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Research Article

IN VITRO CALLOGENESIS AND PHYTOCHEMICAL ANALYSIS OF ARISTOLOCHIA BRACTEOLATA LAM – A MULTIPOTENT MEDICINAL PLANT

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ABSTRACT

The present study was aimed to produce rapid *invitro* callogenesis in *Aristolochia bracteolata* Lam. The protocol has been standardized using leaf explant. *Aristolochia bracteolata* belonging to the family Aristolochiaceae is a multipotent medicinal herb used by traditional healers to treat several diseases as Anti-inflammatory, antipyretic, antimicrobial, antidote and antihelminthic. In order to formulate the micropropagation strategies of this medicinal plants, with various parts of the plant has been used for callogenesis and rapid proliferation was obtained from leaf explants on Murashige and Skoog (MS) medium supplemented with 2-4-D (0.5 mg/l to 1.0 mg/l). The callus possessed a very high regenerative potential was referred to as 'stock callus'. The extract of leaf callus of *Aristolochia bracteolata* Lam revealed the presence of tannins, flavonoids, glycosides, terpenoids, saponins and steroids.

INTRODUCTION

Medicinal plants have been an integral part of life in various regional communities for food and drug both. India has more than 3,000 years of medicinal heritage based on medicinal plants. Medicinal plants are largely used by all divisions of the population either directly as folk medications or indirectly in the preparation of recent pharmaceutical drugs Pushpagandhan *et al.,* (1995). Plants are used medicinally in different countries and are a source of many potent and powerful drugs. The use of medicinal plants to treat human diseases has its roots in ancient days. India is endowed with a rich wealth of medicinal plants, (*Aristolochia bracteolata*) with a view to increasing the wide range of medicinal usages, the present day entails new drugs with more potent and desired activity with no side effects against particular disease.

Aristolochia bracteolata Lam (Aaduthinnapalai – Tamil; Worm killer – English; Gadaparku – Telugi; Bhringi – Hindi) is a herbaceous perennial medicinal plant with cordate leaves and dark purple colour tubular flowers belonging to the family Aristolochiaceae. The whole plant was used as a purgative, antihelminthic, antipyretic and anti-inflammatory. The plant contain Aristolohic acids has many medicinal properties for various diseases Kirtikar *et al.,*(1935). The plant is used in traditional medicine as a gastric stimulant and in the treatment of cancer, lung inflammation, dysentery and snake bites (Negi *et al.,* 2003), antimicrobial activity (Manikandar *et al.,* 2006) antiarthritis activity (Havagiray *et al.,* 2009), antiallergic activity (Chitme *et al.,* 2010) and antioxidant property (Kalpana devi *et al.,* 2011).

Though literature survey showed that much work has not been reported from leaf callus. Multiple uses, rising demand, and destructive harvesting have led to the shrinking of natural population of Ar*istolochia bracteolata* considering the above fact in mind the present study focuses on developing an efficient standardization of leaf explants for callus initiation, rapid massive callus growth, various factors affecting callogenesis and different morphological structure of callus in different concentration of growth regulators and phytochemical analysis of leaf and leaf callus of *Aristolochia bracteolata*.

MATERIALS AND METHODS

The wild plants of *Aristolochia bracteolata* were brought to the herbal garden and grown. The healthier plants of *Aristolochia bracteolata* Lam were collected from the herbal garden of A.V.V.M Sri Pushpam College, Poondi, Thanjavur, Tamil Nadu, India. Different parts like shoot tips, nodal and internodal regions, petioles and young healthy leaves were used as explants. Among the various explants leaf explants were well responded to MS media with different hormones for callus formation.

Aristolochia bracteolata belongs to the family Aristolochiaceae and it is commonly known as worm killer. Tamil name- Aaduthinnapalai.

