Eco. Env. & Cons. 26 (October Suppl. Issue) : 2020; pp. (S33-S38)
Copyright@ EM International
ISSN 0971–765X

Establishment of *in vitro* cultures of valuable medicinal plant *Valeriana jatamansi* jones, its conservation and production of bioactive metabolites

Sushma Pandey[1]* and Bijaya Pant[1]

[1]Central Department of Botany, Tribhuvan University, Kathmandu, Nepal

(Received 17 April, 2020; accepted 9 June, 2020)

ABSTRACT

Valeriana jatamansi Jones is a perennial herb, belonging to the family Valerianaceae. The pharamaceutical significance of this plant is mainly because of the rhizomes and roots. The species has become threatened in its natural habitats due to exploitation of its rhizomes for drug preparation in pharmaceutical industries. *In vitro* plant cell and tissue culture would not only surmount the limitations of vegetative propagation but also can hasten the production of clonal material for field planting and production of unlimited amount of secondary metabolites. In the present study, MS medium supplemented with 2 mg/L of Benzyl amino purine (BAP) produced maximum shoot bud regeneration with shoot numbers (6), shoot lengths (3 cm), root numbers (6.3), and root length (1.4 cm). Methanolic *in vitro* extracts of *V. jatamansi* resulted in the highest phenolic content (55± 1.00 mg GAE/g) compared to the wild sample (49±1.00 GAE/g) and the highest flavanoid content (219±1.00 mg QE/g) as compared to the wild (124±1.00 mg QE)/g). DPPH activity was also highest in *in vitro* extracts (84.33±0.577%) as compared to the wild (78.33±0.569%). GC-MS profiling of the wild plant extracts revealed the presence of important bioactive compounds including valeric anhydride, valerenol. A reproducible protocol was established using shoot bud explants which will be benefitted towards utilization of natural resources for the production of pharmaceutically important compounds at a large scale from *V.jatamansi.*

Key words: *Secondary metabolites, Plant tissue culture, Phytochemical analysis, GC-MS*

Introduction

Valeriana jatamansi, a valuable medicinal plant is an herb, belonging to the family valerianaceae, also known as Sugandhawal have multiple uses in traditional and modern systems of medicine and also a native plant species of Himalaya, distributed widely in the tropical and subtropical regions of the world (Prakash *et al*., 1999). The essential oil and extracts from the roots and rhizomes is used worldwide (Singh *et al*., 2010), and is highly demanded in drug industry (Das *et al*., 2013). In Nepal, this species has been banned for export due to its high medicinal properties and also been prioritized for research and development (Chaudhary *et al*., 2016). The presence of valeopotriates, a group of monoterpenoids having epoxy group and beta-acetoxy isovaleric acids has been a source of high medicinal importance in this plant (Kaur *et al*., 1999). The pharmaceutical

Abbreviations

MS: Murashige and Skoog, 1962; mg/L: milligram per liter; GAE/g: Gallic acid equivalent per gram; QE/g: Quercetin equivalent per gram; DPPH: 2,2-diphenyl-1-picrylhydrazyl; GC-MS: Gass chromatography mass spectrometry; BAP: Benzyl amino purine.

benefits of valeopotriates is due to its tranquilizing properties which play a major role in its its effectivity in leprosy (Singh *et al*., 2010). Roots and rhizomes of the species has been reported to cure obesity, skin diseases, epilepsy, insanity and skin poisoning (Prakash *et al*., 1999). In India this species has been listed in high trade consumption between 100 and 200 metric tons as the plants have been continuously used in industries thus depleting the natural resources (Jugran *et al*., 2015). Thus conservation of this species is very important to meet the needs of the pharmaceutical industries. The roots and rhizomes are continuously being harvested from large quantities from the wild to meet the demand of pharmaceutical industries because of valeopotriates and valtrates which contributes to its multipurpose medicinal value, thus leading to the conservation and sustainable utilization (Kumar *et al*., 2012). Naturally, the herb is propagated through seeds for the propagation, but still has limitation for propagation due to dormant nature of the seeds (Kaur *et al*., 1999). Therefore, plant tissue culture can be a alternative for *in vitro* propagation in a large scale within a small period of time as this method has been reliable and useful for conservation of important medicinal plants and production of important phytochemicals (Pant *et al*., 2014). Previously, the bioactive constituents in north-east India analyzed by GC-MS revealed the presence of twenty-one compounds (Das *et al*., 2011). Though there are reports on propagation of *V. jatamansi* Jones (Das *et al* 2013., Kaur., 1999, Jugran *et al*., 2015), this paper focuses on using shoot bud explants for *in vitro* propagation of *V. jatamansi*. Phytochemical analysis for the total phenolics, flavanoids and antioxidant activity of the wild roots and also the *in vitro* roots was determined and compared followed by GC-MS of the wild plants to characterize the valuable secondary metabolites.

