A Review on Phytochemistry, Pharmacological Properties and Biotechnological Studies in Valeriana officinalis L., An Important Medicinal Herb

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Abstract

Medicinal plants are serving mankind to cure ailments since time immemorial and today they are equally important even with a higher potential, because of the understanding of the mechanism of action with the advent of modern technique and extensive research. The present review looks into the medicinal potentials of *Valeriana officinalis*. To encompass the major activities of this plant, information is collected pertaining to phytochemistry, pharmacological properties and biotechnological investigation carried out in various laboratories world-wide. *Valeriana officinalis*, known as Sumbu-ul-teeb in Unani system of medicine, is an important source of various bioactive compound viz. valerenic acid, valepotriates, alkaloids, flavonoids, lignans etc. Pharmaceutical application of valerian is due to its sedative, anticonvulsant, antidepressant, antihypertensive, hypnotic, antispasmodic, antidysmenorrheal and anxiolytic activity. The literature reviewed on biotechnological approaches include *in vitro* protocols for large scale production of valerian, genetic transformation and elicitation studies undertaken for enhanced production of valerenic acid.

Keywords: *Valeriana officinalis* L., Tagar, Valerenic Acid, Valepotriates, Sedative

Introduction

In recent times an upsurge is witnessed worldwide towards the use of medicinal plants as a whole or in the form of crude drug in traditional systems of medicine such as Ayurveda, Sidha and Unani, which draw the attention of modern science researchers to understand the isolation, application of bioactive compounds and mechanism of action to cure diseases in a more precise way. Now a huge amount of research outcomes are available to be utilised in a judicious way. The present review deals with the application of *Valeriana officinalis* an important medicinal plant of traditional system of medicine to cure various diseases as well as the use of biotechnological tools to conserve its fast depleting population because of indiscriminate collection, and the production of bioactive compounds through hairy root culture, elicitation, and genetic transformation for the overexpression of gene related to desired metabolites.

Botanical Name	:	*Valeriana officinalis* L.
Common name	:	All-heal, Garden heliotrope, Great wild Valerian, Amantilla, Phu, Nard, Theriacaria, Marinella, Genicularis and Terdina.
Unani name	:	Tagar

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The genus Valeriana, with about 200 species, belongs to the Valerianaceae family and grows throughout the world (Piccinelli *et al.*, 2004). *Valeriana officinalis* L. one of the medicinally important species of Valeriana, is a perennial flowering herb commonly known by various names such as all-heal, garden heliotrope, great wild Valerian etc (Cunha *et al.*, 2003). Two other ancient names are "nard" and "phu". "Nard" is derived from a Sanskrit word meaning "strong smell" and "phu" or "fu" refers to the usual exclamation of disgust that attends the experience of smelling the dried root. The word valeriana might have been derived from the Roman province of Valeria, or from Valerianus, a Roman emperor, or from a certain Valerius who first used the herb as medicine, while other writers believe it came from the Latin word *valere* (to be in health). The plant is native to Europe and Asia and has naturalized in eastern North America. It appears in moist places with mild climates, mainly in forests and river margins. It is cultivated in low lying, damp sandy humus with lime fertilizer (Cunha, 2005). Other important species of *Valeriana* includes *V. fauriei* used in Traditional Chinese Medicine and Japanese Medicine, *V. capensis* used in African traditional medicine, *V. edulis* used in Mexico and *V. wallichii* is used in India (Hikino *et al.*, 1975; Iwu, 1993; Huang, 1999). The collective species *V. officinalis* L. is very polymorphic, with a number of naturally occurring subspecies that differ from each other by their degree of ploidy (Bos *et al.*, 1997). This plant grows from a short rhizome to approximately 1.5 m in height, it flowers, and then dies back again in the winter. The rhizome is light greyish brown, about the size of a finger joint, bearing many rootlets. *V. officinalis* L. has pinnately-divided leaves, typically with 6 -10 pairs of lanceshaped leaflets, and bears many small white or pink hermaphroditic flowers in a dense head of several stalked clusters. These heads bear small (5 mm) tapered seeds (Andrews and Basu, 2005). The fresh root has no odor, while the dried root smells distinctly unpleasant, akin to old gym socks, due to isovaleric acid (Bisset, 1994; Flemming, 1998).

V. officinalis (Tagar) is one of the important constituents of many Unani formulations (Anonymous, 1969) which have been reported to possess some interesting pharmacological effects (Ahmad *et al.*, 2012).

Phytochemistry

This plant contains over 150-200 chemical constituents, including the essential oils and their sesquiterpenoid derivatives (valerenic acids), epoxy iridoid esters (valepotriates), alkaloids, flavonoids and lignans which are mainly produced in the root and rhizome (Cramer *et al.*, 2006). Among these constituents, valerenic acids and valepotriates are often regarded as active principles in valerian commercial and medicinal preparations and its crude extract is widely used in many countries (Houghton, 1999).

