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Phytochemical evaluation of leaf callus culture of *Psoralea corylifolia* L. an endangered medicinal plant

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Abstract

Psoralea corylifolia L. belongs to *Fabaceae* family is a traditional medicinal plant in India, commonly known as "Bachi". It is used as a diuretic, diaphoretic, laxative and stimulant and used to improve vitiligo. The powdered seeds were applied externally to cure skin problems. In the present study, the methonalic extract of the leaf callus of *Psoralea corylifolia* L. was investigated for its phytochemicals and quantified by HPLC. Finding of this study revealed that the methanolic extract of leaf callus shows presence of flavanoids, phenolic coumpound and exhibited antioxidant activities, which were calculated by TFC, TPC and DPPH activity is 100 $\mu g^{-ml} QE^{-100g}$, 670 $\mu g^{-ml} GA^{-100g}$ and 77.31%, respectively and HPLC analysis confirms the presence of daidzein (0.0111μg^{-ml})and genistein (0.067μg^{-ml}) in methanolic extract of *P.corylifolia* leaf callus.

Keywords: phytochemical analysis, leaf callus, methanol, HPLC, TPC, TPC, DPPH

1. Introduction

Callus culture one of the technique of plant tissue culture has its importance in pharma industry since the callus has all the phytoconstituent present in original plant. Which is employed for plant based drug production in pharmaceutical. More than 80% of the world's population relies on traditional medicines for their primary health care needs. The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, and phenolic compound and antioxidant activities. Knowledge of the chemical constituents of plants and its scientific validation is desirable, not only for the discovery of therapeutic agents but also for benefit of mankind (Singh, 2012).

Now a day's plant tissue culture considered an important strategy for *in vitro* production of bioactive compounds for drug and food industries (Bougard *et al*., 2001; Mulabagal *et al*., 2004; Srivastava and Srivastava, 2007). Plant flavonoids, phenolics, Antioxidants compounds are needed for both types of industries. Plant phenolics range from simple low molecular weight single ringed compounds to complex tannins and derived polyphenols. They play vital role in plant life being modulators of indol acetic acid catabolism. There is a fair correlation between phenolic content of plant extracts and their antioxidant activities (Kaur and Kapoor, 2002; Ivanova *et al*., 2005; Farrukh *et al*., 2006)

P. corylifolia Linn (Fabaceae) is an endangered herbaceous medicinal plant commonly known as Bachi. It is distributed in tropical and subtropical regions of the world. It is specially recommended in the treatment of leucoderma, leprosy, Psoriasis and inflammatory disease of skin (Anis and Faisal, 2004) Medicinal plants have been given much attention as a source of curative compound by pharma industry so the plant is becoming endangered. Seed dormancy, low germination frequency restricts the propagation of psoralea (Salvi *et al*., 2002) So callus culture is an alternative for meeting their demand of phytoconstituent of *Psoralea corylifolia* L(Hu and Wang, 1983)

The objectives of this study was to induce callus form leaf explants of *Psoralea corylifolia* L. under 16/8 light and dark incubation in plant tissue culture room provided with 500 lux light intensity and calli potential was detected to produce active secondary metabolites. Methanolic calli extracts were subjected to HPLC analysis and phytochemical screening of leaf callus of *P.corylifolia* L.

2. Materials and Methods

Psoralea corylifolia L. seeds were collected from MFP Park, Bhopal. The collected seed were sterilized with 70% alcohol and 0.1% $HgCl_2$ and inoculated in MS (zero) media. Seedling germination was reported in 7days. Young leaves of *in vitro* germinated plantlet were used as explant for callus induction. The leaf callus of *Psoralea corylifolia* was initiated on M S medium supplemented with BAP(0.5)+2,4-D (1.0 mg^{-1}), BAP(1.0) + 2,4-D(2.0mg^{-1}), BAP(2.0)+ 2,4-D(4.0mg^{-1}), BAP(3.0) + 2,4-D(6mg^{-1}),

3. Preparation of callus extract

The Methanolic extract of the leaf callus was prepared. 100mg dry callus induced in MS BAP (2.0) + 2,4-D(4.0mg^{-1}) 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 (Goyal and Ramawat, 2006).

