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# *In-vivo* and *in-vitro* comparative study of primary metabolites and antioxidant activity of *Catharanthus roseus*

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**ABSTRACT**

*The present study describes a comparative study of primary metabolites and antioxidant activity. Various tissues including leaf derived callus of Catharanthus roseus. Fast growing callus was obtainable through leaf explants on MS media supplemented with 2, 4-D 1mg/l. The calluses were fragile and yellowish in colour. Maximum soluble sugars, starch, protein and lipid found in leaf, maximum amount of phenolic contents were found in stem. Leaf extract showed maximum superoxide radical scavenging activity (37.02±1.14%) than other plant parts while root extract have maximum 2,2- diphenyl-1-picryhydrazyl (DPPH) free radical scavenging activity (72.39±1.28%).*

**Keywords:** *Catharanthus roseus*, callus culture, primary metabolites, DPPH, superoxide radical.

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## INTRODUCTION

*Catharanthus roseus* (L.) is an important medicinal plant belongs to the family *Apocynaceae* and possess several pharmaceutically active compounds including alkaloids. *Catharanthus roseus* is an erect handsome herbaceous perennial plant which is a chief source of patented cancer and hypotensive drugs. It is one of the very few medicinal plants which have a long history of uses as diuretic, antidysenteric, hemorrhagic and antiseptic. It is known for use in the treatment of diabetes in Jamaica and India. Prevention of cancer, cancer treatment, anti-diabetic, stomachic, reduces high blood pressure, externally against nose bleeding, sore throat and mouth ulcers. Cell and molecular biological studies have currently been employed to improve alkaloid yield and several key factors that have major control over the biosynthesis of alkaloids have been optimized [1]. Primary products are the substances which are the basic building blocks in the synthesis of other complex substances in the cell. The metabolic pathways that are essential for sustaining life and organization of plant body are the synthesis of primary metabolites viz. starch, proteins, nucleic acids, enzymes, fats etc [2].

## MATERIALS AND METHODS

### Plant material

Healthy plants of *Catharanthus roseus* were collected from herbal garden of Singhania University, Pacheri Bari, Jhunjhunu Rajasthan (India).

**Chemicals**
All the chemicals and growth regulators were used are analytical grade and purchased from Hi Media Pvt. Ltd. Mumbai, India.

**Callus induction**
Leaves were washed through with 5 % Teepol for 15 min followed by their immersion in 70 % ethanol for 1 min and sterilized in 0.1 % mercuric chloride for 3 min. After sterilization the explants were sterile distilled water atleast 4-5 times so that all the traies $HgCl_2$ removed. Fortified with different concentrations of 2, 4-D and IAA. The pH of the media was adjusted to 5.8 before autoclaving. All media were autoclaved at 1.06 kg $cm^2$ and 121ºC for 15 min. The cultures were incubated in growth room at temperature of 25 ± 2 °C and 16-h photoperiod. 10 replicate were taken for each treatment established and each experiment was repeated twice and the cultures were observed at regular intervals.

**Primary metabolite estimation**
Callus, root, stem and leaf parts of *Catharanthus roseus* were evaluated quantitatively to estimate the total levels of soluble sugars, starch, proteins, lipids and phenols following the established methods for the sugars, starch [3], lipid [4], protein [5] and phenol [6]. All experiments were repeated three times for precision and values were expressed in mean ± standard deviation in terms of shade dried material.