Essential Oil

The essential oil contains about 150 constituents which includes monoterpenes, sesquiterpenes, less volatile sesquiterpenic carboxylic acids. Borneol (Carretero, 2000; Cunha, 2005) mainly in the form of ester viz. bornyl acetate and bornyl isovalerate , camphene, á and â-pinene are the major monoterpenes found in essential oils of *V. officinalis* (Carretero, 2000; Barnes *et al.*, 2002; Heinrich *et al.*, 2004). Sesquiterpenes include valeranone (Dewick, 2002; Heinrich *et al.*, 2004), valerenal (Carretero, 2000; Dewick, 2002; Heinrich *et al.*, 2004), valerenol (Barnes *et al.*, 2002; Heinrich *et al.*, 2004), valerianol (Carretero, 2000; Heinrich *et al.*, 2004), bisabolene, caryophyllene, (Heinrich *et al.*, 2004) pacifigorgiol and ledol (Barnes *et al.*, 2002). Valerenic acids, acetoxyvalerenic, hydroxyvalerenic are less volatile sesquiterpenic carboxylic acids present in essential oil (Carretero, 2000; Dewick, 2002; Heinrich *et al.*, 2004; Cunha, 2005). Isovaleric and hydroxyisovaleric acids are liberated when drying and storing the plant which gives the valerian its characteristic smell (an unpleasant aroma) which is not noted in the fresh plant (Weiss, 1988; Dewick, 2002).

Valepotriates

The valepotriates are epoxy iridoid esters (Dewick, 2002) that contain valtrate (Carretero, 2000; Heinrich *et al.*, 2004; Cunha, 2005) which is about 80% of the valepotriates, acevaltrate (Cunha, 2005), dihydrovaltrate (Carretero, 2000; Heinrich *et al.*, 2004; Cunha, 2005) isovaltrate (Carretero, 2000; Dewick, 2002; Heinrich *et al.*, 2004) isovaleroxy-hydroxydihydrovaltrate (Caron and Reidlinger, 1999; Carretero, 2000). The valepotriates are very unstable molecules as they can be easily decomposed by heat, humidity or even pH variations (www.naturaldatabase.com). Therefore, they could only be present in fresh plants. However, small quantities of these compounds exist when the plant material is made to dry at a temperature lower than 40ºC (Carretero 2000). Being insoluble in water valepotriates could be obtained only in non-aqueous preparations. Although valepotriates were once thought to be the active ingredients, these compounds being chemically unstable degrade readily, poorly absorbed and are not found in infusions and tinctures (Foerster *et al.*, 1984; Houghton, 1988; Lin *et al.*, 1991; Reichert, 1998). Instead, their degradation products, baldrinals which are unsaturated aldehydes, are found in such preparations, and may account for much of valerian's sedative effect. The baldrinal and homobaldrinal are the degradation products of valtrate and isovaltrate respectively (von der Hude, 1986; Barnes, 2002; Heinrich, 2004).

Other Constituents

Apart from essential oils and valepotriates, the plant contains other bioactive compounds including amino acids, alkaloids, flavonoids, lignans etc. Arginine,

glutamine, tyrosine, ã-aminobutyric acid (GABA) are the important amino acid constituents. The major flavonoids present in *V. officinalis* are linarin (Fernandez *et al.*, 2004), methylapigenin, hesperidin (Marder *et al.*, 2003; Fernandez *et al.*, 2004). Alkaloidal constituents include chantinine, valerine, valerianine, actinidine, methyl-2-pyrrole ketone (Torssell and Wahlberg, 1967; Franck *et al.*, 1970; Janot *et al.*, 1979; Duke, 1985). Hydroxypinoresinol is an important lignan found in this plant (Houghton, 1999). Other valerian constituents are caffeic and clorogenic acids, â-sitosterol, tannins, choline etc (Barnes *et al.*, 2002)

Pharmacological Properties

Valerian has been used as a medicinal herb since at least the time of ancient Greece and Rome. Hippocrates described its properties, and Galen later prescribed it as a remedy for insomnia. Pharmaceutical application of valerian is due to its sedative, anticonvulsant, antidepressant, antihypertensive, hypnotic effects, antispasmodic and anxiolytic activity. The pharmacological effects of valerian have primarily been attributed to the valepotriates (iridoid esters), volatile oils, monoterpenes, and sesquiterpenes constituents (Wang *et al.*, 2010; Ansari *et al.*, 2013). Lately, the potential cytoprotective effect of aqueous extract of *V. officinalis* on human neuroblastoma cells has also been demonstrated (Madureira de Oliveria *et al.*, 2009). In 1998, valerian was the tenth most popular herbal cure sold in the United States. Now, valerian extracts are available as dietary supplements, which are primarily composed of dried root or extracts from the root, formulated into tablets or soft gelatin capsules are extensively used with an estimated 210 million doses sold annually in the United States and 125 million doses sold annually in Europe (Patocka and Jakl, 2010).

