Phytoestrogens evaluation in root callus of *Psoralea corylifolia* Linn. an endangered medicinal plant .

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Abstract:- Callus culture of *Psoralea corylifolia* Linn. is a potent source for the determination of phytoestrogens. *P.corylifolia* L.an endangered medicinal plant has unique ability of phytoestrogens synthesis responsible for it's over exploitation by pharma companies. In present study HPLC analysis of root derived callus of *P.corylifolia* L. shows presence of phytoestrogens daidzein 70µg^{-dw} of callus and genistein 40µg^{-dw} of callus. Through this technique *P.corylifolia* L. can be conserved and its sustainable management was possible without disturbing natural habitat of plant.

Keywords:- *Psoralea corylifolia* Linn.,HPLC,Conserve, sustainable management,Callus

1. Introduction:-*Psoralea corylifolia* Linn. is a endanagered medicinal plant of Fabaceae family and a important constituent of Indian and Chinese traditional medicine system[8].Pharmaceutically important compounds such as corylifols a-c (prenylflavonoid), phytoestrogens daidzein (4-7 dihydroxyisoflavonoid) and genistein (4,5,7 trihydroxy isoflavonoid) have been indentified in *invitro* callus culture and intact plant [6]. The plant can be cultivated only in winter season because it is a winter season herb and some factors such as seed dormancy, long gestation period, lack of proper cultivation knowledge prevents the commercial cultivation of plant so plant is getting endangered [5]. Phytoestrogens present in *P.corylifolia* L. also known as xenoestrogens shows structures and functions similar to human estrogens [1]. Plant derived estrogens have wide medical applications such as daidzein exhibits antihemolytic, antioxidative, antifugal, estrogenic and anti tumor properties and reported to have biphasic effect on human colon-cancer cell[3].Whereas genistein was found to be potent inhibitors of osteoporosis in female [11]

Callus culture:- Callus unorganized mass of cell originated from any part of plant under the influence of growth hormone, Callus showing organ regeneration, depending on the organ they regenerate called as rooty, shooty, or embryonic callus [12]. Plant callus cell have unique ability of totipotency and to produce secondary metabolite as plant does in natural condition [14]. Through callus culture endangered medicinal plant *P.corylifolia* L. widely used in the treatment of leucoderma, leprosy, Psoriasis and inflammatory disease of skin can be conserved and managed [2].

The objectives of this study was to induce root callus of *Psoralea corylifolia* L. under 16/8 light and dark incubation in plant tissue culture room provided with 500 lux light intensity and HPLC analysis was performed for presence of phytoestrogens.

2. Material and Methods

The Study material of *P.corylifolia* L., seeds was collected from MFP Park, Bhopal. The seed were sterilized under aseptic conditions and inoculated in hormone free MS media. After 7 days seedling germination was reported with young roots. Young roots (1-2 cm) of *in vitro* germinated plantlet were used as a source of explants for callus induction. The root callus of *Psoralea corylifolia* L. was initiated on MS medium supplemented with BAP ($1mg^{-l}$)+2,4-D ($2mg^{-l}$), BAP($2mg^{-l}$) + 2,4-D($4mg^{-l}$), BAP($3mg^{-l}$.)+ 2,4-D(6 mg^{-l})

3. Preparation of Methanolic callus extract.

Callus showing high growth index (GI) and callus induction frequency CI(%) was selected for phytoestrogens extraction according to Khater *et al*., (2013)[9].

The Methanolic extract of the root callus was prepared by using protocol of.Goyal and Ramawat,(2008) 100mg dry callus induced in MS BAP ($2.0mg^{-l}$) + 2,4-D($4.0mg^{-l}$) was homogenized in 5 ml methanol for 5 hrs. Centrifuged at 2000 rpm for 10 min, Suspended was collected. Filtered through 0.45 µm micron filter and used for phytochemical analysis [4].

4. Quantification of phytoestrogens in root callus of *P.corylifolia* L. by HPLC

The HPLC analysis was performed on Thermo Scientific Chromatograph (Model, Accela) equipped with Inertsil C18 (250 mm x 4.6) column with UV wavelength detector 250 nm [6]. The phytoestrogens (daidzein and genistein) were determined by using mobile phase acetonitrile:water: methanol: acetic acid (550:250:200:3 v^{-v}) pH 5.5 was adjusted with triethylamine. The flow rate was 0.8 ml^{-min} and the elution was monitored at 250 nm. Standard daidzein and genistein (Sigma, Aldrich) was prepared in methanol 200μg^{-ml} and chromatograph of callus extract was compared with standard chromatograph. The analytical operation was completed in 14min.

