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Research Article

IN VITRO PROPAGATION OF *SPERMADICTYON SUAVEOLENS* ROXB

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ABSTRACT

Spermadictyon suaveolens Roxb. is an ancient Indian herb used for formation of various medicinal products. In present study successful protocol has been developed for mass propagation of *Spermadictyon suaveolens* Roxb. in Murashige and Skoog (MS) medium. The explants, collected from the Matwan gaon, Taluka Dapoli, District Ratanagiri (Maharashtra) respond well with 1.5 mg/l Benzyl amino-purine (BAP) (14.4 shoots per culture). Shoots were rooted through *in vitro* and *ex vitro* rooting methods with the help of auxins (Indole-3 butyric acid and Indole- 3 Acetic Acid). The plantlets were hardened in greenhouse.

INTRODUCTION

Spermadictyon suaveolens Roxb. (=*Hamiltonia suaveolens* Roxb.) belongs to family Rubiaceae. The family Rubiaceae is widespread and found to be distributed in all major regions of the world except Antarctica.Herbal medicines are the precursors of many common drugs and herbal products are still an important part of the primary health care systems in many parts of the world (Jawahar *et al*., 2008). According to Singh and Jain, 1985 Rubiaceae consists of 500 genera and 6000 species and are mostly found in the tropics. However, some are grow in the temperate zones. A few species like *Gelium sps.* is growing in the frigid regions.

S.suaveolens Roxb. is a branched shrub, growing up to 1-2 m tall. Leaves are oppositely arranged elliptic-lance like, 10-20 cm, and are finely velvety. Leaf stalks are 1-2 cm long. Flowers occur in many-flowered spherical heads, arrange in panicles at the end of branches. The species name *suaveolens* means sweet scented, and refers to the fragrant flowers. The species is commonly known as Forest champa.

Ethno-botanical survey indicated that this plant is being used as an antidiabetic in amchi system of medicine by local bush doctors as well as in the indigenous system of medicines (Naik, 2010). Some of the Vaidoos or herbal practitioners are also using the stem of this plant to cure disease Herpes Zoster commonly known as "Nagin". Roots of this plant are used with mustard oil and applied on wounds. The roots are also used in the treatment of diabetes and rheumatoid arthritis (Sonar, 1968), in diarrhoea and for treating ulcers and wounds (Jain, 1991). Antidiabetic effect of a root extract from *S. suaveolens* also reported by (Sonar, 1968; Farnsworth and Segelman, 1971). The barks of this plant are rubbed on the body when suffering from fever. (Anonymous, 1948-1976). It is potent antidiabetic plant and used in folk, Ayurvedic and homeopathic systems of medicine (Kapoor, 1997; Ravi *et al*., 1995 and Mitra, 1985). The stem bark of this plant is boiled in water and vapors are allowed over the body of a person suffering from fever and also in the case of anemic persons. The patient suffering from flatulence had also given about 20 ml of stem bark decoction. The stem bark decoction of *S. suaveolens* are also used as veterinary medicine, it is given to cattle to increase their appetite (Patil, 2006). The leaves are used as fodder for buffaloes and the woods are used for making charcoal and gunpowder (Watt, 1889-1893).

Active principles, medicinal properties and uses of these plants have been listed by (Kumar *etal*., 2009; Mahmoodreza, 2010; Modupe, 2010; Zhu *et al*., 2010; Sirisha *et al*., 2011). The chemical constituents isolated from *S. suaveolens* by using GC-MS analysis is Azulene, Tetratetracontane, 9-Nonadecane, n-hexadecanoic acid (Palmitic acid), Phenol, 2-methoxy-4-(1-propenyl)-, (E), Tritetracontane and Ergost-5-en-3-ol, 22, 23-dimethyl-, acetate, (3β). The chemicals is used against skin irritation, anti-inflammatory effects and antibacterial properties, anti- ulcergeni and antifungal properties (Kulkarni and Sathe, 2013).

These varied uses have increased utilization and exploitation of *S. suaveolens* for medicinal purposes. The plant grows wild in forests and among other areas. But in the comparison of the other propagation method the *in-vitro* propagation shows the

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results faster and the techniques produced a large number of the plants. Therefore, an effort has been undertaken to develop a reliable protocol for mass multiplication of *S. suaveolens* by using tissue culture in the present investigation.

