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Anatomical study for trichomes growth and number (*In vivo* vs. *In vitro*) in *Hemidesmus indicus* (L.) R. Br

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Abstract
Hemidesmus indicus (L.) R. Br commonly called Anantmool belongs to family Periplocea also known as Indian Sarsaparilla. Plant is recorded all parts of India and it is highly valuable in medicinal aspects of Indian system of medicine. It contains Anti-microbial, Anti-cancer, Anti-viral, Anti-inflammatory, Anti-pyretic, Anti-dysenteric actions with Aromatic fragrance. The medicinal important of secondary metabolites are present. The current investigation is found on the anatomical difference *in vivo* and *in vitro* condition in tissue culture medium. Result shows significance difference in both conditioned cultures plants.

Keywords: *Hemidesmus indicus*, sarsaparilla, medicinal plants, culture medium, tissue culture and anatomy

Introduction
Hemidesmus indicus (L.) R. Br. is a twining shrub of family Periploceae (formerly Asclepidaceae). Plant is found from the upper Gangetic plain eastwards to Assam and throughout central, western and southern India (Nadna *et al.*, 2008) [4]. The Moluccas and Sri Lanka are the other places of its distribution (Neeta *et al.*, 2003) [7]. It is highly valued in Indian system of medicine. Extracted plant materials are used in manufacture of Ayurvedic, Unani and Homeopathic medicine (Sahu *et al.*, 2010) [10]. Roots are used as addition in main treatment of snakebite and scorpion sting (Soma V *et al.*, 2007) [14]. As medicine "Anantmool' holds a reputed place in all systems of medicine in India (Soma V et al., 2003 and Neeta *et al.*, 2005) [13, 6]. Due to multiple use of this plant has been indiscriminately collected from its natural habitat and becoming extremely rare by overexploitation (Vijai *et al.*, 2010) [15]. The natives use the roots internally in treatment of premature graying of hairs, jaundice, eye related diseases (Ravishankar *et al.*, 2002) [9].

Description of Plant Morphology
Root is long rigid, cylindrical, little branched, consisting of aligneous center, a brownish corky bark, furrowed and with annular cracks. Stem and Branches are elongate, narrow, twine anticlockwise are profusely laticiferous, narrow, woody and deep purple or purplish brown color with the surface slightly ridged at the node (Nagarajan *et al.*, 2001) [5]. Leaves are simple, petiolate, exstipulate, opposite, entire, apiculate acute or obtuse, dark green above but paler and sometime pubescent below. Leaves of the basal part of the shoot are linear to lanceolate (Ozgur *et al.*, 2009) [8]. Flowers is Small, greenish yellow to greenish purple outside, dull yellow to light purplish inside, axillary, sessile racemes, imbricate with flowers, followed with scale-like bracts. Fruit are two long slender spreading follicles. Seeds: many, flat, oblong, with a tuft of white silky hairs (Anita *et al.*, 2010) [1].
Description of Plant Anatomy: Anatomically transverse sections of the fresh root are circular with a fairly regular outline. It is slightly porous strand of wood at center (Latiporn *et al.*, 2011) [3]. Shows 3-15 layered cork (thick walled reddish brown), 2-3 rows of colorless phellogen, 1-2 rows of narrow thin-walled cells phelloderm, 2-3 layered thick walled polygonal parenchymatous cells with starch grains, prisms of calcium oxalate crystals (Anita *et. al.* 2010) [1]. Cortex – Wide, contains thin walled, large tangentially elongated cells contains simple and compound starch grains, prisms of calcium oxalate. Phloem – Narrow, with sieve tubes, phloem parenchyma, companion cells and uni-seriate medullary rays (Siddique *et al.*, 2008) [12]. Cambium – Narrow, distinct. Xylem Vessels - Scattered with pitted walls, tracheids, thick walled fibers with uni seriate medullary rays (Shanthi *et al.*, 2010) [11].

Material and Methods
Plant Materials
Plants collected from Satpuda forest Nandurbar District.
Collected plants were washed in tap running water with Twenty 20. stem nodal portions were cut off up to 2-3 cm each node and wash in tap water for 30 min. Cut explants were treated with 1 % of antifungal powder (w/v) for 30 min with continuous shaking followed by 3 time wash with sterilized autoclave distill water. Treatment with $HgCl_2$ was given for 5-6 min with shaking and surface sterilization with 70% alcohol for 15-20 seconds. Explants were washed 5 times with sterile distilled water. All procedure was done in under aseptic condition in laminar air flow cabinet. The explants were inoculated on medium.