Cardiovascular Activity

The coronary dilating and antiarrhythmic effects of valerian extract has been demonstrated in rabbits, mice and cats. Petkov (1979) reported that valepotriates an important component of Valerian, prevented the appearance of acute coronary insufficiency as well as vasopressin-induced arrhythmia, provoked a short-lived increase of coronary blood flow, and had moderate positive inotropic and negative chronotropic effects. In mice, valeranone another bioactive compound found in small quantities in valerian and in larger amounts in its relative, *Nardostachys jatamansii*, exerted weak hypotensive effects (Morazzoni and Bombardelli, 1995). A significant increase in coronary blood flow, a transient fall in blood pressure and a decrease in heart rate was noticed when cats were intravenously injected with valerian extracts (Zhang *et al.*, 1982). Valerian is included in a German heart tonic to maintain neuro-cardiac stability (Mowrey, 1986). In an open, multicenter trial of 2,243 patients with a variety of functional cardiac disorders, an herbal

combination (valerian, hawthorn, cereus and camphor) was associated with improvement in 84% of patients (Busanny-Caspari 1986). Circosta *et al.* (2007) reported significant anticoronaryspastic, antibronchospastic, antihypertensive activities from ethanolic and aqueous extracts of *V. officinalis* L. roots in anaesthetized guinea-pigs and were found similar to those exhibited by nifedipine.

Anxiolytic Activity

Hattesohl *et al.* (2008) evaluated the pharmacological profile of different extracts derived from *V. officinalis* L. including two commercially available extracts and the newly developed preparations VAL SE 35E and phytofin Valerian 368. Therefore tests for sedative, anxiolytic, antidepressant as well as for myorelaxant properties were conducted in rodents. The results revealed that none of the valerian extracts displayed sedative or myorelaxant effects when used up to maximum dosages of 500 or 1000 mg/kg body weight. However pronounced anxiolytic of the 45% methanolic and 35% ethanolic extract as well as of phyotofin Valerian 368 was noticed in the elevated plus maze test in a dose range of 100–500 mg/kg body weight. Additionally and different from its primary extract (35% ethanolic extract) phytofin Valerian 368 showed antidepressant activity in the forced swimming test after subacute treatment. They concluded that anxiolytic and antidepressant activity may contribute to the sleep-enhancing properties of valerian. Further, Murphy et al. (2010) reported primary anxiolytic activity of valerian due to the presence of valerenic acid and the enhanced anxiolytic effect of valerenic acid in the presence of GABA. During their experiment rats were administered either ethanol (1ml/kg), diazepam (1mg/kg), valerian root extract (3ml/kg), valerenic acid (3mg/kg), oral solution of valerenic acid and exogenous GABA (75 mg/kg and 3.6 mg/kg, respectively) and assessed for the number of entries and time spent on the open arms of an elevated plus maze. Results showed that there was a significant reduction in anxious behaviour when valerian extract or valerenic acid exposed subjects were compared to the ethanol control group.

Sedative/anticonvulsant Activity

Rosecrans *et al.* (1961) evaluated the biological activity of the different root extracts of *Valeriana officinalis* wherein two fractions (V103 and VI15) were found to potentiate the pentobarbital sleeping time in rats. In mice, intraperitoneal injections of valerenic acid, valerenal and whole herb extracts produced significant sedation, ataxia and anticonvulsant effects (Veith *et al.*, 1986). Intraperitoneal injections of 100 mg/kg had sedative effects as strong as barbiturates; doses of 400 mg/kg led to death. In comparison with diazepam and chlorpromazine, valerian extract had weak anticonvulsive properties. Valerian root extract (Valdispert) reduced motility and increased thiopental and pentobarbital-induced sleeping time. Even the aroma

of valerian root exerted sedative effects in mice (Buchbauer *et al.*, 1992). Marder *et al.* (2003) reported the sedative and sleep enhancing property of hesperidin, a compound isolated from *V. officinalis*. They also demonstrated the ability of 6-Methylepigenin, another compound from *V. officinalis*, to potentiate the sleep enhancing property of hesperidin. Fernandez *et al.* (2004) further reported the presence of the flavone glycoside linarin and its sedative and sleep-enhancing properties that are potentiated by simultaneous administration of valerenic acid. An intraperitoneal coadministration of linarin (4 mg/kg) and valerenic acid (5 mg/kg) had sedative and sleep-enhancing effects as evidenced by the remarkable reduction in the exploration of holes, the time mice spent head dipping and the number of their rearings as assayed in the holeboard test and also produced a striking increase in the sleeping time induced by sodium thiopental whereas the administration of linarin and valerenic acid independently at the above mentioned doses did not increase the sleeping time induced by sodium thiopental.

