Recent Research in Science and Technology 2011, 3(9): 78-83
ISSN: 2076-5061
Available Online: http://recent-science.com/

# Comparative *In vitro* and *In vivo* study of antioxidants and phytochemical content in *Bacopa monnieri*

N. Mohan*, P.S. Jassal[1], V. Kumar, R.P. Singh[2]

[1]*Department of Biotechnology, Lovely Professional University, Phagwara, Punjab, India*
[2]*Department of Biotechnology, SS PG College, Bulandshar, U.P., India*

Abstract

Medicinal plants are the most important source of life saving drugs for the majority of the world's population. Plant secondary metabolites are economically important as drugs, fragrances, pigments, food additives and pesticides. *Bacopa monnieri* L. Penn. commonly known as "Brahmi" is an important medicinal herb of the family Scrophulariaceae. It is the foremost brain tonic herb of the Indian System of Medicine and other traditional systems, used primarily as a nerve tonic, to treat insomnia and nervous tension. *Bacopa's* antioxidant properties may offer protection from free radical damage in cardiovascular disease and certain types of cancer. *Bacopa monnieri* showed antibacterial effect on both Gram positive and Gram negative bacteria suggests the passage of active phytochemicals through both the bacterial cell wall. Microropagation is rapid, *in vitro* clonal multiplication method of elite clones and also helps in dissemination and *ex situ* conservation of this endangered medicinal plant. The present study is justifiably planned to propagate the valuable medicinal plant *Bacopa monnieri L.* in *in-vitro* condition with various combinations/ concentrations of plant growth regulators, and compare the antioxidant and photochemical content difference in the *in-vivo* and *in-vitro* cultivated plant of *Bacopa monnieri.*

Keywords: *Bacopa monnieri* L., *In vitro*, antioxidant activity, free radical scavenging activity, Alzheimer's disease, medicinal plant extracts

## INTRODUCTION

Medicinal plants are the most important source of life saving drugs for the majority of the world's population. Plant secondary metabolites are economically important as drugs, fragrances, pigments, food additives and pesticides. The biotechnological tools are important to select, multiply, improve and analyze medicinal plants. It is estimated that 70-80% of people worldwide rely chiefly on traditional, largely herbal, medicines to meet their primary healthcare needs. The global demand for herbal medicine is not only large, but growing [1]. In the folklore of Indian medicine, certain herbs have been used traditionally as brain or nerve tonics. One of the most popular of these used in neurotonics is *Bacopa monnieri,* a small, common, amphibious plant growing in marshy areas throughout the Indian subcontinent. *Bacopa* is also called Brahmi, a name derived from Brahma, the creator god of the Hindu pantheon of deities. Since *Bacopa's* primary therapeutic use is to enhance cognitive function, most research has focused on the mechanism behind these properties. The triterpenoid saponins and their bacosides are responsible for *Bacopa's* ability to enhance nerve impulse transmission. The bacosides aid in repair of damaged neurons by enhancing kinase activity, neuronal synthesis, and restoration of synaptic activity, and ultimately nerve impulse transmission [2]. Traditionally, it was used as a brain tonic to enhance memory development, learning, and concentration, [3] and to provide relief to patients with anxiety or epileptic disorders [4]. Research on anxiety, epilepsy, bronchitis and asthma, irritable bowel syndrome, and gastric ulcers also supports the Ayurvedic uses of Bacopa [5]. *Bacopa's* antioxidant properties may offer protection from free radical damage in cardiovascular disease and certain types of cancer. It helps prevent induced lipid peroxidation [6]. *Bacopa monnieri* showed antibacterial effect on both Gram positive and Gram negative bacteria suggests the passage of active phytochemicals through both the bacterial cell wall [7]. Russo suggested that because of its ability to reduce NO-induced cellular alterations, Brahmi has a therapeutic potential in treatment or prevention of neurological diseases [8]. It was also reported that extract of brahmi rich in saponins, is able to induce a dose-related increase in superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities in rat frontal cortex, striatum and hippocampus [9]. *In vitro* research has shown *Bacopa* exerts a protective effect against DNA damage in astrocytes [10] and human fibroblasts [11]. *In-vitro* research suggests an anticancer effect for *Bacopa* extracts, possibly due to inhibition of DNA replication in cancer cell lines [12].

