



IN VITRO PROPAGATION AND ANTI- INFLAMMATORY ACTIVITY OF *SCILLA INDICA* ROXB. - A TRADITIONAL MEDICINAL PLANT

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Abstract

A standard protocol was established for the mass propagation of *Scilla indica*, an important medicinal plant. Bulblets were cultured on MS media supplemented with 0.5-2.5 mg/l BAP and 0.5-1.0 mg/l Kn. Maximum percentage (71%) of shoot induction was observed on MS media augmented with 1.5 mg/l BAP and 1.0 mg/l Kn. The *in vitro* shoots were transferred on MS media augmented with 0.2-1.5mg/l of IBA alone for root induction. Maximum percentage (80%) of root formation was observed on MS medium augmented with 0.8 mg/l IBA alone. The well-developed plantlets were subsequently hardened under greenhouse conditions. For anti-inflammatory activity, ethanol extracts of *S. indica* bulb was evaluated using carrageenan induced experimental model of inflammation in Albino rats. The results showed that maximum dose (400 mg/kg) of this plant extract exhibited a significant reduction in the volume of inflammation. The present results revealed the active constituents of *S. indica* and anti-inflammatory effects against carrageenan-induced paw edema in rats.

Key words: *Scilla indica*, Bulb lets, MS medium, Micropropagation, Anti-inflammatory

Introduction

Scilla indica Roxb., (Liliaceae) commonly known as "Kattu vellai vengayam in Siddha" is a valuable medicinal plant. Synonym of this plant is *Urginea maritima* Baker (South Indian Squill or White Squill) and *Urginea indica* Kunth (Indian Squill). This plant grows wild in the forests of Madhya Pradesh, Gwalior, Bihar, Mahabaleswar and all districts of the Tamil Nadu, except the west coast, up to 4000 feet. The bulb is used as anthelmintic, cardiac stimulant, digestive, diuretic, emmenagogue and expectorant (Anonymous, 1991). It is also used in asthma, cough, bronchitis, paralytic attacks, ailments of the heart, calculous affections, rheumatism and skin diseases. Its pharmacological action is similar to *Digitalis*. It has negative inotropic response and gives strength to the heart by reducing the rate of heart beat (Bashir *et al.*, 2013; Rathabai *et al.*, 2012; Kapoor, 2005; Kar, 2003; Gokhale, 1979).

Clinically, it has been observed that its response time is less than *Digitalis*, because of its quick elimination from the body. Fresh squill yields at least two types of glycosides - Scillarin-A, which is crystalline and Scillarin-B, which is amorphous. Scillarin-A ($C_{36}H_{52}O_{13}$) is sparingly soluble in water, whereas Scillarin-B is freely soluble in water and chloroform, but insoluble in ether and alcohol. Interestingly, the natural mixture of both the glycosides, known as Scillarin, is freely soluble in water (Qadry and Shah, 1990).

Seed viability of *S. indica* is not satisfactory and conventional propagation processes are season dependent and most of the propagation are achieved only during the monsoon period. The application of tissue culture techniques might be of great value as an alternative method to achieve large scale propagation independent of season (Fay, 1994). *De novo* regeneration *in vitro* has been reported for a growing list of medicinal, aromatic, economically important plants (Senthilkumar *et al.*, 2007; Karrupusamy *et al.*, 2006). The present study was aimed at developing a large scale multiplication protocol for *S. indica* using bulb lets culture.

Materials and Methods

The plant *S. indica* (Fig.1) was collected from Thathanuthu village near Gangaikondan Spotted Deer Park, Tirunelveli District, Tamil Nadu, India. Bulblets were washed with running tap water for 10 min and surface sterilized in 0.1 (w/v) $HgCl_2$ solutions for three minutes. After rinsing three to four times with sterile distilled water, bulblets were cut into smaller segments (1.0 cm) and used as the explants. The chosen explants were placed vertically on solid basal MS medium in test tubes (150 x 25 mm) containing 15 ml medium supplemented with 3% sucrose, 0.6% (w/v) agar (HIMEDIA, Mumbai) and different concentration and combination of BAP, Kn, IBA and 2,4-D. For rooting, the *in vitro* raised shootlets were recultured on to half strength MS supplemented with various concentrations and combinations of auxins. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 15 min. The cultures were incubated at 25±2°C under cool fluorescent light (2000 lux 14 hr photoperiod). For hardening, the *in vitro* raised plantlets were removed from culture, washed thoroughly with tap water planted in small polycups filled with sterile garden soil and sand (3:1), covered by unperforated polybags and hardened for four weeks in a mist chamber before transfer to field.