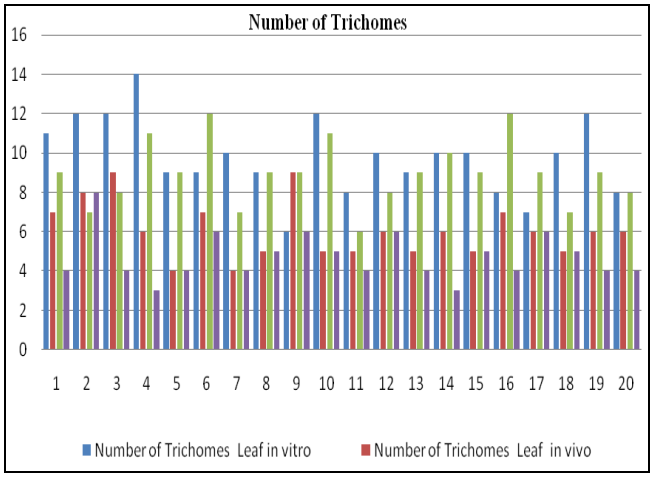
Anatomical Study
The comparative anatomical study was carried out for *in vivo* and *in vitro* plants were 2nd leaf, stem (of 2nd nodal sector), and newly grown root tips of *in vitro* and *in vivo* plants. Transverse sections of leaf, stem, and root were taken and stain with safranine and light green final sections were mount in 50% glycerin. The observations made for microscopic study of leaf trichomes stem trichomes and numbers per microscopic fields. Different 20 field of microscopic, observation of *in vitro* with 20 field of study of *in vivo* was taken.

Result and discussion

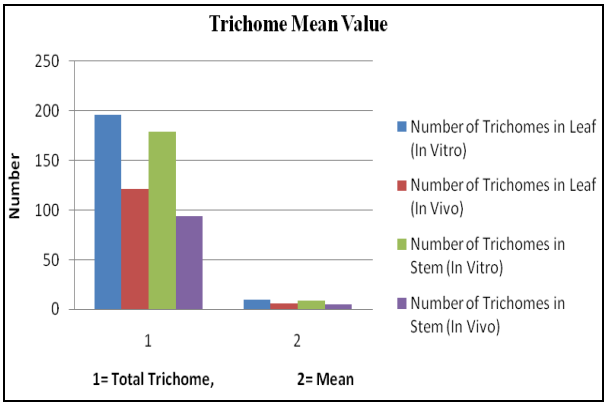
Comparison of number of trichomes *in vivo* and *in vitro*:

Table 1: Number of trichomes in Stem and Leaf of *H. indicus*

No. of Field	Number of Trichomes			
	Leaf		Stem	
	in vitro	*in vivo*	*in vitro*	*in vivo*
1	11	7	9	4
2	12	8	7	8
3	12	9	8	4
4	14	6	11	3
5	9	4	9	4
6	9	7	12	6
7	10	4	7	4
8	9	5	9	5
9	6	9	9	6
10	12	5	11	5
11	8	5	6	4
12	10	6	8	6
13	9	5	9	4
14	10	6	10	3
15	10	5	9	5
16	8	7	12	4
17	7	6	9	6
18	10	5	7	5
19	12	6	9	4
20	8	6	8	4
Tot	196	121	179	94
Mean	9.8	6.05	8.95	4.7



Graph 1: Graphical representation of trichomes in Stem and Leaf of *H. indicus*



Graph 2: Graphical representation for total values and mean value of trichomes in Stem and Leaf of *H. indicus*

In vivo and *in vitro* anatomical observation

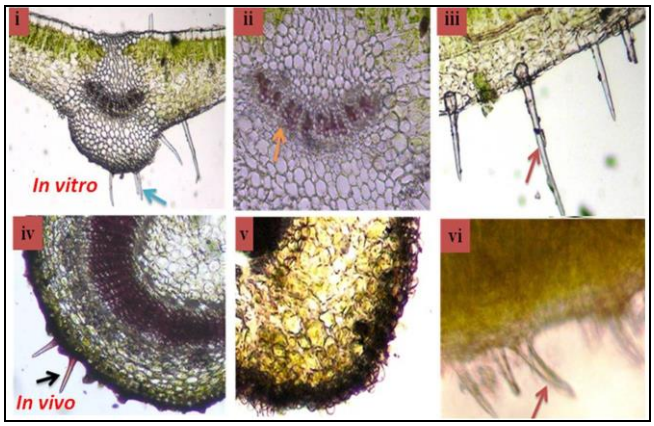


Fig 1: (i, ii, iii) *In vitro* leaf trichomes (iv, v, vi) *In vivo* leaf trichomes of *H. indicus*

Conclusion

Anatomy of leaf, stem and roots are study by transverse sectioning (T.S). Trichomes are of simple unicellular types were observed. The number and size of trichomes are more in leaf and stems of *in vitro* as compared to *in vivo*. This peculiar difference was seen and average numbers of trichomes per microscopic field were recorded. Root of *in vitro* plant shows unicellular, fine outgrowth of hairs roots were *in vivo* not shows such observations.

In this anatomical observation results of more trichomes not are expected in controlled conditions plants. Because increase in leaf pubescence is reduces the level of transcriptions by increasing the reflections of solar radiations, which lower the layer leaf temperature and increase the boundary layer. Were Karabourniotis 1996 [2] reported that the early stages of leaf developments, polyphenol containing trichomes may play a protective role against UV radiation damage.

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