RESEARCH ARTICLE Agricultural Science Digest, Volume 44 Issue 3: 406-413 (June 2024)

Comparison of Antioxidant Properties and Flavonoid of Natural and *in vitro* Cultivated *Nardostachys jatamansi*

Hem Chandra Pant[1], Indra Rautela[2], Harsh Vardhan Pant[3], Arun Kumar[1], Pankaj Kumar[4], Kaneez Fatima[5], Naveen Gaurav[1]

10.18805/ag.D-5654

ABSTRACT

Background: India has very rich diversity of medicinal plants. Medicinal plants are thought to be a rich source of ingredients that can be used in the development of pharmaceutical or synthetic drugs. Aside from that, these plants play an important role in the development of human drug all over the world. Whether in modern or traditional medicine, medicinal plants are used to maintain health, to treat a specific condition, or both. Nardostachys is one of the most important medicinal plant having several therapeutic properties. It is threatened in its natural habitat due to over exploitation for therapeutic purposes and high demand in the traditional medicine system. Keeping these points in mind, we attempted to investigate alternative uses of *in vitro* grown plants in place of wild plants without disrupting plant-based therapeutics and market demand. And then compare the root extract of the *Nardostachys jatamansi* plant's antioxidant and flavonoid levels under *in vitro* and natural growth conditions.

Methods: *Nardostachys jatamansi* is a plant that is widely used in traditional medicine systems. Because of its wide spread use in traditional medicine, this plant is considered endemic. In our study, we compare of antioxidant quality and Flavonoid amount of Natural and *in vitro* propagated *Nardostachys jatamansi*. Firstly we cultivated *in vitro* plants from *Nardostachys jatamansi* nodal explants for comparative analysis. The methanol extract of *in vitro* grown and wild-type plant root extract was then prepared using the maceration method. The extract was subjected to a comparative DPPH method to determine the presence of antioxidant potential in natural and *in vitro* grown plants. Furthermore, the HPLC analysis was used to detect and quantify the amount of Quercetin in both natural and *in vitro* propagated plants.

Result: When grown *in vitro* at a higher concentration, the roots of *Nardostachys jatamansi* have greater antioxidant potential than when they are grown naturally. They demonstrated antioxidant DPPH radical scavenging activity, with an IC50 value of 29.55 µg/ml for *in vitro* generated plants and 24.18 µg/ml for naturally grown plants. The concentration of Quercetin (mg/ml) for natural plant species is 1.95 and for *in vitro* propagate plant is 1.83. The HPLC analysis presents distinct peaks, with the main peaks having retention time for standard Quercetin (10.38) in the natural plant (10.34) and *in vitro* grown plants (10.32). In the end, natural-type species that had been produced *in vitro* were used to obtain the potential of micro propagated plants. The DPPH test and flavonoid were tested on the root extract of natural and *in vitro* plants. Both plants displayed promising antioxidant activity and an HPLC study identified the Quercetin component.

Key words: Antioxidants, Ascorbic acid, DPPH, HPLC, *Nardostachys jatamansi,* Quercetin.

INTRODUCTION

In the region of 3000 to 5000 m high altitude plant is found, *Nardostachys jatamansi* is a fragmented, perennial, flower-patterned plant with a height of 10 to 60 cm. The plant's short, dark grey, woody and thickened rhizome is a modified stem. The herb has rosette-shaped, whole, lanceolate leaves. According to Kumar *et al*., (2011) the plant's flowers have a pale purple color. From the plant's rhizome, essential oil was extracted using the hydro-distillation method. According to Pradhan and Paudel (2014) the oil has a smell that is comparable to musk pod and is greenish in hue. In addition to alpine meadows, juniper scrub, dwarf rhododendron forests and open pine forests, rocky outcrops are the usual habitat type, according to (Ghimire *et al*., 2005). In the Kumaon, Garhwal and Nepal Himalayan regions, the *Nardostachys jatamansi* plant is quite plentiful, but because local herb collectors and traders have taken advantage of it, it is now scarce. When *Nardostachys jatamansi* was first mentioned as being heavily exploited in the Indian

