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AN ANALYTICAL COMPARATIVE STUDY OF ROOTS OF PASHANABHEDA (BERGINIA LIGULATA, WALL) PROCURED FROM NATURAL HABITAT & THROUGH MICROPROPAGATION (TISSUE CULTURE) W R T HPTLC STUDIES

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ABSTRACT

The success of primary health care is the availability & use of suitable herbal drugs. medicinal plants participate in a remarkable role in the primary health care of people around the world. The rich source is disappearing at an alarming rate as a result of over-exploitation. An ever-increasing demand for uniform medicinal plants-based medicines warrants their mass propagation through plant tissue culture technique as a better option when compared with substitutes or adulterants. In this article, an effort is taken to compare the differences between natural habitat-procured plants & tissue culture-obtained plants. This article highlights the studies with both plants with respect to their comparison with HPTLC studies.

Keywords: Pashanabheda (*Berginia ligulata*, *Wall*), Micropropagation, HPTLC

INTRODUCTION

Pashanabheda (*Berginia ligulata*, *Wall*) means the one which breaks or destroys stones. It is a perineal herb that grows wild in stones & rocks mainly found in Himalayan valleys. This plant has been recognized for dissolving kidney stones. Its indications are as follows- Mutrakrichra, ashmari, prameha, Hrudroga,

gulma & Pliharoga [1]. Other diuretics herbs like Aerva lanata Juss, Bryophyllum calycium saslib are also used under the same name [2,3]. While reviewing the literature it was found that the common medicinal plants that are becoming endangered include Katuki, Jatamansi, Pashanabheda, Kushta, etc. So, there is a need to conserve this herb before it becomes extinct. Pashanabheda (*Berginia ligulata, Wall*) belongs to the family Saxifragaceae & is popularly known as "Stone flower or Stone breaker".

Biotechnology has rapidly emerged as an area of activity having a marked, realized as well as the potential impact on virtually all domains of human welfare, ranging from food processing, and protecting the environment to human health [4]. Micropropagation also called "Tissue culture" consists of growing plant cells as relatively organized masses of cells on an agar medium (callus culture) or as a suspension of free cells and small cell masses in a liquid medium. Tissue culture is used for the vegetative multiplication of many species and in some cases for the recovery of virus-free plants. Plant propagation is the process of creating new plants from a variety of sources: seeds, cuttings, and other plant parts. Plant propagation can also refer to the artificial or natural dispersal of plants whereas cultivation means growing them in a better manner [5].

MATERIALS & METHODS:

METHODOLOGY OF PLANT TISSUE CULTURE (PROPAGATION) OF PASHANABHEDA

PROCUREMENT OF PASHANABHEDA *(Berginia ligulata, Wall*)

It was procured from its natural habitat, Rohru, Himachal Pradesh where it is found to be available abundantly along the roadside. Next, these plants were handed over to "Shree Aditya Biotech, Bengaluru" for the purpose of a Tissue culture study. This study was conducted by Mr. S. Balasubrahmanya, M.Sc. Botany, Head- projects (NCS-TCP, National certification system for Tissue culture Raised Plants), Shree Aditya Biotech, Vijayapura, Bengaluru Rural District, Bengaluru, India.

For the initiation of explants of Pashanabheda, the details are mentioned below:

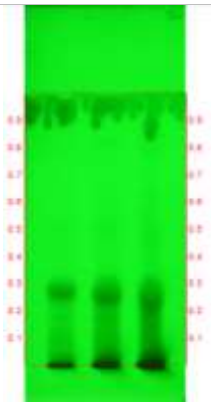
- Explant used – rhizome,
- Media used -MS (1962) solid+5mg 2,4-D.
- Conditions- 25+, 16/8 light, passage time 4-6 weeks.
- Result- callus
- Regeneration of plant lets – Explants- callus.
- Media- MS (1962) solid +0.5 mg BAP, 0.2 mg IAA
- Condition – 25+/-, 16/8, passage time 4-6 weeks

Next, it was subjected to the acclimatization of the plants obtained from the tissue culture method. Acclimatization is the adaptation of plants to a new environment. When tissue culture plants are transferred from the lab to the soil they are exposed to abiotic stresses, like altered temperature, light intensity & humidity conditions & biotic stresses like soil microflora (microbes living in the soil). Then later they were transferred to the soil. The roots for the purpose of analytical study of micro propagated (tissue culture attained) plants of Pashanabheda (*Berginia ligulata, Wall*) was collected at this stage. This was done to compare with an analytical study on roots in between natural habitat plants and tissue culture obtained plants. The HPTLC studies of both roots were conducted at "The SDM centre for research in Ayurveda & allied sciences", Kuthpady, Udupi, Karnataka.