MATERIAL AND METHODS

Collection and Identification of Plant Material

The plant of *Spermadictyon suaveolens* Roxb. was collected from Matwan gaon, Taluka Dapoli, District Ratanagiri. It is a live hedge around orchard of *Anacardium occidentale* Linn. Efforts were made to collect the plant material in flowering and fruiting condition and authenticated from Botanical Survey of India, Regional office, Western circle- Pune, 411001 (Specimen voucher Number-BSI/WRC/IDEN.CER./2016/240). The plant was collected and perpetuated by the method of stem cuttings in the garden of Department of Botany, Savitribai Phule Pune University.

Media and culture conditions

Murashige and Skoog (1962) medium supplemented with different concentration of growth regulators used for the study. The collected nodal explant from the botanical garden were washed with the running tap water. Then the explants were cut and kept in Tween 20 for 10 minutes then thoroughly washed under running tap water. Again the explants is placed in savlon (1%) for 5 min and washed with running tap water. Then the explants are soaked in the antioxidant solution containing PVP (100mg/l), Ascorbic acid (100-500mg/l) and citric acid (50-100mg/l) for 30 minutes. This was washed with the sterile distilled water in laminar air flow for 4-5 times. Then the explants are kept in 0.1% $HgCl_2$ for 2 min. Constant shaking was done during this period to get through sterilization. It was then rinsed with sterilized distilled water for 4-5 times to remove the sterilant from explants. The surface sterilized explants were then inoculated immediately after surface sterilization in the prepared test tubes containing media. The inoculated explants in different concentration of MS media were exposed to 16 hours light and 8 hours dark cycle at 25 ± 2º C.

Multiple shoot induction

To evaluate the influence of culture medium composition on shoot induction, nodal segments were inoculated on MS medium supplemented with different concentrations (0.5-2.5 mg/1) of BAP and Kinetin (Kin) either alone or in a combination of the optimum concentration of IAA (0.5-1.5 mg/1) and NAA (0.5-1.5 mg/1) for shoot induction.

Multiplication of shoots in culture

The shoot cultures were further multiplied by two approaches (i) repeated transfer of mother explants and (ii) subculture of *in vitro* produced shoots. The original explants were repeatedly transferred to fresh MS medium supplemented with 0.5-2.5mg/l BAP and Kin alone or in combination with 0.5-1.5 mg/1 IAA and NAA to yield more shoots. After 2-3 weeks regenerated shoots isolated from explants were cut into segments with 1-2 nodes (1.0-2.0 cm in length) and subcultured on MS medium containing different concentrations of BAP (1.5 mg/1). These experimental sets were maintained for three subcultures, with each passage of 3-4 week intervals.

Rooting of in-vitro produced shoots

Rooting of shoots was attempted on MS medium. The healthy shoots were excised, separated and transferred individually to full MS medium and various concentrations (0.5-1.0 mg/1) of IBA, IAA and NAA.

Ex vitrorooting of regenerated plants

The *in-vitro* regenerated shoots were dipped in different concentration of auxins IAA, IBA and NAA (0.5-1.0mg/l) for 20 minutes under laminar air flow and then it transfer in the pots containing the soil: sand: compost in 1:1:1 ratio. The pots were incubated under the same controlled conditions and the rooting frequency and number of roots obtained per explants was recorded. After root initiation occurred the pots were kept in the greenhouse.

Hardening of plantlets in green house

The *in vitro* grown plantlets with roots were taken out from the medium and culture tubes. The medium sticked to it was rinsed with water to remove the nutrients and agar and transferred to the plastic glass containing a mixture of autoclaved sand: soil: compost (1:1:1) and maintained in culture room condition. The plants were acclimatized by the pot with polythene bags to maintain humidity for 7-8 days and irrigated with water in the interval of two days. After 7-8 days the holes were made in polythene bags and plantlets were irrigated daily at frequent intervals. After acclimatization of plantlets the polythene bags were removed and after 30 days the plantlets were transferred to bigger pots in the greenhouse and were maintained under natural conditions, temperature and humidity. The acclimatized plants were irrigated daily.

Experimental design, data collection and statistical analysis

All the experiments were repeated thrice with 15 replicates for each treatment. The results are expressed as mean ± SD of three experiments.

