Summary and conclusion

Oroxylum indicum and Uraria picta are valuable medicinal plants. The existence of Oroxylum indicum and Uraria picta in natural population is highly threatened and has been categorized as vulnerable by the government of India (Ravikumar and Ved, 2000). Oroxylum indicum and Uraria picta are naturally propagated by seeds. Moreover, the seed set is poor and seed viability and percentage of germination is low. Tissue culture techniques, like micropropagation can be applied to generate large number of clonal propagules and biodiversity conservation, especially for those species which either the roots/rhizome or the whole plant is used in drug preparation. The present studies on Oroxylum indicum and Uraria picta were undertaken for establishing micropropagation protocols as well as medium optimization for the production of important plant pharmaceuticals through tissue culture.

Studies on Oroxylum indicum

The present study was conducted to establish an efficient effect of inorganic nutrients (KNO₃, KH₂PO₄, CaCl₂, ZnSO₄, MnSO₄ and CuSO₄), various precursors (phenylalanine, cinnamic acid, naringenin, proline and p-coumaric acid) and carbon sources (glucose, lactose, fructose and sucrose) on shoot proliferation and root formation on in vitro generated shoots. The results of present study indicate that among various media compositions, medium fortified with 27 mg/l MnSO₄ along with shoot induction medium [1 mg/l BA along with 2 mg/l AgNO₃ in MS medium] found best for the high-frequency regeneration of shoots (5.14 multiples/node), which were higher and greener with broad leaves than control (3.2 shoots/explants). This medium formulation will be beneficial for shoot multiplication of Oroxylum indicum.

Wherever, rhizogenesis of Oroxylum indicum found better in all tested modify media than control experiment. In vitro generated shoots rooted on ½ potential MS medium fortified by 2.5 mg/l IBA and AgNO₃ (2 mg/l) having 5 mg/l concentration of phenylalanine produced highest number of roots (17.5 roots/explants), which is very much higher than control. Roots are healthy, white and having good length.

Determination of total flavonoid content revealed that highest total flavonoid content 10806.99µg QE/g DM was obtained in multiple shoots generated on medium supplemented by 0.60g/l CaCl₂. In multiple shoots of Oroxylum indicum, optimal conditions for flavonoid synthesis were determined for KNO₃, KH₂PO₄, CaCl₂, ZnSO₄, MnSO₄, CuSO₄, phenylalanine, naringenin, p-coumaric acid and cinnamic acid [1.1 g/l, 0.20 g/l, 0.60 g/l, 40 mg/l, 30 mg/l, 0.05 mg/l, 5 mg/l, 1 mg/l, 50 mg/l and 6 mg/l, respectively] as well as carbon sources (sucrose at 3% concentration). Whereas highest total flavonoid contents in in vitro generated roots (8698.94µg QE/g DM) was found at lower of MnSO₄ (18 mg/l). In in vitro generated roots of Oroxylum indicum, optimal conditions for flavonoids synthesis were determined for carbon source (glucose
at 3% concentration), p-coumaric acid (90 mg/l), naringenin, cinnamic acid, phenylalanine, CuSO₄, ZnSO₄, KNO₃, KH₂PO₄ and CaCl₂ (6 mg/l, 6mg/l, 1mg/l, 0.05mg/l, 40mg/l, 1.5g/l, 0.25g/l and 0.60 g/l, respectively).

A HPTLC method has been developed for the qualitative determination of residues of baicalein and chrysin in biological materials. Present study was conducted to optimize production medium for baicalein and chrysin synthesis in differentiated culture of *Oroxylum indicum* and examine the effect of inorganic nutrients (KNO₃, KH₂PO₄, CaCl₂, ZnSO₄, MnSO₄ and CuSO₄), various precursors (phenylalanine, cinnamic acid, naringenin, proline and p-coumaric acid) and carbon sources (glucose, lactose, fructose and sucrose) on shoot proliferation and root formation on *in vitro* generated shoots were conducted.

Result of this study indicate that among various media formulated for shoot proliferation, adapted content of ZnSO₄ (60 mg/l) in basal medium for shoot induction [1 mg/l BA along with 2 mg/l AgNO₃ in MS medium] resulted the highest yield of chrysin (0.927%). Multiple shoots generated on medium having MS salts, 1 mg/l BA, 2 mg/l AgNO₃ and 4 mg/l proline, gave the highest yield of baicalein (0.92%). Baicalein and chrysin both were isolated in high quantity from a methanolic fraction of multiple shoots cultured on respective medium.

