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Research Article

ASSESSMENT OF *IN VITRO* AND *IN VIVO* PHYTOCHEMICAL COMPOSITION OF *ABUTILON INDICUM* (L.) SWEET

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ABSTRACT
Abutilon indicum has been known for its medicinal properties. Various researchers have explored it for chemical constitution. This research is oriented in the same direction beginning with growth of the selected plant species under laboratory conditions by micro propagation on suitable media. Appropriate growth hormones concentration on MS media provides fast induction of callus, shoots and roots removing the barriers faced during *In vivo* growth. Some selected phytochemicals were analyzed in *in vitro* grown plantlets and compared against *in vivo* counterparts. Phytochemical content was more in most cases of *in vitro* samples. Leaf samples showed better results than stem and root samples. It could be said that *In vitro* growth could remarkably enhance the amount of phytochemicals.

Keywords: *Abutilon, Phytochemical Analysis, In Vitro Culture*

INTRODUCTION
By the beginning of 5th century it has been found that various plant material and extracts have medicinal properties (Kay, 1986) which could be due to the presence of one or more of the active chemical components. These could be phytochemicals such as alkaloids, flavonoids and terpenoids lending antimicrobial activities to medicinal plants (Ghosh *et al.,* 2007). Screening of plants for discovery of novel antimicrobial drugs has taken place in recent years**.** Therefore, for further research on medicinal plants, screening for the presence of phytochemicals at primary level can be of great help. The Ayurveda which is traditional system of medicine in India has always emphasized on use of plants and plant derived products for treatment of almost all types of ailments in human and animals. *Abutilon indicum* (Linn) belongs to family Malvaceae and is commonly known as "Country Mallow" (English), "Kanghi (Hindi) and "Atibala" (Sanskrit). Various parts of the plant have been used in treating various human ailments. The plant contains mucilage, tannins, asparagines, gallic acid and sesquiterpenes (Khare, 2004).
Muthu *et al.,* (2006) has reported the use of paste of whole plant which is applied topically to treat cuts and wounds. Jain *et al.,* (2005) reported the use of seed extract and stem powder in birth control and sexual diseases. The methanolic extract of *A. indicum* exhibited some estrogenic potential of antifertility substances (Johri *et al*., 1991). The roots are useful in treating uterine heamorrhagic discharges. Similarly, seeds are used in the treatment of bronchitis, gonorrhoea and piles. Leaves are useful in toothache, lumbago, piles and all kinds of inflammation. Bark is used as antihelmentic, diuretic and alexeteric (Kirtikar and Basu, 1991). The purpose of present study was *in vitro* culturing of *Abutilon indicum,* analysis of phytochemical content of root, stem and leaf extract of plant as well as comparative account of *in vitro* and *in vivo* results.

MATERIALS AND METHODS
Collection of Sample
In vivo plant material was collected randomly from its natural habitat in Sri Ganganagar and *In vitro* was collected from plantlets grown in Plant Tissue Culture laboratory, Department of Biotechnology at Seth G.L. Bihani S.D. (P.G.) College, Sri Ganganagar.
In Vitro Culture
The nodal parts were surface sterilized by preliminary cleaning with tap water, followed by treatment of 0.1% $HgCl_2$ (w/v) for 2-3 minutes. Finally, the nodes were thoroughly rinsed with deionised water from 3-5 times and grown on MS media. Different concentrations of growth regulators were tried. The callus

obtained was grown in Erlenmeyer's flasks of 250 ml capacity containing 50 ml medium having same composition as that of former but growth regulator concentration varied for shooting and rooting.

Chlorophyll Estimation

The pigment content was estimated according to the method of Arnon (1949). Fresh leaves, stems and roots were collected and cleaned. 1g of sample was taken, rapidly crushed and grinded with 80% acetone in pestle and mortar. The homogenate was centrifuged at 5000 rpm for 5 minutes and supernatant was collected.

The procedure was repeated with the pellet obtained and continued till the residue was completely devoid of chlorophyll. The final volume of extract was made up to 10 ml by adding 80% acetone. Absorbance was taken at 645 nm, 652 nm and 663 nm. Amount of different pigments was calculated by Arnon equations.

