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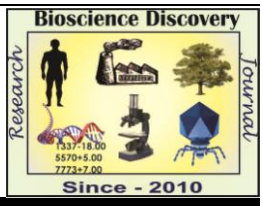

In vitro rhizome induction of *Hedychium coronarium* Koenig., a rhizomatous medicinal and aromatic plant

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Abstract

Rhizomes are inferred to be the part of importance for plants under Zingiberaceae family due to their tremendous medicinal value in traditional as well as modern medicine. Therefore the present study highlights a protocol on *in vitro* rhizome induction and its qualitative phytochemical screening in *Hedychium coronarium*, a rhizomatous medicinal and aromatic plant. Zygotic embryos were used as explants for establishment and multiplication of *in vitro* cultures. Shoot and root development occurred simultaneously in 15 µM IAA with maximum shoot number (4.17) and root number of (6.50) in 6 weeks of culture. Two different carbons sources, a stress hormone and a polyamine were assessed for rhizome formation of which 7 % sucrose in MS medium displayed best results with an average rhizome length of (2.92 cm), rhizome weight (1.36 g) and rhizomatous buds (8.50) in 8 weeks of treatment. Phytochemical screening of methanolic extract of *in vitro* induced rhizomes shows the presence of major secondary metabolites such as alkaloid, flavonoid, glycoside, saponin, steroid and terpenoid.

INTRODUCTION

Hedychium coronarium J. Koenig commonly known as 'white ginger lily' is an erect perennial and aromatic rhizomatous plant of Zingiberaceae family, distributed in tropical and sub-tropical Asia. It possesses tremendous medicinal properties with anti-cancerous, anti-oxidant, anti-microbial, anti-fungal, anti-hypertensive etc also characterized by the presence of volatile oils and oleoresins of export value (Endringera *et al*., 2014). The traditional knowledge about the use of natural products and their derivatives for preparation and administration as novel therapeutic agents and valuable drugs has historically been exploited (Kakati and Barthakur, 2017). Such natural products also play significant role in modern medicine (Vasait and Khandare, 2017). Essential oil from rhizomes is used in the treatment of body aches, cold, contusion, diabetes, headache, inflammation, lancinating pain, osteoclastogenesis and rheumatic pain (Morikawa *et al*., 2002). Flower essential oil has significant anti-inflammatory activity and is valued in high grade perfumes (Matsumoto *et al*., 1993). The medicinal value of this plant is mentioned in Ayurveda, CharakaSamhita and Sushruta Samhita for treatment of a large number of human ailments (Shekhar and Anju, 2015).

Due to innumerable medicinal properties, the plants have been exploited from its natural habitat. *In vitro* rhizome induction technology can be incorporated as a research implement to protect these plants from severe exploitation since their development is independent of seasonal fluctuation. *In vitro* produced rhizomes can be used as an

alternative source for the production of bioactive compounds. Keeping in view the above mentioned points we aim to induce rhizomes and test the major phyto chemicals in *H. coronarium* under *in vitro* conditions.

MATERIALS AND METHODS

Seeds were treated with copper oxychloride 5 % WP and washed under running tap water. Surface sterilization were carried out under sterile condition with tween-20, 70% alcohol and 0.12% sodium hypochlorite for 10 min each, followed by rinsing with sterile double distilled water for 4-5 times. Zygotic embryos were extracted carefully and cultured in MS (Murashige and Skoog, 1962) medium containing 3% (w/v) sucrose, 0.8% (w/v) agar and 0.1% (w/v) activated charcoal and supplemented singly with different concentrations (5, 10, 15, 20, 25 µM) of 6-benzylaminopurine (BAP), Kinetin (Kn), Indole-3-acetic acid (IAA) and α-naphthaleneacetic acid (NAA) (Himedia).

The *in vitro* raised plantlets were sub-cultured three times in MS medium devoid of plant growth regulators (PGRs). Explants of single shoot were cultured in MS medium, 0.8% Agar and 0.1% activated charcoal with different rhizome induction treatments.