**Antioxidative assay**
The antioxidative activity of the extracts was elucidated by 2, 2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging assay [7]. Experiments were initiated by preparing a 0.1 mM solution of DPPH in methanol. Two ml of this solution was added to a sample solution (0.1ml, 1mg/ml in methanol). After 30 min, absorbance at 515nm was measured and the percentage of radical scavenging activity was calculated from the following equation: % Radical scavenging = (1-Abs.sample/Abs.control)×100 Abs. control is the absorbance of the DPPH solution without sample and Abs. sample is the absorbance of the tested sample. The superoxide radical scavenging capacity of plant extract was analyzed using a modified method of [8] as described by [9]. The 2ml of reaction mixture containing 3x10-6 mol/l riboflavin, 1x10-2 mol/l methionine and 1x10-4 mol/l nitrobluetetrazolium (NBT) in 0.05 M phosphate buffer (pH 7.8) was illuminated with two 20W fluorescent lamps at 25ºC for 25min in an aluminium foil-lined box. The photochemically reduced riboflavin generated $O_2$ which reduced NBT to blue formazan. The unilluminated reaction mixture was used as a blank. The absorbance (A) was measured at 560nm. The plant extracts (0.1 ml, 1mg/ml in methanol) were added to the reaction mixture, which scavenged $O_2$ generation, thereby inhibiting the NBT reduction. Absorbance ($A_1$) was measured and the decrease in $O_2$ was calculated by $A–A_1$. The degree of the scavenging was calculated by the following equation:

Scavenging (%) = $(A – A_1 / A) \times 100\%$.

## RESULTS AND DISCUSSION

**Callus induction:**
MS medium was supplemented with different concentrations of 2, 4- D and IBA for callus induction. Leaf showed maximum callus formation on MS medium with 2, 4- D at the concentration of 1.0 mg/liter. On this medium a moderate growth in callus was achieved which was cremish green in colour and counterpart in nature. On increasing the concentration of 2, 4-D (1.5 mg/l) callus produced was fragile and yellowish in colour. The calli raised on 2, 4-D (1 mg/l) for 8 weeks were used for the analysis of primary metabolites (Figure 1). Several researchers observed that the 2, 4-D was the best auxin for callus induction for monocot and dicot plants [10, 11, 12, 13]. Similarly the present study 2, 4-D alone showed better effect for callus.

**Primary metabolites**:
In the present investigations amongst the different in vivo tissues, the leaves showed maximum amount of primary metabolites presence such as total sugar (50 mg/gdw), total level of starch (36 mg/gdw), total proteins (35 mg/gdw) and total lipids (46 mg/gdw) as compared to other tissues studied the phenols which was found to be maximum (40 mg/gdw) in stem (Shown in table 2 and graph 1). Sugar has large numbers of stereo-isomers, because they contain several asymmetric carbon atoms. However, phenols were found to be maximum 40 mg/gdw in stem as compared to other tissues (Graph-1), phenol have immunomodulating, anti-tumors and antibacterial activities. Phenolic compounds and intermediates of phenylpropanoid metabolism may stimulate the inactivation of IAA. Comparative

*in vitro* and *in vivo* biochemical performance has been evaluated in *Adhatoda vasica* [14]. However in the present study callus was showed highest soluble sugars (42 mg/gdw) and proteins (28 mg/gdw) but less phenolic (22 mg/gdw) contents starch (32 mg/gdw) and lipids (26 mg/gdw) than *in vivo* plant parts. *In vitro* cells accumulate more sugar due to its easy availability in culture medium and these cells are in highly proliferating stage so they accumulate more primary metabolites than storage metabolites (starch, lipid) and secondary metabolites (phenolic contents).

**Antioxidant activity**

The antioxidant activity of callus and other plant parts of *Catharanthus roseus* was measured using 2, 2-diphenyl-1-picryhydrazyl (DPPH) free radical and superoxide radical scavenging assays. The results showed that all the parts exhibited antioxidative activity as well as the callus raised the leaf explants on MS + 2, 4 – D (1 mg/l). In DPPH assay at 0.1mg/ml (in methanol) displayed comparable activity and the highest radical scavenging activity (72.39±1.28%) was achieved in root and lowest in the callus (28.35±1.21%). While the highest superoxide radical scavenging activity was shown by in leaf (37.02±1.14%) and lowest in root (31.10±1.02%). However, callus also showed significant antioxidant activities as superoxide radical scavenging (35.09±1.32%) and DPPH radical scavenging (30.23±1.20%) (Shown in graph 2). As in the present study the different parts of the plant and in vitro raised callus tissues in superoxide radical scavenging on their phenolic content, also reported by [15] Indian herbal tea .