Various technologies have been adopted for enhancing bioactive molecules in medicinal plants [13]. Biotechnological tools are important for the multiplication and genetic enhancement of the medicinal plants by adopting techniques such as *in vitro* regeneration and genetic transformation [14]. Plant tissue culture is the process of small pieces of living tissues (explants)

Received: August 17, 2011; Revised September 20, 2011; Accepted September 21, 2011.

*Corresponding Author

N. Mohan
Dept. of Biotechnology, Lovely Professional University, Phagwara, Punjab, India

Tel: +91-9897063474; Fax: +91-5732230757
Email: nitinbiotechss@gmail.com

isolated from a plant and grown aseptically for indefinite periods on a semi defined or defined nutrient medium [15]. It is considered in wide sense which comprises the various culture methods of plant organs, tissues which facilitates experimental approach with a large objective of developmental biology and crop modification. It provides new possibilities for *in-vitro* propagation and manipulation of plants and also recognized as an efficient tool for rapid clonal propagation [16]. Murashige and Skoog's medium is commonly used for plant tissue culture studies [17]. The present study is justifiably planned to propagate the valuable medicinal plant *Bacopa monnieri* L. in *in-vitro* condition with various combinations/ concentrations of plant growth regulators, and compare the antioxidant and photochemical content difference in the *in-vivo* and *in-vitro* cultivated plant of *Bacopa monnieri.*

## MATERIAL AND METHODS

### Collection of plant sample

*Bacopa monnieri* plant was collected from Ghatgarh hills, Ghatgarh (District Nainital) India. The plants were put under Poly bags and planted in to a shade area.

### Preparation of Media

Murashige and Skoog's medium was used for the cultivation of *B. monnieri*. at *in vitro* condition. The MS medium was prepared by adding required amounts of stock solutions and final volume was made up with distilled water. The pH of the medium was adjusted to 5.8 using 1 N NaOH/KCl. About 50 ml of the medium was poured into sterile culture bottles. The culture bottles with MS medium was autoclaved at 121°C for 20 min. at 15 lbs pressure and transferred to the media storage room where they were kept under aseptic condition for further experimental study.

### Selection of explants & sterilization

Auxiliary nodes, young leaves and internodes of *Bacopa* were used as explants. The explants were first washed with running tap water for 30 min. to remove the soil particles and other extraneous fine particles. The explants parts such as nodal segment, stem, leaf and root will be cut from the healthy plant of *B. monnieri* and washed with tap water for 5-10 times, and they was soaked in 0.2-0.5% bavistin and 0.03% streptomycin aqueous solution for 10 min. It was gently washed twice in sterile double distilled water. The explants were immersed in aqueous solutions of savlon (1.5% v/v chlorohexidine gluconate solution & 3% w/v cetrimide) for 10 min. Then the explants were washed twice thoroughly with sterile double distilled water. After this treatment, the explants were surface sterilized with 0.01% $HgCl_2$ aqueous solution for 1 min. and rinsed thoroughly with sterile double distilled water.

### Initiation of cultures

There is a high risk of contamination of the MS medium at the time of transfer of the explants into the culture medium. Therefore, surface sterilized explants were transferred aseptically to sterile glass plate. Then undesirable and dead portions of both basal and the top portion of the explants will be removed. The nodal explants were placed in an erect position in the culture bottle containing MS medium with the help of sterile forceps. Then lid would be closed carefully and sealed with Klin film. The same procedure was used for all the explants. The culture bottles would be kept in the growth room at 25±2°C, with a photoperiod of 16 h daylight and 8 h night breaks under the cool white fluorescent light.

### Establishment of cultures

The explants with bud proliferation cultures were transferred to culture tubes containing fresh MS medium. After 21-25 days of incubation the initiated plants would be taken out from the culture tubes and transferred into fresh semi-solid MS media. Then the bottles were kept in culture room at 25±2°C for 8-16 h of day and night under the low temperature with white fluorescent light [18]. After experimental days, the full matured culture would be obtained and they were further subculture in MS medium supplemented with different plant growth hormones at different concentrations for regeneration of shoots.

