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Research Article

IN VITRO PROPAGATION OF *BARLERIA PRIONITIS* LINN AND ITS ANTIBACTERIAL ACTIVITY

Shukla P[1], Singh A*.[1], Gawri S[1], Alexander A.[2] and Sonwane S[1]

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1) G.D. Rungta College of Science and Technology, Bhilai (C.G) (India)
2) Rungta College of Pharmaceutical Sciences and Research, Bhilai (C.G) (India)

Abstract
Protocol for plant propagation through shoot tips and leaf were established for Vajradanti- *Barleria prionitis* L. (Acanthaceae). Vajradanti is a known medicinal plant and its value in medicine is known since ages. The tissue culture of vajradanti has not been established and therefore the work was undertaken to develop the protocol. Murashige and Skoog's (MS) medium with following 2,4-D, Kinetin, BAP and IAA were tested for growth. In 0.0125mg/l concentration of 2,4-D and 0.0125mg/l concentration of Kinetin callus induction from leaves was the fastest i.e. in 3 days. In shoot culture the fastest callus induction was in 4 days with 2,4 D -.025mg/ml and Kinetin -.025mg/ml. Antibacterial activity was also measured and it was found that in ether extract callus gave most antibacterial activity.

Keywords: - *Barleria prionitis* L., Antibacterial activity.

Introduction
Barleria prionitis L of Acanthaceae family enjoys a long history of clear acceptance as important herb possessing healing & curative qualities. It is known as varjradanti. because its use to make the teeth (in Hindi-dant) strong and free from all diseases. *Barleria prionitis* Linn (Acanthaceae) is widely distributed throughout Africa, India, Sri Lanka and tropical Asia [1]. The juice of the leaf is used in cataract and fever. The dried bark is used in cough treatment and the leaves chewed to relieve toothache. The paste of the root is applied to disperse boils and glandular swellings [2]. The leaves are chewed to relieve toothache. Juice of the leaves is used in ulcer and fever. Paste of the roots is applied to disperse boils and glandular swellings. Leaves are also used by some tribal communities for the treatment of piles and to control irritation. Plant is also used in stiffness of limbs, enlargement of scrotum and sciatica [3,4,5]. From the literature it is evident that very few works have been done on this plant and any literature if present was not available to the authors in the case of tissue culture of this plant even though antimicrobial activity of this plant has been studied [1].

Correspondence Address:
A.Singh
Rungta College of Pharmaceutical Sciences and Research, Bhilai (C.G.), India.
PH: 09907333846
E-mail: inwines_j@yahoo.co.in

Materials and Methods

Plant Material
Plant material was collected from medicinal garden of Rungta College of Pharmaceutical Sciences and Research, Bhilai. Shoot tip and leaves were taken as explants. These explants were washed with distilled water and sterilized by 0.1% solution of mercuric chloride for 2 minutes. After sterilization the explants were thoroughly washed with distilled water.

Establishment of In vitro culture
Explants were inoculated in MS basal medium [6] which contained different combinations of 2,4 D, Kinetin, BAP and IAA. The explants were inoculated in the autoclaved medium containing different concentrations of hormones and kept under white fluorescent light.

Preparation of extracts
The field grown *Barleria prionitis* plant and calluses from in-vitro propagated shoot tips & leaves were used for preparation of extracts. Each were washed, dried and reduced to powder. The powdered callus was extracted using chloroform, ether and ethanol using Soxhlet apparatus. Aqueous extract was prepared by crushing the callus in distilled water using pestle mortar.

Test organisms
The bacteria used for the antibacterial assay were isolated

with bacterium were used for the assay. Discs of six millimeter were dipped into the test extracts and introduced into the plates inoculated with the bacterium. The plates were incubated overnight at 37 degree Celsius. Antibacterial activity was determined measuring the inhibition zones formed around the discs.

.Result and Discussion

The in vitro propagation of *Barleria prionitis* was performed using different hormones concentrations. Callus from shoot tip showed fastest callus induction (4 days) with 0.025 mg/ml concentration of 2,4 D and 0.025 mg/ml concentration of Kinetin. Similarly fastest callus induction from leaves (3 days) was with 0.0125mg/l concentrations of 2,4 D and Kinetin. IAA and BAP did not show callus induction from shoot tip while callus was induced in leaves using IAA and BAP (Table 1, 2).