Table 1: Different treatments for *in vitro* rhizome induction in *H. coronarium*

Sl. no.	Treatments
1	(4-10%) Sucrose
2	(3-7%) Glucose
3	3% Sucrose + (5, 10, 15, 20, 25 µM) Abscisic acid
4	3% Sucrose + (5, 10, 15, 20, 25 µM) Spermine

The pH of the medium were adjusted to 5.8±0.03 with 1N NaOH or 1N HCl prior to autoclaving at 121ºC, 18 psi for 15 min. Cultures were incubated in growth room with 23±2ºC temperature, 14 h photoperiod with photosynthetic photon flux density of 60.0 µmol m^{-2} s^{-2} provided by cool white fluorescent tubes. The experimental data were analyzed through Tukey's test at 5% probability level ($P<0.05$) using Origin8.

Plantlets with well developed roots were washed thoroughly under running tap water and transferred into thermocol pots containing a mixture of soil, litter, charcoal and brick pieces in 4:2:1:1 ratio and covered with perforated plastic bags for a week.

In vitro rhizomes were collected separately for all the treatments, chopped, oven dried at 50ºC for 24 h and ground into powder form. Extraction was carried out in an orbital shaker at 150 rpm for 24 h using methanol as solvent (Himedia). Solution was filtered and supernatant was used for phytochemical screening following standard procedures (Singh, 2012).

RESULTS AND DISCUSSION

The embryos cultured in MS medium with PGRs initiates' growth within 1 week from inoculation. However, optimum number of shoots (4.17 ± 0.31) and roots (6.50 ± 0.43) were recorded in medium containing 15 µM IAA in 6 weeks (Table 2; Fig 1). The development of shoots and roots occur simultaneously which was supported by earlier findings in Zingiberaceae (Chirangini *et al*., 2005; Purohit *et al*., 2017).

MS medium supplemented with TDZ and Kn is reported best for shoot multiplication and IAA for root formation in *H. coronarium* from axillary bud (Parida *et al*., 2013). The decrease in shoot number with an increase in the concentration of all PGRs treated might be due to supra-optimal concentration which is not desirable for plant growth.

Different lowercase letters in superscript indicate significant differences; SE-standard error

Various concentrations of sucrose (Table 1) significantly affect *in vitro* rhizome formation in *H. coronarium*; while 7% displayed maximum rhizome length (2.92) rhizome weight (1.36 g) and rhizomatous buds (8.50) (Table 3; Fig 2). The efficiency of sucrose on enhancement of *in vitro* rhizome formation may be due to presence of high carbon energy in the form of sucrose (Nayak, 2000). There are reports on *in vitro* rhizome formation at 7.5% sucrose and propagation efficiency at 8% in *Zingiber officinale* (Sharma and Singh, 1995; Zheng *et al*., 2008), 5% in *Zingiber cassumunar* (Chirangini and Sharma, 2005) and 9% in Curcuma *longa* (Islam *et al*., 2004).

