WWW.IJAPC.COM

IJAPC

e ISSN 2350 0204

VOLUME 12 ISSUE 1 2020

GREENTREE GROUP PUBLISHERS (GGP)

Int J Ayu Pharm Chem

RESEARCH ARTICLE

www.ijapc.com

e-ISSN 2350-0204

Comparative Pharmacognostical Studies of Naturally Grown and Tissue Cultured Brahmi – *Bacopa monnieri Linn*

J M Chethana[1*], Seema Pradeep[2] and Shiva Manjunatha M P[3]

[1-3]Sri Sri College of Ayurvedic Science and Research, Bangalore, Karnataka, India

ABSTRACT

Background: *Brahmi* a well-known *Medhya* drug is in great demand for its medicinal use. It has a great demand by the pharmaceutical industry sector. To cater this need, various studies have been taken up for its propagation through tissue culture as the conventional means of propagation takes a long time for multiplication.

Methodology: The study deals with macroscopic parameters, microscopic study of transverse sections physicochemical, phytochemical analysis and powder microscopy of both naturally grown and tissue cultured brahmi

Results: There was no significant difference in the microscopic structure of the two cultivars. HPLC quantification showed marked difference in quantification of bacosides.

KEYWORDS

Brahmi, Bacopa monnieri, Medhya, Neerabrahmi, Water Hyssop

Greentree Group Publishers

Received 15/11/19 Accepted 08/01/20 Published 10/01/2020

INTRODUCTION

Brahmi- Bacopa monnieri is a well-known *Medhya* drug. It is commonly known[1] as Neerabrahmi in Kannada and *Water Hyssop* in English. Acharya Charaka has mentioned it as *Aindri* while explaining *Aindri rasayana*[2]. Though it is well-known for its memory enhancing action, it is also a very effective anti-depressant[3], anti-diabetic[4], anti-aging and anti-oxidant[4] plant. Recent researches have proven it to be an excellent Nootropic drug. Due to all these activities it has been used in various formulations, hence has a great demand in the pharmaceutical industry. To cater this need, various studies have been taken up for its propagation. The conventional means of propagation takes a long time for multiplication and plant tissue culture can be a potential method to solve increasing demand. To consider using the tissue cultured Brahmi as an alternative to naturally grown Brahmi, both the cultivars need to be evaluated for its similarities or dissimilarities. This study is intended to compare the two cultivars.

MATERIALS AND METHODS

Botanical Identification:

Collection and Identification of Plant Material:

1. Collection of drug:

a. Natural Brahmi was grown in specifically allotted plots at the garden of Sri Sri College of Ayurvedic sciences and research.

b. Tissue cultured Brahmi was cultured at Tissue culture Laboratory; PG studies Dept. of Dravyaguna, Sri Sri College Of Ayurvedic Sciences And Research. These were further set up for hardening in specifically allotted plots at the garden of Sri Sri College of Ayurvedic sciences and Research.

Both the Samples were botanically identified and authentified as *Bacopa monnieri* Linn by taxonomist.

Macroscopic Evaluation[5]: The morphology of the whole plant of both cultivars was studied with the help of available literature, and was observed for the following features- colour, texture, length and diameter of the roots. (Fig no. 1)

Microscopic evaluation[5]: The cross section of root, stem, and leaf of *Bacopa monnieri* Linn was done and observed under compound microscope and captured using camera microscope and compared.

Physicochemical Evaluation[5]: The two samples of brahmi were subjected to Physico-chemical tests and Phyto-chemical parameters such as total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive, total alkaloids were

determined according to standard procedures done for medicinal plants.

Phytochemical Evaluation[6]: The qualitative chemical tests carried out for the identification of the natural phytoconstituents present in the water and alcohol extract of the two powdered crude drugs. The tests were carried out using conventional protocols. Estimation of total alkaloids and HPLC for Bacosides was done using standard protocol.

OBSERVATION AND RESULTS

Macroscopic Features:

The two samples had similar organoleptic characters except that the tissue cultured sample had a slight tinge of brown colour. [The details are as listed in Table No 1]

Table 1 Organoleptic Evaluation of the powder of two samples:

Sl No.	Organoleptic characters	Natural *Brahmi* Powder	Tissue cultured *Brahmi*
1	Colour	Dark green	Slightly brownish Green
2	Touch	Smooth	Smooth
3	Odour	Characteristic odour	Characteristic odour
4	Taste	Bitter	Bitter

Morphological Study

The morphological characters can be diagnostic parameters for the plant.