For anti-inflammatory activity, bulblets were air-dried and pulverized using mechanical grinder. 250g of air-dried powdered barks were coarsely powdered and subjected to successive solvent extraction with 95% ethanol. An oily extract was obtained after complete elimination of solvent under reduced pressure. The ethanol bulblets extract gave 8.71%.

Adult Wister rats (100-120g wt) were kept under standard laboratory conditions. They were divided into five different treatment groups of six animals each 0.2 ml equivalent of 50, 100 and 200 mg/kg of the ethanolic extract were injected intraperitoneally (i.p.) into the rats. 0.2 ml saline solution and 0.2 ml equivalent of 5 mg/kg indomethacin were administered as negative and positive controls, respectively. The paw volume of each of the rats was measured by dipping the right hind paw in the cell compartment of the plethysmometer and the volume of fluid displaced by the paw was noted. 0.1 ml (1%) carrageenan was injected into the right hind paw of all the rats under the sub-planter region to induce edema that is intra-dermal. The measurement of the paw volume was repeated using the

plethysmometer an hour after carrageenan injection. The latter was repeated within same interval till the fifth hour after the carrageenan injection. The ability of the extract to reduce swelling (oedema) was taken as a measure of anti-inflammatory activity. The experiment was carried out in quadruplicate and the percent inhibition was calculated with the reference to the 0.2 ml saline control.

$$\% \text{ of inhibition} = \frac{(C_t - C_o) \text{ control} - (C_t - C_o) \text{ treated}}{(C_t - C_o) \text{ control}} \times 100$$

Results were expressed as the mean value \pm standard error of the mean. Treated groups were compared with the controls for statistical significant differences ($P < 0.005$) using paired Student's *t*-test.



Results and Discussion

In many cases, explants determined the relative success of *in vitro* culture. In the present study also the bulblets explants showed maximum frequency of regeneration compared to the leaf ones. Three minutes sterilized nodal segments with 0.1% HgCl₂ treatment and showed shoots initiations and elongation of axillary buds within four days. Less or more than three minutes failed to regenerate, less than three minutes sterilized bulb segments were contaminated by fungal and bacterial colonies, more than three minutes sterilized cultures showed the browning on the wound surface and showed high percentage of mortality. Shoot initiation from bulb segments was mainly a cytokinin effect, because the explants in cytokinin free medium did not respond. The role of BAP in bud breaking has been recorded in many medicinal plants such as *Wadelia calendulacea* (Emmanuel *et al.*, 2000), *Vitex trifolia* (John Peter Arulanandam & Ghanthikumar 2011), *Wattakaka volubilis* (John Peter Arulanandam *et al.*, 2011). Reports of the previous workers agree with present reports. Effect of cytokinin and auxin on shoot multiplication from nodal segments is shown in Table 1. MS augmented with 1.0 mg/l of BAP in combination with 0.5 mg/l of Kn showed maximum percentage (71.3 ± 0.69) of shoot formation. Callus induction was observed on the cut surface of nodal segments on MS augmented with BAP in combination with Kn. The degree of callus production varied with reference to the supplementation of the plant growth regulators on the medium. Highest degree of callus induction was observed on MS augmented with 2.5 mg/l of BAP in combination with 1.0 mg/l of Kn. The *in vitro* raised shoots

transferred to 0.2-1.5 mg/l of IBA. Among the concentration 0.8mg/l of IBA showed maximum number of roots. The well-developed plantlets were subsequently hardened under greenhouse conditions. Previous literature suggest that BAP is most active at combination of 1.0 mg/l to 2.0 mg/l in many plant systems (Senthilkumar *et al.*, 2007, Karrupusamy *et al.*, 2006, John Peter Arulanandam & Ghanthikumar 2011, John Peter Arulanandam *et al.*, 2011).