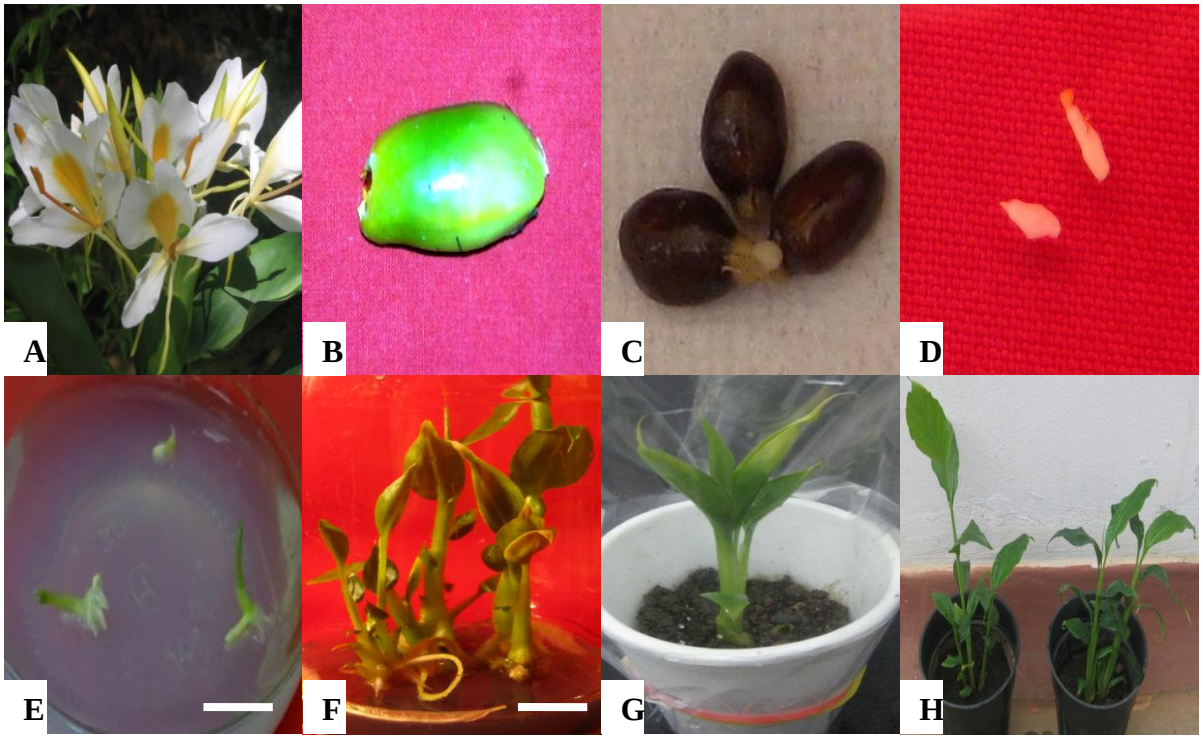

Fig. 1: *Hedychium coronarium*: A - flower in its habitat; B - fruit; C - seeds; D - embryos; E - initiation of growth after 1 week of culture; F - *in vitro* raised plantlets in MS medium containing 15µM IAA; G&H - hardened plants (Bar = 0.5 cm)