Roots: Creamish yellow in colour, thin tapering, wiry, small, branched and arising from the nodal region of the stem.

Stem: Greenish colour, prostrate, thick and fleshy, herbaceous, soft, with prominent nodes and internodes

Leaves: Simple, sessile, glabrous, opposite and decussate, obovate -oblong to spathulate in shape, apex is obtuse and margins are completely entire, 1-3 nerved, faint green in colour [Photo 2D].

Flowers: White with violet and green bands and spangled with shining dots while fresh, short lived and colour lightens gradually, actinomorphic, solitary, axillary, bracteoles are shorter than pedicel, pedicel is slender in shape [Photo 1, 2A,].

Photo 1 Plant- Brahmi- *Bacopa monnieri* Linn

Photo 2 Morphological parts of Plant- Brahmi- *Bacopa monnieri*
A. Flower, B-Root, C-Stem, D- Leaf

Table 2 Observations on the morphological characters of the two sample of *Brahmi*

SL.No	Plant part	Natural Brahmi	Tissue cultured Brahmi
1	Length of Stem	16-20 cm	10-15cm
2	Thickness of stem	0.4-0.5 cm	0.4-0.5cm
3	Width of Leaf	0.6-0.8 cm	0.4-0.6cm
4	Length of leaf	1.5-1.7cm	1.2-1.5cm
5	Length of Root	4.5-5cm	8-10cm

The morphological characters of the two sample of *Bacopa monnieri* had above measurements, it was found that the Natural brahmi seemed more robustly grown than the tissue cultured brahmi. Except the root of tissue cultured brahmi measured comparatively more than that of the natural brahmi, as it got easily uprooted from soil [Photo 4].

SL.No	Plant part	Natural Brahmi	Tissue cultured Brahmi
1	Length of Stem	16cm	10cm
2	Thickness of stem	0.4 cm	0.45
3	Width of Leaf	0.8cm	0.6cm
4	Length of leaf	1.7cm	1.2cm
5	Length of Root	4.5-5cm	10cm

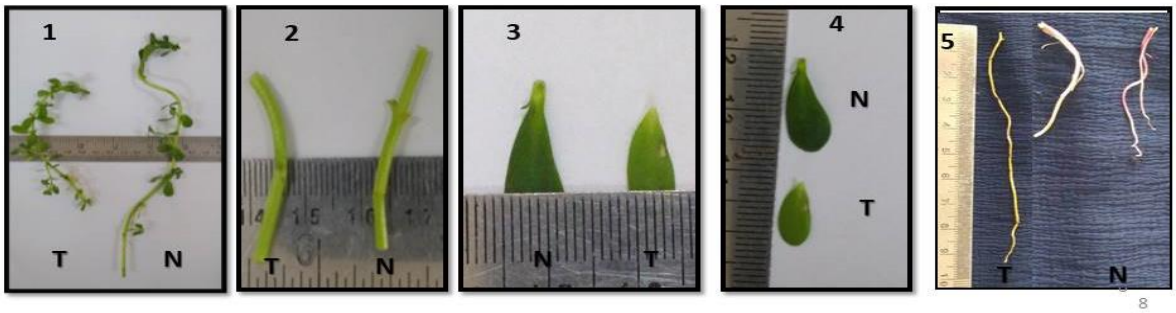


Photo 4

MICROSCOPIC EVALUATION

- The morphological parts of plant like: stem, leaf and root of the sample where conducted and observed that there was no difference in its microscopic structure.
- The powder Microscopy also showed no difference in the two sample.
- The following structures were observed under compound microscope and captured using camera microscope

Transverse section (T.S.) of stem showed single layer of epidermis, cortex with chlorenchymatous aerenchyma or air spaces, cortical cells with starch grains, single layered endodermis, 1-2 layered pericycle, continuous vascular ring composed of a narrow zone of phloem towards periphery and a wide ring of xylem towards centre, centrally located parenchymatous pith with simple, round to oval starch grains [Figure1].