Materials and Methods

Plant material and surface sterilization

The fresh plants of *V. jatamansi* were collected from Dabur Nepal, Kathmandu and planted in the departmental garden of Tribhuvan University. Shoot buds explants were excised and rinsed in running tap water followed by sterilization with few drops of Tween 20 (Himedia, India) and rinsing in sterile water for 30 min. The explants were then treated with 0.1% $HgCl_2$ (mercuric chloride) for 5 min followed by rinsing five times with sterile water to remove traces of $HgCl_2$. The explants were dried using a Whatman filter paper and inoculated onto Murashige and Skoog (MS) (Murashige and Skoog 1962) medium containing 3% sucrose (w/v), 0.1% myoinositol supplemented with different combination of BAP, prior adjusting the pH of the medium to 5.8 prior to autoclaving (121 ºC, 20 min), solidified with 0.8% plant tissue culture tested agar-agar (w/v) (Himedia, India) The cultures were maintained at 25 °C ± 2 °C and 1000 flux lux under 16/8 h photoperiod with 75% relative humidity (RH).

Inoculation in culture medium and Shoot Proliferation, Elongation and root formation

Explants (shoot buds) were cultured on MS basal medium supplemented with different concentrations BAP (0.5 to 2 mg/L). All cultures were incubated under 16h photoperiod with a light intensity of 55 $\mu molm^{-2}s^{-2}$ provided by cool white fluorescent lamps (Phillips, India) at 25 ± 2 °C. Subcultures were carried out after 4-weeks and number of shoots were calculated.

Acclimatization and Hardening

After 5-6 weeks, the *in vitro* raised plantlets were taken out from the culture medium, rinsed with distilled water. The plantlets were subsequently transplanted into plastic pots containing a mixture of coco peat: soil (3:1), and placed in green house.

Phytochemical analysis

Plant material and extract preparation

The fresh roots of wild plant as well as *in vitro* plants were washed under running tap water, dried with filter paper and shade dried at room temperature. 100 mg of dried tissue was homogenized in 100 mL of methanol and extractions were carried on an orbital shaker (REMI, India) with constant stirring at 180 rpm for 24 h followed by centrifuging at 10,000 rpm for 15 min and the supernatant ltered through lter paper. Measurements for biochemical parameters were taken in Lamda-35 double beam spectrophotometer (Perkin-Elmer, USA).

Determination of total polyphenols, total flavanoid

Quantitative estimation of total phenolic content (TPC), and total flavanoid content (TFC), using the extracts were carried out according to the methods

described by Singleton and Rossi (1965) and Chang *et al.,* (2002). The results of TPC, TFC, were expressed in mg standard gallic acid equivalent (GAE) and quercetin equivalent (QE) per gram of dry tissue respectively.

Antioxidant activity assay

DPPH radical scavenging assay

The DPPH radical activity was estimated using the method of Liyana-Pathiranan and Shahidi, (2005). A solution of 0.135 mM DPPH in methanol was prepared, 1.0 mL of this was mixed with 1.0 mL of different concentrations (0.02 – 0.1 mg) of the extract prepared in methanol, following the reaction mixture vortexed in dark at room temperature 27 ºC for 30 min The absorbance was measured spectrophotometrically at 517 nm. DPPH radical scavenging activity was calculated as follows

DPPH radical scavenging activity (%) = [(Abs control – Abs sample)]/(Abs control)] x 100 where Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract /standard.

GC MS analysis

The extracts of the wild roots were analyzed using gas chromatography and mass spectrometry GC-MS 4000 (Varian, USA)system with a HP-5MS agilent column (30m × 0.25 mm, 0.25 5 film thickness). Injector temperature was 28 C. Oven temperature programme used was holding at 50 ºC for 5min, heating to 28 ºC at 3 ºC/min, and keeping the temperature constant at 28 ºC for 7min. Helium was used as a carrier gas at a constant flow of 1.0 mL/min and an injection volume of 0.20 5L was employed. The important bioactive compounds were studied according to previous literatures and their biological properties.