5. Result

Callus induction in root explants of *P.corylifolia* L. is due to synergetic effect of two hormones BAP leads to division of cells and 2-4,D leads to enlargement of cells(Fig:1).The interaction of 2,4-D and BAP was also studied on medicinal plant like *Tridax procumbens* and *Stevia rebaudiana* [10]. Nodular brown callus induction with maximum callus induction frequency (60± 2) and growth index (1.0 ±0.04) was reported in root explants of *P.corylifolia* L. in presence of 2BAP + 4 2,4-D after 20 days of incubation (Table:1). Whereas higher concentration of PGR $3mg^{-ml}$BAP+ 6 mg^{-ml} 2,4-D shows no callus induction in root explants(Fig:1). Tested 2,4-D/BAP combinations showed different responses like the degree of compaction of callus, unfriable/friable callus, and these effect were influenced by auxin and cytokinin concentrations during cell division, cell elongation and cell

differentiation, although exactly how they are involved in each process is not completely understood[13].

Table:-1.Fresh and dry weight of callus cultured from root explants of *Psoralea corylifolia* L. after 20 days of incubation.

S.No	MS+growth regulators (mg^{-L})	Callus,Colour, texture	FW(gm)	DW(gm)	CI (%)	GI
1.	Control	**----**	**----**	**----**	**---**	**----**
2	1 BAP+2 2,4-D	Small,brown compact	0.7 ±0.25*	0.21±0.12*	40±3.0	1.0 ±0.3
3.	2BAP+4, 2,4-D	Huge,brown,compact	1.6 ±0.35*	0.6±0.12*	60±2.0	1.3 ±0.04
4.	3 BAP+6,2,4-D	No response	----	----	---	---

Note: MSO = MS media without growth hormones, gm= weight in gram, *= Significant value compared with each group at (P< 0.001); a = indicates non-significant value compared with each group at (P< 0.001)

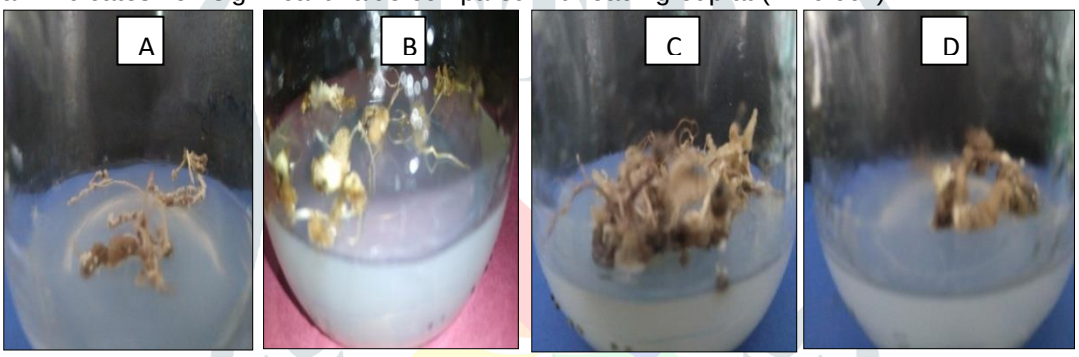
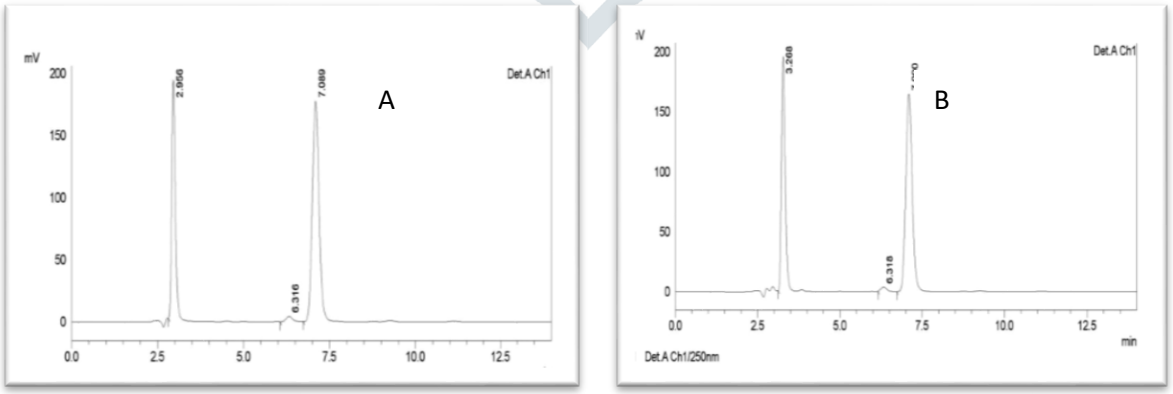


Fig 1**:** Nodular **c**allus formation from root explants of *P.corylifolia* L.(16 X Magnification)
A) MSO, B) 1BAP+2 2,4-D,C) 2BAP+4, 2,4-D, D) 3 BAP+6,2,4-D

HPLC analysis of Methanolic extract of *P.corylifolia* L. rooty callus shows the presence of phytoestrogens daidzein at RT 2.3 min and genistein at RT 3.2 min when compared with standard chromatogram (Fig:2).