### Auxiliary shoot proliferation

The stem explants were inoculated on MS medium supplemented with different plant growth regulators at different concentrations and multiplication of shoots were carried out by repeated sub-culturing in MS medium. Multiple shoots and cluster was transferred from the culture bottle to a sterile glass plate and the debris parts were removed. These nodal segments were transferred to the multiplication media with 0.5 mg/l IAA/0.5 mg/l KIN as growth regulators. These culture bottles were incubated at 25±2°C. These steps would be repeated at every 25-30 days intervals and shoot induction rate was observed [19].

### Callus induction

Leaf explants were taken from established cultures of B. monnieri L. for callus induction. The MS basal medium was supplemented with 0.5 mg/l NAA and 0.25 mg/l TDZ. After inoculation with established culture, the culture bottles were sealed properly, labeled and the triplicates were maintained. Then they were transferred to the incubation room and kept in appropriate condition. After two weeks, the callus induction rate was recorded [20].

### Antioxidant Assays

There are following antioxidant assays viz. DPPH free radical scavenging, Nitric oxide radical scavenging assay.

### DPPH radical scavenging assay [21]

DPPH [1,1-diphenyl-2-picryl hydrazyl] is a stable free radical with purple color, the intensity of which is measured at 510 nm spectrophotometrically. Antioxidants reduces DPPH to 1,1-diphenyl-2-picryl hydrazine, a colorless compound.

### Procedure

Various concentrations of test solution and 1ml of DPPH (0.3 mM) solution were incubated at 25°C for 20 min. Following which the absorbance is read at 510 nm. A control reaction was carried out without the test sample. The % inhibition was calculated according to the following equation

$$\% \text{ Inhibition} = (A_0 - A_t) / A_0 \times 100$$

Where $A_0$ is the absorbance of the control (blank, without extract) and $A_t$ is the absorbance in the presence of the extract.

### Nitric oxide radical scavenging assay [22]

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions, which can be measured at 540 nm spectrophtometrically in the presence of Griess reagent (1% sulphanilamide, 0.1% naphthyethylene diamine dihydrochloride is

dissolved in 2% phosphoric acid).

### Procedure

2ml Test solution of various concentrations and 3ml of 10 mM sodium nitroprusside was added and incubated at room temperature (25-30°C) for 1 hr. 5ml of Griess reagent was added and incubated for 10 min at room temperature. The color developed was measured at 540 nm. The % inhibition was calculated according to the following equation

% Inhibition = $(A_0-A_t) / A_0 \times 100$

Where $A_0$ is the absorbance of the control (blank, without extract) and $A_t$ is the absorbance in the presence of the extract.

### Phytochemical study

The phytochemical study deals with the estimation of phenolics, flavonoids and carotenoids, present in the aqueous and ethanolic extracts.

### Estimation of Phenolics [23]

- 5 gm sample was homogenized in acetone and kept overnight in a flask.
- Supernatant was collected and residues were extracted with acetone, filter and centrifuge.
- Supernatant was used for estimation of phenolics.
- DW was added to extract and add ferric ammonium sulphate kept at room temperature.
- Potassium ferricyanide was added and absorbance was measured at 720 nm (concentration in μg/ml of extract.)

### Estimation of Flavonoids [24, 25]

- 5 gm sample was acid hydrolyzed with sulphuric acid and neutralized with sodium hydroxide.
- Ethyl acetate was added and shake well, ethyl acetate portion is collected.(repeat thrice)
- Ethyl acetate was palled and evaporated to dryness residues are reconstituted with methanol and assayed for flavonoids content.
- Extract was mixed with methanolic $AlCl_3$ and absorbance was measured at 430 nm.

### Estimation of carotenoids [26]

- 5 gm sample was homogenized in acetone, filter this step is repeat until extract is not free from pigments.
- Filtrate was pooled and partitioned with equal vol. of peroxide free ether thrice using the separating funnel.
- Ether phase containing the carotenoids, evaporated and residues dissolved in ethanol.
- KOH was added to particles twice into peroxide free ether.
- Ether was evaporated and dissolved in ethanol; carotenoids were measured by spectrophotometer at 450 nm absorbance.