[1]Department of Biotechnology, Shri Guru Ram Rai University, Dehradun, Dehradun-248 001, Uttarakhand, India.
[2]Department of Biotechnology, Uttaranchal University, Dehradun-248 001, Uttarakhand, India.
[3]Shri Guru Ram Rai P.G. College, Dehradun-248 001, Uttarakhand, India.
[4]Department of Microbiology, Dolphin (PG) Institute of Biomedical and Natural Sciences, Dehradun-248 007, Uttarakhand, India.
[5]Department of Microbiology, Galgotias University, Greater Noida-201 310, Uttar Pradesh, India.

Corresponding Author: Naveen Gaurav, Department of Biotechnology, Shri Guru Ram Rai University, Dehradun, Patel Nagar, Dehradun-248 001, Uttarakhand, India. Email: naveensri17@gmail.com

How to cite this article: Pant, H.C., Rautela, I., Pant, H.V., Kumar, A., Kumar, P., Fatima, K. and Gaurav, N. (2024). Comparison of Antioxidant Properties and Flavonoid of Natural and *in vitro* Cultivated *Nardostachys jatamansi.* Agricultural Science Digest. 44(3): 406-413. doi: 10.18805/ag.D-5654.

Submitted: 10-08-2022 **Accepted:** 25-01-2023 **Online:** 06-04-2023

Himalayas, it was suggested that its systematic cultivation was required to preserve the plants for the Himalayan region. According to Airi *et al*., (2000) it is a plant species that is critically endangered in the Kumaon region of Uttarakhand. The study of *Nardostachys jatamansi* includes a thorough description of the plant, its development patterns, applications and other relevant information that may be useful in establishing the species' long-term cultivation. It is intriguing to notice that the chemical compositions of the essential oils from *Nardostachys jatamansi* and Indian valerian have been compared in published studies. State that India, Nepal and Bhutan have banned the export of *Nardostachys jatamansi* (Bhattarai *et al*., 2002).This plant reproduces vegetative and by its seeds, which are presumably pollinated by tiny insects like flies. Nautiyal *et al*., (2003) review that seed germination rates between 10 and 20%. According to a study from Dolpo, Nepal, adult plants have higher survival rates (88-100%) than seedlings do (68-90%). The plant species may also take 3-4 years before the boom of seedlings to reproductive size. Nepal has determined survival prices of adults to be high (88-100%), at the same time decreasing for juveniles and seedlings (68-90% and 46-78%) (Ghimire *et al*., 2008). According to Sharma *et al*., (2016) and Malik*et al*., (2018) the plant roots contain a variety of phytochemicals, including sesquiterpenes, jatamansic acid, jatamansinone, nardostachone, jatamansinol, jatamol, nardostachyin, nardosinone, angelicin, jatamansin, calarene, atchoulense. Singh *et al*. (2009) and Rahman *et al*. (2011) describe that the plant's rhizomes contain a variety of substances, including gum, sugar, starch, ketone, corrosive, lupelol, malliene, calarenol, coumarin, propionate and cyclohexanal.

The month of October sees the ripening of seeds that may later be sown following the winter. Jatamansi rhizomes can therefore be harvested around October, typically following Nepal's Dashain festival. Even in November, if green plants are still visible, the rhizome can be taken. However, after November, the plants dry out and become hidden by snow, making it impossible to harvest the rhizome. So, according to Pant *et al*. (2021) October is the ideal month for collecting Jatamansi rhizomes. Both conventional and contemporary therapies have employed *Nardostachys jatamansi*. This study's goal is to gather naturally growing *Nardostachys jatamansi* plants from various Himalayan regions and compare their antioxidant activity and flavonoid (Quercetin) content to that of *in vitro* cultivated plants.

MATERIALS AND METHODS

Selection of the plant

The *Nardostachys jatamansi* plant was obtained from Pithoragarh and Baghwesher districts of Kumaun region of Uttarakhand, India. Plants authenticated by the Botanical survey of India, Dehradun which will provide a certificate with the number 702 on it.