RESULTS AND DISCUSSION

Bud breaking and initiation of cultures

The explants with fresh shoot segments of length 2 to 3 cm with 1-2 nodes were found to be most suitable for culture initiation.100% bud break occurred after 7-10days of inoculation on MS medium. On MS medium supplemented with 1.5 mg/l BAP, a maximum of 14.4±0.25 shoots were obtained (Fig. 2 and Table 1). Shoots (13.2 ± 0.36) were differentiated on MS medium with 1.0 mg/l concentration of Kin.

In vitro multiplication of shoots

Shoots initiated from activation of axillary meristem were further amplified by two ways (i) repeated transfer of mother explant (Boulay 1985, Franclet and Boulay 1989, Deora and Shekhawat 1995) or (ii) subculturing of *in vitro* rejuvenated shoots. Repeated transfer was carried out on MS medium supplemented with 1.5 mg/1 of BAP. On this medium, 11.2 shoots were obtained after 15-20 days. This medium was supportive for shoot development up to fourth culture cycle. Multiplication of cultures was best achieved by sub-culturing shoots on MS solid medium supplemented with 1.5 mgl/1 of BAP for mass multiplication of shoots. About 14.2 ± 0.77

shoots were obtained after 3-4 weeks of inoculation (Fig. 2 and Table 1).

Fig. 1- Habit of *S. suaveolens*

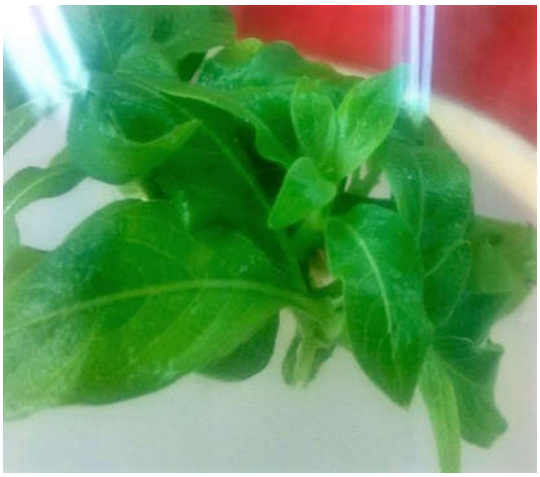

Fig. 2 MS + 1.5 mg BA

Table 1 Effects of cytokinin (BAP) and (Kin) on induction of shoots from explants of *S. suaveolens* on MS medium.

Cytokinins(mg/l)		Nodal explants	
BA	Kin	Explants forming shoots %	No. of shoots per explants.
Control		-	-
0.5		75.3 ± 1.85^{c}	4.4 ± 0.29^{c}
1.0		98.3 ± 0.21^{b}	7.2 ± 0.17^{b}
1.5		100 ± 00^{a}	14.4 ± 0.25^{a}
2.0		25.7 ± 3.37^{d}	0.95 ± 0.14^{d}
2.5		19.04 ± 2.52^{d}	0.75 ± 0.12^{d}
	0.5	72.0 ± 5.39^{b}	3.3 ± 0.46^{c}
	1.0	100 ± 00^{a}	13.2 ± 0.36^{a}
	1.5	97.0 ± 0.63^{a}	9.5 ± 0.45^{b}
	2.0	21.4 ± 5.43^{d}	1.8 ± 0.53^{d}
	2.5	13.3 ± 3.82^{d}	0.93 ± 0.33^{d}

Data recorded after 4 weeks of culture incubation.

The value represent the mean ± SE calculated on three independent experiments, each based on minimum of 15 replicates. Values followed by the same letter were not significantly different at 5% level (DMRT).

Among the cytokinins BAP was found superior to Kinetin (Bonga and Von-Aderkas, 1992) when placed in 12 h photoperiod (Rathore *et al*., 2005) (Table 1). It was also observed during the investigation that culture medium containing more than the average cytokinin, the number of shoots was reduced (Rathore *et al*., 1993, Shekhawat *et al*., 2011) as shown in Table 1.

Table 2 Effects of BA and Kin in combination with auxins on shoot multiplication in nodal explants of *Spermadictyon suaveolens* Roxb.