Table 1. Effect of PGRs on multiple shoot proliferation from Bulblets explants of *S. indica*.

MS + Plant growth regulator in mg/l		% of shoot response \pm S.E	Degree of callus formation*
BAP	Kn		
0.5	-	20.8 \pm 0.34	NIL
1.0	-	24.2 \pm 0.61	NIL
1.0	0.5	31.3 \pm 0.32	+
1.5	-	64.8 \pm 0.48	NIL
1.5	1.0	71.3 \pm 0.67	++
2.0	1.0	70.8 \pm 0.54	+++
2.5	1.0	50.4 \pm 0.34	++++

* + - Low callus; ++ - Average/ medium; +++ - High.

Table 2. Rooting response of excised shoots of *S. indica*

MS + Plant growth regulator in mg/l	% of root response \pm S.E	Degree of Callus produced
IBA		
0.2	-	NIL
0.4	30.3 \pm 0.64	NIL
0.8	80.3 \pm 0.53	++
1.0	78.4 \pm 0.48	++
1.5	64.3 \pm 0.54	++

* + - Low callus; ++ - Average/ medium ; +++ - High.

Anti-inflammatory activity of ethanolic extract of *S. indica*

The percentage inhibition at 50, 100,200 and 400mg/kg/ day body weights of the tested rats for the anti-inflammatory study gave 7.17%, 38.01%, 45.17%, and 68.84% respectively, at six hour post-carrageenan administration. The ethanolic extract showed moderate anti-inflammatory activity. The value was lower than that of indometacin the reference drug that gave 71.96% at 5 mg/kg dose (Table 3).

Carrageenan induced oedema of rat foot is widely used model of inflammation in the anti-inflammatory agents (Emir *et al.*, 1994). It is developed from discovery of Indomethacin, the anti-inflammatory drug (Winter, 1964). The oedema which develops in rat paw after carrageenan injection is a biphasic event (Schreiber and Hug, 1969). The initial phase is attributed to the release of histamine and serotonin. The oedema maintained between the first and second phase to kinin, and the second phase to prostaglandin (Di Rosa and Willoughby, 1963). All the mediators appear to be dependent upon an intact complement system for their activation and release (Di Rosa and Willoughby, 1963).

The present study gave maximum (68.84%) inhibition produced by the ethanol extract of *S. indica* bulblets extract and effective dose 400mg/kg of effective anti-inflammatory time was observed (4-5hr). Anti-inflammatory effect may be due to the active constituent Scillarin-A and Scillarin-B. Further investigation is necessary to identify the exact mode of action of individual constituents of *S. indica* bulblets.

Table : 3 Percentage inhibition of *S. indica* bulb lets extract on carrageenan rat paw induced oedema.

Treatment	Dose (mg/kg)	Mean paw- size in cm(Hr)					Inhibition (%)
		0	1	2	3	5	
Normal saline		2.16±0.01	2.51±0.05	3.17±0.01	4.22±0.01	3.21±0.02	-
Extract (I)	50	3.92±0.01	4.55±0.01	4.26±0.01	3.53±0.01	2.98±0.01	7.17
Extract (II)	100	3.44±0.01	2.92±0.02	2.54±0.01	2.15±0.01	1.99±0.01	38.01
Extract (III)	200	2.23±0.01	2.15±0.01	2.42±0.01	2.52±0.01	1.76±0.01	45.17*
Extract (IV)	400	2.46±0.01	2.15±0.01	1.55±0.01	1.23±0.01	1.00±0.02	68.84**
Indomentacin	5	1.25±0.01	1.47±0.01	1.27±0.01	0.99±0.01	0.90±0.01	71.96**

No. Animals = 6; Values are expressed as Mean ± SE; * $P < 0.05$; ** $P < 0.01$

Conclusion

In the present study, conservation and anti-inflammatory activity of *S. indica* is revealed. An efficient protocol for micropropagation of medicinal plant *S. indica* has been successfully developed which can be employed for large scale multiplication at a commercial sector. Anti-inflammatory studies is very much useful for drug formulation.