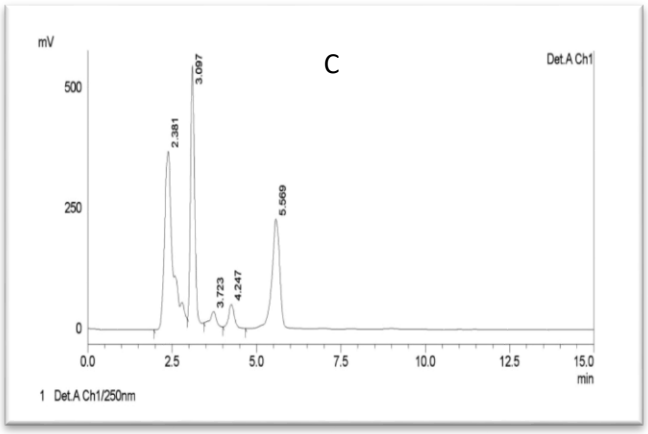


Fig:2. HPLC chromatograph A) Standard daidzein B) Standard genistein C) Root callus of *P. corylifolia L.*

In the present study root derived callus of *P.corylifolia* L. show daidzein 0.07 % dry wt of callus and genistein 0.04 % dry weight of callus. The presence of daidzein (2.28% dry wt) and genistein (0.21% dry wt) in root derived callus and leaf derived callus of *P.corylifolia* L. was also reported by Shinde *et al*.,(2010) through HPLC. Isoflavone synthase (IFS) mainly present in *P.corylifolia* L. responsible for synthesis of phytoestrogens[7].

Conclusion: - HPLC analysis of root derived callus of *P.corylifolia* L. confirmed the presence of phytoestrogens. Through callus culture concentration of phytoestrogens can be enhanced by manipulating the hormones concentration which can fulfill growing demand of phytoestrogens by humans and on the other hand conserve the endangered medicinal plant.

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References

1. H. Adlercreutz, W. Mazue, “Phyto-oestrogen and Western diseases”, Annal of Medicine, vol.29, no. 2, pp 95-120, 1997.

2. M, Anis, M, Faisal, “ In *vitro* regeneration and mass multiplication of *Psoralea corylifolia* L. an endangered medicinal plant”, Indian Journal of Biotechnology, vol 4, pp261-264, 2004

3. J.M.Guo, B.X.,Xiao, D.Liu, M.Grant., S.Zhang, Y.F. Lai, Y.B. Guo, Q.Liu, “Biphase effect of daidzein on the cell growth of human colon cancer cell,” Food Chemistry,vol 46, pp1641-1646, 2004

4. S.Goyal., K.G. Ramawat “Ethrel treatment isoflavoniods acculumation in cell suspension culture of *Pueraria tuberose”,* Acta Physiol Plant*,* Vol 30,pp 849-853, 2008.

5. N.G.Nabi, M. Shrivastava, Phytochemical screening and antioxidant activity of ethanolic extract of *Psoralea corylifolia* seed,United Kingdom Journal of Pharmaceutical and Bioscience, vol 5, no.2, pp3-7,2017 .

6. A.N. Shinde, N. Malpathak, P.F. Devanand, “Enhancement strategies for *phytoestrogens production* in shake flasks by *suspension cultures of Psoralea corylifolia L”.,* Biosource technology, vol 100,no3, pp 1833-1839.2009

7. A.N. Shinde, N. Malpathak, P.F. Devanand,“Determination of isoflavone content and antioxidant activity in *P.corylifolia* L. callus culture”,Food Chemistry, vol 118 no.1,pp128-132. 2010

8. S.K.Jain,“ Ethanobotany and research in medicinal plant in India,” CIBA Symposium185, in Ethanobotany and the search of new drugs, edited by J Chadwick and J Marsh (JohnWilly and Sons,Inc.,UK), pp 153-168,1994.

9. M.A.Khater, S.S.A. Soliman, .S. Abdel-Hady, ,A.H. Fayed,“Tropane alkaloid production via new promissing *A.belladonna* L. lines by *invivo* and *invitro”*, Nature and Science *,vol* 11,no. 3,pp 49-57, 2013.

10. S. Teshome, T.Feyissa,“ In *vitro* callus induction and shoot regeneration from leaf explant of *Glinus lotoides* L. An important medicinal plant,” American Journal of Plant Science*,vol* 6,no.9, pp1329-1340, 2015.

11. M. Yamaguchi, “Osteoporosis treatment with new osteogenic factors Osteoporosis”, Journal of Molecular and Genetic Medicine, vol 7,no.2,pp 1-11, 2013

12. M.Fank, H.M. Rupp, E,Prinsen , V. Motyka, H.VanOnckelen, T. Schmuling, “Hormone autotrophic growth and differentiation identifies mutant lines of Arabidopsis with altered cytokinin and auxin content or signaling”, Plant Physiol. vol 122,pp 721–729,2000

13. A.P.K. Ling, K.P.Tan, K.P., S.Hussein, “Comparative effects of plant growth regulators on leaf and stem explants of *Labisa pumila var.”* Alata, Journal of Zhejiang University Science B, vol 14,no.7,pp 621−631,2013.

14. M.Ikeuchi, K.Sugimato,A.Iwase,Plant callus:mechanism of induction and repression, Plant Cell,vol 25,no.9,pp 3159-73, 2013.