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Evaluation of phytochemical constituents and In-vitro regeneration of *Angelonia angustifolia* (L.): A rich source of secondary metabolites.

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ABSTRACT

In this study, evaluation for the phytoconstituents was carried out. The protocol for callus induction and regeneration in *Angelonia angustifolia* L. was standardized. Young apical leaves and nodes were used as explants for callus induction on Ms Medium containing 2, 4-D and Kinetin, 2, 4-D and BAP, IAA and BAP and IBA and Kinetin in different concentrations. The maximum percentage of callusing was observed on the medium supplemented with 0.5mg/L IBA and 0.5mg/ Kinetin was found to be 100% for leaf & 100% for node explants. The calli in most of the cultures were whitish green and soft in nature. Initiation of shooting of *Angelonia angustifolia* established from leaf explants on MS medium supplemented with combination of hormones IAA 0.4 mg/L & IBA 0.4 mg/L. This study was aimed to develop standard protocol for callus induction, protocol for organogenesis & standardization of media and growth hormonal concentrations, which may helps in conservation and cultivation of this species. This plant is also the ware house of secondary metabolites and therefore callus will be the source of extraction of these many secondary metabolites for the therapeutic drugs.

Keywords: *In-vitro*, Regeneration, Organogenesis, Phytochemicals, *Angelonia angustifolia*..

INTRODUCTION

The present century has witnessed the emergence of herbal therapy as a branch of modern medicines. Herbal plants have become a fascination for the present day Homo sapiens. They had a good knowledge of herbals. This knowledge is now exploited for manufacturing of the herbal medicines. The history of herbal medicines is as old as human civilization and their dependency on the plants is for their survival and well being. Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits.

In-vitro Micropropagation is an important tool from rapid multiplication of medicinal plants [1-2]. *In-vitro* culture is one of the best and most successful examples of commercial application of plant tissue culture technology. The capability to regenerate and propagate plants from cultures cells and tissues is one of the most exciting and useful aspects of *In-vitro* cell and tissue culture. Increasing demand of those plants, which are specially use for the food and medicine, is one of the cause of their rapid depletion from the natural habitats. *In-vitro* micropropagation provides a great potential for conservation and large-scale multiplication of such useful species and subsequent exploitation as well as for the extraction of active ingredient. Thus, the exploration of tissue culture technique in medicinal plant is indeed desirable. Therefore, the whole world is diverting towards the multiplication of these plants. Besides preventing from depletion of stocks of wild plants, the contamination of plant material may lead to inferior quality of product. Tissue culture is one way by which plant material can be supplied in a pure from and continuously throughout the year [3].

Angelonia angustifolia L. is a probe for anti-inflammatory properties due to its uses in traditional latin American medicine. Its importance as a medicine ranges from anti-inflammatory, analgesic, anti-hyperlipidemic etc.The high concentration of Lupeol found to be present in aerial parts of this plant. Although this plant is a rich source of several secondary metabolites. Therefore it has attracted the attention of Botanists, Chemists, and Pharmacologists because of its medicinal importance in Ayurvedic mixture. In nature, seed production in this plant is irregular, with a low germination percentage due to the impermeability of the integument. It is highly demanded by the different Pharmaceutical companies. Little work done on *in-vitro* regeneration of *this plant*. Keeping entire importance of taxa in mind decided to do *In-vitro* Micropropagation of it. The present study was undertaken to examine the potential of different explants with different concentrations of hormones in combination, to rapid initiation of callus and regeneration.

METHODOLOGY

Angelonia angustifolia L . plant used in the present study was collected from the wild population from Nawegaon Bandh, dist. Bhandara (MS). More ever the medicinal importance of the plant has also been documented. Different explants were used for establishing callus including apical leaf and nodes. Inoculation was done on agar solidified MS[4] medium Supplemented with different concentration of IBA, Kinetin & BAP in combination. All media contained 3% sucrose & 1% agar with pH 5.8 adjusts before sterilization. All cultures were maintained at 27 ^{0}C with 16-18hr. photoperiod.

Preliminary phytochemical analysis.