Data Analysis

All the samples were analyzed in triplicates. The values were expressed as means of triplicate analysis of the samples (n = 3) ± SD. All the data were further analyzed using a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test ($p< 0.05$) with the aid of SPSS (17 version) statistical package program. $p< 0.05$ was considered as indicative of significance and the percentage of response was scored on the basis of DMRT analysis.

Results and Discussion

Establishment of *in vitro* cultures from shoot buds

Das *et al*., (2013) reported efficient callus-mediated shoot regeneration system for the large-scale production of *V. jatamansi* Jones. The effect of composition and contents of growth regulators in culture media on the accumulation of secondary metabolites in *in vitro* cultures is well known Maurmann *et al*., 2006. In our study, the shoot bud, explants inoculated on MS media supplemented with various concentration of BAP (Table 1), for shoot initiation and proliferation. Shoot initiation was observed in the medium supplemented with BAP (Fig 1B), particularly among the various concentrations of BAP tested 2 mg/L induced maximum shoot initiation (6.0 shoots per explant) after 7 days of culture (Fig 1C, D). The average shoot length (3.0 cm), average root numbers (6.3) and average root length (1.4 cm) were also highest compared to other tested concentration of BAP (Table 1). High multiplication and rooting rate was achieved using combination of BAP (1.5µM) along with either NAA or IAA (0.5 µM), and GA_3 by Purohit *et al*., 2015. This combination of BAP and NAA has been used for high-frequency shoot multiplication for several species including *V. jatamansi.* Kaur *et al*., (1999) also reported

Table 1. Statistical analysis of all the treatments using ANOVA. Data were taken after 5 weeks of culture.Data presented as mean±SD

Plant Growth Regulators BAP (mg/L)	Average shoot number	Average shoot length (cm)	Average root number	Average root length (cm)
0	1.0±0.00	0.5±0.00	0.3±0.33	0.16±0.16
0.5	2.3±0.33	1.4±0.06	2.0±0.57	0.3±0.33
1	4.0±0.57	2.0±0.00	3.667±0.33	0.2±0.03
1.5	5.0±0.00	2.1±0.05	1.667±0.33	0.1±0.057
2	6.00±0.00	3.0±0.10	6.33±0.33	1.4±0.17

Table 2. Contents of total phenolic and flavonoid of *in vitro* and wild extract. Values are expressed as mean ± SD (n=3).

Sample	Extract	Total phenol (mg gallic acid g^{-1})	Total flavonoids (mg quercetin g^{-1})
In vitro	Methanol	55± 1.00	219±1.00
Wild	Methanol	49±1.00	124±1.00

that shoot buds cultured on medium fortified with BA and IAA or NAA induced shoots, and subsequently produced roots on the same medium. During our experiments using BAP, root formation was also observed (Fig 1C, D). Previous literatures reported that cytokinins BA, TDZ, zeatin or Kinetin had no effect on callus induction, shoot organogenesis Das *et al*., 2013. But in our experiment BAP produced plantlet regeneration. The production of roots in *Valeriana* species is very important because the valuable bioactive metabolites is accumulated in roots and roots are harvested to meet the demands of industries. The acclimatized plantlets were established showed and 80 % survival rate (Fig 1E,F). Being one of the medicinally rich plant, the knowledge of the biochemical constitution of *V. jatamansi* is essential. The methanolic *in vitro* root extracts of *V. jatamansi* possessed the highest phenolic, flavanoid content and antioxidant activity when compared with wild extracts (Table 2, 3). The phenolic content of all the extracts was considerably high, which could be a major contributing factor to the antioxidant activity of this plant extracts. According to Asekun *et al*., (2007) high phenolic content of plant extracts could be responsible for their enhanced antioxidant activity. Flavonoids, a groups of natural compounds also classified as natural phenolics by Agrawal *et al*., (1989). Both valepotriates and valerenic acids were detected in root tissues of wild plants when profiled using GC-MS (Table 4). Thus this protocol can be useful for isolation and characterization of medicinally important metabolites valepotriates. The proposed tissue culture system will be able to maximize the production of these plants.