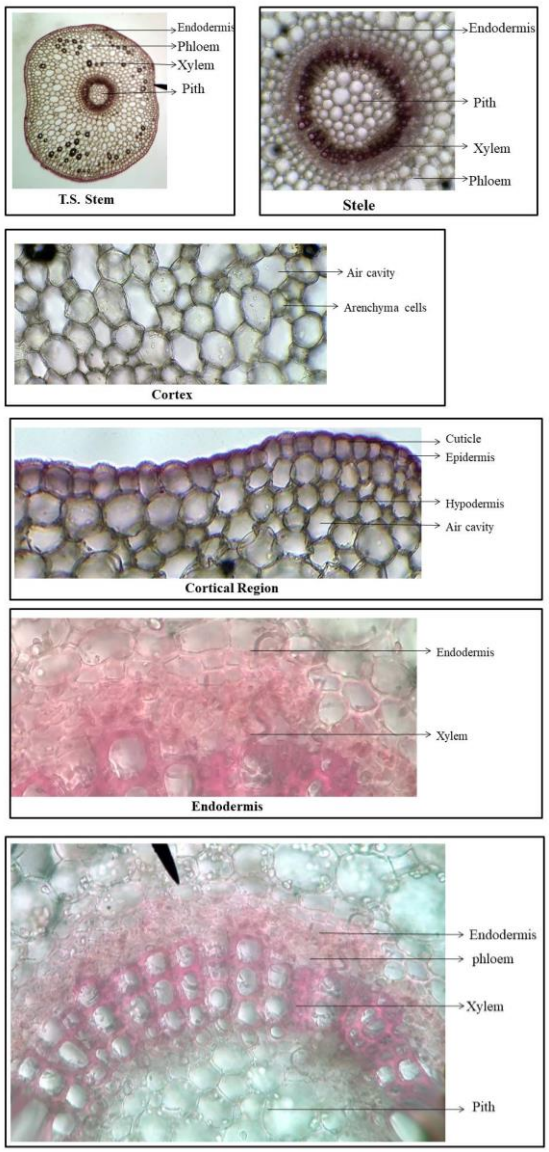


Figure 1 showing microscopic V.T.S. LEAF

Transverse section (T.S.) of leaf showed distinct upper and lower epidermis, cells of upper epidermis were comparatively larger than the cells of lower epidermis and covered with striated cuticle. Presence of sub - epidermal foliar idioblasts (found in the form of empty cavity) and a centrally located conjoint, collateral vascular bundle encircled by a parenchymatous sheath were observed [Figure 2]. Few crystals of calcium oxalate were seen embedded in the undifferentiated mesophyll tissue [Figure 2].

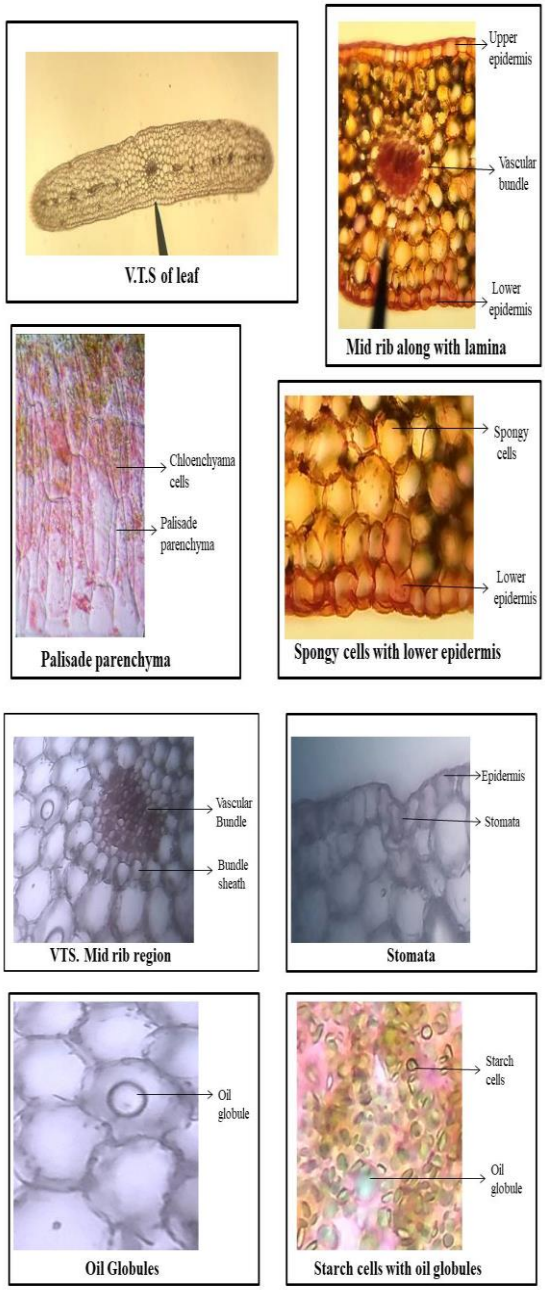
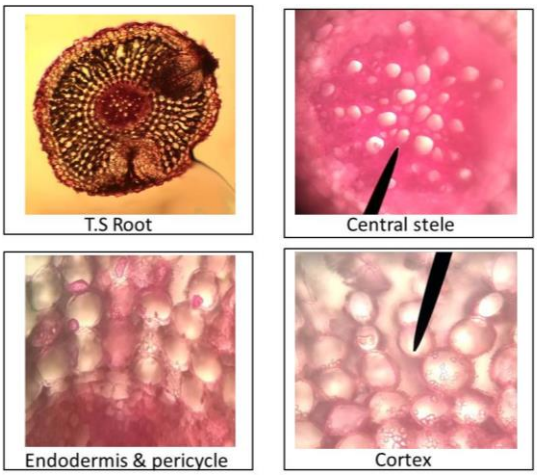


