Plant growth promoting activity of *Stevia rebaudiana* Hemsl.

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Introduction: Allelopathic studies on the aerial parts of *Stevia rebaudiana* Hemsl. were carried out following a specific protocol derived from the definition of allelopathy. *S. rebaudiana* is a perennial shrub of Asteraceae (Compositae) family, native to certain regions of South America (Paraguay and Brazil). It is often referred to as "the sweet herb of Paraguay". Stevia plant, its extract and stevioside are being used since long as a sweetener in South America, Asia, Japan, China, and in different countries of Europe. Bioactivity guided extraction and chromatographic techniques were used to isolate and purify the allelochemicals from the aqueous methanolic extract of stevia.

Methods and Materials: Dried leaf powder of *S. rebaudiana* (1.5 kg) was soaked in 80% methanol for 2 weeks. The extract was filtered to obtain a dark brown gummy material (296.9 g). A portion of the gummy material (37.1 g) was partitioned with various organic solvent. All these fractions, along with the crude extract, were subjected to bioassays using lettuce and cucumber seedlings as test species. Aqueous fraction and the hexane fraction of crude extract were found to be active (Figure 1 a-b & Figure 2a-b). The aqueous fraction (Fr-3) was subjected to charcoal column chromatography and eluted with water (Fr-3-1), water-methanol (1:1) (Fr-3-2), methanol (Fr-3-3), acetone (Fr-3-4) and finally with ethyl acetate (Fr-3-5). The bioassay results indicate that the major activity was separated into the acetone-eluted fraction. Fraction 3-4 was further subjected to bioassay directed silica gel chromatography to obtain pure compound-C (500 mg, 0.3% of the dried leaves). The structure of compound-C is being elucidated by detailed spectroscopic analysis of EIMS, FABMS, $^1$H-NMR, COSY, $^{13}$C-NMR, DEPT, HMQC and HMBC etc. The second active fraction (Hexane fraction) is also being purified.

Results and Discussion: Bioactivity guided extraction and chromatographic procedures were used to isolate and purify the allelochemicals from the aqueous methanolic extract of stevia. The pure compounds are being identified by extensive mass and NMR spectroscopic methods. Comparison of the growth promoting activity of extracts on cucumber/lettuce root/shoot growth revealed that the major activity of the original extract was fractionated into the hexane and water fractions. Chromatographic purifications of the most active aqueous fraction gave compound-C. The growth promoting activity of the original water fraction was not greatly lost, and compound-C accounted for almost all of the activity observed in the original water fraction. Compound-C enhanced the growth of cucumber and lettuce shoots up to 147% and 140%, respectively (Figure 3 & 4). It is inferred that compound-C is the cause of the growth promoting activity of the water fraction. Spectroscopic studies are in progress for finalization of the structure of compound-C.
Figure 1-a: Effect stevia extracts on the root growth of cucumber seedlings

Figure 1-b: Effect stevia extracts on the shoot growth of cucumber seedlings

Figure 2-a: Effect of stavia extracts on root growth of lettuce

Figure 2-b: Effect of stavia extracts on shoot growth of lettuce

Figure 3: Effect of Compound C on the root and shoot growth of cucumber seedlings

Figure 4: Effect of Compound C on the root and shoot growth of lettuce seedlings