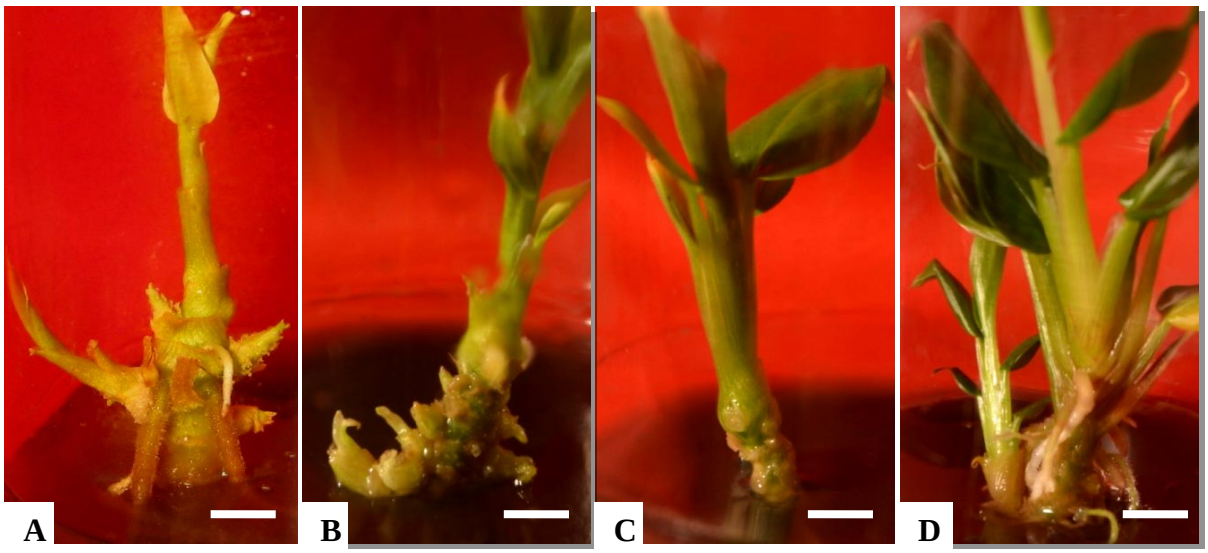

Fig. 2: *In vitro* induced rhizomes of *Hedychium coronarium*: A - Treatment 1(MS medium+7% sucrose); B - Treatment 2 (MS medium+5% Glucose); C – Treatment 3 (MS medium+3% sucrose+15µM ABA); D – Treatment 4 (MS medium+3% sucrose+5µM Spermine) (Bar = 0.5 cm)

Table 2: Effects of PGRs on propagation of *H. coronarium* after 6 weeks of culture

PGRs (µM)				Shoot number ± SE	Shoot length (cm) ± SE	Root number ± SE	Root length (cm) ± SE
BAP	Kn	IAA	NAA				
5				1.83 ± 0.31^{b}	3.67 ± 0.44^{a}	1.67 ± 0.21^{b}	1.33 ± 0.17^{ab}
10				2.17 ± 0.17^{b}	4.08 ± 0.27^{a}	1.83 ± 0.31^{b}	1.50 ± 0.13^{ab}
15				2.67 ± 0.21^{ab}	4.17 ± 0.28^{a}	2.83 ± 0.31^{ab}	1.83 ± 0.17^{a}
20				3.67 ± 0.21^{a}	4.50 ± 0.37^{a}	3.83 ± 0.48^{a}	1.75 ± 0.21^{a}
25				2.17 ± 0.31^{b}	4.25 ± 0.48^{a}	2.17 ± 0.31^{b}	1.08 ± 0.08^{b}
	5			2.83 ± 0.31^{abc}	3.25 ± 0.38^{a}	3.83 ± 0.40^{ab}	1.75 ± 0.31^{a}
	10			3.83 ± 0.31^{a}	4.50 ± 0.26^{a}	4.17 ± 0.31^{a}	1.42 ± 0.15^{ab}
	15			3.33 ± 0.33^{ab}	4.18 ± 0.39^{a}	2.67 ± 0.33^{bc}	1.08 ± 0.08^{ab}
	20			2.50 ± 0.22^{bc}	3.50 ± 0.18^{a}	2.33 ± 0.21^{c}	0.95 ± 0.14^{b}
	25			1.83 ± 0.31^{c}	3.50 ± 0.26^{a}	1.67 ± 0.21^{c}	0.82 ± 0.09^{b}
		5		2.83 ± 0.31^{b}	3.58 ± 0.27^{b}	3.83 ± 0.60^{b}	3.25 ± 0.17^{b}
		10		3.67 ± 0.33^{ab}	4.08 ± 0.33^{ac}	5.33 ± 0.76^{ab}	4.58 ± 0.30^{a}
		15		4.17 ± 0.31^{a}	4.92 ± 0.24^{a}	6.50 ± 0.43^{a}	4.25 ± 0.34^{ab}
		20		3.67 ± 0.33^{ab}	4.17 ± 0.25^{abc}	5.50 ± 0.50^{ab}	3.92 ± 0.24^{ab}
		25		3.50 ± 0.22^{ab}	3.67 ± 0.25^{bc}	4.0 ± 0.63^{b}	3.50 ± 0.18^{b}
			5	3.83 ± 0.31^{a}	3.83 ± 0.49^{a}	5.50 ± 0.43^{a}	4.33 ± 0.34^{a}
			10	3.33 ± 0.21^{ab}	3.92 ± 0.37^{a}	5.0 ± 0.58^{a}	4.25 ± 0.38^{a}
			15	3.0 ± 0.26^{ab}	4.08 ± 0.33^{a}	4.67 ± 0.80^{a}	3.67 ± 0.25^{a}
			20	2.83 ± 0.31^{ab}	4.25 ± 0.49^{a}	3.50 ± 0.34^{a}	3.33 ± 0.36^{a}
			25	2.67 ± 0.21^{b}	3.42 ± 0.40^{a}	3.50 ± 0.34^{a}	3.17 ± 0.28^{a}