Figure 2 showing microscopic V.T.S. LEAF

Transverse section (T.S.) of root showed single layered epidermis with wide cortical arenchymatous region. Endodermis was distinct and single layered while pericycle was not differentiable. Central region was occupied by stele consisted of 1-5 layers of

peripheral phloem and centrally located xylem vessels [Figure 3].



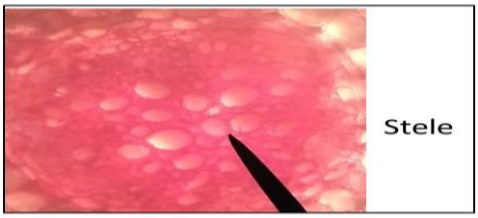


Figure 3 Showing microscopic T.S. ROOT

POWDER MICROSCOPIC STUDY: Powder microscopy of the whole plant of *Bacopa monnieri* showed the presence of: Epidermal cells, Sclerenchyma cells, Parenchyma cells, Epidermal Parenchyma, Spongy Parenchyma, Xylem Element, Xylem fibre, Tracheids, Chlorophyll, Stomata, Palisade cells, Oil Globule, Pollen grain, Starch grains. [Figure: 4].

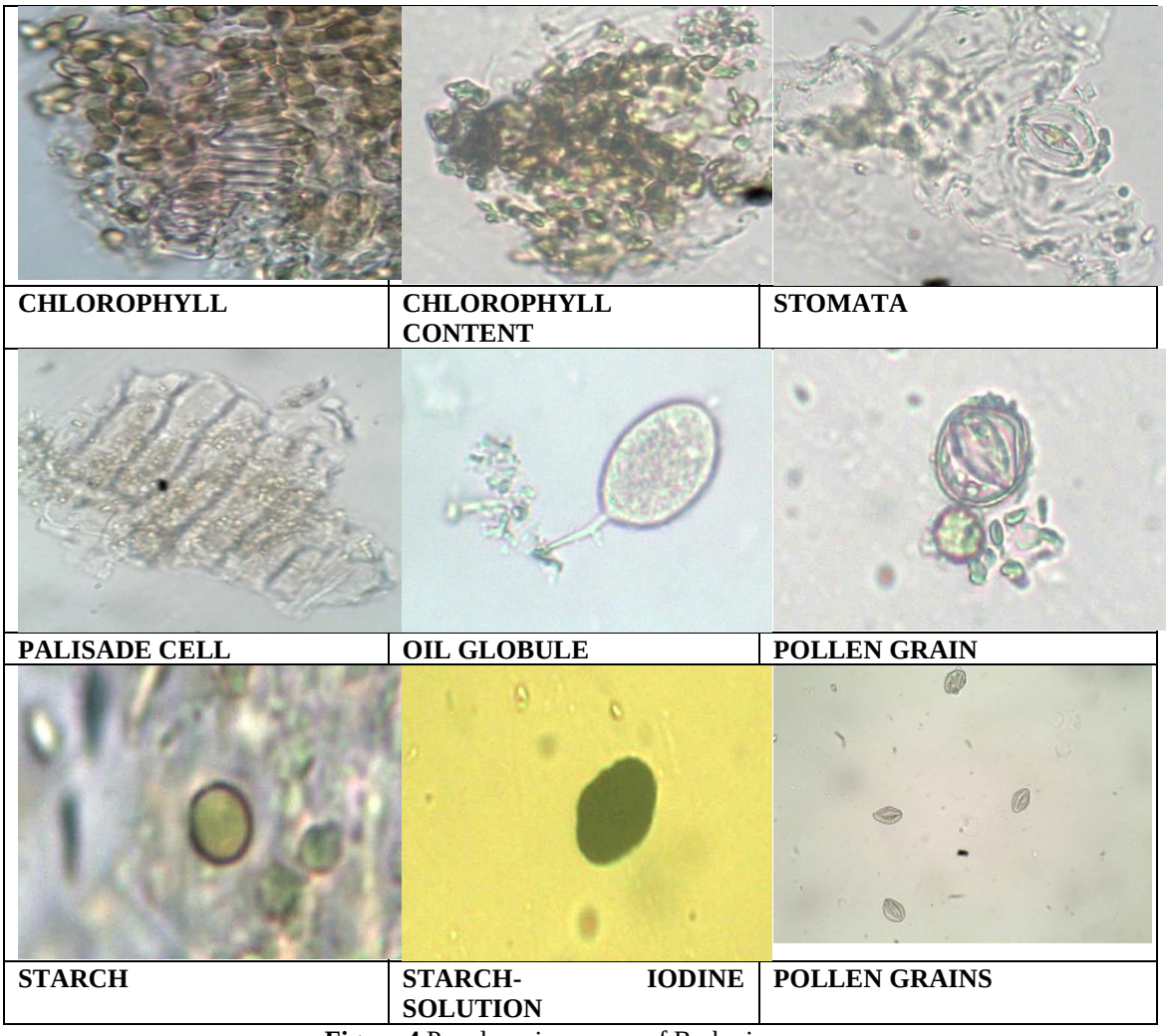


Figure 4 Powder microscopy of Brahmi

QUANTIFICATION OF THE PHYTO-CONSTITUENTS:

Table 3 Analysis of organic constituents of Natural and tissue cultured *brahmi*:

Sl No.	Method adopted	Organic constituents	Natural Brahmi		Tissue cultured	
			Aqueous Extract	Methanol Extract	Aqueous Extract	Methanol Extract
1	Wagners	Alkaloids	+	+	+	
2	Foam test	saponins	-	+	-	+
3	Molischs test	carbohydrates	+	+	+	+
4	Iodine test	Starch	+	+	+	+
5	Benedicts test	Non reducing sugar	-	-	-	-
6	Molischs test	glycosides	-	-	-	-
7	Salkowskis test	Steroids	+	+	+	+
8	Biurets test	proteins	-	-	-	-
9	Ferric chloride test	Phenols	+	+	+	+
10	Benedicts test	Reducing sugar	+	+	+	+
11	Anthocyanin test	-	+	+	+	+
12		Tannins	-	-	-	-

Table 4 Quantification of the Phyto-Constituents

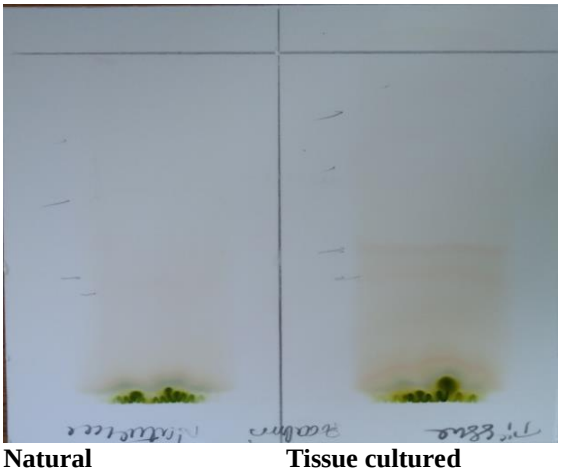
RAW MATERIAL ANALYSIS REPORT
Name of the material: Brahmi
1. Description - Macroscopic - (As per API)
Small branched, creamish, yellow, wiry, thin, simple leaf, opposite decussate, green powder, slightly bitter