Preliminary phytochemical screening of plant was done following the standard procedures adapted by the various workers like Daniel M. (1991)[5], Harborne (1998)[6] Phillipson (2000)[7] and Kokate et. al. (2004)[8].

Quantitative and Qualitative Phytochemical screening

Quantitative and Qualitative Phytochemical screening of plant was done according to standard procedures. Qualitative analysis of some phytochemicals such as alkaloids, flavanoids, phenolics, saponins, steroids, glycosides were done by employing Thin Layer Chromatographic technique Wagner *et. al.,* (1996)[9] Harborne (1998)[6]).Whereas quantitative chemical

analysis of Alkaloids, Phenolics, Flavonoids and Saponins were done by different methods [10,11].

RESULTS AND DISCUSSION

In this investigation, many phytochemicals have been found to be present. Thin layer chromatographic study was done for confirmation of exact chemical compound present in the plant. In present investigation, after preliminary investigation, TLC of the samples was carried out (Table 1). Thus, in all the species, the variation in number of various phytochemicals was observed. This variation was reflected in number of bands in the TLC plates. This might be due to the fact that different environmental conditions affect the synthesis of different chemical constitution of a species[12].

Important phytochemicals like flavonoids, phenolics, saponins, and alkaloids were detected qualitatively so, an effort was made to quantify the same in the different plant parts (leaf, stem and root). In leaf of plant flavonoid content was found to be highest i.e., 319 and it was followed by stem and then by root. Phenolic was also more in leaf i.e. 216 mg/g in comparison to stem and root (Table 2). Saponin was well distributed in all parts; highest content of 290mg/g was found in leaf. However, alkaloid was only 298 mg/g in leaf, 97 mg/g in stem and 110 mg/g in root. The content of the phytochemicals can be enhanced with the use of elicitors and can be made commercially available.

The MS medium supplemented with various concentration of 2, 4-D and Kinetin, 2, 4-D and BAP, IAA and BAP and IBA and Kinetin in different concentrations inducing callusing. The MS medium supplemented with all this combination showed brown and soft and brown white callus induction. The maximum percentage of callusing was observed at the medium supplemented with 0.5 mg/L IBA and 0.5 mg/L kinetin was found to be 100% for apical leaf & 100% for node explants.

Table 1: Quantitative data of various phytochemicals in different plant parts.

Plants → / Sec. metabolyte	*Angelonia angustifolia* **L.**		
	Leaf	Stem	Root
Flavonoids (mg/g)	319 mg	210 mg	175 mg
Phenolics (mg/g)	216mg	116mg	75mg
Saponins (mg/g)	290 mg	210 mg	245 mg
Alkaloids(mg/g)	298mg	97 mg	110mg

Table 2: Data on TLC analysis of some phytochemicals in different plant parts of *Angelonia angustifolia* L.

Chemical name	**Plant part**	**No. of bands**	**Rf values**
Alkaloids	Leaves	3	0.794, 0.971, 0.758.
	Stem	3	0.794, 0.971, 0.758.
	Root	1	0.971.
Flavonoids	Leaves	2	0.103, 0.275.
	Stem	4	0.096, 0.331, 0.965, 0.448.
	Root	3	0.110, 0.344, 1.
Phenolics	Leaves	5	0.038, 0.053, 0.184, 0.469, 0.869.
	Stem	4	0.038, 0.146, 0.184, 0.869.
	Root	3	0.038, 0.869, 0.876
Saponins	Leaves	2	0.669, 0.848.
	Stem	2	0.669, 0.712.
	Root	1	0.669.

Table No.3: Induction of callus on MS media supplemented with different concentration of hormones.