Similarly, glucose at 5% contributes significantly on *in vitro* rhizome formation with maximum rhizome length (2.41 cm), rhizome weight (0.34g) and rhizomatous buds (4.08) (Table 3; Fig2).

Maximum rhizome length (1.37 cm), rhizome weight (0.25 g) and rhizomatous buds (2.16) was recorded in 15 µM Abscisic acid (Table 3). ABA promoting *in vitro* rhizomes, tubers, bulbs and corms formation has been reported (Xu *et al*., 1998; Cheethaparambil *et al*., 2013). Different lowercase letters in superscript indicate significant differences; SE-standard error

Polyamines occur ubiquitously in most cells of plant and influences several physiological and developmental processes (Valero *et al*., 2002). *H. coronarium* showed maximum rhizome length (2.70), weight (0.63 g) and rhizomatous buds (5.0) at 5 µM spermine (Table 3). Spermine has been reported to promote *in vitro* tuber formation in potato and bulbs in tulip (Mader, 1995; Podwyszynska *et al*., 2015).

The acclimatized *in vitro* raised plantlets showed 90% survivability (Fig 1) as the compost mixture used might have provided all the essential nutrients for proper growth of plantlets and aeration for root respiration.

Screening of phytochemical for detection of their presence in a plant may be significant for pharmacological formulations (Nandagoapalan *et al*., 2016). The phytochemical screening of *in vitro* rhizomes shows the presence of alkaloid, flavonoid, glycoside, saponin, steroid and terpenoid (Table 4). This proves the retention of major phyto chemicals in the induced rhizomes as compared to *in vivo rhizomes* of *H. coronarium* as reported earlier by Singh and Bag, 2013; Dash and Sheikh, 2015.

Table 3: Effects of different treatments on *in vitro* rhizome induction after 8 weeks of culture