. Tests for Identify, Purity and Strength - (As per API)			
TEST RESULT	**RESULT**		**STANDARD**
	Tissue cultured brahmi	**Natural brahmi**	
a. Test for Total ash	17.30%	14.90%	NMT 18%
b. Test for Acid In-soluble ash	2.70%	2.00%	NMT 6%
c. Test for Alcohol soluble extract	7.20%	10.80%	NLT 6%
d. Test for water soluble ext	22.80%	20.00%	NLT 15%
e. Loss on drying @ 110°C (LOD):	9.70%	11.40%	NMT 12%

Observation on Thin layer Chromatography

Table 5 TLC: Of Natural and Tissue cultured Brahmi

Tissue Cultured Brahmi		***Natural Brahmi RF values***	
Band Colour	***RF values***	**Band Colour**	***RF values***
Yellow	0.62	Yellow	0.68
Orangish red	0.56	orangish red	0.62
Grey	0.31	Grey	0.41
Grey	0.17	Grey	0.23



Natural **Tissue cultured**

Figure 4 Showing TLC plate

The bands were found to be more clearly visible in the tissue cultured sample.

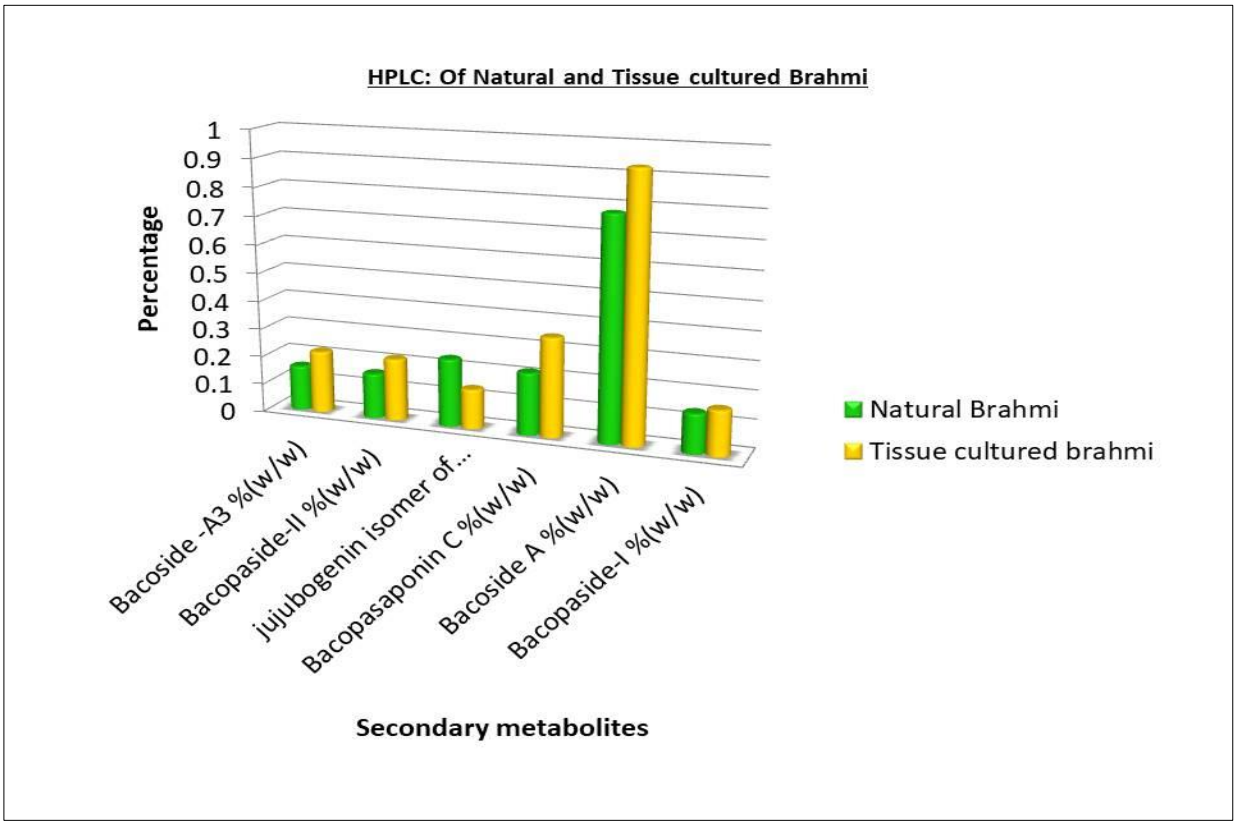
Observations on HPLC reports are as follows:

Both samples were analyzed for HPLC:

1. Natural Brahmi
2. Tissue culture Brahmi collected from open field.

Table 6 HPLC: Of Natural and Tissue cultured Brahmi

	COMPONENTS Analyzed	Natural Brahmi	Open Field TCB
1	Bacoside -A3 %(w/w)	0.16	0.22
2	Bacopaside-II %(w/w)	0.16	0.22
3	jujubogenin isomer of bacopasaponin C %(w/w)	0.24	0.14
4	Bacopasaponin C %(w/w)	0.22	0.35
5	Bacoside A %(w/w)	0.78	0.93
6	Bacopaside-I %(w/w)	0.14	0.16



Graph 1 HPLC: Of Natural and Tissue cultured Brahmi

DISCUSSION

1. Discussion on plant Tissue culture:

Micropropagated plants are observed to establish more quickly, grow more vigorously and taller, have a shorter and more uniform production cycle, and produce higher yields than conventional propagules[7].

At the same time, the chemical synthesis of plant-derived compounds is often not economically feasible because of their highly complex structures and specific stereo-chemical characteristics. The production of valuable secondary metabolites in plant cell cultures is an

attractive alternative to the extraction of the whole plant material[8].

2. Discussion on the Phyto-chemical analysis and HPLC:

The values of bacoside A content was higher in the tissue cultured brahmi. The probable reason for the difference could be that the naturally grown had lesser bacoside A content when compared to the tissue cultured brahmi. This could be because the tissue cultured variety received enough quantity of nutrients through the media during its growth phase, which the natural brahmi was deprived of from the soil.

Thus this source can also be used for fractionation of saponine from the crude drug.

CONCLUSION

The morphological parts of plant like: stem, leaf and root of the sample where conducted and observed that there was no difference in its microscopic structure. The powder microscopy also showed no difference in the two samples. HPLC quantification gave the percentage of bacosides & other metabolites, and showed significantly higher values in tissue cultured brahmi. Thus the tissue cultured brahmi can be a potential source plant that can be substituted for the conventionally grown brahmi.

REFERENCES

1. Kirtikar.K.R. and Basu.B.D., Indian Medicinal plants with Illustrations, Revised by E.Blatter, J.F.Caius and K.S.Mhaskar, Second edition 2001, Oriental Enterprises, Tpg- 1724

2. Acharya Agnivesha, Charaka samhitha, Vol-2, translated by P.V.Sharma, Published by Chaukhambha Orientalia, Varanasi, Pp-878.

3. Sairam K, Dorababu M, Goel RK, Bhattacharya SK. Antidepressant activity of standardized extract of Bacopa monniera in experimental models of depression in rats. Phytomed 2002;9:207-11.

4. Ghosh, T., Maity, T., Sengupta, P., Dash, D., Bose, A. Antidiabetic and In Vivo Antioxidant Activity of Ethanolic Extract of Bacopa monnieri Linn. Aerial Parts: A Possible Mechanism of Action. *Iranian Journal of Pharmaceutical Research*, 2010; Volume 7(Number 1): 61-68. doi: 10.22037/ijpr.2010.745

5. The Ayurvedic pharmacopoeia of India, Government of India Ministry of health and family welfare Department of ISM $ H.Published by Chaukhamba publications, New Delhi, First edition, Part-1, Volume-2, Pp-367, Page no-2

6. The Ayurvedic pharmacopoeia of India, Government of India Ministry of health and family welfare Department of ISM $ H.Published by Chaukhamba publications, New Delhi, Third edition, Part-1, Volume-8, Pp-65.

7. Smetanska, Iryna. (2008). Production of Secondary Metabolites Using Plant Cell Cultures. Advances in biochemical engineering/biotechnology. 111. 187-228. 10.1007/10_2008_103.

8. ***Web sites*- refereed from following sites:**

- www.bbc.com
- www.med-help.com
- www.pubmed.com
- www.medscape.mcg.edu`