Sr. No.	Hormone concentrations	Explants Used	% of Callus induction	Duration of induction Of callus in days	Colour and Nature of the callus
1	0.4 mg/L 2,4-D + 0.4 mg/L Kinetin	Apical Leaf	72 %	16	Brown and Soft
		Node	-		
2	0.5 mg/L 2,4-D + 0.5 mg/L Kinetin	Apical Leaf	100 %	15	Brown and Soft
		Node	20 %	15	Brown and Soft
3	0.6 mg/L 2,4-D + 0.6 mg/L Kinetin	Apical Leaf	90 %	12	Brown and Soft
		Node	-		
4	0.6 mg/L 2,4-D + 0.6 mg/L BAP	Apical Leaf	90 %	13	Brown and Soft
		Node	-		
5	0.4 mg/L IAA + 0.4 mg/L BAP	Apical Leaf	100 %	9	White and Soft
		Node	-		
6	0.5 mg/L IBA + 0.5 mg/L Kinetin	Apical Leaf	100 %	15	White and Soft
		Node	100 %	15	White and Soft

Table 4: Effect of different concentration of hormones on shoot regeneration.

Sr. No.	Hormonal concentration	No of shoot per treatment	Shoot length in cm.	Shoot morphology
1	0.4 mg/L IAA + 0.4 mg/L BAP	6	2.5	Green & long
		5	2.0	Green & long
2	0.5 mg/L IBA + 0.5 mg/L Kinetin	2	1.5	Thin Short
		3	2.0	Thin Short

100% callus induction for the apical leaf was found to be in MS medium supplemented with 0.5 mg/L 2,4-D + 0.5 mg/L Kinetin, 0.4 mg/L IAA + 0.4 mg/L BAP and followed by MS medium supplemented with 0.6 mg/L 2,4-D + 0.6 mg/L Kinetin i.e. 90% and 0.4 mg/L 2,4-D + 0.4 mg/L Kinetin i.e. 72% respectively. Apical leaf explants were found to be more responsive for the induction of the callus than that of the nodal explants in all hormonal combination which were tested (Table 3 and photo plate 1).

Toker. *et. al.,[13]* studied the formation of callus using different type of explants like stem, root, leaf and seed of *Ecbollium elaterium* where seed and root explants did not yield callus at all while, stem node and leaf explants formed the callus to a lesser extent. Thus the differential response of various explants can be attributed to differences in cultural requirements of explants and also the variation in endogenous hormone level [14].

Further studies were carried out for shoot regeneration capacity by using apical leaf explant callus. Shoot were initiated from indirect organogenesis. The best result of shooting (2.5 cm) was observed with MS medium supplemented with the combination of 0.4mg/L IAA and 0.4mg/L BAP after 13th day with good and long morphology in which 36 shoot per treatment wear recorded. Followed by shoot length (2 cm) was recorded (Table 4). Tissue culture provides the best approach for preservation and multiplication of medicinal herbs. Bera and Roy [15], proposed the plant tissue culture as a tool for rapid multiplication of plants. Advantages of *in vitro* culture method lie in its ability to produce huge number of true type individuals in a short time and limited space. Tissue cultural techniques a means for conserving and multiplying medicinal plants have been reported by Le, Nin, *et. al.,* and Wawrosch *et.al.,*

CONCLUSION

The Phytochemical analysis exhibited the medicinal potential of *Angelonia angustifolia* L.

Medicinal properties of the plant are due to their active principles which are nothing but chemical compounds of importance present in them. In the present investigation it was found that phenols, alkaloids, tannins, flavonoids, saponins, steroids are present in leaves, stem and root of the plants. TLC analysis also confirmed these results. Quantitative analysis of stem, leaves and root indicated the concentration of phenols to be maximum in the leaf part of the plants. However, alkaloids, saponins and flavonoids are present in all parts, but their quantity is variable. The content of these metabolites can be enhanced further commercially with elicitors. The present investigation also support the plant to be "a warehouse of chemo-diversity", which can be used economically as a useful source of medicine.

Plant having medicinal importance where collected from wild condition. This lead to gradual depletion due to in discriminate collection. This wild population depletion can be prevented by cultivating such plant for commercial use through In-vitro micropropagation. From the all types of explants collected from *Angelonia angustifolia* that is apical leaf, and nodes, apical leaf was found to be better for callusing on 0.5 mg/L IBA and 0.5 mg/L kinetin supplemented in MS medium. Shoot were induced at concentration 0.4mg/L IAA and 0.4mg/L BAP after 13th day from apical leaf explants callus indirectly.

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