Gastrointestinal Activity

Valerian is traditionally used in the treatment of intestinal spasms, colic, and "nervous stomach". Valerian has a bitter flavor, and bitters have historically been used to enhance appetite and digestion. Valerenic acid, valtrate and valeranone exert spasmolytic effects in guinea pig ileum through direct effects on smooth muscle (Hazelhoff *et al.*, 1982; Busanny-Caspari, 1986).

Antidysmenorrheal activity

Valerian seems to be an effective treatment for dysmenorrhea, probably because of its antispasmodic effects. In a double-blind, randomized, placebo-controlled trial, 100 students were randomly assigned to receive valerian (dose 255 mg) 3 times daily for 3 days beginning at the onset of menstruation, for 2 consecutive menstrual cycles. At baseline and during the intervention cycles, the pain severity was evaluated with a visual analog scale and the systemic manifestations were assessed using a multidimensional verbal scale. The pain severity at baseline did not differ significantly between the groups. After the intervention, the pain severity was significantly reduced in both groups, but the extent of the reduction was larger in the valerian group, with the difference between the 2 groups being statistically significant. The total scores of the systemic manifestations associated with dysmenorrhea decreased after the intervention, but there was no significant difference between the groups, with the exception for syncope (Mirabi *et al.*, 2011).

Attention enhancing activity

In Germany valerian is sometimes used to treat attention deficit hyperactivity disorder (ADHD) in children (Cavazzuti, 1969). German studies during 1960's

reported that valerian could antagonize the hypnotic effects of alcohol, enhancing concentration and coordination (Mowrey, 1986). In a randomized, placebo-controlled, double-blind study, valepotriates demonstrated a dose-dependent increase in concentration abilities in 24 healthy volunteers. Also, valeropotriates when given in combination with alcohol did not affect blood alcohol levels, sedative effects or effects on driving performance (Mayer and Springer, 1974). There are no controlled trials evaluating valerian's use in treating attention deficit hyperactivity disorder (ADHD) (Mowrey, 1986).

Other Neurological Activity

Unlike diazepam, valerian did not affect spontaneous ambulation and rearing or approach-avoidance conflict in mice in a water-lick conflict test. On the other hand, valerian and imipramine significantly inhibited immobility induced by a forced swimming test in rats and significantly reversed reserpine-induced hypothermia in mice, leading researchers to conclude that valerian may be a useful antidepressant (Morazzoni and Bombardelli, 1995). Among 80 hospitalized geriatric patients enrolled in a placebo controlled trial for 14 days, those assigned to an aqueous valerian extract had significant improvements in mood and behavioral disturbances as well as sleep (Kam-kohl *et al.*, 1984). Among 121 patients with sleep disturbances enrolled in a controlled trial, those assigned to an alcoholic extract of valerian (600 mg daily for 28 days) had a significant improvement in depression, mood and global functioning as well as sleep; no significant side effects were reported (Vorbach *et al.*, 1996).

Propagation

Conventional Methods

The plant is conventionally propagated through seeds as well as from root cuttings. Although Valerian can grow in any ordinary soil, but prefer moist humus rich loam having pH of 6-7. It is often found to flourish in damp and shady places, though some drought resistant forms also occur in chalk and limestone hills. The plant may easily be propagated by portions of the old rootstocks either in the autumn months or in spring. The divisions of the rootstock are spaced 30 cm × 60-90 cm. A good crop of the drug may be harvested in the autumn season if the rootstock divisions are planted very early in autumn in time to become well established before the onset of frost. The summer cultivation requires the weeding of beds and cutting off all floral stalks to avoid exhaustion and promote the formation of larger rootstocks. Soil should be rich in phosphorous (or supply a fertilizer) as the leaves turn purple when the plant is grown in phosphorous-deficit soil. Seeds are directly sown by simply pushing them into the soil during spring season. Avoid

covering them as they require light and a temperature of 68 to 70°F to germinate. Their germination takes one to two weeks. The nursery raised seedlings need consistent moisture, grow slowly, need protection from faster growing weed and are first transplanted at a spacing of 18-20 cm in rows 30 cm apart. The seedlings are then re-transplanted to their permanent sites at the same spacing as used for root stock divisions. Application of farmyard manure with proper mixing into the soil before the transplanting of seedlings favours the good growth of the plant. The formation of large root stock is promoted by raising a low ridge of soil along the bases of plant. For the better development of the rhizome flowering tops must be cut off as they appear. Many of the young plants do not flower in the first year, but produce a luxuriant crop of leaves, and yield rhizome of good quality in the autumn. The rhizomes and roots of the plants, propagated from the divisions of the rootstock may be harvested in the autumn of the first year's growth, though the yield is generally low. The bud-raised plants do not attain a size suitable for harvesting before the end of second growing season (Anonymous, 2003).