4. Phytochemical Study

Preliminary phytochemical screening was performed (Harborne, 1987). The presence of phytoconstituents such as phenolic, flavonoid were confirmed by the following procedure

4.1 The total phenolic content was estimated by the Folin-Ciocalteu method (Singleton and Rossi, 1965).
Callus extract was mixed with equal amount of Folin-Ciocalteu reagent and 7.5 ml of distilled water was added. It was kept at room temperature for 5 min, 5 ml of 7% sodium carbonate was added in it. It was incubated for 90 min at room temperature. The absorbance against the reagent blank was determined at 760 nm.

4.2 Total Flavonoid Content (TFC) (Dewanto *et al.*, 2002).
Callus extract was diluted with 2 ml water.0.15 ml of 5% $NaNO_2$ solution was added. It was kept for 5 min. 15 ml of 10% $AlCl_3$ was added in it and 1 ml of 1.0 M NaOH was added and mixed well. Absorbance of the reaction mixture was read at 510 nm.

4.3 Determination of antioxidant activity (DPPH Method)
The DPPH (2, 2-diphenyl- 2- picrylhydrazyl) antioxidant activity test (Mensor *et al.,* 2010).The antioxidant activity of the extract was observed from the decrease in absorbance of the DPPH at 514 nm after addition of the extract. 5ml of 0.1mM DPPH was added to 0.5 ml solution of the callus extract. Kept at room temperature for 1 hour. DPPH get decolourized (absorbance was recorded) the antioxidant activity was compared with ascorbic acid (asc) as standard. Different concentrations 0.1, 0.01, 0.03, 0.05, 0.07, 0.09 mg^{-ml} of ascorbic acid were used as standard. The absorbance (Abs) of the resulting mixture was measured at 514 nm and converted to percentage antioxidant activity AA %, using the formula:

$$AA\% = [(\text{Abs control} - \text{Abs sample})/\text{Abs control}) \times 100]$$

Table 1: Fresh and dry weight of callus cultured from leaf explant of *Psoralea corylifolia* L

S.No	MS+Plant Growth Regulators(mg^{-l})	Type of Callus	Fresh Weight(gm)	Dry Weight(gm)
1	BAP 0.5+2,4-D 1	Hard callus	0.57±0.021*	0.010±0.02*
2	BAP 1+2,4-D 2	Friable small	2.15±0.99*	0.29±0.2[a]
3.	BAP 2+2,4-D 4	Friable huge	4.4±0.34*	0.38±0.04[a]
4	BAP 4+2,4-D 6	Small callus	1.24±0.59*	0.22±0.35[a]

Note: 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)

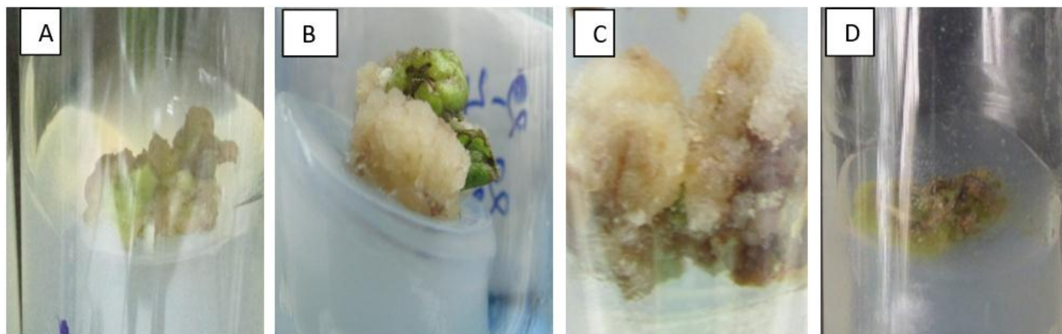

Fig 1: Different types of callus formation from leaf explants of *P.corylifolia* L.(16 X Magnification)
A Hard callus **B**. Friable small **C**. Friable huge **D.** Small callus.