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Traditional uses of plant parts

Leaves	Anti-inflammatory, Dermatitis, rashes, skin disease, Scorpion sting, antipyretic, snake bite, antiulcer, Amenorrhoea, antihelmintic, antiplasmodial
Whole plant	Dermatitis, leprosy, jaundice, worms, fever, mosquito repellent, anodyne, purgative, emmenagogue
seeds	antibacterial, anti-inflammatory, analgesics, toxic of goats
roots	syphilis, gonorrhea, skin diseases, eczema

Initiation of aseptic cultures

Sterilization

The leaf explants were washed with running tap water for 4 – 5 times for 15 minutes. The leaves were surface sterilized with 0.1% solution of mercuric chloride (sigma chemicals Co, St Louis, Mo) for 10 minutes, stirred for 2 minutes and then washed in tap water.

Leaves are then taken in petriplates into laminar air flow chamber and surface sterilized by 2% sodium hypochloride solution for 1 minutes and rinsed in double distilled sterile water. Finally leaves were cut into small pieces of 1 cm and trimmed.

The sterilized and trimmed leaf explants were inoculated on MS medium with 3% sucrose and various combinations of growth regulators: pH was adjusted to 5.7 with 1N NaOH or 1N HCl was solidified with 0.8% of agar. This medium is used for callus propagation. The medium was autoclaved at 120°C for 20 minutes. All the cultures were incubated at 23°C ± 2°C under 16 hrs photoperiod by cool white fluorescent light and 70 ± 5% relative humidity various phytohormones were used .

Transfer of explants as well as subculturing was done aseptically in the laminar air flow chamber. Fresh weights of the callus at every five days interval upto the cessation of callus growth was measured. The mean of ten replications expressed with standard deviation was considered to prepare growth curve.

Phytochemical screening (Harborne, 1988)

The phytochemical analysis were carried out to know the chemical constituents and compared them between leaves and leaf callus of *Aristolochia bracteolata* Lam. The methanolic extracts of leaves of the plant and callus obtained from leaf explants in MS media supplemented with 2-4-D (0.50 mg/L) were subjected to following different chemicals tests separately to find out the nature of phytochemical constituents.

Test for alkaloids

0.5 ml of methanolic extracts was mixed with 8ml of 1% HCl, warmed and filtered. 2ml of filterate were treated separately with Maeyar's reagent and Dragendroff's reagent. After which it was observed whether the alkaloids were present in the appearance of cream and orange colour precipitates in response to the above reagents respectively.

Test for tannins

About 20ml of filtered extract was taken in a test tube and 2ml of ferric chloride added. The presence of blue – black coloured precipitate indicates the presence of tannins.

Test for saponins

To 0.5ml of extract was added 5ml of distilled water in a test tube. The solution was shaken vigorously and observed for stable persistent forth.

Test for terpenoids (Salkowski test)

To 0.5ml each of the extract was added 2ml of chloroform. Concentrated sulphuric acid 3ml was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Test for cardiac glycosides

To 2ml of extract 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayered with 1ml of sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Test for anthraquinone

0.5ml of the extract was boiled with 10ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipetted into another test tube observed for colour changes.

Test for flavonoids

Dilute ammonia 5ml was added with the extract. Concentrated sulphuric acid 1ml was added. A yellow colouration that disappears on standing indicates the presence of flavonoids.

Test for steroids

To 1ml extract added with 10ml of chloroform was added. 10ml of concentrated sulphuric acid was added carefully to form coloured layer. Upper layer turns red, sulphuric acid layer form yellow with green fluorescence, indicates presence of steroids.

Test for phenolic

2ml of extract 1ml of ferric chloride was added, a blue or green colour indicates presence of phenols

Table-1 Qualitative phytochemical analysis of leaf (*invitro*) and leaf callus of *Aristolochia bracteolata* Lam. obtained on MS medium supplemented with 2,4-D (0.50 mg/l)

S.No	Tests	Leaf	Leaf Callus
1.	Alkaloids	+	+
2.	Tannins	+	-
3.	Saponins	+	+
4.	Terpenoids	+	_
5.	Cardiac Glycosides	_	_
6.	Anthraquinone	+	_
7.	Flavonoids	+	+
8.	Steroids	+	+
9.	Phenol	+	_

RESULT AND DISCUSSION

Among the various explants used young moderate sized leaf explants were well responded for rapid callogenesis after incubation period of about three weeks.

Table 2 – Effect of auxins and cytokinins on the growth (fresh weight) and nature of leaf callus in *Aristolochia bracteolata* Lam.