Lipid Estimation

1g of fresh sample was taken and crushed in pestle and mortar with 4-5 ml of chloroform: methanol (2:1) mixture. Crushed sample was kept undisturbed overnight. The contents were filtered through a sintered glass funnel (Grade 3).

The residue was given 2-3 washings with same mixture. All the filtrates and washings were pooled to give a final volume of 20 times the volume of sample. Crude extract of lipids was obtained and subjected to Folch washings (Folch *et al.,* 1957) for removal of water soluble impurities.

The lower layer contained lipids and the percentage of total lipids was determined by evaporating 1ml of aliquot of this extract.

Protein Estimation

500 mg of fresh sample was extracted with 5 ml of 5% TCA. The homogenate was centrifuged at 2000 rpm for 20 minutes and supernatant discarded. The residue was dissolved in 10 ml of 0.1 N NaOH. To 0.1 ml of this solution, distilled water was added to get 1 ml of diluted sample. Protein content was estimated according to the method of Lowry *et al.,* (1951).

Starch Estimation

Fresh sample was prepared by grinding 500 mg of plant material with 10 ml of 80% ethanol followed by centrifugation at 2000 rpm for 20 minutes. Supernatant was collected and starch content was estimated by Anthrone - Sulphuric acid method (Hedge and Hofreiter, 1962).

Phenol Estimation

Phenol content was estimated with Folin-Ciocalteau's reagent method given by Singleton *et al.,* (1999). Briefly, the reaction mixture was prepared by mixing 0.5 ml of methanolic solution (1 mg/ml) of extract, 2.5 ml of Folin-Ciocalteau's reagent (10%) and 2.5 ml of $NaHCO_3$ (7.5%).

The samples were incubated at 45˚C for 15 min. The absorbance was determined at 765 nm taking gallic acid as standard.

Carbohydrate Estimation

500 mg of fresh tissue was homogenized with 10 ml of 80% ethanol and centrifuged at 2000 rpm for 20 minutes. The supernatant was collected and carbohydrate content was estimated by method of Dubois *et al.,* (1956).

IAA Estimation

500 mg fresh tissue was homogenized with 10 ml of 80% ethanol and centrifuged at 2000 rpm for 20 minutes. The supernatant was collected and estimated by method of Mahadevan and Chandra Mohan (1966).

Aliphatic Amino Acids Estimation

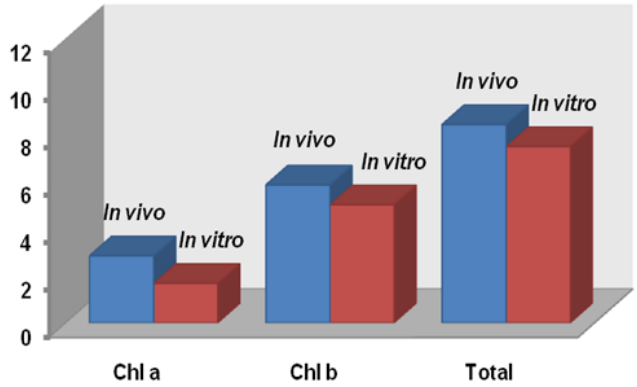
500 mg fresh tissue was homogenized with 10 ml of ethanol: water (1:1) mixture followed by centrifugation at 2000 rpm for 20 minutes. To 1 ml of supernatant, ethanol: water was added to make up volume to 5 ml.

To 1ml of diluted sample, 2 ml of ninhydrin reagent (0.005%) was added, heated in water bath for 15 minutes until purple color appears. Optical density was measure at 575 nm after cooling the tubes. Standard curve was prepared with 0.5 mg/ml of Glycine made in same mixture.