For the determination and analysis of total phenolic content, total flavonoid content and antioxidant activity a standard curve is needed which is prepared from standard such as Gallic acid, Quercetin and Ascorbic acid. Different concentration series were prepared (10-1000µg^{-ml})

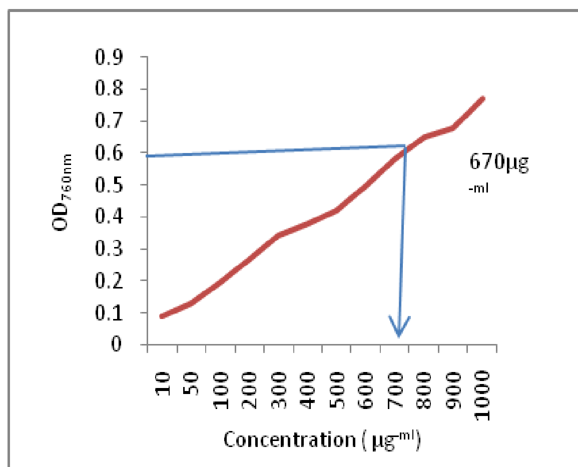

Fig 2: Total TPC in callus tissue extract after one month of incubation

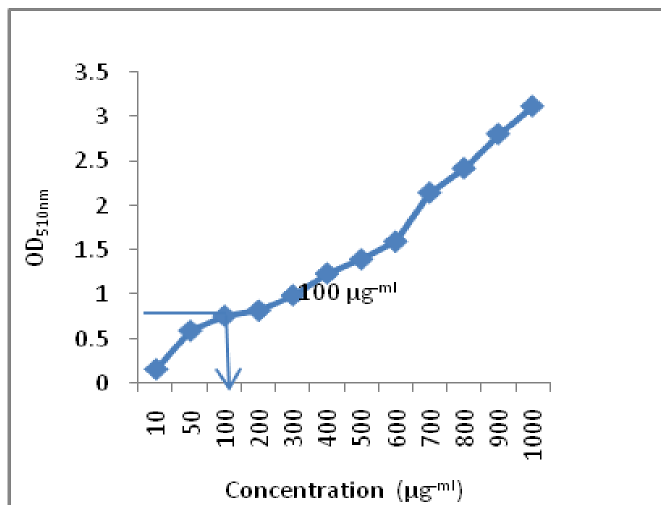

Fig 3: Total TFC in callus tissue extract after one month of incubation

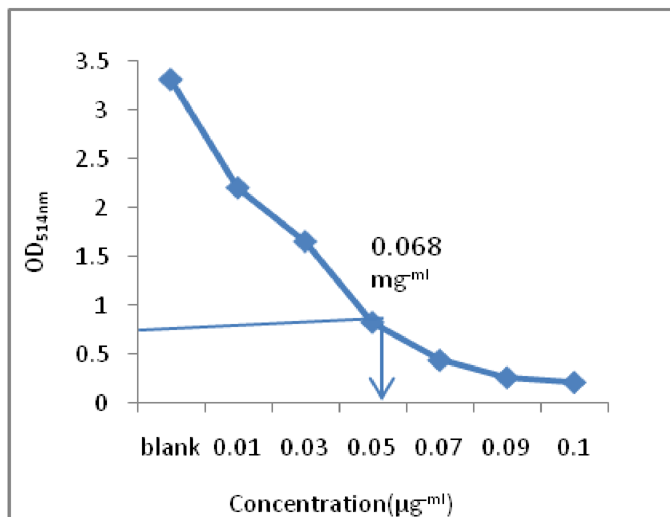

Fig 4: Antioxidant activity in callus extract after one month of incubation

Table 2: Calibration curve employed for photochemical analysis

S.no	Phytochemical analysis	Absorbance (nm)	Concentration of plant extract (µg^{-ml})$^{-g}$ and antioxidant activity
1	TPC	760	670 µg^{-ml}GA^{-100g}
2	TFC	510	100 µg^{-ml}QE^{-100g}
3	DPPH activity	514	77.31(%)

