ABSTRACT

The present research work was carried out for callus formation and to analyze secondary metabolites in *in vitro* cultures of *Viola odorata* L. Secondary metabolites in callus cultures were extracted, cleaned up and thereafter analyzed by reverse phase high performance liquid chromatography (RP-HPLC) and accessible and suitable techniques such as Fourier transform infrared spectroscopy (FTIR) and Spectrophotometery etc

Different concentrations of BAP, BAP/NAA, 2,4-D/kin, 2,4-D/BAP, 2,4-D /BAP/NAA and NAA/Kin were employed in MS medium to study the response of explants like leaf, stem and root of *V. odorata* for callus induction. Callogenesis was observed in leaf and stem explants of *V. odorata* while root failed to respond. Best results were obtained in stem explants at 0.25mg/L BAP and with combination of 3mg/L BAP and 2mg/L NAA with 100% callus induction and callus index of 300 with yellow brown callus. In leaf with 2mg/L 2,4-D and 2mg/L Kin callus induction was observed 33% with callus index of 33 and granular green callus was formed. In the combination of 2,4-D/BAP/NAA callus production was good and large amount of callus was achieved.

Secondary metabolites from stem, leaf tissues and callus of stem, leaf explants of *V. odorata* L. were analyzed by FTIR. Two significant compounds of *V. odorata* L. salicylic acid and eugenol were determined in contol as well as in callus cultures through HPLC and spectrophotometer. In these analyses total 17 samples were analyzed for determination of salicylic acid and eugenol. Out of all samples, 15 were of callus cultures while 2 samples of control plant of *V. odorata* L.; stem and leaf explants which were named as sample"7" and sample "B". The highest concentration of salicylic acid is present in sample SA "7" and SA "3"; whereas good amount of salicylic acid is also present in samples like SA"4", SA "6", SA"10", SA"12", SA"A", "Star*, SA'B", and SA"1". The highest concentration of eugenol is present in sample EU "A" and EU "B"; whereas good amount of eugenol is also present in samples like EU"7", EU "6", EU"10", EU"12", EU"13",EU"Star*and EU"1".

In spectrophotometric Analysis of callus cultures from stem and leaf explants of *V.odorata* L. for antioxidant activity through DPPH; the scavenging action of DPPH is present in all samples including callus cultures as well as control plant parts. The highest scavenging action of DPPH is present in samples "9", "7" and "2"; whereas good amount of scavenging action is also present in samples like "10", "6", "8", and "1".

Secondary metabolites from leaf, stem tissues and their respective callus were analyzed by FTIR. The common secondary metabolites present in leaf and stem tissues were Salicylic acid, Eugenol, Toluene, α-sphinosterol, Quercetin, Tetradecane, Pentadecane, Hexadecane, Octadecane, Hentriactone, 9,12-Octadecadienoicacid and n-Hexadecanoic acid. Quercetin a flavonoid found from stem, leaf tissue and callus can prove to be an important secondary metabolite of *V. odorata* L. From the comparative analysis of secondary metabolites, it is concluded that most of the secondary metabolites from stem and leaf tissue were present in their respective callus.