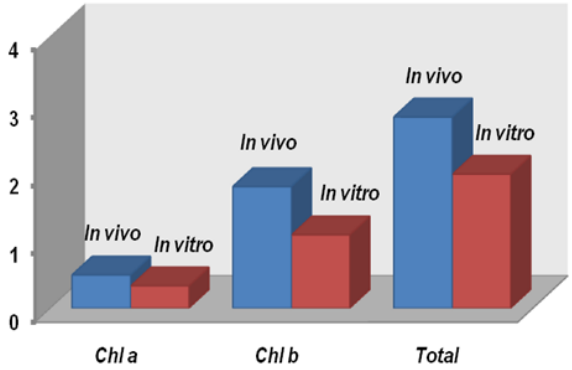
RESULTS AND DISCUSSION

Chlorophyll Estimation

The chlorophyll content was estimated using stem, leaf and root of both *In vivo* and *In vitro* grown plants. *In vivo* samples showed higher amounts of each type of chlorophyll, viz. chla, chlb and total chlorophyll (Graph 1, 2). Total chlorophyll was maximum in *In vivo* leaf (8.37 mg/g) and minimum in *In vitro* stem (1.97 mg/g). Chlorophyll *a* is the molecule found in all plant cells and therefore, its concentration is to be reported during chlorophyll analysis. Chlorophyll *d* is found only in marine red algae, but chlorophylls *b* and *c* are common in fresh water (Carlson and Simpson, 1996).



Graph 1: Comparison of Chlorophyll Content (mg/g) in Leaf of *In Vivo* and *In Vitro* Grown *Abutilon*



Graph 2: Comparison of Chlorophyll Content (mg/g) in Stem of *In Vivo* and *In Vitro* Grown *Abutilon*

Lipid Estimation

Graph 3 reveals that lipid contents vary with the plant material taken as well as conditions of growth. *In vitro* grown leaf had highest percentage of 8.3 while *In vivo* grown root had lowest lipid content of 0.7 %. Despite the early use of chloroform in extracting lipids (Bornmann, 1931), the greatest improvement of the extraction of polar lipids from animal tissues was made when Folch (1957) his classical extraction procedure.

Protein Estimation

Tables 1 and 2 show variation of protein quantity in different plant samples under different growth conditions. Highest protein was observed when plant was cultured *In vitro* and stem sample was processed for analysis. The protein content ranged from 0.23 mg/ml of root extract to 0.814 mg/ml of stem extract. Yadav and Dogra (1992) analyzed the seed protein of twelve cultivars of Indian wheat

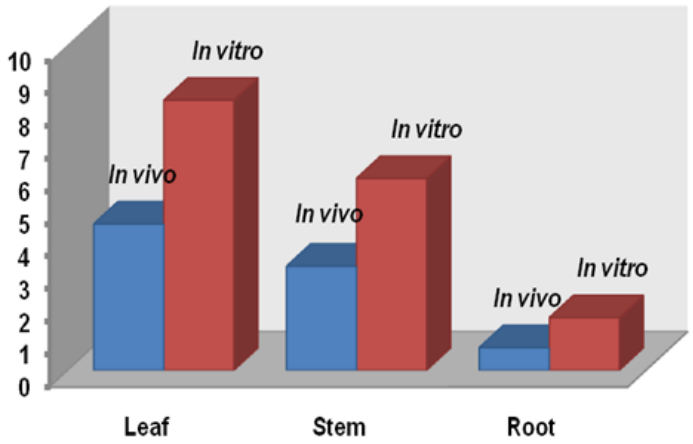
(*Triticum aestivum* L.) using polyacrylamide gel electrophoresis which made possible the classification of these cultivars.

Table 1: Phytochemical Analysis of *In Vivo* Grown *A. indicum*

Phytochemical Analyzed (Units)	Leaf	Stem	Root
Chlorophyll (mg/g of fresh wt.)			
Chl. a	2.83	0.49	-
Chl. b	5.82	1.79	-
Total	8.37	2.81	-
Lipids (%age)	4.5%	3.2%	0.7%
Proteins (mg/ml)	0.5	0.7	0.23
Starch (mg/ml)	0.035	0.043	0.019
Phenol (mg/ml)	2.1	1.0	0.6
Carbohydrates (mg/ml)	4.3	3.8	3.2
Indole acetic acid (mg/ml)	0.0083	0.078	0.056
Aliphatic amino acids (mg/ml)	0.47	0.18	0.14