Conflict of interest

No conflicts declared

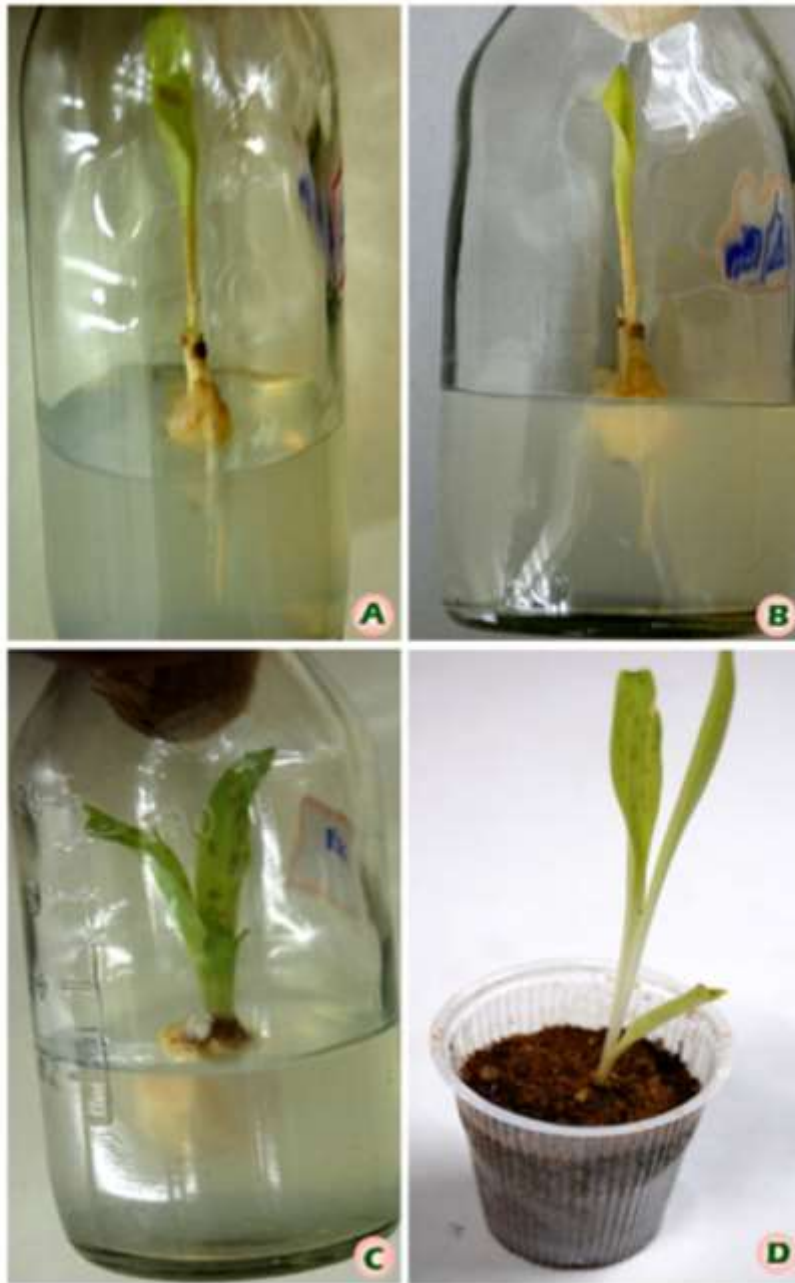
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Plate 1

IN VITRO PROPAGATION OF *SCILLA INDICA* ROXB.

A) Bulblets induced shoots ; B-C) Shoot induced root from 0.8 mg/l IBA alone ;
D) Hardening.