S.No	MS + Hormones (mg/L)	DAI	Fresh weight of callus in gms.	Nature of callus
1.	MS + IAA (2.0 mg/L) + BAP (1 mg/L)	28	0.5	Y, S and S
2.	MS + 2,4-D (0.50 mg/L)	20 - 25	3.0	Y, T and S
3.	MS + 2,4-D(0.50 mg/L)	25 - 35	5.0	LGR, Y and H
4.	MS + 2,4-D(0.50 mg/L)	35 - 45	12.0	Y and H
5.	MS + 2,4-D(0.50 mg/L)	45 - 55	15.0	LGR and N
6.	MS + 2,4-D(0.50 mg/L)	55 - 65	19.0	DGR and H
7.	MS + 2,4-D(0.50 mg/L)	65 - 75	22.0	DGR and H
8.	MS + 2,4-D(0.50 mg/L)	75 - 85	24.2	DGR and H
9.	MS + 2,4-D(0.50 mg/L)	85 - 95	25.0	DGR and H
10.	MS + 2,4-D(0.50 mg/L)	95 and above	25.0	H and BR
11.	MS + 2,4-D (1.0 mg/L)	20 - 25	3.0	Y, C and S
12.	MS + 2,4-D(1.0 mg/L)	25 - 35	4.5	Y,T and S
13.	MS + 2,4-D(1.0 mg/L)	35 - 45	7.0	Y and H
14.	MS + 2,4-D(1.0 mg/L)	45 - 55	12.0	Y and H
15.	MS + 2,4-D(1.0 mg/L)	55 - 65	16.0	LGR and N
16.	MS + 2,4-D(1.0 mg/L)	65 - 75	17.0	H,N and DGR
17.	MS + 2,4-D(1.0 mg/L)	75 - 85	17.0	H,N and DGR
18.	MS + BAP (0.5 mg/L)	20 - 50	1.0	C and DGR
19.	MS + Kinetin (1.0 mg/L)	20 - 25	2.0	C and DGR
20.	MS + Kinetin (1.0 mg/L)	25 - 35	3.0	S, C and LGR
21.	MS + Kinetin (1.0 mg/L)	35 - 45	4.5	C, SD and LGR
22.	MS + Kinetin (1.0 mg/L)	45 - 55	5.0	C, SD and DGR
23.	MS + Kinetin (1.0 mg/L)	55 - 65	5.5	C, SD and DGR
24.	MS + Kinetin (1.0 mg/L)	65 - 75	5.7	C, SD and DGR
25.	MS + Kinetin (1.0 mg/L)	75 and above 85	5.7	C, SD and DGR
26.	MS + Zeatin (2.0 mg/L)	20 - 75	10.0	C, SD, DGR and N

MS – Murashige and Skoog nutrient media; 2,4-D-2,4-Dichlorophenoxyacetic acid; IAA – Indole 3 Acetic acid; BAP – Benzyl amino purine; DAI – Days after inoculation; C – Compact; S – Soft; SD – Solid; Y – Yellow; H – Hard; LGR – Light green; T – Translucent; G – Granular; DGR – Dark green; N – Nodular; BR – Browning

A pale yellow colour callus was observed after 10th day of inoculation (DAI) at the trimmed regions on MS media supplemented with 2, 4-D at 0.50 mg/L concentration. For successful callus initiation, substantiation of the basal media (MS with 3% sucrose) with 0.50 mg/L of 2, 4-D was found to be more suitable than other growth regulators like NAA, IAA, BAP, cytokinins, kinetin and zeatin separately and in different concentrations and combinations. In *Aristolochia bracteolata* an anticarcinogenic herb a nodular callus was obtained from leaf explants of MS with BA (2.22 – 4.44 µM) Alice chara *et al.,* (1997). It has been demonstrated in many cases that 2, 4-D is usually the choice of auxin for callus induction and subculture of grasses and herbs Bhaskaran *et al.,* (1990). But in *Tylophora indica* optimal callus was developed from leaf explants on Murashige and Skoog (MS) basal medium supplemented with 10 µM 2, 4, 5-T Mohammed Faisal *et al.,* (2003). In a rare medicinal plants *Ceropegia candelabrum* L. and *Saposhnikovia divaricata* a friable callus was developed from leaf explants grown on Murashige and Skoog (MS) medium supplemented with 4.52 µM 2,4-D reported by Beena *et al.,* (2002). Callus induction of *Thymus persicus* was observed on MS medium supplemented with different concentrations of 2,4-D and NAA, alone or in combination with BAP and KN(Ziba Bakhtiar *et al*., 2016).The reports are available for the seed – derived callus induction in *Hybanthus enneaspermus* on MS medium supplemented with NAA (2.6 WPM) and BA (2.2 WPM) (Prakash *et al.,* 1999; QiQiao *et al.,* 2009; Bidwell *et al.,* 2001) reported the necessity of medium containing half strength MS medium with 5 µM N-BA for callus formation in *Hybanthus floribundus* was achieved from seed explants Bidwell *et al.,* (2001). In present study the callus is growing enormously in many fold upto 10 weeks on MS medium with 2, 4-D (0.50 mg/L). After 10 weeks the growth of the callus is decreasing.