Table 2: Phytochemical Analysis of *In Vitro* Grown *A. indicum*

Phytochemical Analyzed (Units)	Leaf	Stem	Root
Chlorophyll (mg/g of fresh wt.)			
Chl. a	1.67	0.33	-
Chl. b	4.98	1.08	-
Total	7.44	1.97	-
Lipids (%age)	8.3%	5.9%	1.62%
Proteins (mg/ml)	0.69	0.814	0.36
Starch (mg/ml)	0.047	0.055	0.027
Phenol (mg/ml)	2.9	1.46	0.922
Carbohydrates (mg/ml)	5.74	4.43	3.68
Indole acetic acid (mg/ml)	0.009	0.084	0.060
Aliphatic amino acids (mg/ml)	0.588	0.24	0.216



Graph 3: Comparison of Lipid Content (%) in *In Vivo* and *In Vitro* Grown *Abutilon*

Starch Estimation

The results are depicted in Tables 1 and 2. In *In vivo* plant, stem showed maximum content (0.043 mg/ml) whereas root showed minimum content (0.019 mg/ml). In *In vitro* plant, stem showed maximum content (0.055 mg/ml) whereas root showed minimum content (0.027 mg/ml).

Phenol Estimation

The content was more in each plant part taken from *In vitro* grown *Abutilon.* The leaf extract from micropropagated plant had maximum phenolic concentration of 2.9 mg/ml (Table 2).

Carbohydrate Estimation

The content ranged from 3.2 mg/ml in root to 4.3 mg/ml in leaf of *In vivo* cultured plant. In case of *In vitro* culturing, content ranged from 3.68 mg/ml in root to 5.74 mg/ml in leaf indicating the photosynthetic process going in leaves (Tables 1, 2). Many sugars were detected in developing and maturing seed of pigeonpea. Sucrose was the predominant sugar at early stages and glucose level rise steadily with development (Khattra and Kaur, 1998).

IAA Estimation

The result of IAA estimation has been summarized in tables 1 and 2. Each of leaf, stem and root samples showed marginal variation in content of Indole Acetic Acid when taken from natural habitat and when grown by tissue culture. Stem had highest IAA content followed by root and lastly by leaf extract.

Aliphatic Amino Acids Estimation

Aliphatic amino acids content in *In vivo Abutilon* was estimated to be 0.47 mg/ml (leaf), 0.18 mg/ml (stem) and 0.14 mg/ml (root) and in case of *In vitro* growth, content was 0.588 mg/ml, 0.24 mg/ml and 0.216 mg/ml, respectively (Table 1, 2). Goswami and Yadav (1994) evaluated nutritive content in *Capparis decidua* and *Ziziphus mauritiana* indicating the seasonal variations. Amino acid and IAA contents were noted to be maximum in stem and leaf, respectively.

Conclusion

Abutilon In vivo and *In vitro* phytochemical results showed different variations regarding different parameters. *In vivo* samples had high amounts of each type of chlorophyll when comparison was made with corresponding *In vitro* samples. 8.3% total lipids were found to occur in leaf samples of micropropagated plant showing maximum content among all sample types. Leaf, stem and root extracts obtained from *In vitro* growth were found to possess greater protein and starch content than *In vivo* samples and stem samples had highest content 0.814 mg/ml and 0.055 mg/ml, respectively. Likewise, leaf extracts were observed to have highest phenol and carbohydrate content during both types of growth but *In vitro* growth showed higher content in comparison to *In vivo*. *In vitro* stem had highest IAA content (0.084 mg/ml) followed by root (0.060 mg/ml) and lastly by leaf extract (0.009 mg/ml). Aliphatic amino acids content ranged from 0.14 mg/ml to 0.47 mg/ml in *In vivo Abutilon* and from 0.216 mg/ml to 0.588 mg/ml in case of *In vitro* growth. *In vitro* growth could remarkably enhance the amount of phytochemicals. Except the chlorophyll content, *In vitro* growth gave better results than *In vivo* growth.

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