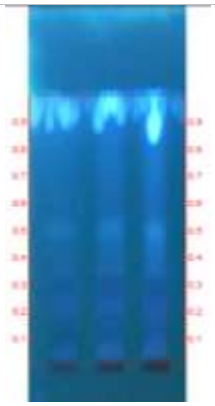
HPTLC:

1gm of a sample of Pashanabheda (*Berginia ligulata, Wall*) roots of both samples was dissolved in 10.0ml of alcohol kept overnight, and filtered. 3, 6, and 9µl of each of the above extracts were applied on a precoated silica gel F254 on aluminium plates to a band width of 7 mm using Linomat 5 TLC applicator. The sample plates were developed in Toluene: Ethyl Acetate: (9.7: 0.3). The developed plates were visualized in short UV, long UV and then derivatised with Anisaldehyde sulphuric acid reagent subsequently scanned under UV 254nm, 366nm, and 620nm (after derivatisation). Rf, the colour of the spots, densitometric scan, and 3-D chromatograms were recorded.

HPTLC photo documentation of ethanol extract of Root of *Bergenia ligulata natural habitat*



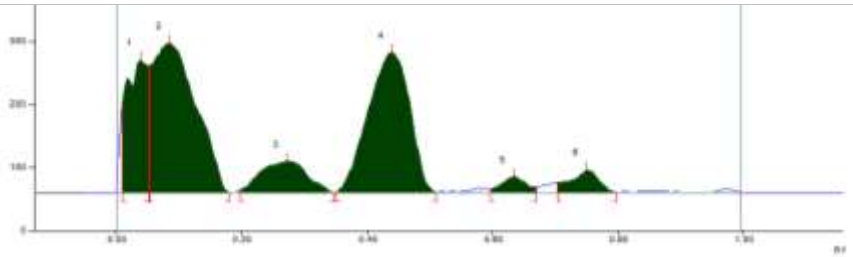
Short UV



Long UV

Post derivatisation

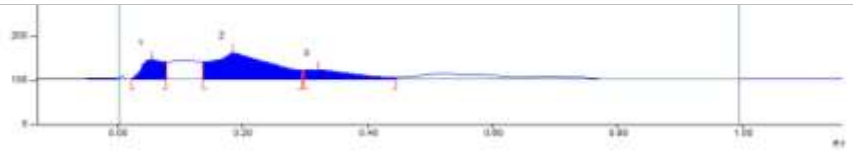
TRACK 1 -Root of *Bergenia ligulata*– 3µl
TRACK 2 -Root of *Bergenia ligulata*– 6µl
TRACK 3 -Root of *Bergenia ligulata*– 9µl
Solvent system – Chloroform: Methanol: Acetic acid (8.0: 1.0:1.0)
Densitometric scan of Root of *Bergenia ligulata*



Track 3, ID: B.ligulata root

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	138.7 AU	0.04 Rf	209.3 AU	26.93 %	0.05 Rf	01.1 AU	4999.2 AU	15.69 %
2	0.05 Rf	201.2 AU	0.09 Rf	235.8 AU	30.33 %	0.18 Rf	0.2 AU	11826.2 AU	37.11 %
3	0.20 Rf	4.6 AU	0.27 Rf	49.9 AU	6.42 %	0.35 Rf	0.0 AU	2648.2 AU	8.31 %
4	0.35 Rf	0.1 AU	0.44 Rf	221.7 AU	28.52 %	0.51 Rf	0.5 AU	10557.0 AU	33.13 %
5	0.60 Rf	5.2 AU	0.64 Rf	25.1 AU	3.23 %	0.67 Rf	8.7 AU	698.9 AU	2.19 %
6	0.70 Rf	15.4 AU	0.75 Rf	35.5 AU	4.57 %	0.80 Rf	0.2 AU	1137.8 AU	3.57 %