In vitro cultivation of the mother plant

The plant hormones BAP (6-benzyl amino purine) and IBA (Indole butyric acid) were utilized in MS (Murashige and Skoog) media at various concentrations to initiate shoot growth in the *Nardostachys jatamansi* plant. Before inoculation, the explants were surface sterilized to completely eradicate fungus, bacteria and spores from the plant. When a shoot was first noticed in the plant, the plant hormones cytokinin, BAP (6-benzyl amino purine) and kinetin (6-furfural amino purine) were used to promote the growth of the shoot. After that, half-strength MS Medium with various concentrations of NAA and BAP was used to promote rooting in the plant. Sucrose was employed as a carbon and energy source at 0.8% (w/v), while agar was used as a freeze to MS Medium at 0.5% (w/v). Explants injected with MS medium were then moved into a tissue culture facility at 25°C for a 16-hour photoperiod of light and dark after the MS Medium's pH was fixed at 5.8. After two weeks of incubation, routine data collection was started, including the number of plant shoots and root length.

Antioxidant activity

Because of their calming and relaxing effects on the central nervous system, *Nardostachys jatamansi's* roots and rhizomes have been employed in Ayurvedic medicine. This investigation focused on the methanolic extract of *Nardostachys jatamansi's* anti-stress abilities. By using a DPPH scavenging assay with an IC50 value of 10 g/ml, the antioxidant activities of *Nardostachys jatamansi* were examined.

DPPH radical scavenging activity

The free radical scavenging capacity of phytochemicals was assessed by adding 0.1 mM DPPH to 1.0 ml of standard/ extract solution in methanol at various concentrations. After that, let the combination sit for 20 minutes before measuring it at 517 nm. The log dose inhibition curve was used to determine the sample's IC 50 value, or the concentration of the sample needed to inhibit the DPPH free radical 50% of the time. Higher levels of free radical activity were indicated by the reaction mixture's lower absorbance. Blosis *et al*., (1958) used the following equation to compute the percentage of DPPH that was scavenged.

A positive control was ascorbic acid. The DPPH radical scavenging activity was calculated using the formula:

$$\text{Per cent scavenging} = \frac{(Ao - At) \times 100}{Ao}$$

Where,
At = The absorbance of the sample.
Ao = Absorbance of the control (without the extract).
The tests were run three times for a total of 30 minutes.

Preparation of DPPH

4 mg of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) was dissolved in 100 ml of 95% methanol in the absence of light to create

a 0.004% (w/v) DPPH solution. Three milliliters of various extracts in methanol were combined with one milliliter of this solution at concentrations of 10, 15, 20, 25, 30 and 35 µg/ml (Fig 2). Here, only those extracts that could be dissolved in methanol were used and different quantities of those extracts were made using the dilution approach. After shaking, the mixture was left to sit at room temperature for 30 minutes. The sample's absorbance was then measured at 517 nm with a UV-VIS spectrophotometer. Ascorbic acid was employed as the reference standard chemical and three copies of the experiment were run.

HPLC outline of *Nardostachys jatamansi*

For the HPLC analysis of the plant, *Nardostachys jatamansi's* methanolic extract was used. Using an extract of *Nardostachys jatamansi*, the flavonoid Quercetin was produced; a straight forward HPLC method was developed and verified for this. Due to the quick chromatographic run time, the new method can be applied to *in-vivo* research as well as routine quantification and quality control of Quercetin. The previously described HPLC method for Quercetin was outperformed by this technique. As a mobile phase, the experiment used acetonitrile, water and methanol. A UV-VIS detector with a variable wavelength of 242 nm was used for the detection. The amount of Quercetin in the plant material ranged from 1 to 2.0 mg/ml with a correlation coefficient. The method of standard addition was used to test both the HPLC method's accuracy and the correctness of the complete extraction process. The Quercetin isolated from various extracts and preparations can be standardized using this method. The limit of quantification was determined to be ten times this ratio (LOQ = 10•SD/slope) and the limit of detection to be three times the ratio between the SD and the slope of the low concentration curve (LOD = 3•SD/slope) (Stefova *et al*., 2001).

