Title: SECONDARY METABOLITES IN CALLUS CULTURES OF *Vitex negundo* L.
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Statement of the Problem:

This work sought to develop a tissue culture method for *Vitex negundo* L., a popular medicinal herb with many known biological activities, and for which a number of biologically active metabolites have been identified and isolated from intact plant samples.

The major objectives were (a) to determine conditions for the production and maintenance of callus and cell suspension cultures of *V. negundo* and (b) to study the secondary metabolites produced by the tissue culture, to compare these with the whole plant, and to determine whether the metabolites in the intact plant could be replicated in culture.

Procedure and Treatment of Data:

Callus cultures derived from leaf samples were established in Murashige and Skoog (MS) medium and studied to determine the effects of nutrients, plant growth regulators, aeration and illumination on development and secondary metabolite production. The compounds produced by the calli were compared with those found in intact plants by thin layer chromatographic analysis.

A callus line maintained in MS medium supplemented with 2,4-dichlorophenoxyacetic (2,4-D) acid and benzyladenine (BA) and then analyzed by various chromatographic and spectroscopic methods to determine the identities of the metabolites accumulated in culture.

Findings:

The calli grown under our experimental conditions exhibited fast growth and a metabolite profile that persisted under various culture conditions and over several subcultures. The profile of compounds produced in callus cultures was different from that of the intact plant. The callus however exhibited phenylalanine ammonia-lyase activity that was comparable to that found in extracts obtained from leaves of *V. negundo*.

Ethyl acetate extracts of the callus yielded the following triterpenes: oleanolic acid; 2α, 3α-dihydroxyolean-12-en-28-oic acid; 2,3,23-trihydroxyolean-12-en-28-oic acid, as well as its methyl ester. Acetylation of the ethyl acetate extract followed by GC-MS analysis gave the propyl and butyl esters of 3-acetoxy-11-oxoolean-12-en-28-oic acid. From the hexane extract, palmitic acid, ethyl oleate, sitosterol, stigmasterol and β-amyrin were detected.

Conclusions:

We have demonstrated the possibility of applying the technique of plant tissue culture for the production of secondary metabolites. From leaf explants of *Vitex negundo* a stable callus culture was established in Murashige and Skoog medium supplemented with the hormones 2,4-D and BA. The callus produced interesting metabolites, among them triterpenes, which have so far not been reported in the intact plant. None of the biologically active components of *V. negundo* (e.g. flavonoids) were detected in callus. The significant phenylalanine ammonia-lyase activity observed in callus extracts however may indicate that the biosynthetic pathway leading to phenylpropanoid derivatives may still be capable of operating in the tissue culture.

Many of the compounds found in callus were closely related triterpenes. A possible biosynthetic route for the production of triterpenes in *V. negundo* tissue culture is proposed.

Recommendations:

1. Develop a culture medium that will duplicate the secondary metabolism of the whole plant and then find the conditions needed for the enhanced accumulation of important plant products.
2. Isolate and identify the other metabolites present in the callus line established in this study. This could lead to more triterpene derivatives and other interesting metabolites.
3. Determine potential medical and other applications for the triterpene derivatives and other metabolites in this callus culture.
4. Study other enzymes involved in the biosynthetic pathways leading to compounds present in the parent plant to establish and explain variabilities between the secondary metabolism in the intact plant and the tissue culture.