**[Table: 1] Percentage of the callus induction from *Catharanthus roseus* leaf under different levels of 2, 4-D after 8 weeks of culture**

| S. No | Growth regulators | Concentration (µM/liter) | Percentage of the callus induction | Nature of callus |
|---|---|---|---|---|
| 1 | **2,4-D** | 0.5 | 30±1.8 | Healthy creamish green coloured |
| | | **1.0** | **80±0.4** | |
| | | 1.5 | 72±1.2 | |
| | | 2.0 | 65±1.1 | |
| | | 2.5 | 55±1.4 | |
| | | 3.0 | 50±1.3 | |
| | | 3.5 | 40±1.2 | |

**[Table: 2] Estimation of primary metabolites in different parts and in vivo raised tissue of *Catharanthus roseus***

| Material | Root | Stem | Leaf | Callus |
|---|---|---|---|---|
| **Protein** | 20.5±1.24 | 32.8±1.32 | **35.2±1.34** | 28.6±0.32 |
| **Starch** | 18.3±1.17 | 30.1±1.40 | **35.0±0.16** | 32.5±1.28 |
| **Sugar** | 26.0±0.32 | 34.5±1.12 | **50.0±1.25** | 42.0±0.24 |
| **Phenol** | 36.1±0.22 | **40.2±1.35** | 28.1±0.62 | 22.15±1.18 |
| **Lipid** | 24.2±1.52 | 34.2±0.21 | **46.0±0.24** | 26.2±1.15 |