## RESULT AND DISCUSSION

Plant tissue culture techniques for ornamental as well as herbaceous plants have been well established. *In vitro* propagation technique is a powerful tool for plant germplasm conservation. Hence tissue culture is the only rapid process for the mass propagation of plants. The ability to generate plants directly for explants is fundamental to clonal multiplication of elite germplasm via micropropagation [27]. Plant biotechnology is considered in a wide sense which comprises the various culture methods of plant organs and explants to facilitate experimental approaches with a large objective of developmental biology in grain legumes for crop modification [28]. In the present study, to raise stock culture, nodal explants were taken from the field growing wild plants. The auxiliary bud was found initiated from both leaf and nodal explants on hormone free MS medium within 9 days Shoot buds of *B. monnieri*. were also initiated on the MS basal medium supplemented with 0.5 mg/l IAA and 0.5 mg/l KIN within 25-30 d from the nodal explants. Correspondingly, Thejavathi et al. [29] has also been used shoot tip and nodal explants for the micropropagation studies of *B. monnieri*. Most of the other research studies for other medicinal plant species have shown the use of cytokinin alone or in combination with other different concentrations for plant culture initiation. For eg. In Paederia foetida and *Centella asiatica* multiple shoots were obtained in MS medium supplemented with BAP 1.0 mg/l [30] and *Rauwolfia serpentina* on MS medium supplemented with benzyladenine and NAA [31].

### Effect of growth regulators on callus induction

Production of callus and its subsequent regeneration are the prime steps in crop plant to be manipulated by biotechnological means and to exploit somaclonal variation [32]. In the present study remarkable callus induction rate was observed with leaves were used as explants and the appearance of *B. monnieri*. callus was globular and pale yellow in colour. The explants were enlarged within 12-14 days of inoculation; however callus formation was started after 20-25 days. Rapid callus growth (90%) response was observed in the MS medium with 0.5 mg/l NAA, 0.5 mg/l 2,4-D and 0.25 mg/l TDZ individually and 2,4-D 0.5 mg/l with TDZ 0.15 mg/l, TDZ 0.05 + NAA 0.4 mg/l and TDZ 0.05 + NAA 0.5 mg/l in combinations of growth regulators. Minimum callus formation rate (10%) was noted in the MS medium containing 0.05 mg/l of TDZ.

### Antioxidant Assays

### DPPH free radical assay

The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517 nm induced by antioxidants. The scavenging effects of extract increased with their concentrations to similar extents. The percentage inhibitions of Gallic acid (taken as standard), plant and callus extract is shown in table. The comparison of plant and callus extract is shown in figure 1; the histogram showing that the plant extract is able to inhibit the DPPH 76.55% in the concentration of 50 μg/ml the callus is able to inhibit 71.17%.

### Nitric oxide free radical assay

Nitric oxide or reactive nitrogen species formed during its reaction with oxygen or with superoxide such as $NO_2$, $N_2O_4$, $N_3O_4$, nitrate and nitrite are very reactive. These compounds alter the structure and function of many cellular components. Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this damage. Extract of *Bacopa monnieri* plant and *in vitro* grown callus shows reduction in nitric oxide, as shown in table. The curcumin was taken as standard. The comparable scavenging effect of plant and callus is shown in figure 2; the histogram showing that the plant extract is able to inhibit the Nitric oxide 77.85% in the concentration of 100 μg/ml the callus is able to inhibit 68.94%.

## Phytochemical estimation:

### Estimation of Phenolics

For the estimation of Phenolics the Quercetin was taken as standard. The absorbance of Quercetin in different concentration was taken at 720 nm for preparing the standard curve (Fig. 3). The comparison of phenolic compound in plant and callus extract is shown in Fig. 6.

- Absorbance of the plant extract = 1.509
- Absorbance of the callus extract = 1.457
- The concentration of the phenolics (in µg/ml) was estimated by the standard curve.
- The phenolic content in plant extract = 686.5 µg/ml
- The phenolic content in callus extract = 660.5 µg/ml

### Flavonoids Content Estimation

For the estimation of flavonoids the Quercetin was taken as standard. The absorbance of Quercetin in different concentration was taken at 430 nm for preparing the standard curve (Fig. 4) as shown in Table. The comparison of flavonoids content in plant and callus extract is shown in Fig. 6.