Quantification of isoflavones by HPLC

The HPLC analysis was performed on Thermo Scientific Chromatograph (Model, Accela) equipped with Inertsil C_{18} (250 mm x 4.6) column with UV wavelength detector 250 nm. The 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.

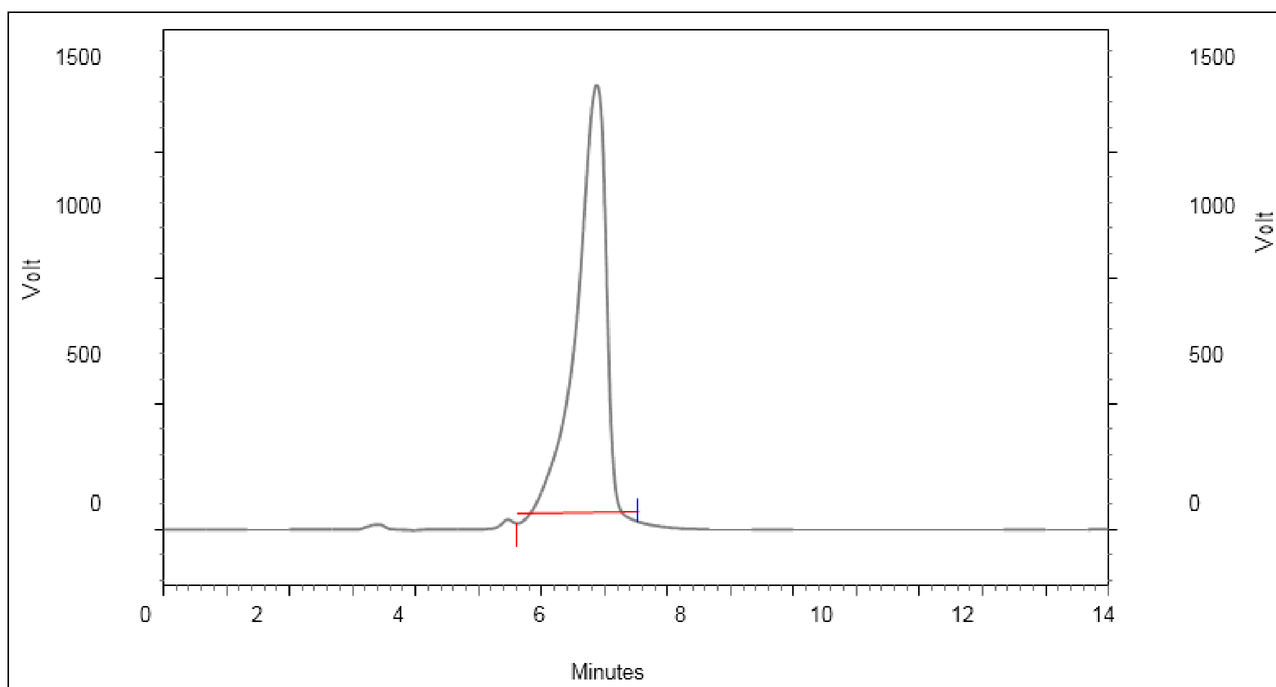

Fig 5: HPLC chromatogram of daidzein and genistein (200µg^{-ml}) as standard

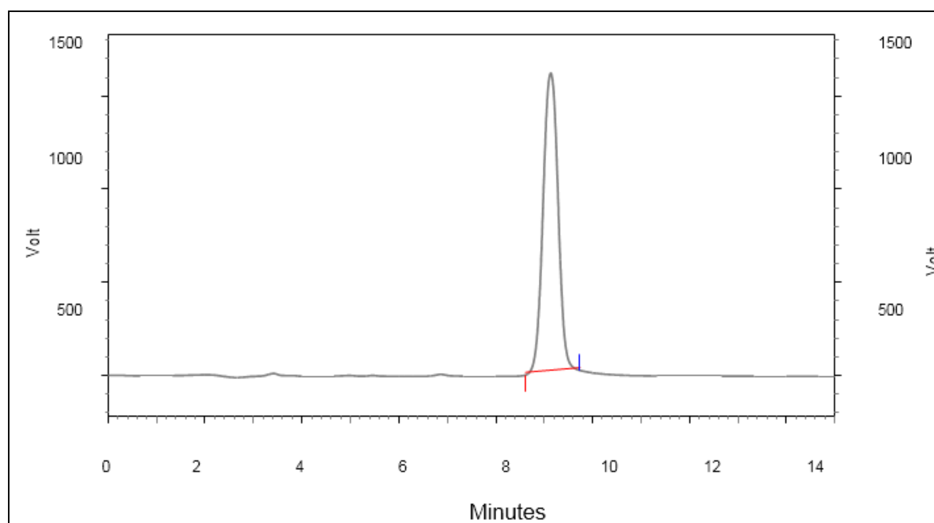

Fig 6: HPLC chromatogram of standard Genistein ($250\mu g^{-ml)}$

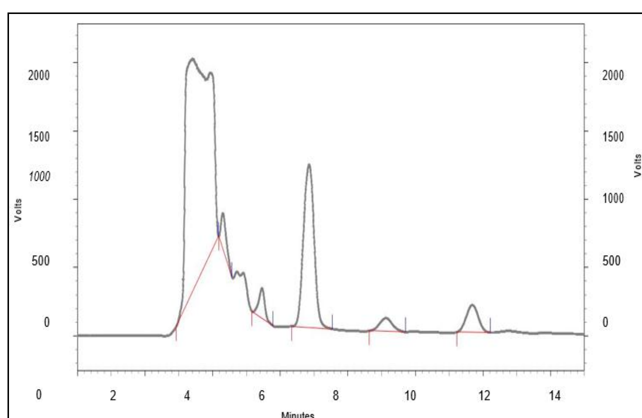

Fig 7: HPLC chromatograph of daidzein and genistein in leaf callus tissue extract of *P. corylifolia*

5. Result and Discussion

Callus is a growing mass of unorganized plant parenchyma cells. In the present study callus was induced after 30 days of incubation in MS media supplemented with combination of auxin and cytokinin this is due to the interaction of auxin with DNA to forms a complex with receptor callus was induced after 30 days of incubation in MS media supplemented with combination of auxin and cytokinin this is due to the interaction of auxin with DNA to forms a complex with receptor protein in cell wall. These protein increases the concentration of H^+ion in the cell wall. There is dramatic increase in specific m RNA content of cell wall due to increased H^+ion in the cell wall and it produces cell wall loosening enzyme β-1, 3-glucanase which increases the plasticity of cell wall and cell division starts to form callus (Key, 1969). Huge friable callus induced in MS+BAP 2 mg^{-1} +2,4-D $4mg^{-1}$ (Fig:1 A to D) This is used for phytochemical analysis in present study.

In the present study methanolic extract was used for phytochemical studies because it is polar in nature and most of the plant bioactive compounds are soluble in it. The dissociation contant (pka) of daidzein and genistein in different solvents water, methanol, ethylethanote, propanone, trichloromethane were determined at 298.2K by UV spectroscopy method. Methanol found to be best solvent for daidzein and genistein (Nan *et al*.,2014). Methanolic extract of *P.corylifolia* L. leaf extract shows presence of maximum flavonoid content (Farooqui,2016).In present study HPLC analysis confirms the presence of daidzein and genistein in methanolic leaf callus extract of *P.corylifolia* L.