Cytokinins (mg/l)	Auxins (mg/l)		Nodal explants	
BA	Kin	IAA NAA	Explants forming shoots %	No. of shoots per explants.
Control			-	-
		0.5	100 ± 0.0^{a}	12.8 ± 0.36^{a}
		1.0	91.8 ± 1.6^{c}	7.2 ± 0.24^{b}
		1.5	73.9 ± 0.57^{e}	5.7 ± 0.11^{c}
1.5		0.5	98.9 ± 0.33^{a}	12.3 ± 0.31^{a}
		1.0	96.2 ± 0.59^{b}	7.4 ± 0.51^{b}
		1.5	83.5 ± 0.56^{d}	4.9 ± 0.36^{c}
		0.5	100 ± 0.0^{a}	8.8 ± 0.23^{a}
		1.0	96.5±0.36^{b}	5.3 ±0.31^{b}
		1.5	85.6± 0.88^{c}	5.1 ±0.23^{b}
	1.0	0.5	100 ± 0.0^{a}	8.1 ± 0.21^{a}
		1.0	85.4 ± 0.96^{c}	4.0 ± 0.45^{c}
		1.5	74.6 ± 0.30^{d}	2.6 ±0.16^{d}

Data recorded after 4 weeks of culture incubation.

The value represent the mean ± SE calculated on three independent experiments, each based on minimum of 15 replicates. Values followed by the same letter were not significantly different at 5% level (DMRT).

Rooting of shoots

In-vitro rooting of individual shoots was achieved with solid MS medium containing a different concentration of IAA, IBA and NAA. A 100% response was recorded in terms of *in vitro* rooting on the concentration 0.5mg/l IBA. About 22.1 number of roots were obtained after 3-4 weeks of inoculation (Fig. 3 and Table. 3).

Fig.3-*In- vitro* root induction MS + 0.5 mg/l IBA

Table 3 Effects of auxins on root induction from *in-vitro* regenerated shoots of *Spermadictyon suaveolens* Roxb.

Auxins (mg/l)			*In vitro* regenerated shoots	
IAA	IBA	NAA	Root Regeneration %	No. of roots per explant. (Mean ± SE)
0.5			96.0 ± 0.59^{b}	17.4 ± 0.35^{b}
0.75			93.8 ± 0.46^{c}	15.6 ± 0.75 ^{c}
1.0			56.6 ± 1.10^{e}	7.0 ± 0.26^{f}
	0.5		100 ± 00^{a}	22.1 ± 0.60^{a}
	0.75		83.6 ± 0.50^{c}	11.4 ± 0.55d^{e}
	1.0		70.2 ± 0.93^{d}	8.0 ± 0.46^{f}
		0.5	88.3 ± 0.18^{ab}	12.5 ± 0.40^{d}
		0.75	85.2 ± 0.87^{c}	10.6 ± 0.42^{e}
		1.0	55.8 ± 1.67^{e}	6.9 ± 0.26^{f}

The *in vitro* root induction was low on medium supplemented with higher concentration (1.0 mg/l) of IAA, IBA and NAA (6-8 roots per shoot). Diffused light (10 -20 µmolm-2s-1 PPFD) also favored *in vitro* root induction. Delayed rooting was observed under high light intensity (30 to 50 µmolm-2s-1 PPFD).

In vitro produced shoots also rooted under *ex-vitro* conditions. Shoots of length 3 to 4 cm having 2-3 nodes were harvested and treated with 0.5-1.0 mg/1 of IAA, IBA and NAA for 20 min, 100% of the shoots rooted after 3-4 weeks of this treatment. A maximum number of roots obtained by using MS + 0.5 mg/l IBA (19.8 ± 0.49) (Fig. 4 and Table. 4). IBA is more effective than NAA and IAA in promoting rooting of a wide variety of plants, and it is used commercially for rooting of many plant species worldwide (Phulwaria *et al.*, 2011a).

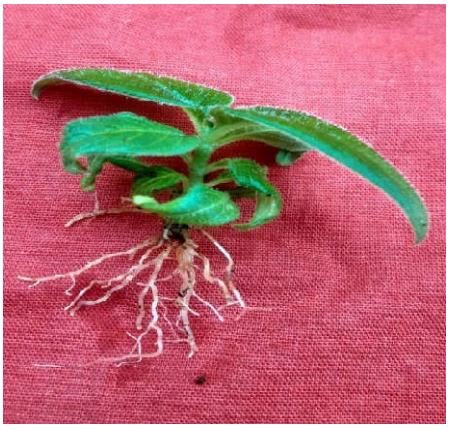

Fig.4-*Ex- vitro* root induction
MS + 0.5 mg/l IBA

Table 4- Effects of auxins on *Ex- vitro* root induction from *in-vitro* regenerated shoots of *Spermadictyon suaveolens* Roxb.