The growth of the callus ceases after 14 weeks of duration. It was found that the callus is remaining viable even up to 18 to 20 weeks of time. Subculturing of the fresh callus on MS medium with 2, 4-D (0.55 mg/L) and measuring at the end of 98 DAI, the callus nearly showed about 50 ± 2 fold increase of fresh weight.

It was observed that leaf callus turned to light brownish from 98 DAI onwards. Browning of calli was perhaps due to the synthesis of phenolic compounds. The transfer of calli to fresh medium was found to be effective in reducing the browning was observed. This practice was used to control the blackening of the cultures in a considerable number of species such as *Euphorbia lathyris* Ripley *et al*., (1986), Jagadish Chandra, (1999) and *Pisonia alba*. This approach is used in present study to prevent the browning and to maintain the callus as a source of explants for future in vitro culture and considered as 'stock callus'. Reduction in growth is because of stress imparted due to depletion of medium leading to cessation of growth has been considered as one of the physiological parameters for differentiation of the callus as found in *Cicer arietinum* Chandra *et al.,* (1993). However in the present study there is no such growth retardation was observed in MS with 2, 4-D hormone during this phase.

Different morphological forms of the callus (Table - 2) were observed in media with various combination of auxins and cytokinins. Callus is potent to produce roots and shoots. Leaf callus is potent to produce shoots on MS medium amended with NAA (0.4 mg/L) + kinetin (2.0 mg/L) (Fig -) and produce roots on MS containing kinetin (4.0 mg/L) (fig -)

Thirumal *et al*., (2012) studied various phytochemical in *Aristolochia bracteolata*. In the present study photochemical investigation on leaf and leaf calli were carried out to know the

chemical constituents and compared them between *invivo* and *invitro* plants. The methanolic extracts of the leaves and leaf calli were subjected to the different chemical tests to find out the nature of chemical constituents. The leaf showed that the presence of alkaloids, steroids flavonoids, terpenoids and absence of reducing sugar and the leaf callus showed the presence of alkaloids, saponins and steroids and absence of terpenoids, phenols were recorded(Table:1)

CONCLUSION

The leaf and leaf callus consists of various secondary metabolites (alkaloids, steroids, saponins, terpenoids, flavonoids) present in the *invitro* plants, the rapidly growing leaf callus may be used for the production of medicinally important drugs like antimicrobial drugs, anti-inflammatory drugs, anti venom drugs etc., in large scale by using bioreactors. It provides a commercially realistic alternative to whole plant for the important chemicals or drugs and minimizes the exploitation of this species. This is an effective tool for conservation of the species and to make the source of drugs available throughout the year.

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References

Alice Clara Augustine and D'souza, L., (1997). Regeneration of anticarcinogenic herb, *Curciligo orchioides* (GAERTN.), *In Vitro cell Dev. Biol – Plant*, 33: 111 – 113.

Beena, M.R. and Martin, K.P., (2002). *Invitro* propagation of the rare medicinal plant *Ceropegia candelabrum* L. through somatic embryogenesis, *Invitro* cell. Dev. Biol. Plant, 39: 510 – 513.