The TPC concentration and TFC concentration in callus extract after one month incubation was 670 $\mu g^{-ml}GA^{-100g}$ and 100 $\mu g^{-ml}QE^{-100g}$ respectively. (Table:2). TFC of *Momordica charantia* was analyzed through Quercetin method. It was found to be $1.83mg^{-1}$ dry wt of callus (Agarwal and Kamal,2007). Most of secondary metabolites are derivative of phenolic compound therefore TPC was determined. It is well known that, the concentrations of phenolic content usually reported to be higher than the concentrations of flavonoids in most of medicinal plant such as *Citrullus colocynth* and *Psoralea corylifolia* L (Farouk *et al*.,2010).These observations indicate that the flavonoids, phenolic were more prominently present in *P.corylifolia* L. Genistein and daidzein phytoestrogen were derived from isoflavonoid therefore TPC,TFC were determined in *P.corylifolia* L. Among many flavonoids daidzein has reported to be present in *in vitro* callus culture of *Fabaceae* family plant like *Pueraria candollei, Glycine max* and *Pueraria lobata*.(Brenda, 2001).

DPPH test performed in callus extract which shows 77.31% antioxidant activities (Fig:4). This is due to the fact that phenolic and flavonoid compounds have been reported to be responsible for the antioxidant activities of medicinal plants *Psoralea corylifolia* L and *Citrullus colocynth* (Mohamed *et al.,* 2010).Methanolic extract of different plant material (Stem,leaf, bark,root) of *Cullen Glandulosa* L.were analyzed for DPPH activity. Methanolic leaf extract shows highest antioxidant activities (Alejandro *et al*., 2012). Photochemical screening of Methanolic extracts of *P.corylifolia* has shown the presence of large numbers of phenolic, flavonoids compound and high antioxidant activities.These results could be related to the protective role of phenolics,flavonoid against pathogenic agent and stress in *P.corylifolia* L. These molecules have mechanism to cope with stress such as increase availability of soluble sugar which is latent source of H^+ donor which increases the antioxidant activity of plant. (Nabi and Shrivastava, 2017). Thus *P.corylifolia* L. has its importance as a source of new useful drugs along with antioxidant activities (Gupta *et al*.,2013). It has therapeutic

importance in pharmaceuticals reported by several authors (Prasad *et al*.,2001).

HPLC analysis of different extract (Petroleum ether, Chloroform, Ethanolic, Aqueous) of *P.corylifolia* L shows presence of different compound steroids and triterpenoids are presnt in petroleum ether, chloroform extract where as flavonoids and saponins are present in ethanolic and aqueous extract (Pandey, 2013). HPLC analysis reveals that root derived callus and leaf derivd callus of *P.corylifolia* L. produced maximum amount of daidzein (2.28% dry wt) and genistein (0.21% dry wt) respectively(Shinde *et al*., 2010). Higher concentration of daidzein in callus culture of five different species of Psoralea (*P.Cinerea, P.tenax, P.Obtuslifolia, P.Bituminosa, P.Macrostachyma*) were analyzed through HPLC (Bourgard *et al*.,1995). In the present study HPLC analysis of standarad compound daidzein and genistein (Fig:5) 200μg^{-ml} shows retention time 6.873min and 9.14min respectively, Where as methanolic callus tissue extract shows the presence of phytoestrogen daidzein and genistein (Fig:6) with retention time 6.8min and 9.12 min. Calculated phytoestrogen concentration was 0.0111μg^{-ml} and 0.067μg^{-ml} respectively. This study confirms that methanolic extract of leaf callus culture of *P.corylifolia* L. can be optimized for the production of daidzein and genistein.

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7. References

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