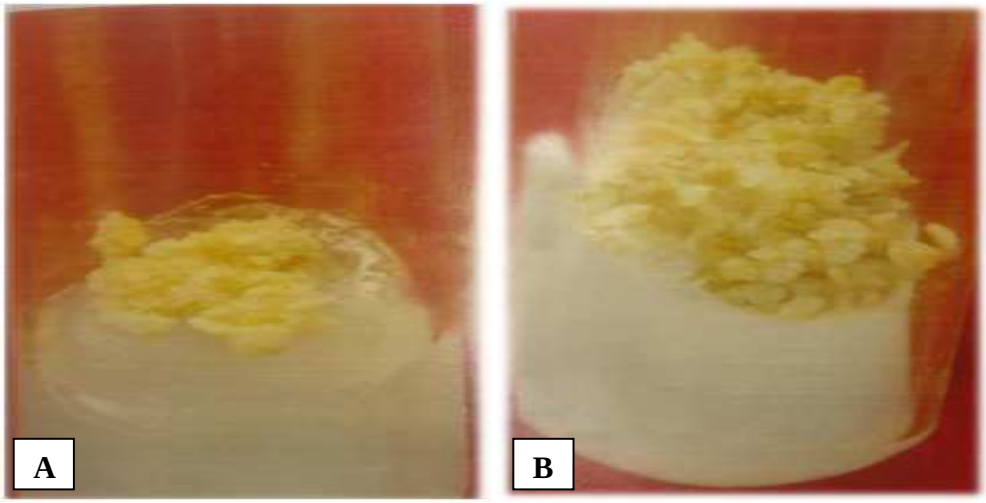
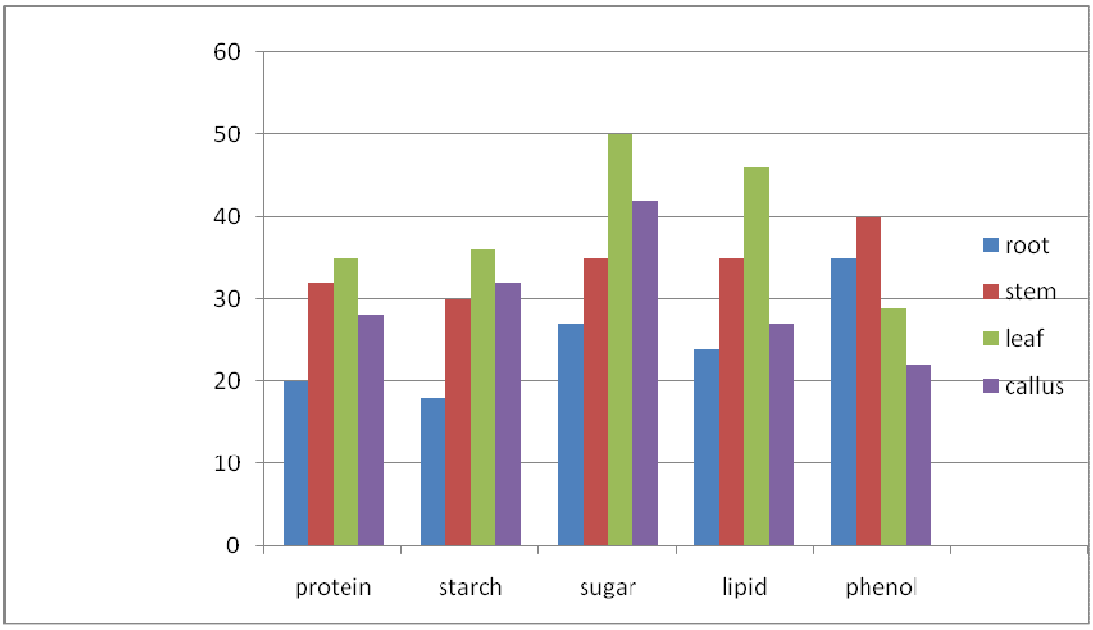
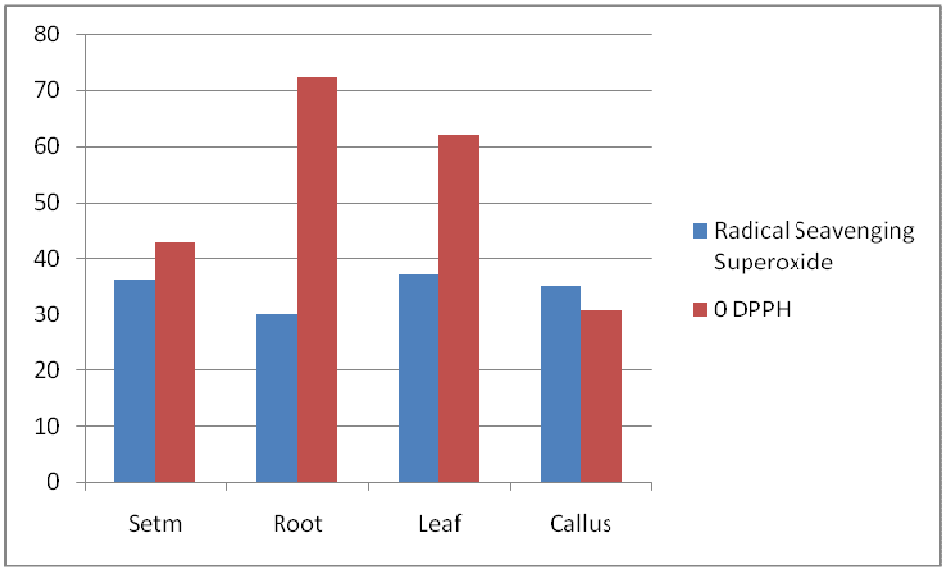



**Figure: 1 Induction and proliferation of callus from leaf of *Catharanthus roseus.* A. Leaf explants on MS medium after 7 days of inoculation B. Callus after 8 weeks (on MS medium supplemented with 2, 4-D 1.0 mg/l)**

**[Graph: 1] Estimation of primary metabolites in different parts and in vivo raised tissue of *Catharanthus roseus* in mg/ gram dry weight**




**[Graph: 2] Antioxidant activities of callus, roots, stem and leaves of *Catharanthus roseus***

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