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Evaluation of anti-oxidant Activity of Mother and *in vitro* raised Plants of *Valeriana jatamansi* Jones

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ABSTRACT: *Valeriana jatamansi* Jones (family Caprifoliaceae) is a pharmaceutically important, North-Western Himalayan medicinal and aromatic herb which possess neuroprotective, antidepressant, anti-tumor, gastrointestinal, cytotoxic, anti-virus, antioxidant, sedative and other activities. The demand for this rare plant is increasing day by day which leads to dwindling of herbs availability in forest. Therefore, plant tissue culture provides a more powerful and promising tool for plant propagation of this important medicinal plant. In the present study, methanolic and di-ethyl ether extracts of *Valeriana jatamansi* were prepared and analyzed for their polyphenols and flavonoid contents. Antioxidant activity of different extracts of *Valeriana jatamansi* was determined by DPPH radical scavenging method. The results indicate that in methanol extract, mother plant leaf samples showed higher (63.07 ± 0.92) DPPH scavenging antioxidant activity in comparison to *in vitro* raised plants (58.50 ± 1.06). Whereas, in diethyl ether extract DPPH radical scavenging activity (%) of mother plant(41.35 ±1.88) was also higher than *in vitro* raised plants (36.84 ± 1.10).

Keywords: *Valeriana jatamansi*, antioxidant, biomolecules, methanol, flavonoids, medicinal plants

INTRODUCTION

The therapeutic potential of medicinal plants, as a source of phytochemicals, is gaining momentum all over the world. Among the phytochemicals, polyphenols are largely being used in different health related activities as best antioxidants, which are known to reduce the risk of several degenerative diseases including coronary heart disease and cancer (Marchioli *et al.*, 2001). The demand for this rare plant is increasing day by day in pharmaceutical as well as perfumery industries, which lead to dwindling of herb's availability in the forest (Shukla *et al.*, 2021). Because of increasing demand and over-exploitation of *V. jatamansi* roots and rhizomes for medicinal usages and the biotic interferences in its distribution range have caused habitat degradation thus creating nearly extinct condition of the herb (Patan *et al.*, 2018). Thus, convention on International Trade of Endangered Species notified *V. jatamansi* in its schedule for conservation and has been enlisted as anendangered species in the list of National Medicinal Plant Board, New Delhi, India (Nawchoo *et al.*, 2012). Phytochemical study of *Valeriana jatamansi* Jones afforded 45 compounds, including twenty-three iridoids, five sesquiterpenes, three steroids and fourteen lignins (Wang *et al.*, 2021). About more than 39 Ayurvedic formulations are available in the market (Jugran *et al.*, 2019). Enrichment of metabolites of *V. jatamansi* was reported by Partap *et al*., (2020) when cultivated under aeroponic conditions and concluded that aeroponic cultivation gives quality biomass production and easy root harvesting in *V. jatamansi* to meet the demand of the pharmaceutical industries. Antioxidant activity is defined as, "limitation of oxidation of proteins, lipids, DNA or other molecules that occurs by blocking the propagation stage in oxidative chain reaction" and primary antioxidants directly scavenge free radicals, while secondary antioxidants indirectly prevent the formation of free radicals through Fenton's reaction (Huang *et al.*, 2013; Jugran *et al.*, 2020). Polyphenolic compounds such as reservetol, lignans, phenolic acid, flavonoids, tanninsact as persuasive antioxidant as they have an ability to scavenge free radicals like hydroxyl (OH), peroxyl (RO_2), alkoxyl (RO) etc. In plants, reactive oxygen species (ROS) are produced as a product of cellular metabolism and through environmental stresses. Electron transport chain in mitochondria, microsomal oxidation in endoplasmic reticulum, lipogeneses in plasma membrane, myeloperoxidase in lysosomes are

the major endogenous sources of ROS production which lead to oxidative stress, cell damage and cause various diseases such as cardiovascular diseases, asthma, skin diseases etc. Antioxidants are substances that prevent, delay or remove the oxidative damage by reducing the level of ROS or free radicals even at low concentration (Ma *et al*., 2021).

So, it is necessary to analyses antioxidant activity of medicinal plants. Thus, for the analysis of antioxidant activity of mother plant and *in vitro* raised plants DPPH is used. DPPH (1, 1-Diphenyl-2-picrylhydrazyl) is a stable free radical with deep purple color which gets decolorized on gaining hydrogen atom from a corresponding donor (extract) which acts as an antioxidant. The degree of discoloration indicates the radical scavenging activity of antioxidant. *Valeriana jatamansi* has different forms of free radical scavenging molecules such as phenols, flavonoids, etc. with high antioxidant properties. The objective of this research was to measure and evaluate the *in vitro* antioxidant properties of *Valeriana jatamansi* in mother plant and*in vitro* raised plant samples in various solvents such as methanol and diethyl ether.