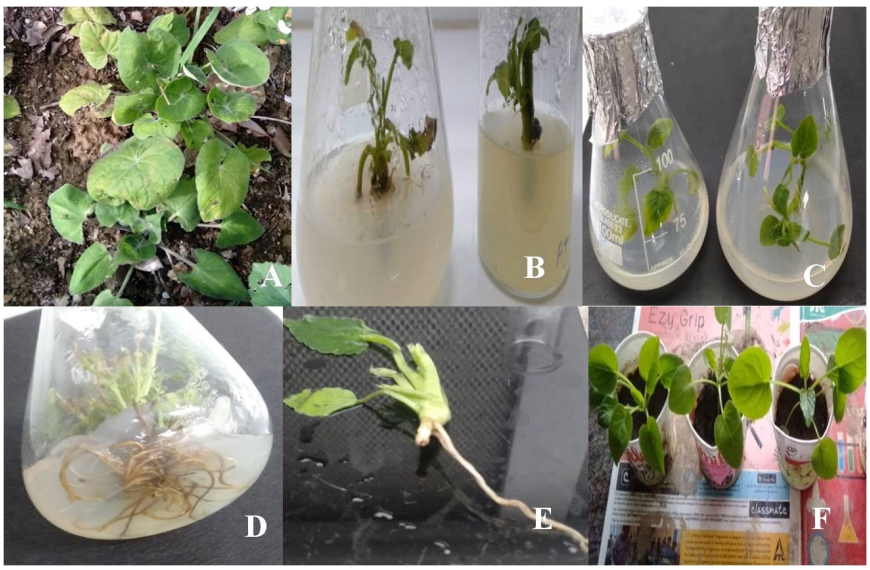
RESULTS AND DISCUSSION

Nardostachys jatamansi is a threatened plant species and necessary for many types of medicine (Bhatt *et al*., 2015). In this research study firstly a tissue culture technique can be utilized to grow *Nardostachys jatamansi* plant in *in vitro* condition without damaging the plant species.

In vitro propagation of *Nardostachys jatamansi*

According to Pant *et al*., 2021 MS (Murashige and skoog) medium containing phytohormone BAP + IBA (2.0 mg/l +1.5 mg/1) appear to have the maximum shoot growth. The largest shoots are seen in the Cytokines Carry MS medium (2 mg/l BAP and 1.5 mg/l KN) for the plant's post-subculture shoot proliferation. Rooting branches that were between 1.0 and 5.5 cm long were cut from the culture plant and transferred to a rooting media containing various concentrations of NAA combined with BAP. For inducing roots in plants, NAA and BAP (1.5 mg/l+1.0 mg/1) in the 1/2 Strength of MS medium works very well (Fig 1). Then, after being carefully taken from the culture flask and being well cleaned with distilled water, the better-developed rooted plants were transplanted to a small pot filled with a unique soil mixture.

Nardostachys jatamansi plant is over bleeding from the Himalayan region of India Purohit *et al*. (2012). *In vitro* clonal propagation, which produces genetically stable and disease-resistant clones of plants while limiting cultural variation, is the more powerful tool for plant propagation. The current



A. Mother plant of *N. jatamansi* where explant taken for further propagation process; B. Shoot Initiation in the explants; C. Shoot multiplication occur in the plant after subculture of *in vitro* raised plant; D and E. Rooting is obtained inthe plant after the multiplication; F. Hardening of the plantsin the mixture of different soil.