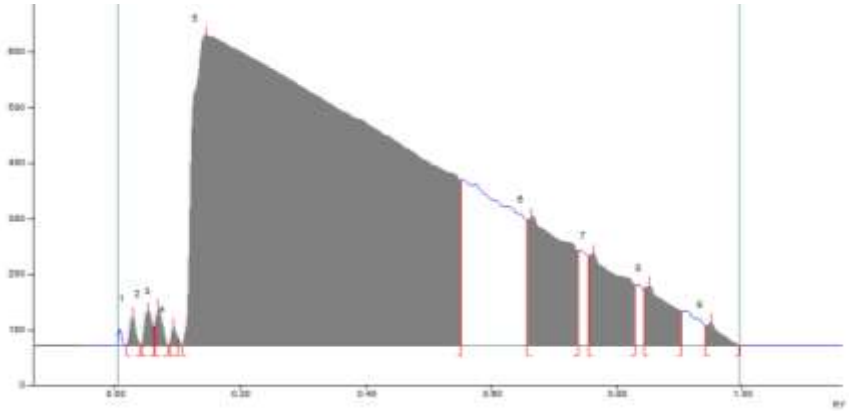
Fig 3a. At 254nm



Track 3, ID: B.ligulata root

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.5 AU	0.05 Rf	43.4 AU	35.06 %	0.08 Rf	38.2 AU	1123.3 AU	17.82 %
2	0.14 Rf	38.7 AU	0.19 Rf	59.0 AU	47.65 %	0.30 Rf	19.9 AU	3990.3 AU	63.30 %
3	0.30 Rf	19.9 AU	0.32 Rf	21.4 AU	17.29 %	0.45 Rf	4.0 AU	1190.1 AU	18.88 %

Fig 3b. At 366nm

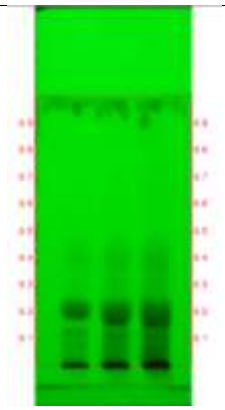


Track 3, ID: B.ligulata root

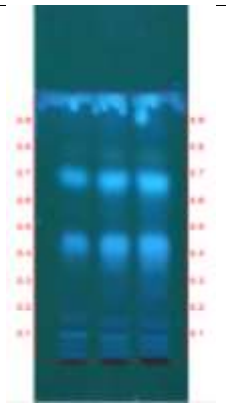
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.9 AU	0.03 Rf	54.0 AU	4.06 %	0.04 Rf	0.6 AU	366.4 AU	0.26 %
2	0.04 Rf	3.6 AU	0.05 Rf	63.2 AU	4.75 %	0.06 Rf	32.0 AU	542.4 AU	0.39 %
3	0.06 Rf	38.5 AU	0.07 Rf	67.0 AU	5.04 %	0.08 Rf	2.0 AU	518.5 AU	0.37 %
4	0.09 Rf	1.4 AU	0.09 Rf	35.0 AU	2.63 %	0.10 Rf	12.2 AU	203.4 AU	0.15 %
5	0.11 Rf	0.8 AU	0.15 Rf	558.9 AU	42.01 %	0.55 Rf	97.7 AU	117468.4 AU	83.99 %
6	0.66 Rf	226.7 AU	0.67 Rf	233.8 AU	17.58 %	0.74 Rf	70.8 AU	10462.3 AU	7.48 %
7	0.76 Rf	162.8 AU	0.76 Rf	167.2 AU	12.57 %	0.83 Rf	09.4 AU	6387.4 AU	4.57 %
8	0.85 Rf	103.4 AU	0.85 Rf	108.4 AU	8.15 %	0.90 Rf	63.3 AU	3189.4 AU	2.28 %
9	0.94 Rf	36.9 AU	0.95 Rf	42.7 AU	3.21 %	1.00 Rf	0.7 AU	715.7 AU	0.51 %

Fig 3c. At 620nm

HPTLC photo documentation of ethanol extract of Tissue culture Root of *Bergenia ligulata*