MATERIALS AND METHOD

The present study was conducted in Department of biotechnology, College of Horticulture and Forestry, Neri Hamirpur.

A. Extract preparation

The mother plant sample (leaf) was washed thoroughly under tap water to remove dust particles and kept on blotting paper to remove moisture. Afterwards, samples were shade dried at room temperature for 5 days and grinded to convert it into powder form for extraction. *In vitro* plant sample (leaf) was taken from culture flask and washed thoroughly with distilled water to eradicate trace amounts of agar, and the water on the surface of the explant was dried with the aid of filter paper. For extraction, 5 gram of each sample was crushed with mortar or grinder. Dry content (5 gram powder of each mother plant and *in vitro* sample) was collected in 100 ml of two specific solvents, i.e. 99 per cent (v/v) methanol and 99 per cent (v/v) diethyl ether, in a flask for 48-hour with continuous shaking, at room temperature (25°C). The resulting suspensions were filtered using the Whatman No. 1 filter paper. The final concentration of the extract (mother plant and *in vitro*) was prepared and used to detect antioxidant activity in both the mother plant and *in vitro* raised plants.

B. Antioxidant activity by DPPH assay

The antioxidant activity of the extracts and the standard was evaluated on the basis of the radical scavenging behaviour of the stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) according to William *et al* (1995) with some modifications. The experiment was performed in triplicates.

Preparation of plant samples with methanol and di-ethyl ether extracts

Step 1. Prepare a solution series (10µL, 20µL, 30µL-100µL) in test tubes by using extracted solution of methanol and di-ethyl ether separately.

Step 2. Add 2ml of methanol and di-ethyl ether solutions in each test tube.

Step 3. Take 1 ml solution from each test tubes.

Step 4. Add 1 ml DPPH solution in each test tube.

Step 5. Add 1 ml methanol solution and di-ethyl ether in each test tube again.

Step 6. Cover the whole test tube with aluminum foil and sealed with parafilm.

Step 7. Leave the test tube sample in dark condition for 30 minutes.

Step 8. After incubation in dark condition quantitative analysis was done at 517nm by using UV- vis spectrophotometer

C. Quantitative estimation of total antioxidant activity

Absorbance of sample was measured in UV-Vis spectrophotometer at 517nm. After that percentage inhibition IC_{50} coefficient was measured. IC_{50} (mg / ml) is the inhibitory concentration at which the DPPH radicals were scavenged by 50 percent and obtained graphically (percent inhibition versus corresponding sample concentration) by interpolation from a linear regression study. Ascorbic acid (0.5mg/ml) has been used as standard. Absorption of the DPPH reagent was taken for monitoring without the addition of sample extract. The percent inhibition of free radical DPPH was determined as follows:

$$\text{Inhibition } \% = A_C - A_S/A_C \times 100$$

A_C =Absorbance of control A_S = Absorbance of sample

D. Statistical Analysis

For all the experiments, three samples were analysed and all the assays were carried out in triplicates. The results were expressed as mean values with standard deviation.

RESULTS AND DISCUSSION

A. DPPH Radical Scavenging Activity

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) is a stable free radical with deep purple color which gets decolorized on gaining hydrogen atom from a corresponding donor (extract) which acts as an antioxidant. The degree of discoloration indicates the radical scavenging activity of antioxidant. In the present investigation, methanol and diethyl ether extract of mother plant and *in vitro* raised plant of *Valeriana jatamansi* showed dose dependent relationship between DPPH radical scavenging activity (%) and concentration of extract.

B. Quantitative estimation of total antioxidant activity of Valeriana jatamansi

Methanolic and diethyl ether extract of leaves of mother plant and *in vitro* raised plants of *Valeriana jatamansi* showed variations in antioxidant activity

