SUMMARY AND CONCLUSION
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The present investigation was carried out in two threatened plants *Tragia involucrata* and *Oxystelma secamone* which play an ideal role in traditional medicine. The plants used to cure various ailments by different tribal communities of India.

The present *in vitro* studies reveals that MS medium was effective supporting medium for morphogenic expression, callogenesis and maintenance than Gamborg’s B-5 and White’s medium of both leaf and stem explants. In our study the auxins 2,4 D, NAA and IAA favours the callusing in leaf explants of both the species while cytokinins like BAP and Kn induced callus only in *O.secamone*, NAA at higher concentration (4.0 & 5.0 mg/l) does not show any response where as IAA (5.0 mg/l) induced root initials.Among cytokinins BAP (2.0 mg/l) favored high percentage of callus. In Kn supplied medium also callogenesis was observed at all concentration but survival rate of callus was very low.

Different combinations of cytokinin and auxin such as BAP+ 2.4-D, BAP+NAA, Kn+2.4-D, Kn+NAA and Kn+IAA were tried on leaf explants of *O.secamone*. Luxuriant proliferation of callus was observed on MS medium supplemented with 2,4 D in combination with either BAP or KN. But maximum callogenesis was noticed in BAP+ 2,4D (3.0+1.0 mg/l). Browning of both leaf and stem explants of *Tragia involucrata*, avoids the callogenesis and leads to necrosis. Addition of ascorbic acid (2.0 mg/l) to the MS medium neutralized the exudation of phenolics and supports better callogenetics in both the explants.

Stem explants of *T.involucrata*, exhibit highest callogenesis in 2,4D and Kn fortified medium. Higher concentrations of growth regulators lead to decreasing
individually callossing. In *O. secamone* internodal explants produced greenish compact nodular callus in 2,4 D supplemented medium. 2,4 D (1.5 mg/l) showed highest percentage of response. Nodal explants on BAP (3.0 mg/l) fortified medium induced bud sprouting and induces multiple shoots along with formation of callus but at higher concentration (5.0 mg/l) only multiple shoots were formed. without callusing. Formation of callus was observed lower concentration of BAP (1.0 & 2.0 mg/l). The callus induces white soft translucent callus. Kn induces only callus at all concentrations.

The leaf and stem derived callus induced from 2,4D (2.0 mg/l) and Kn 2.0 (mg/l) was subcultured on MS medium supplemented with BAP+NAA, at different concentrations luxuriant proliferation was produced.

The callus when subcultured on MS+IAA (2.5 & 5.0 mg/l) and MS+IBA (2.5 & 5.0 mg/l) induces roots along with proliferation of callus. Luxuriant proliferation of primary callus of *T. involucrata* was also observed on MS+ Coconut water (5-15%) and MS+ casein hydrolysate. Repeated subculture of primary callus exhibited a good proliferation on all tested growth regulators either alone or in combinations. No regeneration was observed in *T. involucrata*.

Subculture of leaf or stem derived callus of *O. secamone*, on different concentration and combination of BAP or Kn with NAA or IAA, exhibited indirect organogenesis. The leaf derived callus of *O. secamone* induced from 2,4 D (2.0 mg/l) was subcultured on MS+BAP (1.0-10.0 mg/l) and on MS+BAP (1.0-5.0 mg/l) in combination with NAA (0.5-1.0 mg/l) or IAA (0.5-1.0 mg/l) produced embryogenic callus with multiple shoots.
Among BAP and Kn, BAP was found to be effective in the induction of shoot buds from callus. The stem callus induced highest percentage of multiple shoots on MS+BAP (2.0 mg/l) than Kn (55%). It was also observed that, at higher concentrations there is decrease in the percentage of shoot buds.

The primary callus derived from leaf explants, was subcultured on MS+BAP or Kn alone in different concentration (1.0-10 mg/l). The callus proliferated luxuriantly but no shoot bud formation takes place. Highest proliferation of primary callus was observed on MS+BAP (2.0 mg/l) during subculture. The proliferation rate of callus was very low in Kn fortified MS medium. BAP along with NAA & IAA induces embryogenic callus with multiple shoots. Maximum percentage of shoot regeneration was observed in BAP (2.0 mg/l) + NAA (0.5 mg/l) containing medium which elicited a better response than BAP+IAA. Maximum shoot length was also observed in this combination.

The stem derived callus cultured on MS medium fortified with BAP in combination with NAA or IAA or Kn +NAA showed better response in multiple shoot induction than cytokinin alone tested. MS+BAP (3.0 mg/l) + NAA (1.0 mg/l) favors the maximum number of shoots formation than BAP+IAA combination and maximum shoot length was also achieved in same concentration. The primary stem callus on BAP+IAA also promotes the multiple shoot formation but produced shoots with very short internodes.