Auxins (mg/l)			*In vitro* regenerated shoots	
IAA	IBA	NAA	Root regeneration %	No. of roots per explants. (Mean ± SE)
0.5			98.2 ± 0.14[a]	18.7 ± 0.48[a]
0.75			82.6 ± 1.25[bc]	12.1 ± 0.54[c]
1.0			75.8 ± 0.51[c]	6.2 ± 0.48[d]
	0.5		100 ± 00[a]	19.8 ± 0.49[a]
	0.75		88.0 ±0.73[b]	15.5 ± 0.45[b]
	1.0		75.8 ± 0.86[c]	8.2 ± 0.48[d]
		0.5	35.5 ± 7.8[d]	5.2 ± 1.16[e]
		0.75	23.6 ± 4.77[e]	3.7 ± 0.92[e]
		1.0	21.6 ± 5.51[e]	3.6 ± 0.71[e]

Data recorded after 4 weeks of culture incubation.

The value represent the mean ± SE calculated on three independent experiments, each based on minimum of 15 replicates. Values followed by the same letter were not significantly different at 5% level (DMRT).

Hardening in greenhouse

After 25-30 days of acclimatization plantlets measuring 5-6 cm long were successfully transferred from bottles to pots containing soil: sand: compost (1:1:1) ratio. The cloned plantlets were hardened successfully in the greenhouse and transferred to the pots (Fig. 1D). The hardened plantlets of *S. suaveolens* were transferred to the nursery after 45 days of hardening in the greenhouse.

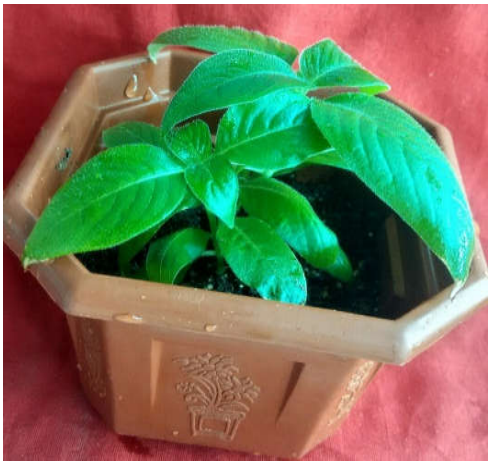

Fig.5- Hardened plant in green house

This is the first report on *in vitro* propagation of *S. suaveolens* at mass scale from mature somatic tissues. The cloning process developed and described in this paper offers a quick method of propagation as well as *ex-situ* conservation of this medicinal plant.

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CONCLUSIONS

The outcome of the present investigation will be helpful for Pharmaceutical industries as over increasing demand this plant. In addition, it is also helpful for the traditional practitioners and researchers.

Reference

Anonymous, (1948-1976): The Wealth of India: A dictionary of Indian Raw materials and Industrial products. Publication and information directorate, CSIR, New Delhi **1**-11.

Bonga, J. M. and Von Aderkas P. (1992): *In vitro* cultures of Trees. Kluwer Academic Publishers, the Netherlands.

Boulay, M. (1985): Some practical aspects and applications of the micropropagation of forest trees. Proc.

International Symp. on "*In-vitro* propagation of forest Tree species" Bologna. A.R.F. Bologna. 53-81.

Deora, N.S. and Shekhawat, N.S. (1995): Micropropagation of *Capparisdeciduas* (Forsk.) Edgew - A tree of arid horticulture. Plant Cell Rep., **15**: 278-281.

Franclet, A. and Boulay, M. (1989): Rejuvenation and clonal silviculture for *Eucalyptus* and forest species harvested through short rotation In: Pereira, J. S. and Landsberg J. J. (eds) Biomass Production by Fast-Growing Trees. Kluwer Academic Publishers, the Netherlands, pp. 267-274.

Fransworth, N.R and Segelman, A.B. (1971): Hypoglycemic plants. Tile Till., **57**: 53-55.

Jain, S.K. (1991): Dictionary of Indian folk medicine and ethnobotany. Deep publication, New Delhi, India.**1-**223.