performed by using DPPH assay. Among methanol extract, mother plant leaf samples showed higher (63.07 ± 0.92) DPPH scavenging antioxidant activity in comparison to *in vitro* raised plants (58.50 ± 1.06) (Fig. 1). Whereas, in diethyl ether extract DPPH radical scavenging activity (%) of mother plant (41.35 ±1.88) was also higher than *in vitro* raised plants (36.84 ± 1.10) (Fig. 2, 3). This might be due to the presence of more polyphenolic content in mother plant leaves as compared to the leaves of *in vitro* raised plants. Polyphenols have higher antiradical activity than monophenols and also the compounds those having hydroxyl group on ortho and para position have higher antiradical activity than Meta position (Williams *et al.*, 1995). These finding are accordance with the results obtained from *Valeriana alliariifolia* (Utsukarci *et al.*, 2019) in whichethanol extract (IC_{50} = 17.69±0.34) had highest antioxidant activity. Reddy and Grace (2016) observed that in *Bruguier gymnorrhiza* and *Aegialitis rotundifolia* DPPH scavenging activity was more in methanol extract (IC_{50} = 72 ± 1.63 and 75 ± 1.63 respectively) than the other solvent extracts (ethyl acetate, acetone and chloroform). Similar findings are also reported in *Valeriana dioscodii* (Sarikurkcu *et al.*, 2020) where methanol extract (IC_{50} = 4.81± 0.12) showed higher antioxidant activity than water extract (IC_{50} = 4.35 ±0.12).

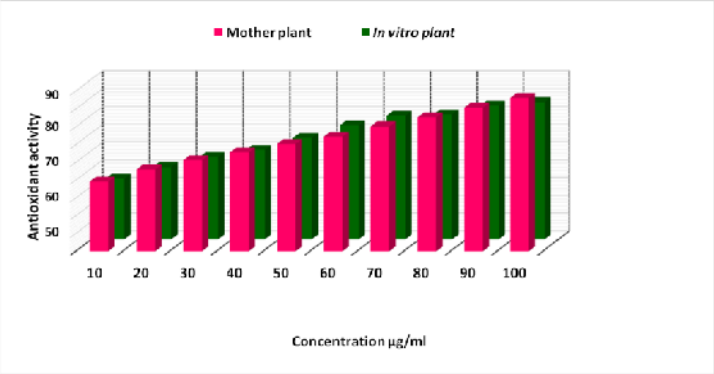

Fig. 1. Comparison of antioxidant activity of methanolic extract of mother plant and in vitro raised plant of *Valeriana jatamansi* using DPPH assay.

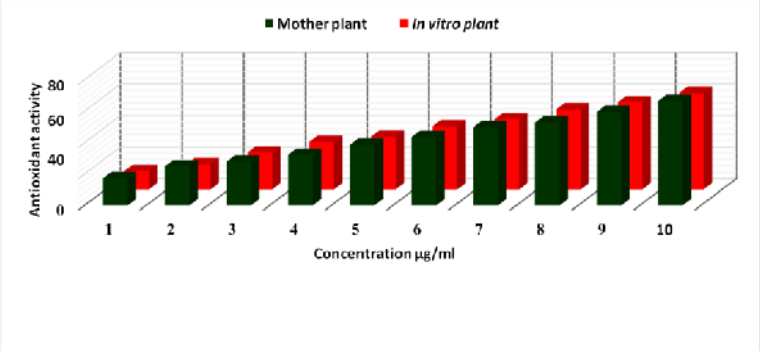

Fig. 2. Comparison of antioxidant activity of Diethyl ether extract mother plant and in vitro raised plant of *Valeriana jatamansi* using DPPH assay.

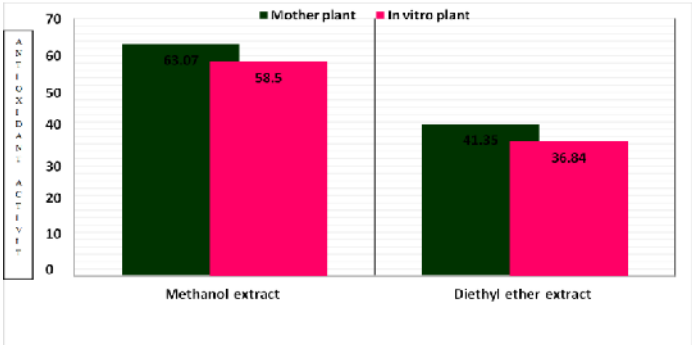

Fig. 3. Comparison of antioxidant activity of methanol and diethyl ether extract of mother plant and in vitro raised plant using DPPH assay.

CONCLUSION

Antioxidant activity can further be utilized for identification of best provenances for large scale production through biotechnological intervention. In the present study quantification of antioxidant potential is recommended in order to achieve maximum benefits from high value medicinal plants in the region. Methanol extract of *Valeriana jatamansi* possesses high antioxidant activity as compared to di-ethyl ether, whereas mother plant samples showed higher DPPH scavenging activity in comparison to *in vitro* raised plants in both the solvents.

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Conflict of interest: The authors declare that there is no conflict of interest.

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