Treatment no.	Sucrose (%)	Glucose (%)	ABA (µM)	Spermine (µM)	Rhizome length (cm) ± SE	Rhizome weight (g) ± SE	Rhizomatous buds ± SE
		Control			0.07 ± 0.03^{b}	0.06 ± 0.02^{b}	0.50 ± 0.22^{b}
	4				0.55 ± 0.09^{bce}	0.45 ± 0.04^{bc}	2.83 ± 0.30^{bc}
	5				0.67 ± 0.08^{bce}	0.59 ± 0.05^{c}	3.67 ± 0.33^{cd}
	6				1.25 ± 0.21^{cd}	0.91 ± 0.09^{cd}	5.67 ± 0.61^{de}
1	7				2.92 ± 0.24^{a}	1.36 ± 0.17^{a}	8.50 ± 1.09^{a}
	8				1.75 ± 0.28^{d}	1.12 ± 0.12^{ad}	7.0 ± 0.71^{ae}
	9				1.03 ± 0.17^{df}	0.80 ± 0.06^{cd}	5.0 ± 0.36^{ce}
	10				0.65 ± 0.08^{bcf}	0.64 ± 0.04^{c}	4.0 ± 0.25^{cd}
		3			0.91 ± 0.18^{c}	0.16 ± 0.02^{bd}	2.41 ± 0.45^{acd}
		4			2.0 ± 0.27^{a}	0.22 ± 0.02^{cd}	3.08 ± 0.57^{acd}
2		5			2.41 ± 0.16^{a}	0.34 ± 0.01^{a}	4.08 ± 0.35^{a}
		6			1.66 ± 0.21^{ac}	0.21 ± 0.01^{d}	2.58 ± 0.35^{ad}
		7			1.12 ± 0.18^{c}	0.20 ± 0.02^{d}	1.58 ± 0.41^{bd}
			5		0.88 ± 0.14^{a}	0.13 ± 0.02^{bc}	0.66 ± 0.22^{b}
			10		1.02 ± 0.13^{a}	0.14 ± 0.01^{bc}	0.75 ± 0.21^{b}
3			15		1.37 ± 0.17^{a}	0.25 ± 0.02^{a}	2.16 ± 0.40^{a}
			20		1.19 ± 0.20^{a}	0.23 ± 0.03^{a}	1.08 ± 0.19^{b}
			25		0.82 ± 0.16^{a}	0.14 ± 0.02^{bd}	0.75 ± 0.25^{b}
				5	2.70 ± 0.15^{a}	0.63 ± 0.02^{a}	5.0 ± 0.42^{a}
				10	1.50 ± 0.21^{c}	0.40 ± 0.05^{c}	3.66 ± 0.5^{ac}
4				15	1.33 ± 0.21^{cd}	0.26 ± 0.03^{cd}	2.50 ± 0.43^{ce}
				20	1.29 ± 0.20^{cd}	0.21 ± 0.03^{bd}	1.91 ± 0.54^{cde}
				25	0.62 ± 0.12^{bd}	0.17 ± 0.02^{bd}	1.50 ± 0.31^{de}

Table 4: Results of qualitative phytochemical screening

Treatments	Alkaloid	Flavonoid	Glycoside	Phenol and Tannin	Saponin	Steroid and terpenoid
Control	+	++	++	-	+	++
Sucrose	+	++	++	-	+	++
Glucose	+	+	+	-	+	++
ABA	+	+	+	-	+	++
Spermine	+	+	+	-	+	++

(++) Strong positive; (+) Positive; (-) Negative

CONCLUSION

Rhizome of *H. coronarium* produces an essential oil which is of great importance in medicinal, pharmaceuticals and ayurvedas. Therefore, the technology of inducing rhizome in large scale can be implemented under laboratory conditions. Till date, to the best of our knowledge there are no reports on *in vitro* rhizome induction in *H. coronarium* and other *Hedychium* species. The present study reveals the first report on *in vitro* rhizome induction in *H. coronarium* and also screens its quality in phytochemistry. However, further investigations are still warranted on in depth research on photochemistry.

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REFERENCES

Cheethaparambil A, Pillai GS and Balachandran I, 2013. Comparative studies on *in vitro* microrhizome induction in three varieties of *Curcuma longa* (Turmeric) – The role of two stress hormone. *Int. J. Sci. Res. Manag.*, **1**(4):230-237.

Chirangini P and Sharma GJ, 2005. *In vitro* propagation and microrhizome induction in *Zingiber cassumunar* (Roxb.) – an antioxidant - rich plant. *J. Food Agric. Environ.*, **3**(1):139-142.

Chirangini P, Sinha SK and Sharma GJ. 2005. *In vitro* propagation and microrhizome induction in *Kaempferia galanga* Linn. and *K. rotunda* Linn., *IJBT*, **4**:404-408.

Dash PR and Sheikh Z, 2015. Preliminary studies on phytochemicals and cytotoxic activity of methanolic rhizome extract of *Hedychium coronarium. J. Pharmacogn. Phytochem.*, **4**(1):136-139.

Endringera DC, Taveira FSN, Kondratyuk TP, Pezzuto JM and Braga FC, 2014. Cancer chemoprevention activity of labdane diterpenes from rhizomes of *Hedychium coronarium*. *Rev. Bras. Farmacogn.*, **24**(4):408-412.