Jawahar, M.,Karthikeyan, A.V.P.,Vijai, D.,Maharajan, M.,Ravipaul, S. and Jeyseelan, M. (2008b): *In vitro* plant regeneration from different explants of *Cardiospermumhelicacabum* L. *The International Journal of Biological and Chemical Sciences***, 2**:14-20.

Kapoor, L.D. (1997): Availability of Medicinal and aromatic plants in north West Indian. In: Atal C.K. and Kapus B.M. (edn.) Cultivation and Utilization of Medicinal and Aromatic Plants. Regional Research Laboratory, Jammu-Tawi, 439-448.

Kulkarni, M.G. and Sathe, P.S. (2013): Phytochemical and GC-MS analysis of *Hamiltonia suaveolens* (ROXB). *International Journal of Chem. Tech Research*. **5**:212-219.

Kumar, A., Jayachandran, T., Aravindhan, P., Deecaraman, D.,Iiavarasan, R. and Padmanabhan, N. (2009): Neutral components in the leaves and Seeds of *Syzygium cumini*. *African Journal of Pharmacy and Pharmacology*, **3:**560–561.

Mahmoodreza, M., Foro, K., Hossein, T. and Ghasemia. Y. (2010): Composition of the essential oil of *Rosa Damascene* mill. From south of Iran. Iranian Journal of Pharmaceutical Sciences Winter,**6**:59-62.

Mitra, R. (1985): Bibliography on Pharmacognosy of Indian Medicinal Plants, National Botanical Research Institute Lucknow. **86.**

Modupe, O., Wesley, O., Morufu, A. and Elizabeth, A.O. (2010): Analysis of Essential Oil from the stem of *Chansmantheradependens*. *Journal of Natural Products*, **3**:47-53.

Murashige, T. and Skoog F. (1962): A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant., **15**: 473-497.

Naik, V.N. (2010): Flora of Marathwada, Amrut Prakashan, Aurangabad. **1**:450.

Patil, D.A. (2006): Ethnobotany of Nasik district Maharashtra. Daya Publishing House, 186.

Phulwaria, M., Rai, M.K., Harish, Gupta, A.K., Ram, K. and Shekhawat, N.S. (2011a): An improved micropropagation of *Terminalia bellerica* from nodal explants of mature tree. Acta PhysiologiaePlantarum, (in press) DOI 10.1007/s11738011-0828-3.

Rathore, J.S., Rathore, M.S. and Shekhawat, N.S. (2005): Micropropagation of *Maeruaoblongifolia* -a liana of arid areas. Phytomorphology, **55**: 241-247.

Rathore, T.S., Deora, N.S., Shekhawat, N.S. and Singh, R.P. (1993): Rapid micropropagation of a tree of arid forestry *Anogeissusacuminata*. Biol. Plant., **35**: 381-386.

Ravi, J. and Wahi, A.K. (1995): Antidiabetic studies of *Daemiaextensa*. *International seminar on recent trends in pharmaceutical sciences, Ootacamund*, 44.

Shekhawat, M.S., Shekhawat, N.S., Harish, K.R., Phulwaria, M. and Gupta, A.K. (2011): High frequency plantlet regeneration from nodal segment culture of female *Momordicadioica* (Roxb.). *J. Crop Sci. Biotech*., **14**(2): 133-137.

Singh, V. and Jain, D.K. (1985): Taxonomy of Angiosperm. 2nd edition, 1985,1981, V. Singh and D.K. Jain, Rastogi Publications, Meerut-250002, India, 303, 306.

Sirisha, N., Sreenivasulu, M., Sangeeta, K. and Madhusudhana, C.C. (2011): Antioxidant Properties of *Ficus* Species- A review. *International Journal of Pharm-Tech Research,* **24**:2174-2182.

Sonar, V.G. (1968): Chemical composition of the roots of *Hamiltonia suaveolens*. *Journal of Shivaji University*, **1:**85-90.

Watt, George. (1889-1893): A dictionary of Economic products of India. Periodical expert, Shahadara, Delhi, India. **1-**6.

Zhu, S., Zhang, Q., Chen, Q., Zhou, T. and Yao, R. (2010): Study on the Chemical constituents of *Dehrocephala integrifolia*. *Journal of Chinese Medicinal Materials*, **1**.

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