Fig 1: Different stage of *In vitro* clonal propagation of *Nardostachys jatamansi* from nodal ex plants.

work provides a possible *in-vitro* propagation method for *Nardostachys jatamansi* that is large-scale, prolific, fast and affordable through proliferation shoot growth. So by the *in vitro* method the *Nardostachys jatamansi* plant was multiplied in a lab. In order to create shoots and multiple shoots in plants, a combination of BAP, IBA and kinetin appears to be the best response. Next, a concentration of BAP, NAA and half-strength MS medium demonstrated the most successful rooting, after two weeks of the culture, healthy plants were collected for future research study, according to (Dhiman *et al.*, 2021). This study can be applied to the cost-effective *in vitro* production and preservation of this medicinal plant that is in grave danger of extinction.

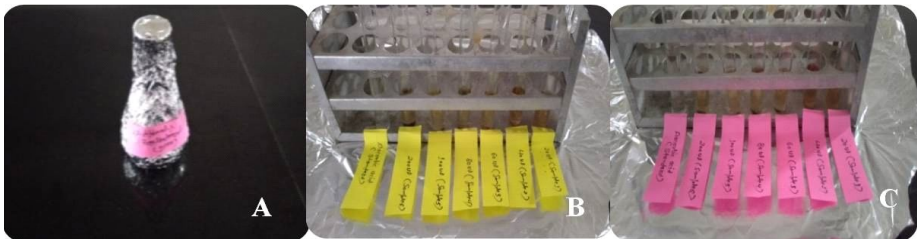
DPPH activity in *Nardostachys jatamansi*

After the *in vitro* propagation of the plant both natural and *in vitro* cultivated plant extracts are used to compare for the DPPH test. In the DPPH test, varied time durations between 10 and 60 min were needed for the plant extracts to reach chemical equilibrium (Sarbhov *et al.*, 1978). This is a characteristic predicted because the reactivity of the antioxidant molecules is not constant (Berghe *et al.*, 2012). The investigation began with the top phases or unambiguous solutions (Rahman *et al.*, 2007). The final DPPH level was expressed in mole per cent and plotted against the mass of dried plant (*i.e.*, the theoretical mass of dried plant needed to make the volume of extract utilized for a measurement) in the cuvette when equilibrium was attained at various doses of the extracts. The roots of methanol extract of naturally and *in vitro* showed better antioxidant potential when compared to standard ascorbic acid by DPPH scavenging assay method. The absorbance at 517 nm by UV visible spectrophotometer were found to be as 0.174±0.075 and % free radical inhibition 56.25±11.34 for the wild extract and for 0.063±0.022 *in vitro* methanolic extract and % free radical inhibition 66.83±6.81, IC 50 value obtained were as 24.18 for naturally species and 29.55 µg/ml for *in vitro* propagated species (Choudhary *et al.*, 2007; Hasan *et al.*, 2008). The inhibition values of all the different plant extracts both naturally and *in vitro* have been provided in Table 1 and Table 2 and Fig 3 and Fig 4. It determined that methanolic extract of plants at higher concentration captured more free radicals formed by DPPH resulting in decrease in absorbance and increase in IC 50 Value (Sharma *et al.*, 2020).

This study discovered that *Nardostachys jatamansi* species methanolic extract shown superior antioxidant capability by DPPH radical scavenging method, indicating that this plant has antioxidant activity (Kinsella *et al.*, 1993; Mishra *et al.*, 1995). This determining the possible antioxidant activity is found all plant extracts, both natural and *in vitro*and by the comparison with naturally plant *in vitro* propagated plant show better results (Sharma *et al.*, 2008).

HPLC detection of phytochemicals of *N. jatamansi*

The phytochemical constituents *i.e.* flavonoid (Quercetin) was evaluated using HPLC (Yu-ling *et al.*, 2017). By this technique identification and other types of impurities was detected. Any form of deviation from the norm in the peaks number, peaks area, or retention time indicates that the



A. Preapration of the free radical DPPH; B. Different concentration of wild species of *N. Jatamansi;* C. Different concentration of *in vitro* propagated *N. jatamansi.*

Fig 2: Preparation of the DPPH and various different concentrations of wild and *in vitro* propagated extract of *N. jatamansi.*