Islam MA, Kloppstech K and Jacobsen HJ, 2004. Efficient protocol for *in vitro* microrhizome induction in *Curcuma longa* L. (Zingiberaceae) - A medicinal plant of tropical Asia. *Plant Tissue Culture*, **14**(2):123-134.

Kakati D and Barthakur SK, 2017. Study on indigenous knowledge and approach for conservation of *Brucea mollis* Wall. ex Kurz- an RET plant of NE India. *Bioscience Discovery*. **8**(2):119-124.

Mader JC, 1995. Polyamines in *Solanum tuberosum in vitro*: free and conjugated in hormone-induced tuberization. *J. Plant. Physiol.*, **146**:115-120.

Matsumoto F, Idetsuki H, Harada K, Nohara I and Toyoda T, 1993. Volatile components of *Hedychium coronarium* Koenig flowers. *J. Essent. Oil Res.*, **5**(2):123-133.

Morikawa T, Matsuda H, Sakamoto Y, Ueda K and Yoshikawa M, 2002. New franesane type sesquiterpenes, hedychiols a and b 8, 9-diacetate and inhibitors of degranulation in rbl-2h3 cells from the rhizome of *Hedychium coronarium*. *Chem. Pharm. Bull.*, **50**(8):1045–1049.

Murashige T and Skoog F, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*, **15**(3):473-497.

Nandagoapalan V, Doss A and Marimuthu C, 2016. Phytochemical analysis of some traditional medicinal plants. *Bioscience Discovery*. **7**(1):17-20.

Nayak S, 2000. *In vitro* multiplication and microrhizome induction in *Curcuma aromatic* Salisb. *Plant Growth Regul.*, **32**(1):41-47.

Parida R, Mohanty S and Nayak S, 2013. *In vitro* propagation of *Hedychium coronarium* Koen. through axillary bud proliferation. *Plant Biosyst.*, **147**(4):905-912.

Podwyszynska M, Kosson R and Treder J, 2015. Polyamines and methyl jasmonate in bulb formation of *in vitro* propagated tulips. *Plant Cell Tiss. Org. Cult.*, **123**(3):591-605.

Purohit S, Nandi SK, Paul S, Tariq M and Palni LMS, 2017. Micropropagation and genetic fidelity analysis in *Amomum subulatum* Roxb.: a commercially important Himalayan plant. *JARMAP*, **4**:21-26.

Sharma TR and Singh BM, 1995. *In vitro* micro rhizome production in *Zingiber officinale* Rosc. *Plant Cell Rep.*, **15**:274-277.

Shekhar TC and Anju G, 2015. A comprehensive review on *Hedychium coronarium* J. Koenig. (Dolanchampa/ Kapurkachri). *Int. J. Res. Ayurveda Pharm.*, **6**:98-100.

Singh AP, 2012. Identification of chemical constituents of plant *Hedychium spicatum*. LAP LAMBERT Academic Publishing, Germany.

Singh KL and Bag GC, 2013. Phytochemical analysis and determination of Total Phenolics Content in water extracts of three species of *Hedychium*. *Int. J. Pharm. Tech. Res.*, **5**(40):1516-1521.

Valero D, Martinz-Romero D and Serrano M, 2002. The role of polyamines in the improvement of the shelf life of fruit. *Trends in food Science & Technology*, 13:228-234.

Vasait RD and Khandare K, 2017. Preliminary assessment of phytochemical constituents and antibacterial activity of crude leaves extracts of *Simarouba glauca*. *Bioscience Discovery*. **8**(1):30-34.

Xu X, van Lammeren AAM, Vermeer E and Vreugdenhil D, 1998. The role of Gibberellin, Abscissic Acid and Sucrose in the regulation of Potato tuber formation *in vitro*. *Plant Physiol.*, **117**:575-584.

Zheng Y, Liu Y, Ma M and Xu K, 2008. Increasing *in vitro* microrhizome production of ginger (*Zingiber officinale* Roscoe). *Acta Physiol. Plant.*, **30**(4):513-519.

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