In Vitro Methods

Abdi and Khosh-khui (2007) established a highly reproducible micropropagation protocol through adventitious shoot bud regeneration from leaf explants. They reported influential role of size as well as orientation of leaves, type of plant growth regulators on shoot bud induction. Maximum shoot regeneration (74 %) was observed from abaxial side of leaves with the production of about 7.4 mean number of shoot on MS (Murashige and Skoog, 1962) supplemented with 1 mg/L 6-Benzyl adenine (BA). Multiplication of shoots was further achieved by transferring shoots regenerated from leaves on MS medium supplemented with Isopentenyl Adenine (2-ip) and indole-3-acetic acid (IAA) alone or in combination. The highest multiplication rate (93.3 %) was observed on combination of 2-iP (6 mg/L) and IAA (0.3 mg/L) which induce the formation of about 13.7 mean shoot number. á-naphthalene acetic acid (NAA) at 1 mg/L was the optimal treatment for *in vitro* root induction in their study.

Abdi *et al.* (2008) further evaluated the potential of silver nanoparticle (35 nm average size) for removal of bacterial contaminants from in vitro cultures of *V. officinalis.* Their experiment involved the treatment of explants with different concentration (25, 50 and 100 mg/L) of silver nanoparticle before and after surface sterilization for three different time periods (30, 60 and 180 min). Explants were cultured on MS medium supplemented with 5 mg/L KN and 0.1 mg/L NAA. Their results revealed the highest percentage (89 %) of disinfected explants by using 100 mg/L of silver nanoparticle solution for 180 min after surface sterilization while same amount of silver nanoparticle solution used for the similar duration resulted in only 32 % disinfected explants when the nano silver solution was used

