants of the family Boraginaceae, produce pyrrolizidine alkaloids, which are quite toxic and have shown antitumor activities. Comfrey is being used as cover mulch in fields as it produces large amount of biomass. However its plant growth inhibitory activity has not so far been investigated. To the best of our knowledge, this is the first report on the isolation and identification of allelochemicals from comfrey.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5