Table 1 Callus induction in Shoot tips

S.No.	Hormones	Concentration	No. of days for induction of callus
1	2,4 D + Kinetin	0.0125mg/ml+0.05 mg/l	10
2	2,4 D + Kinetin	0.05mg/ml+0.1mg/ml	15
3	2,4 D + Kinetin	0.025mg/ml+0.025 mg/ml	4
4	2,4 D + Kinetin	0.026mg/ml+0..026 mg/ml	7
5	BAP + IAA	0.025mg/ml+0.025 mg/ml	-

Antibacterial assay revealed that *Barleria prionitis* has antibacterial properties. The ethanol, ether and chloroform extracts showed clear inhibition zones while no or slight inhibition zones were present around the discs of aqueous extracts (Fig.No.2).

With ethanol, ether and chloroform extracts inhibition zones were present in all the types of test organisms tried as well as for all types of extract material i.e. field grown plant, callus from shoot tips (Fig.No.1a) and callus from root tips (Fig. No.1b). While no inhibition zones were present (Fig.No.1)

Table 2 Callus induction in leaves

S. No.	Hormones	Concentration	No. of days for induction of callus
1	2,4 D + Kinetin	0.0125mg/ml+0.05 mg/l	11
2	2,4 D + Kinetin	0.0125mg/ml+0.0125mg/ml	3
3	Kinetin	0.0250mg/l	4
4	2,4 D + Kinetin	0.026mg/ml+0..026mg/ml	7
5	BAP + IAA	0.025mg/ml+0.025mg/ml	9

FIG:-2

FIG:-1a

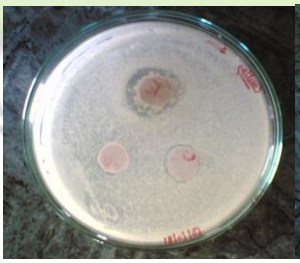

FIG:-1b

Fig 1

Table 3 Antibacterial activity of leaves of field grown plant

S.No.	Type of Organism	Ether extract	Ethanol extract	Chloroform extract	Aqueous extract
1	Gram +	++	+	+	-
2	Gram +	+++	+	+	-
3	Gram +	++++	+	++	-
4	Gram +	++	-	+	-
5	Gram +	++	+	++	-
6	Gram +	+++	+	++	-
7	Gram +	++	+	+	-
8	Gram +	++	+	+	-
9	Gram +	++	+	+	-
10	Gram +	++	+	+	-

Table 4 Antibacterial activity of callus from shoot tip

S.No.	Type of Organism	Ether extract	Ethanol extract	Chloroform extract	Aqueous extract
1	Gram +	++++	++	+++	-
2	Gram +	++++	+	+++	-
3	Gram +	+++++	+	++	-
4	Gram +	+++	-	+	-
5	Gram +	+++	+	++	-
6	Gram +	++++	+	+++	-
7	Gram +	++++	++	++	-
8	Gram +	+++	+	+	-
9	Gram +	+++	+	+	-
10	Gram +	++++	++	+	-

It is evident that callus induction is early when the concentrations of the hormones are equal while it takes longer time to induce callus when the concentrations of hormones was different (Table 1, 2). With antibacterial activity it is clearly evident from the results that ether extract have more antibacterial properties than ethanol or chloroform extracts while aqueous extracts have o or minimum antibacterial properties. Our results are very much in common with the results obtained by Chavan et al. (2010).

Table 5 Antibacterial activity of callus from shoot tip

S.No.	Type of Organism	Ether extract	Ethanol extract	Chloroform extract	Aqueous extract
1	Gram +	+++	++	++	-
2	Gram +	++++	+	+++	-
3	Gram +	+++++	+	+++	-
4	Gram +	+++	+	+	-
5	Gram +	+++	+	++	-
6	Gram +	+++	++	+++	-
7	Gram +	++++	++	++	-
8	Gram +	+++	-	+	-
9	Gram +	++++	+	+++	-
10	Gram +	++++	++	+	-

Conclusion

Equal concentrations of 2,4 D and Kinetin can be used for fast in vitro propagation of *Barleria prionitis*. Also it has been shown by us and is also available in other literature that *Barleria prionitis* has antibacterial properties. In view of this it can be used for development of new antibacterial drugs. As callus of shoot tips as well as leaves showed more antibacterial activity than field grown plants, in vitro propagated plants would be more beneficial for drug development than filed grown plants.

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