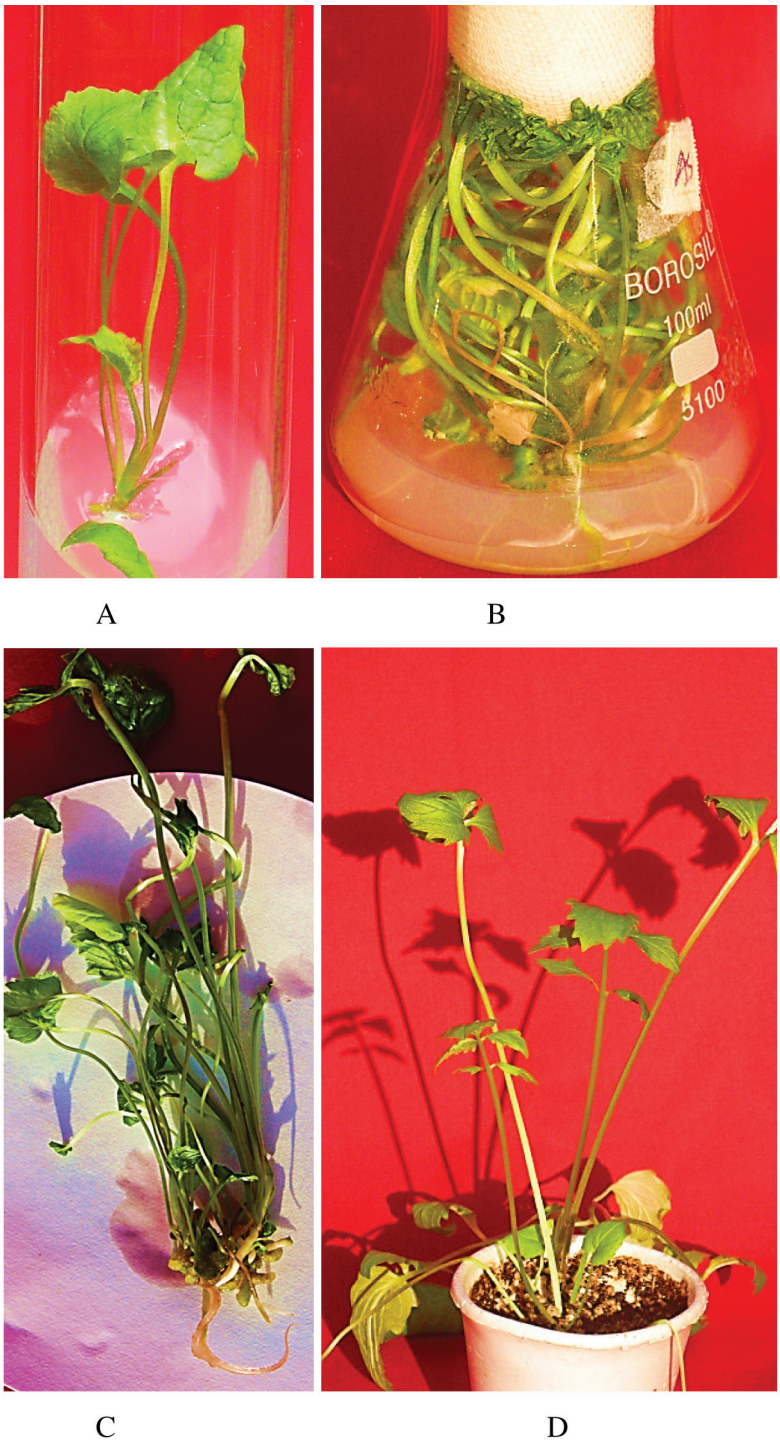

Figure 1: Steps involved in micropropagation protocol of *Valeriana officinalis* L.

Step A. In vitro induction of shoots from nodal explant in culture media in *V. officinalis;* Step B. Multiplication and proliferation of cultures for enhanced production of *V. officinalis*; Step C. In vitro rooting in tissue culture raised plantlets*;* Step D. Acclimatised in vitro raised plantlet ready to transfer in field

before surface sterilization. However, no affect on contamination was observed when silver nanoparticle solution was used without surface sterilization of explants. However, the authors did not notice any affect of nano silver solution on the characters measured. On the basis the above mentioned experiment, it was concluded that NS had a good potential for removing of the bacterial contaminants in plant tissue culture procedures.

Muntean (2008) reported somaclonal variation among the valerian (*V. officinalis*), micropropagated plantlets. During their experiment optimum callus induction was observed on Murashige and Skoog (MS) medium supplemented with 10 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) while maximum regeneration was achieved upon transferring the callus to MS medium containing combination of 2.5 mg/L BA and 0.5 mg/L IAA. In vitro rooting of regenerated shoots was obtained on MS medium containing 1 mg/L indole-3-butyric acid (IBA). About 19 plantlets regenerated from the callus were successfully hardened followed by their transfer to the greenhouse. The genetic analysis through RAPD marker was further performed during their study in order to investigate any detectable variation among the parent plant and the regenerants. They found four somaclonal variants out of 19 regenerants and the obtained somaclones were different from the parental plants by at least one polymorphic amplification product. However, the remaining regenerants were found to be genetically stable as compared to the elite donor plants. The seven primers generated 13 new RAPD markers in the somaclonal variants that were not found in the donor plant fingerprints. Moreover, two out of four somaclonal variants were polymorphic, compared with the donor plant, with two or more primers.

Reza *et al.* (2009) reported indirect shoot regeneration from leaf and petiole derived callus. In their study callus was developed on MS medium supplemented with different combinations of different auxins (2,4-D, NAA, picloram) and N^6-furfuryladenine (KN) from petiole and leaf segments wherein highest callus induction frequency was obtained on 1.0 mg/L 2,4-D for petiole (98 %) and leaf (100 %) explants. For shoot regeneration the calli were transferred to a medium supplemented with various concentrations of BA or KN and they observed maximum shoot regeneration frequency on 0.2 mg/L BA or KN with the production of about 13 adventitious shoots per callus segments derived from leaves. MS medium supplemented with 1 mg/L NAA was reported to be the optimal rooting media during their investigation on which 100 % regenerated shoots developed roots with an average of 10.7 roots per shoot within 3 weeks. The in vitro raised plantlets were further successfully hardened and transferred to greenhouse with 80 % survival rate.

Zebarjadi *et al.* (2011) developed in vitro regeneration protocol via indirect organogenesis from leaves, stems and roots explants excised from 35 day old in

vitro germinated seedlings. The highest callus formation frequency was achieved on MS medium augmented with 0.1 mg/L KN and 2 mg/L 2,4-D for leaf and root explants (100 %) while MS medium containing 0.1 mg/L KN and 10 mg/L 2,4-D induce maximum callus (100 %) from stem explants. Their results further revealed maximum shoot regeneration frequency of 60 % on MS medium containing 0.5 mg/L NAA and 2 mg/L BA in leaf-derived calli. Regenerated shoots were subsequently transferred to medium lacking growth regulators and then MS medium supplemented with 2 mg/L IBA for in vitro rooting.

Tansaz *et al.* (2014) investigated the effects of three different culture media formulations viz. MS, B5 (Gamborg et al. 1968) and MB (new combined medium containing half MS and B5 salts and vitamins, 10 ml/L Fe EDTA) and various combinations of plant growth regulators (BA and NAA) on *in vitro* regeneration and growth parameters of *V. officinalis*. Excised crown with 2-3 segments having one bud was used as explants. Among the different media tried during their study, MB medium exhibited highest regeneration frequency alongwith maximum induction of mean number of microshoots. However, highest percentage of regenerated plantlet (80 %) with maximum mean shoot number (9.43) and mean shoot length (7.04 cm) was obtained on MB medium supplemented with BAP (0.5 mg/L) in combination with NAA (0.25 mg/L). B5 medium without any PGR exhibited the lowest regeneration frequency of 40 % which increased to 75 % on supplementation with 1.5 mg/L BA and 0.25 mg/L NAA.