Bhaskaran, S. and Smith, R.H., (1990). Regeneration in cereal tissue culture: A Review. *Crop Sci.,* 30: 1328 – 1336.

Bidwell, S.D., Pederick, J.W., Sommer Knudsen and Woodrow, I.E., (2001). Micropropagation of the Nickel hyperaccumalator, *Hybanthus floribundus* (family – Violaceae), Plant cell, tissue and organ culture, 67(1): 89 – 92.

Chandra, R., Chatrath, A., Polisetty, R. and Khetarpal, S., (1993). Differentiation of *invitro* grown explants of chickpea (*Cicer arietinum* L.). *Indian J. Plant Physiol.,* 36: 121 – 124.

Harborne, J.B., (1988). Phytochemical methods, Third Edition, Chapman and Hall, London. 117.

Kirtikar, K.R. and Basu, B.D., (1935). Indian Medicinal plants. International Book Publishers, Dehra Dun. 1: 88 – 139.

Mohammed Faisal and Mohammad Anis, (2003). Rapid mass propagation of *Tylophora indica* Merrill via leaf callus culture, Plant Cell, Tissue and Organ Culture, 75: 125 – 129.

Prakash, E., Sha Vallikhan, P.S., Sairam Reddy, P. and Rao, K.R., (1999). Regeneration of plant from seed – derived callus of *Hybanthus enneaspermus* (L) Muell., a rare ethnobotanical herb. *J of plant cell reports,* 18(10): 873 – 878.

Pushpangandan, P., (1995). Ethnobotany in India – A status report all India Co-ordinated research project, Ministry of Environment and forests, Government of India, New Delhi.

Qi Qiao, Fu-Wu Xing, Ya-Ping Xiao and Hong – Feng Chen, (2009). Somatic embryogenesis and in vitro flowering in *Saposhnikovia divaricata. J. Plant Growth Regul,* 28: 81 – 86.

Wealth of India, (2003). Raw materials, CSIR, New Delhi,1: 88.

Ripley, K.P. and Preece, J.E., (1986). Micropropagation of *Euphorbia lathyris* L. *Plant Cell, Tissue and Organ Culture,* 5; 213 – 218.

Jagadish Chandra, K.S., Rachappaji, S., Gowda, K.R.D. and Thara Saraswathi, K.J., (1999). *In vitro* propagation of *Pisonia alba* (Linn.) Sapanogae (Lettuce Tree), A threatened species. *Phytomorphol.,* 49: 43 – 47.

Chitme, H.R., Mallikarjun Malipatil, Chandrashekhar, V.M. and Prashant, P.M., (2010). Antiallergic activity of *Aristolochia bracteolata* Lam in animal model. *Indian Journal of Experimental Biology,* 48: 46 – 52.

Havagiray, R., Chitme, and Nitin Patel, P., (2009). Antiarthrities activity of *Aristolochia bracteolata* extract in experimental animals. *The Open Natural Products Journal*, 2: 6 – 15.

Kalpana Devi, Kanimozhi, S. and Suganyadevi, P., (2011). Phytochemical screening and biological property of *Aristolochia bracteolata. Journal of Pharmacy Research,* 4(5): 1509 – 1514.

Manikandar, R.V., Selvamani, P. and Latha, S., (2006). Antibacterial activity of leaf extracts of *Aristolochia bracteolata* Retz. *Indian Journal of Pharmaceutical Sciences,* 68(4): 509 – 510.

Negi, P.S., Anantharamakrishnan, C. and Jayaprakash, G.K., (2003). Antibacterial activity of *Aristolochia bracteolata* roots extracts. *J. Med. Food,* 6: 401 – 403.

Thirumal, M., Vadivelan, R., Kishoree, G. and Brahmaji, V.S., (2012). *Aristolochia bracteolata*: An overview on pharmacognostical, phytochemical and pharmacological properties. *Critical Review in Pharmaceutical Science,* 1(1): 70 – 82.

Ziba Bakhtiar ., Mohammad H.M. , Ali Sonboli (2016). *Invitro* callus induction and micropropagation of *Thymus persicus* (Lamiaceae), an endangered medicinal plant *Crop Breed. Appl. Biotechnol*, 16 (1).1984-1988.

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