As per present results it is suggested that baicalein may be obtained in high quantity (1.74%) from acetone fraction of *in vitro* roots generated on the modified concentration of KNO₃ (1.7 g/l) in basal medium for root induction [MS medium with ½ potency fortified with 2 mg/l IBA and 2 mg/l AgNO₃]. Chrysin may be obtained in high quantity (0.963%) from methanol fraction of *in vitro* roots generated on the modified concentration of KNO₃ (1.5 g/l) in root induction medium. So, baicalein and chrysin production can be increase in differentiated cultures of *Oroxylum indicum* (*in vitro* generation of shoots and roots). The results might lay the foundation for further transgenic study.

**Studies on Uraria picta**

Two approaches have been focused in the present work for increase production of natural drugs from plant cell culture of *Uraria picta* are (A) micropropagation and (B) callus culture.

The nodal explants of *Uraria picta* from 35 days old seedlings were generated on media having MS salts and various concentrations [1 to 4 mg/l] of phytohormones [BA;NAA] as well as TDZ and 2 mg/l AgNO₃. Greatest numbers of shoots were obtained on medium having MS salts along with 1 mg/l concentration of BA. Root induction on *in vitro* generated shoots was achieved in medium having ½ potency of MS salts and 1.5 mg/l IBA. Through this procedure about 196 plantlets were generated from single nodal explant over two subcultures. Micropropagation would be useful to generate a large number of clonal propagules and biodiversity conservation.
Callogenesis studies on *Uraria picta* were undertaken in establishing a protocol for improving the production of important plant pharmaceuticals in callus culture through optimizing composition of culture media by study the effect of plant growth regulators, inorganic nutrients, carbon sources, precursors and optimized culture conditions for incubation. Various types of explants such as root, stem and leaf segments were implanted on media having MS salts fortified with NAA in variety of concentrations [0 to 2 mg/l] alone as well as in combination with 0.5 mg/l BA. Maximum rate of callus induction and proliferation was observed on medium formulation having MS salts, 2 mg/l concentration NAA along with 0.5 mg/l BA. From all types of explants, among them root explants exhibited the maximum response of callus induction (100%).

To optimized physical factors for growth and secondary metabolites production of *Uraria picta* callus culture, calli generated from *Uraria picta* root explants were cultured on basal medium for callus proliferation [MS medium fortified with NAA in 2 mg/l concentration along with BA in 0.5 mg/l concentration] underneath the various colors of light, at different temperatures and on different physical state of culture. As a result it was found that white light illumination; semi-solid medium and 25°C temperature were optimum for the callus proliferation of *Uraria picta* individually. Whereas the individual application of white light, semi-solid medium and 8°C temperature were found advantageous for the increase the production of flavonoids in *Uraria picta* callus culture. It is recommended that application of these optimized culture conditions in combined manner may prove beneficial to increase the callus proliferation as well as flavonoids production in *Uraria picta* callus culture.

For establishment of an efficient protocol for growth and production medium of *Uraria picta* callus culture, calli generated from *Uraria picta* root explants were cultured on basal medium for callus proliferation [medium fortified with MS salts, NAA in 2 mg/l concentration along with BA in 0.5 mg/l concentration] and basal media supplemented with various concentrations of ABA, inorganic nutrients (KNO₃, KH₂PO₄, CaCl₂, ZnSO₄, MnSO₄ and CuSO₄), various precursors of shikimic acid pathways including p-coumaric acid, cinnamic acid, phenylalanine, naringenin and proline along with carbon sources (glucose, lactose, fructose and sucrose) at 3% concentration and various concentrations of sucrose. Results of this study indicate that among various media formulated, basal medium with 5% sucrose exhibited highest-frequency of callus proliferation in *Uraria picta* (3.36±1.81g fresh wt.). Moreover, basal media for callus proliferation of *Uraria picta* individually supplemented with KNO₃, KH₂PO₄, ZnSO₄, CuSO₄, phenylalanine, cinnamic acid, naringenin and proline [1.5 g/l, 0.20 g/l, 60 mg/l, 0.1 mg/l, 1 mg/l, 6 mg/l, 2 mg/l and 6 mg/l, respectively] were also found beneficial for callus growth of *Uraria picta*.

An HPTLC phytochemical profile and quantification of the total flavonoid content revealed that among various media formulated, highest contents of total flavonoids (7451.08µg QE/g DM) was obtained from callus proliferated on the modified concentration of KNO₃ (1.5 g/l) in basal
medium for callus proliferation. Whereas the individually application of modified concentration of MnSO₄ (18 mg/l), phenylalanine (3 mg/l), p-coumaric acid (70 mg/l), proline (6 mg/l) and sucrose (3%) in basal medium for callus proliferation were found advantageous for flavonoid synthesis in *Uraria picta* callus culture. Addition of ABA could not prove beneficial for increase the secondary metabolites in callus culture of *Uraria picta*.

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