In *O. secamone*, NAA along with BAP enhanced the shoot bud formation than IAA+BAP combination. Micro shoots of *O. secamone* induced roots after 15 days on MS half strength liquid medium fortified with IAA (1.0 mg/l). After 3-4 weeks of incubation the well grown shoots with roots were transferred to pots for hardening.
The survival rate was 75-80% and regenerated plantlets morphology similar to *in vivo* plant.

Histological studies were carried out in both the plants at different stages of development starting from the initiation of the callus up to the induction of embryos and shoot buds.

The microscopic sections of both plants of leaf calli derived from 2,4 D supplemented medium revealed the origin of callus from parenchymatous tissue present around vascular bundle. The sections of callus that developed on MS medium supplemented with BAP+NAA showed the meristematic centre which are embryogenic and the cells are densely cytoplasmic. The cells meristematic centre are embryogenic and densely cytoplasmic. These centers are subepidermal in position and later sections reveal the presence of surface meristem due to continuity of division. The peripheral region showed globular or spindle shaped embryoids with suspensor and the embryoids differentiated into somatic embryo with a shoot apex consisting of dome with a pair of leaf primordia and root apex. Shoot buds were also differentiated from subepidermal meristematic centres. In stem explants callus formation occurred both from the perivascular tissues and also from cortical cells. The sections also reveal the presence of non embryogenic cells along with meristematic centres. The stem calli cultured on BAP+NAA showed the presence of dome shaped apex with well developed leaf primordial.

Root formation and xylogenesis was observed from the meristematic centers of stem calli and leaf calli of *T. involucrata* on IAA fortified medium.

Suspension culture of *T. involucrata* and *O. secamone* were established from friable callus induced from primary culture.
In *O. secamone* the growth curve of suspension cultures based on settled cell volume showed gradual increase in the cell biomass. The large clumps were formed after 4 weeks of culture. The clumps were embryogenic induces different types of embryoids. Microscopic examination of cultures revealed the presence of a large amount of embryogenic and non embryogenic cells in very negligible amount. The embryogenic cells are densely cytoplasmic and are aggregated as small clumps. The clumps exhibited various types of embryoids such as globular, cordate and torpedo stages. In the suspension bicelled and four celled embryos were also observed.

In *T. involucrata*, callus fails to produce organogensis either directly or indirectly on semisolid medium. But in liquid medium, the callus induced the formation of embryoids. Different solvent extract of dried cells were phytochemically analysed and reveals the presence of active secondary metabolites. Such as alkaloids, phenol, flavanoids, steroids sugars and saponins.

The *in vitro* cultures of *T. involucrata* revealed the presence of polyphenols in high content. Concentrated methanolic extract was subjected to TLC to confirm the presence of phenolics. The same extract, was used for HPLC analysis to identify phenolic compounds. HPLC peak reveals the presence of ferulic acid along with gallic acid, chlorogenic acid, protocatechuic acid and vanillic acid. Ferulic acid is a *de novo* compound prominently present in cell suspension extracts but in *in vivo* extract, the compound was in very negligible concentration.

In both the plants phytochemical analysis of the leaf and leaf calli extracts in different solvents were analysed. Acetone and methanol extracts of *T. involucrata* indicates the presence of alkaloids, glycosides, carbohydrate saponins, phytosterols,
phenolic and tannins. Chloroform extract showed the presence of phytosterols and sugars.

The methanol extract of leaf and leaf calli of *T. involucrata*, was subjected to HPLC analysis and reveals the presence of polyphenolic antioxidants like gallic acid, vanillic acid, protocatechuic acid and chlorogenic acid in leaf extract. But in *in vitro* extract a *de novo* compound ferulic acid was observed along with other four phenolics. In leaf extract ferulic acid was observed in very negligible concentration.

To conclude, the increase in demand for the medicinal plants by herbalists, pharmacologists and disciplines of other allied naturopathy relies upon the limited supply of herbal resources growing world in nature. The exhaustive extraction procedures involved in natural herbal technology are also posing severe threats for natural resources. The means for replenishment of the depleting sources by convention propagation methods are not promising because of less adaptability of elite plants and their low survival rate. The manipulation of the medicinal plants for the better yield of the bioactive compound is feasible in the *in vitro* propagative conditions.

Keeping these points in view, the established protocols (*in vitro*) for the production of plantlets and embryoids has been achieved in *O. secamone* and *T. involucrata* and their phytochemical analysis may be useful tool to provide an alternative source for the extraction of drugs, maintenance of the quality and quantity of active component and the propagation technique helpful to improve the status of both the plants.