In-vitro CULTURE AND ALKALOIDS PRODUCTION IN *Rauvolfia serpentina* AND *Rauvolfia canescence*

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Dissertation submitted in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE of the UNIVERSITY OF COLOMBO, SRI LANKA.

DECEMBER 1997

ABSTRACT

In Sri Lanka genus *Rauvolfia* is represented by two indigenous species *Rauvolfia serpentina* and *Rauvolfia densiflora*. In addition *Rauvolfia canescence* is found as an introduced naturalized species.

Due to the presence of tranquilizer alkaloids such as reserpine and ajmalicine in . *R.serpentina* it has attracted worldwide attention. In Sri Lanka *R.serpentina* is not cultivated systematically. The roots and other parts of the plant are being collected from wild sources, leading to the plant being threatened.

Although at present several conventional methods are used for propagation of *R.serpentina* there are some drawbacks in these methods such as poor seed viability, delayed rooting in seedlings and cuttings etc. Therefore there is an urgent need to apply non conventional propagation methods.

Although in Ayurvedic system *R.canescence* is used as a substitute for *R.serpentina*, there are no scientific records pertaining to the medicinal properties of *R.canescence.*

Therefore this research project was carried out to,

1. Micropropagate *R.serpentina* and *R.canescence* by multiplication through callus and proliferation of apical and axillary buds.
2. Compare the alkaloids present in different plant parts of *R.serpentina* and *R.canescence,* to determine the suitability of *R.canescence* as a substitute for *R.serpentina.*
3. Compare the quality and quantity of alkaloids present in original plant materials with that of callus.

Explants form both species were tested in basic MS medium with auxins and cytokinins at different concentrations. The best callus formation was observed in leaf at 3.5 mg/l 2,4-D and 0.5 mg/l BAP in light. Stem, seed and petiole explants also produced callus. Greening of the callus was observed in the medium containing 4.0 mg/l BAP and 1mg/l NAA whereas shoot bud production from callus was observed in the medium containing 5 mg/l BAP and 2mg/l NAA.

Apical and axillary buds from both species were tested as the explant for bud proliferation. Proliferation of buds were observed in the medium containing 4.0 mg/l BAP and 1.0 mg/l NAA.

Different plant parts from both species were screened for the alkaloids with authentic markers. Presence of different identified as well as unidentified alkaloids were observed in both species. The total alkaloid concentration in roots, leaves, stem, stem calli and leaf calli were higher in *R.serpentina.* However the total alkaloid concentration in seeds and seed calli were higher in *R.canescence.*

Extracts from different plant materials from both species were quantified for the two alkaloids reserpine and ajmalicine. The comparative study between *R.serpentina* and *R.canescence* showed distinct differences in their reserpine and ajmalicine contents. *R.serpentina* plant materials showed higher amount of reserpine and ajmalicine than *R.cansecence* except in seeds. In both species, roots contained higher amounts of reserpine and ajmalicine than other parts.

The concentrations of alkaloids in different calli were also studied. According to the results stem, leaf and seed calli, from both species contained reserpine and ajmalicine. But their levels were very lower than their respective explant. Among the two species *R.serpentina* calli showed higher amount of reserpine and ajmalicine except in seed calli.

Finally this study suggest that there is a potential for propagating *R.serpentina* and *R.canescence* through *in vitro* culture and also the potentionl exist to produce alkaloids through callus and cell cultures.

Also this project work reveals that *R.canescence* contained alkaloids which are similar to alkaloids of *R.serpentina*. But their levels were very lower than in *R.serpentina.*