Short UV

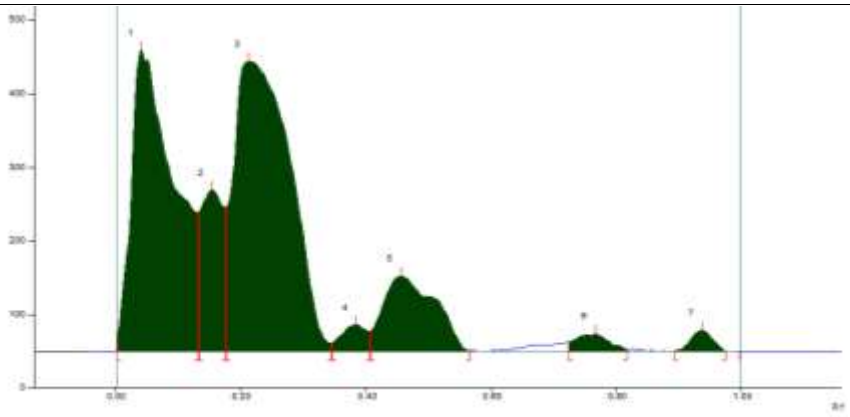


Long UV

Post derivatisation

TRACK 1 -Root of *Bergenia ligulata*– 3µl
TRACK 2 -Root of *Bergenia ligulata*– 6µl
TRACK 3 -Root of *Bergenia ligulata*– 9µl
Solvent system – Chloroform: Methanol: Acetic acid (8.0: 1.0:1.0)

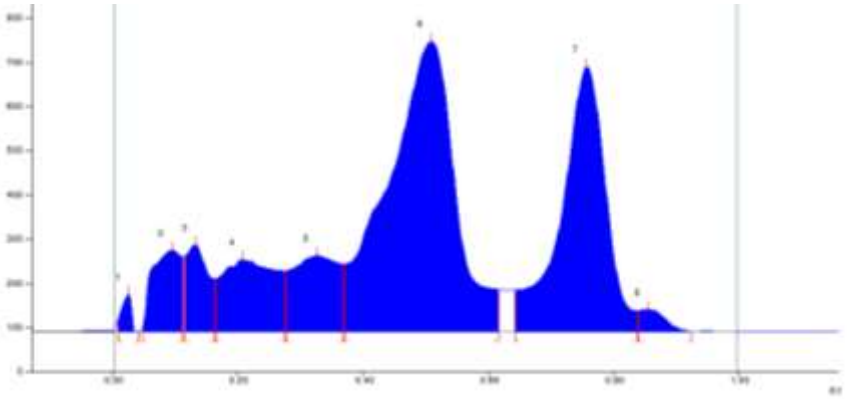
Densitometric scan of Tissue culture root of *Bergenia ligulate*



Track 3, ID: B.ligulata tissue culture

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	12.5 AU	0.04 Rf	409.5 AU	33.75 %	0.13 Rf	88.5 AU	20176.8 AU	33.47 %
2	0.13 Rf	189.1 AU	0.15 Rf	219.3 AU	18.08 %	0.18 Rf	96.0 AU	5566.2 AU	9.23 %
3	0.18 Rf	196.2 AU	0.21 Rf	393.7 AU	32.45 %	0.35 Rf	11.5 AU	25734.4 AU	42.69 %
4	0.35 Rf	11.6 AU	0.39 Rf	35.8 AU	2.95 %	0.41 Rf	27.5 AU	1045.2 AU	1.73 %
5	0.41 Rf	27.6 AU	0.46 Rf	102.4 AU	8.44 %	0.57 Rf	1.2 AU	6141.3 AU	10.19 %
6	0.73 Rf	12.5 AU	0.77 Rf	24.2 AU	2.00 %	0.82 Rf	2.8 AU	907.3 AU	1.51 %
7	0.89 Rf	0.2 AU	0.94 Rf	28.5 AU	2.35 %	0.98 Rf	0.1 AU	715.0 AU	1.19 %