- Absorbance of the plant extract = 0.928
- Absorbance of the callus extract = 0.827
- The concentration of the flavonoids (in µg/ml) was estimated by the standard curve.
- The flavonoids content in plant extract = 585.5 µg/ml
- The flavonoids content in callus extract = 535 µg/ml

Table 1: % of inhibition of DPPH by the plant and callus extract w.r.t. Gallic acid as standard

| Concentration (µg/ml) | Gallic Acid % of inhibition | Plant extract % of inhibition | Callus extract % of inhibition |
|---|---|---|---|
| 10 | 50.72 | 15.08 | 6.90 |
| 15 | 57.18 | 47.16 | 26.22 |
| 25 | 61.80 | 50.55 | 45.10 |
| 30 | 65.92 | 58.12 | 48.71 |
| 35 | 67.98 | 64.49 | 51.28 |
| 40 | 73.94 | 70.54 | 57.79 |
| 45 | 76.55 | 73.21 | 63.86 |
| 50 | 80.17 | 76.55 | 71.15 |

Table 2: % of inhibition of Nitric oxide by the plant and callus extract w.r.t. curcumin

| Concentration (µg/ml) | Curcumin % of inhibition | Plant extract % of inhibition | Callus extract % of inhibition |
|---|---|---|---|
| 20 | 19.17 | 5.03 | 2.09 |
| 40 | 33.56 | 19.86 | 9.81 |
| 60 | 57.30 | 45.43 | 26.71 |
| 80 | 79.68 | 59.36 | 39.95 |
| 100 | 85.15 | 77.85 | 68.94 |

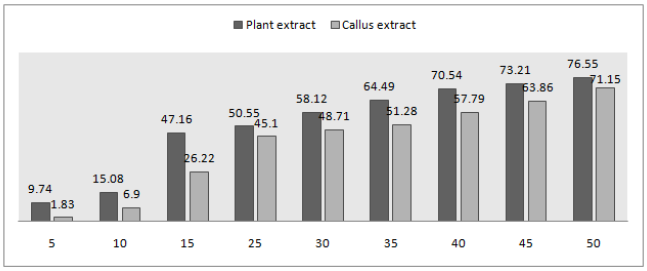


Fig 1: Histogram showing the comparative % of inhibition of DPPH by the plant and callus extract

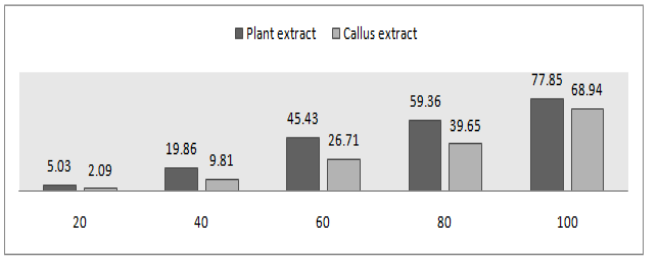


Fig 2: Histogram showing the % of inhibition of Nitric oxide by the plant and callus extract