Ghadheri and Jafari (2014) for the first time reported efficient and rapid propagation protocol of *V. officinalis* for large-scale production of uniform raw materials for future pharmaceutical compound extraction as well as the quantification of the two industrially important bioactive compounds (i.e. valerenic acid and valtrate) accumulated in tissue culture-raised plants. Petiole and leaf explants were used for the callus induction and the three types of callus could be recognized by color (creamy green, light or dark green) and texture (compact, nodular and friable) depending on the PGR concentrations/combinations used. Compact, nodular, yellowish green callus usually formed through leaf and petiole explants on medium containing 4.65 or 9.30 ìM KN in combination of 2.26 ìM 2,4-D showed the highest regenerative capacity with the formation of maximum shoot buds in both types of explants. IBA at a concentration of 2.46 ìM was found to be the best rooting treatment, which promoted the highest frequency of rooting (98 %). Successful acclimatization of rooted plantlets was achieved in potting medium containing 5:1 mixture of peat and perlite with 95.34 % survival rate. The contents of valtrate and valerenic acid in biomass extracts from petiole-derived plants were significantly higher than leaf-derived plants as well as the seed-raised field-grown plants. Maximum valtrate (6.98 mg g”1 DW) and valerenic acids (3.02 mg g”1 DW) contents were quantified in root tissue of petiole derived plants raised on medium

with higher concentration of Kn (9.30 ìM) in combination with 2.26 ìM 2,4-D. Further they also conducted RAPD analysis to screen tissue culture induced genetic variations in high level valerenic acid-accumulated plants using 15 RAPD primers (OPA 1-15) wherein only 4 primers viz. OPA-3, 5, 8, and 12 produced reproducible and scorable bands ranging from 3 to 8, with the generation of 104 amplification products of 200 to 1400 bp. Their results showed no differences between the plants raised from in vitro cultures but a slight variance in only one electrophoresis band, produced by OPA-8 primer, with seed-raised field grown plant was reported by them which demonstrates significant difference of these plants with seed raised plants from the metabolite content point of view. They lastly concluded that tissue culture-raised plants had higher genetic stability than did the seed-propagated plants.

Bhat and Sharma (2015) evaluated the effect of light and low temperatures as well as pretreatment of seeds with GA_3 on seed germination along with the development of in vitro propagation protocol through shoot tip explants. Their results revealed increased germination rate (48 %) in unchilled seeds pretreated with GA_3 (200ppm) for 24 hr in dark as compared to non pretreated seeds with only 10 % germination under dark conditions. Further maximum germination rate of 60 % was obtained through pre-chilling treatment of seeds for 72 hr and keeping them in light conditions during their study. MS medium supplemented with BA (1mg/L) and IAA (0.5 mg/L) was found to be the optimal treatment for shoot multiplication and elongation with 50 % response.

Genetic transformation and Elicitation studies for enhanced metabolites production

Gränicher *et al.* (1992) established hairy root cultures in *V. officinalis* var. *sambucifolia* by infection of sterile plantlets with *Agrobacterium rhizogenes* strain R1601. Different hormone-free liquid media were used for the growth of transformed roots and the yield of different valepotriates viz. isovaltrate, valtrate, didrovaltrate, isovaleroxyhydroxydidrovaltrate content was quantified through HPLC. The highest overall valepotriate content (10.3 % dry wt) which was about 4 times the amount found in the roots of 9-month-old nontransformed plants, was observed in half strength Gamborg B5 medium supplemented with 2 % sucrose. They further demonstrated the production of about 44 mg/g dry wt valepotriates from hairy roots cultured in MS liquid medium supplemented with 2 % sucrose for 50 days.

Zebarjadi *et al.* (2011) carried out genetic transformation studies in *V. officinalis* under in vitro conditions by co-cultivating leaf, stem and root derived callus with two different strains of *A. rhizogenes* viz. AR15834 and LBA 9402 for the

induction of hairy root cultures. During their experiment, leaf, stem segments (1 cm^2) and calli were immersed in a suspension of *A. rhizogenes* (OD_{600} = 0.6-1) for 5 to 7 min followed by subsequent co-cultivation on solidified MS medium without any hormone and MS medium containing (0.1 mg/L NAA+1 mg/L BAP), (0.5 mg/L BAP+0.5 mg/L NAA+0.5 mg/L 2,4-D) for 2 days in darkness. Explants were then transferred onto selective medium containing 250 mg/L cefotaxime and 20 mg/L rifampicin, and further subcultured onto fresh selective medium every 15 days. Un-inoculated explants were used as a control and after 28 days of culture, the number of produced roots on selective medium to the number of cultured explants was determined. The results obtained demonstrated LBA9402 strain to be better than AR15834 strain in terms of both mean number of hairy root formation (68.62%) and production of the respective hairy root (90%) while leaf and root calli were found to be the best explants for transformation with both the strains. The optimal growth of the hairy roots occurred on selective MS medium containing 20 mg/L rifampicin and 250 mg/L cefotaxime without any plant growth regulators. Genomic DNA of putative transgenic and non-transgenic (control) regenerated hairy roots were analyzed by PCR for presence of *rol* B gene, using specific primers.