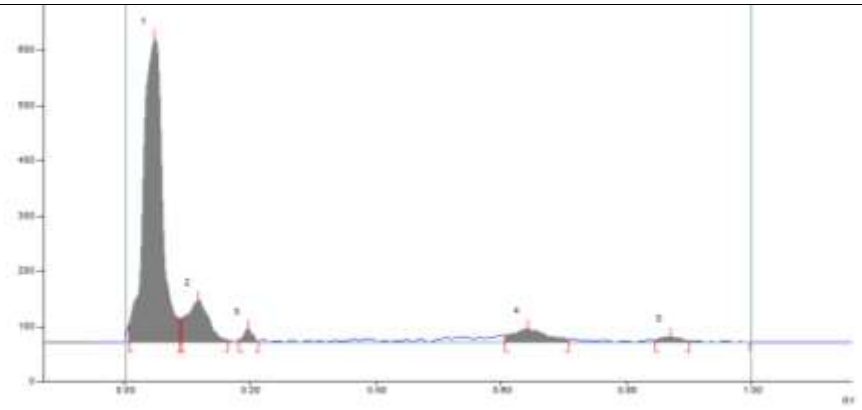
At 254nm



Track 3, ID: B.ligulata tissue culture

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	26.0 AU	0.03 Rf	85.5 AU	4.06 %	0.04 Rf	1.3 AU	948.0 AU	0.83 %
2	0.05 Rf	0.4 AU	0.10 Rf	183.9 AU	8.73 %	0.11 Rf	69.9 AU	6172.8 AU	5.40 %
3	0.11 Rf	170.5 AU	0.13 Rf	197.0 AU	9.36 %	0.16 Rf	19.1 AU	5072.6 AU	4.44 %
4	0.16 Rf	119.1 AU	0.21 Rf	162.5 AU	7.72 %	0.28 Rf	36.6 AU	10151.6 AU	8.88 %
5	0.28 Rf	137.1 AU	0.33 Rf	171.7 AU	8.16 %	0.37 Rf	52.4 AU	9166.9 AU	8.02 %
6	0.37 Rf	152.5 AU	0.51 Rf	656.3 AU	31.18 %	0.62 Rf	95.4 AU	50552.1 AU	44.21 %
7	0.64 Rf	93.9 AU	0.76 Rf	598.2 AU	28.42 %	0.84 Rf	46.8 AU	30770.2 AU	26.91 %
8	0.84 Rf	46.9 AU	0.86 Rf	50.1 AU	2.38 %	0.93 Rf	0.7 AU	1511.0 AU	1.32 %

At 366nm



Track 3, ID: B.ligulata tissue culture

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	32.1 AU	0.05 Rf	551.2 AU	79.82 %	0.09 Rf	42.4 AU	11881.4 AU	77.54 %
2	0.09 Rf	42.8 AU	0.12 Rf	77.4 AU	11.21 %	0.16 Rf	3.6 AU	1941.0 AU	12.67 %
3	0.18 Rf	4.6 AU	0.20 Rf	25.4 AU	3.67 %	0.21 Rf	0.6 AU	264.6 AU	1.73 %
4	0.61 Rf	11.0 AU	0.64 Rf	25.7 AU	3.72 %	0.71 Rf	4.2 AU	975.3 AU	6.37 %
5	0.85 Rf	2.6 AU	0.87 Rf	10.9 AU	1.58 %	0.90 Rf	2.2 AU	259.9 AU	1.70 %

At 620nm

DISCUSSION

The need for the study was to compare the findings between the plants procured from their natural habitat & tissue culture obtained from plants. HPTLC- photo documentaion of ethanol extract of natural habitat roots & tissue culture root of Pashanabheda (*Berginia ligulata, Wall*) is provided with the image at different tracks as 3µl, 6µl & 9µl. The R_f values of the sample of both the roots of Pashanabheda (*Berginia ligulata, Wall*) are highlighted with the solvent system – chloroform-methanol acetic acid (8.0:1.0:1.0). The detailed description of both the roots of Pashanabheda (*Berginia ligulata, Wall*) densiometric scan at 254nm, 366nm & 620 nm is mentioned with graphical presentaion. From the above studies with the comparison of both roots it is found that there is a slight variation with the natural habitat plant & tissue culture obtained plants root w r t HPTLC, R^f values & densiometric scanning. The spots indicate the presence of secondary metabolites present in Berginia ligulata also as berginin.

CONCLUSION

The studies in relation to the comparison between natural habitat & tissue culture obtained from both Pashanabheda (*Berginia ligulata, Wall*) plants from the HPTLC studies it was found that there was slight

variation with respective studies like HPTLC, R_f values & densiometric scanning with variations in secondary metabolites. Tissue culture obatined plants did not show much variation as compared with natural habitat plants with respect to all the studies conducted & henceforth tissue culture plants can opt as the best option when compared with substitues & adulteration. Micropropagation of plants proves to be a boon with respect to conservation, propagation of rare & endangered plant species

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