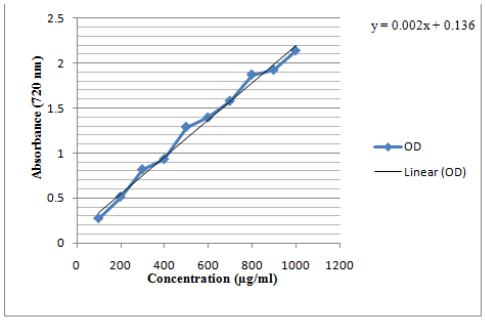


Fig 3: Standard curve of Quercetin at 720nm

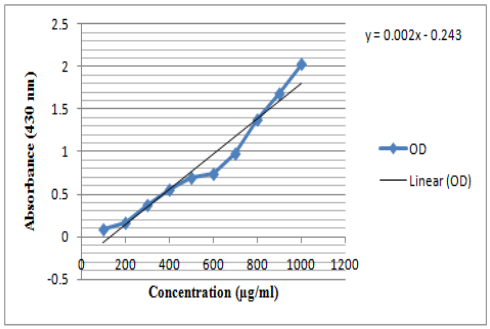


Fig 4: Standard curve of Quercetin at 430nm

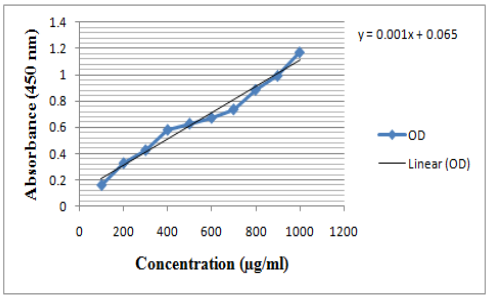


Fig 5: Standard curve of Quercetin at 450nm

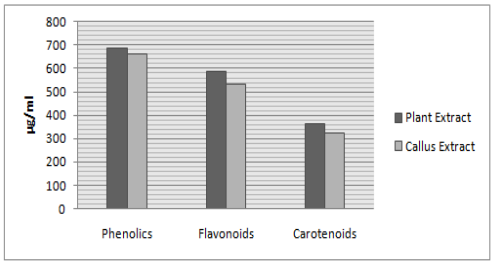


Fig 6: Comparison of phytochemical content in plant extract and callus extract

Carotenoids content estimation: For the estimation of carotenoids the Quercetin was taken as standard. The absorbance of Quercetin in different concentration was taken at 430 nm for preparing the standard curve (Fig. 5) as shown in Table. The comparison of carotenoids content in plant and callus extract is shown in Fig. 6.

- Absorbance of the plant extract = 0.428
- Absorbance of the callus extract = 0.387
- The concentration of the carotenoids (in µg/ml) was estimated by the standard curve.
- The caraotenoid content in plant extract = 363 µg/ml
- The caraotenoid content in callus extract = 322 µg/ml.

## Conclusion

The present study shows the antioxidant and phytochemical contents of *Bacopa monnieri* and also shows that these bioactivities

differ between *In vitro* and *In vivo* grown plants. The antioxidant and phytochemical content of *In vivo* grown plant was comparatively higher than *In vitro* grown callus. *Bacopa monnieri* is known to be a plant having antioxidants and various phytochemical constituents which has been studied in the work. The comparison of antioxidant and phytochemical constitutes in *In vivo* and *In vitro* grown plant is not having any high difference. So advances in plant tissue culture will enable rapid multiplication and sustainable use of *Bacopa monnieri* for future generations.

ACKNOWLEDGEMENT

We are thankful to authorities of Lovely Professional University for providing laboratory facility and very thankful to every person who involve in this work directly and indirectly.

REFERENCES:

[1] Farnsworth NR & Soejarto DD (1991), Global importance of medicinal plants in conservation of medicinal plants, edited by O Akerele, V Hegwood & H Synge, (Cambridge university press), 1991, 25-51.

[2] Singh HK, Dhawan BN. Neuropsychopharmacological effects of the Ayurvedic nootropic *Bacopa monniera* Linn. (Brahmi). *Indian J Pharmacol* 1997;29:S359-S365.

[3] Mukherjee DG, Dey CD. Clinical trial on Brahmi. I. *J Exper Med Sci* 1966;10:5-11.

[4] Chopra RN. *Indigenous Drugs of India*. 2nd ed. Calcutta, India: U.N. Dhur and Sons; 1958:341.

[5] Shakoor A, Akram M, Asharaf CM and Siddiqui MR (1994) Pharmagonistic study and chemical /pharmacological evaluation of Brahmi-buti. Hamdard Medicus. 37, 92-109.

[6] Tripathi Y B, Chaurasia S, Tripathi E, Upadhyay A and Dubey G P (1996) *Bacopa monnieri* Linn. as an antioxidant mechanism of action. Indian Journal of Experimental Biology 4(6): 523-526.

[7] S.R. Ayyappan, R. Srikumar and R. Thangaraj (2010), Phytochemical and antibacterial activity of *Bacopa monniera* against the common bacterial isolates from human, International journal of Microbiological Research 1 (2); 67-71,2010.

[8] Russo A, Borrelli F, Campisi A, et al. Nitric oxiderelated toxicity in cultured astrocytes: effect of *Bacopa monniera. Life Sci* 2003;73:1517-1526.

[9] Bhattacharya SK, Bhattacharya A, Kumar A, Ghosal S. Antioxidant activity of *Bacopa monniera* in rat frontal cortex, striatum, and hippocampus. *Phytother Res* 2000;14:174-179.