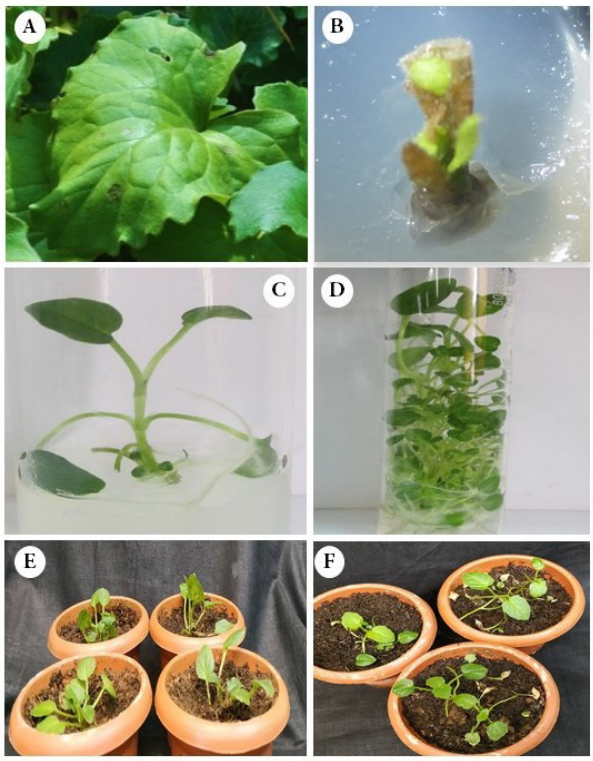

Fig. 1. *In vitro* regeneration of *V. jatamansi* in MS+ BAP (2mg/L) (**A**) Mother plant of *V. jatamansi*; (**B**) shoot bud explants post 3 days incubation; (**C**) Shoot initiation after 8 days of culture (**D**) Shoot elongation after 30 days of culture. (**E,F**) Plants of *V.jatamansi*, hardened on Coco peat : soil (3:1).

Table 3. Total antioxidant capacity of the extracts. Values are expressed as mean ± SD (n=3).

Sample	Extract	DPPH(%)
In vitro	Methanol	84.33±0.577
Wild	Methanol	78.33±0.569

Conclusion

In the present research, an efficient protocol for *in vitro* propagation of *V. jatamansi* using shoot bud as explants was established. This protocol will be useful for the conservationof this valuable medicinal plant. This paper presents the phytochemical potential of *in vitro* propagated plants to produce high amounts of phenolics, flavanoids and antioxidants. Important bioactives compounds including Valerenol and Valeric anhydride, a class of valepotriates was also detected from the wild roots of *V.jatamansi*, which is a pharmaceutically important compounds and simultaneously the *in vitro* cultures can also be used for the production of the same without disturbing the natural habitat.

Table 4. List of major compounds detected in GC-MS analysis of in wild roots of *V. jatamansi*with biological importance.

Name of compound	Retention Time (min)	Molecular Formula	MW (g/mol)	Biological importance	References
Valeric anhydride	22.727	$C_{10}H_{18}O_3$	186	Neuroprotective	Raina *et al*.,2015
Valerenol	27.276	$C_{15}H_{24}O$	220	Essential oil	Raina *et al*.,2015
Hexadecanoic acid	34.360	$C_{17}H_{34}O_2$	270	Antimicrobial activity, anti cancer activity	Chowdhary *et al*., 2015
DL-Proline	20.939	$C_6H_9NO_3$	143	Drought-stress tolerant	Hayat *et al*., 2012
Octadecanoic acid	38.158	$C_{19}H_{36}O_2$	296	Anti-inflammatory	Senguttuvan *et al*., 2014

Recommendation

From the present research, since the pharmaceutical industries harvest all the wild resources, the present protocol can be useful to produce the plant irrespective of season, which can be highly benefitted to the farmers to produce the plants throughout the year. Supplementation of BAP as a growth hormone can aid in producing more plants in short duration, which will be a great advantage for the pharmaceutical industries as well as the farmers.

Acknowledgement

The authors would like to thank University Grants commission, Nepal for Fellowship support (award no PhD/73-74/S&T-10) and also would like to thank Dabur Nepal Pvt Limited, Kathmandu, Nepal for providing the plant material.

References

Agrawal, P. K. (Ed.). 2013. *Carbon-13 NMR of flavonoids*. Elsevier.