Torkamani and Samadi (2014) studied the influence of four different levels of Calcium and Potassium (half, full, two and four fold concentrations) on enhanced accumulation of valerenic acid in hairy root cultures of *V. officinalis*. Hairy root cultures were established via *A. rhizogenes* A13 strain using various explants derived from 42-day-old sterile seedlings and the transgenic status of the roots were confirmed by the presence of *rol* B and *rol* D amplified products during Polymerase chain reaction (PCR) analysis. The induced hairy root cultures of *V. officinalis* were maintained in the media and after 35 days were used for investigations of biomass accumulation and valerenic acid production through high-performance liquid chromatography (HPLC). The results showed highest valerenic acid accumulation of about 0.69±0.03 mg/g DW from hairy roots cultured on media with two-fold calcium, which was 1.92 times higher than normal culture (0.36± 0.01 mg/g DW). The study also demonstrated the positive effect of double concentration (880 mg/L) of calcium in media on growth of transformed hairy roots. However, they further reported decrease biomass accumulation but 1.2 fold increase in valerenic acid production than control roots on the media with 3.32 mg/l (four times of control concentration) potassium concentration. Their results revealed the positive role of calcium in intensification of the valerenic acid production in *V. officinalis* hairy roots cultures.

Tokramani *et al.* (2014a) also studied the effect of exposure time (3 and 7 days) of different calcium and magnesium concentrations (2 to 6 fold than basal MS

medium) on the growth of hairy roots and valerinic acid production in hairy root cultures. They used hairy root cultures grown in MS liquid medium without elicitors for 8 weeks for the quantification of valerinic acid through HPLC. Their results revealed highest production of valerenic acid (1.83 ± 0.06 mg/g DW) in hairy root cultures exposed to 6-fold calcium for 7 days which was 7.9 times higher than that of control culture (0.23 ± 0.01 mg/g DW) while (6-fold) of magnesium at exposure time of 7 days resulted in the accumulation of valerenic acid at (1.11 ± 0.03 mg/g) DW which is 4.2-fold higher than that of non-elicited control.

Torkamani *et al.* (2014b) further increased valerenic acid production in the selected hairy root line 'LeVa-C4' through the use of three different elicitors' viz. *Fusarium graminearum* extract (FE), methyl jasmonate (MJ) and salicylic acid (SA). They use wild-type strain 'A13' of *A. rhizogenes* for the induction of hairy roots and 23-day-old hairy root cultures were further inoculated into 25 ml MS liquid medium supplemented with various concentrations of MJ (50, 100, 200 ìM), SA (50, 100, 150 ìM) or FE (0.5, 1, 2% v/v) for different exposure times (3 and 7 days). They found 1% FE and 100 ìM L-1 MJ highly efficient for increased levels of valerenic acid after 7 days of elicitation which were 12.31 (3.02 mg/g DW) and 6 times (2.296 mg/g DW) higher than that of non-elicited control (0.24 mg/g DW) respectively. They further demonstrated that FE did not exert any negative effects on biomass yield of hairy root and no significant increase in valerenic acid content was noticed through the use of salicylic acid as elicitor.

Parizi *et al.* (2014) established hairy root cultures through *A. rhizogenes* (strain ATCC 15834) mediated genetic transformation and further determine the effect of different media viz. MS, B5 media (full and half strength), N6 medium and a modified MS medium without phytohormones, on the growth parameter of hairy roots. They also studied the effect of different NH_4^+/NO_3^- ratios (00:20, 10:20, 20:20, 20:10, 20:00, 20:40) in MS medium on hairy root growth and the effect of these treatments were evaluated after 21 days of culture in relation to hairy root growth. Their results revealed B5 and ½ B5 media to be the best basal medium with the production of maximum dry weight of hairy roots i.e. 3.85 and 3.67 g/L respectively while N6 medium exhibited lowest hairy root growth with the production of only 0.86 g/L dry weight after 21 days. Further among the different NH_4^+/NO_3^- ratios in MS media tested, MS medium supplemented with a 20:20 mM and 20:40 mM ratio of NH_4^+ to NO_3^- produced the maximum biomass of 1.80 and 1.62 g/L respectively after 21 days.

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