[10] Russo A, Izzo A, Borrelli F, et al. Free radical scavenging capacity and protective effect of *Bacopa monniera* L. on DNA damage. *Phytotherapy Res* 2003;17:870-875.

[11] Russo A, Borrelli F *(2005) Bacopa monniera, a reputed nootropic plant: an overview. Phytomedicine 12: 305-317.*

[12] Elangovan V, Govindasamy S, Ramamoorthy N, Balasubramaanian K. *In vitro* studies on the anticancer activity of *Bacopa monnieri. Fitoterapia* 1995;66:211-215.

[13] Abdin MZ (2007), Enhancing bioactive molecules in medicinal plants, in natural product-essential recourse for human survival, edited by Y Zhu, B Tan & C Lin, (World scientific publishing Co. Pvt. Ltd. Singapore) 2007, 45-57.

[14] Abdin MZ & Kamaluddin (2006), Improving quality of medicinal herbs through physio-chemical and molecular approaches in traditional systems of medicine, edited by M Z abdin & Y P Abral (Narora Publishing House Pvt. Ltd. India) 2006, 30-39.

[15] Ignacimuthu S (1997) Plant Biotechnology, Oxford and IBH publishing Co. Pvt. Ltd, p. 180.

[16] Negrutiu I, Jacobs N and Caboche M (1984) Theor. Appl. Genet. 67, 289-304.

[17] Murashige T and Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15, 473-497.

[18] Murashige T and Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15, 473-497.

[19] Ray T, Saha P and Roy SC (2005) In vitro plant regeneration from young capitulum explants of Gerbera jamessonii. Plant Cell Biotechnol. Mol. Biol. 6, 35-40.

[20] Nagaraja YP, Krishna V and Maruthi KR (2003) Rapid micropropagation of Andrographis alata Nees. Through leaf callus culture. Plant Cell Biotechnol. Mol. 4, 117- 124.

[21] McCune LM, Johns T (2002). Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous peoples of the North American boreal forest. J. Ethnopharmacol. 82: 197-205.

[22] Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR (1982). Analysis of nitrate, nitrite and 15N nitrate in biological fluids. Anal. Biochem. 126: 131-138.

[23] Price, M.L., A.D. Hagerman and L.G. Bilture, 1980.Tannin content of cow peas, chick peas, pigeon peas and mung beans. J Agriculture and food Chemistry, 28: 459-461.

[24] Harborne, J.B., 1975, Biochemical systeamatics of flavonoids. In the Flavonoids, J.B. Harborne, H. Mabry, (Eds). Academic Press: New York, San Fransisco, pp 1056-1095.

[25] Lamaison, J.L and A. Carnt, 1990. Teneurs en acid resmarinique en derivers hydroxycinnamiquest totaud et activities antioxidets chez les Apiacees, les Borrabinacees et less Lamiacees medicinal. Phaemaceutica, Acta Helvetiae, 65:315-320.

[26] Narayanaswamy, P. and A. Palanisami, 1973. Studies on yellow mosaic disease of soybean, Effect of virus infection on plant pigments. Experimental. Biol., 29: 1165-1167.

[27] Ignacimuthu S (1997) Plant Biotechnology, Oxford and IBH publishing Co. Pvt. Ltd, p. 180.

[28] Ramawat KG (2003) Plant Biotechnology, S. Chand and Co. pp:1-37.

[29] Thejavathi DH, Sowmya R and Shailaja KS (2001) Micropropagation of Bacopa monnieri using shoot tip and nodal explants. J. Trop. Med. Plants. 2, 39-45.

[30] Singh S, Ray BK, Mathew S, Buragohain P, Gogoi J,Gogoi S, Sharma BK and Deka PC (1999). Micropropagation of a few important medicinal plants. Ann. Biol. 15, 1-7.

[31] Sehrawat AR, Sanjogta U and Anita P (2001) In vitro culture and multiplication of Rauwolfia serpentine – a threatened medicinal plant. Crop Res. 22, 68-71.

[32] Monirul Islam M, Ahmed M and Mahaldar D (2005) In vitro callus induction and plant regeneration in seed explants of rice (*Oryza sativa* L.). Res. J. Agri. Biol. Sci.1, 72-75.