Asekun, O. T., Grierson, D. S. and Afolayan, A. J. 2007. Effects of drying methods on the quality and quantity of the essential oil of *Mentha longifolia* L. subsp. Capensis. *Food Chemistry*. 101(3) : 995-998.

Chang, C. C., Yang, M. H., Wen, H. M. and Chern, J. C. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*. 10(3).

Chaudhary, R. P., Uprety, Y. and Joshi, S. P. 2016. Plant Biodiversity In Nepal: Conservation and Legal Status. Frontiers of Botany. Central Department of Botany (CDB), Tribhuvan University, Kathmandu, Nepal. 224-268.

Chowdhary, K. and Kaushik, N. 2015. Fungal endophyte diversity and bioactivity in the Indian medicinal plant *Ocimum sanctum* Linn. *PLoS One*. 10(11): e0141444.

Das, J., Mao, A. A. and Handique, P. J. 2013. Callus-mediated organogenesis and effect of growth regulators on production of different valepotriates in Indian valerian (*Valeriana jatamansi* Jones.). *Acta Physiologiae Plantarum*. *35*(1) : 55-63.

Das, J., Mao, A. A. and Handique, P. J. 2011. Terpenoid compositions and antioxidant activities of two Indian valerian oils from the Khasi Hills of north-east India. *Natural Product Communications*. 6(1): 1934578X1100600131.

Hayat, S., Hayat, Q., Alyemeni, M. N., Wani, A. S., Pichtel, J. and Ahmad, A. 2012. Role of proline under changing environments: a review. *Plant Signaling & Behavior*. 7(11) : 1456-1466.

Jugran, A. K., Bhatt, I. D. and Rawal, R. S. 2015. Identification of ISSR markers associated with valerenic acid content and antioxidant activity in Valeriana jatamansi Jones in the West Himalaya. *Molecular Breeding*. 35(2) : 73.

Kaur, R., Sood, M., Chander, S., Mahajan, R., Kumar, V., and Sharma, D. R. 1999. In vitro propagation of *Valeriana jatamansi*. *Plant Cell, Tissue and Organ Culture*. 59(3) : 227-229.

Kumar, A., Shirkot, P., Thakur, A. K. and Raina, R. 2012. Assessment of genetic diversity in *Valeriana jatamansi* Jones. germplasm using RAPD markers. *National Academy Science Letters*. 35(3) : 221-226.

Liyana-Pathirana, C. M. and Shahidi, F. 2005. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. *Journal of Agricultural and Food Chemistry*. 53(7) : 2433-2440.

Maurmann, N., De Carvalho, C. M., Silva, A. L., Fett-Neto, A. G., Von Poser, G. L. and Rech, S. B. 2006. Valepotriates accumulation in callus, suspended cells and untransformed root cultures of Valeriana glechomifolia. *In vitro Cellular & Developmental Biology-Plant*. 42(1) : 50-53.

Pant, B. 2014. Application of plant cell and tissue culture for the production of phytochemicals in medicinal plants. In *Infectious Diseases and Nanomedicine II* (pp.

25-39). Springer, New Delhi.

Prakash, V. 1999. *Indian Valerianaceae: a monograph on medicinally important family*. Scientific Publishers.

Purohit, S., Rawat, V., Jugran, A. K., Singh, R. V., Bhatt, I. D. and Nandi, S. K. 2015. Micropropagation and genetic fidelity analysis in Valeriana jatamansi Jones. *Journal of Applied Research on Medicinal and Aromatic Plants*. 2 (1) : 15-20.

Raina, A. P. and Negi, K. S. 2015. Essential oil composition of Valeriana jatamansi Jones from Himalayan regions of India. *Indian Journal of Pharmaceutical Sciences*. 77(2) : 218.

Senguttuvan, J., Paulsamy, S. and Karthika, K. 2014. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, Hypochaeris radicata L. for in vitro antioxidant activities. *Asian Pacific journal of Tropical Biomedicine*. 4 : S359-S367.

Singh, R. D., Meena, R. L., Sharma, B., Singh, B., Kaul, V. K. and Ahuja, P. S. 2010. Seasonal variation of bioactive components in Valeriana jatamansi from Himachal Pradesh, India. *Industrial Crops and Products*. 32(3) : 292-296.

Singleton, V. L. and Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*. 16(3) : 144-158.