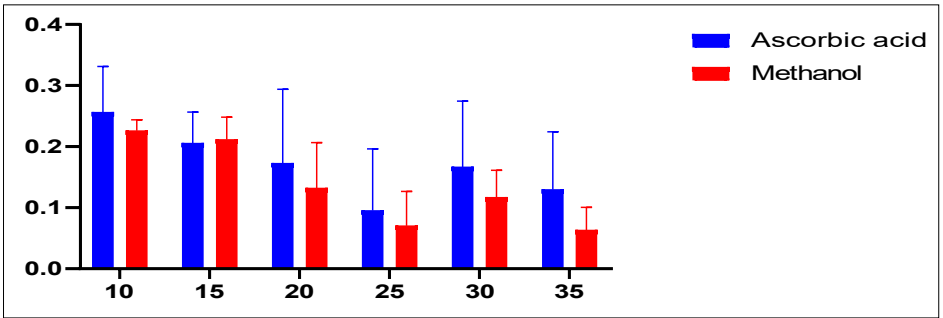


Fig 3: *N. jatamansi* (wild) root extract with standard ascorbic acid.

medication is impure or failing (Rahman *et al*., 2013). The *Nardostachys jatamansi* HPLC examination produced a number of peaks, however the most important ones with high area concentration were recorded as 29619399 for the naturally species with a retention time of 10.34 2.8 min and 27876969 and 10.32 min for the *in vitro* cultivated plant. UV detector used to study the concentration of Quercetin in the natural type and *in vitro* grown plants for the identification of Quercetin, liquid phase acetonitrile, methanol and water used and absorbance of Quercetin measured at 242 nm by using UV detector. Concentration of Quercetin in mg/ml is 1.95 and 1.83 for natural and *in vitro* grown plant species Fig 5 and Table 3. For the identification, standardization of methanolic extract of plant roots, HPLC is an impressible and authentic tool that widely used for the standard estimation of plant extract and it obtain formulation (Mahendra *et al*., 2011). Results of HPLC analysis of *Nardostachys jatamansi* methanolic extract of natural species at 242 nm mobile phase: acetonitrile, water and methanol in ratio (40:30:30) various constituents were recorded as evidenced by the chromatogram obtained at various retention times (10.341, 11.002, 14.058, 16.074, 17.347, 23.507 and 25.183) are the constituents found in *Nardostachys jatamansi* roots mainly. The methanolic extract of *Nardostachys jatamansi* root extract of *in vitro* chromatogram shows different constituents at various retention times (10.321, 10.999, 14.034, 16.027, 17.303, 25.867 and 28.773) (Rashid *et al*., 2018). According to Márquez *et al*., (2005) these peaks indicate the chemical components found in plant roots.

Currently, research is being done on the isolation and characterization of bioactive compounds. Quantitative results show that HPLC analysis is crucial for determining the quantity, legitimacy and identification. The effectiveness of *Nardostachys jatamansi* in treating various bodily ails is also demonstrated by this analytical methodology for quality

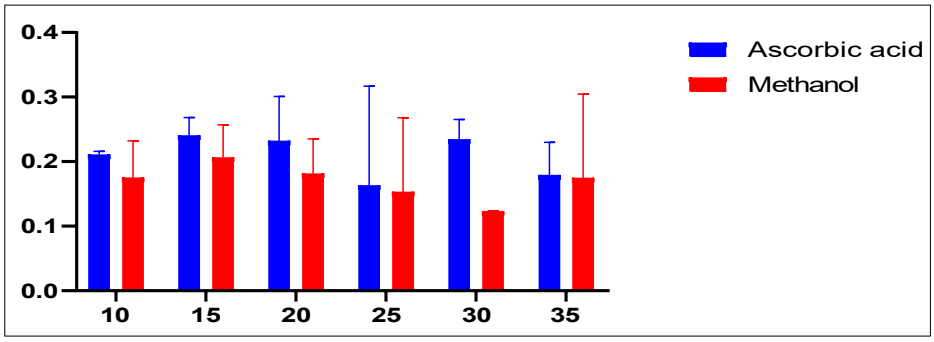


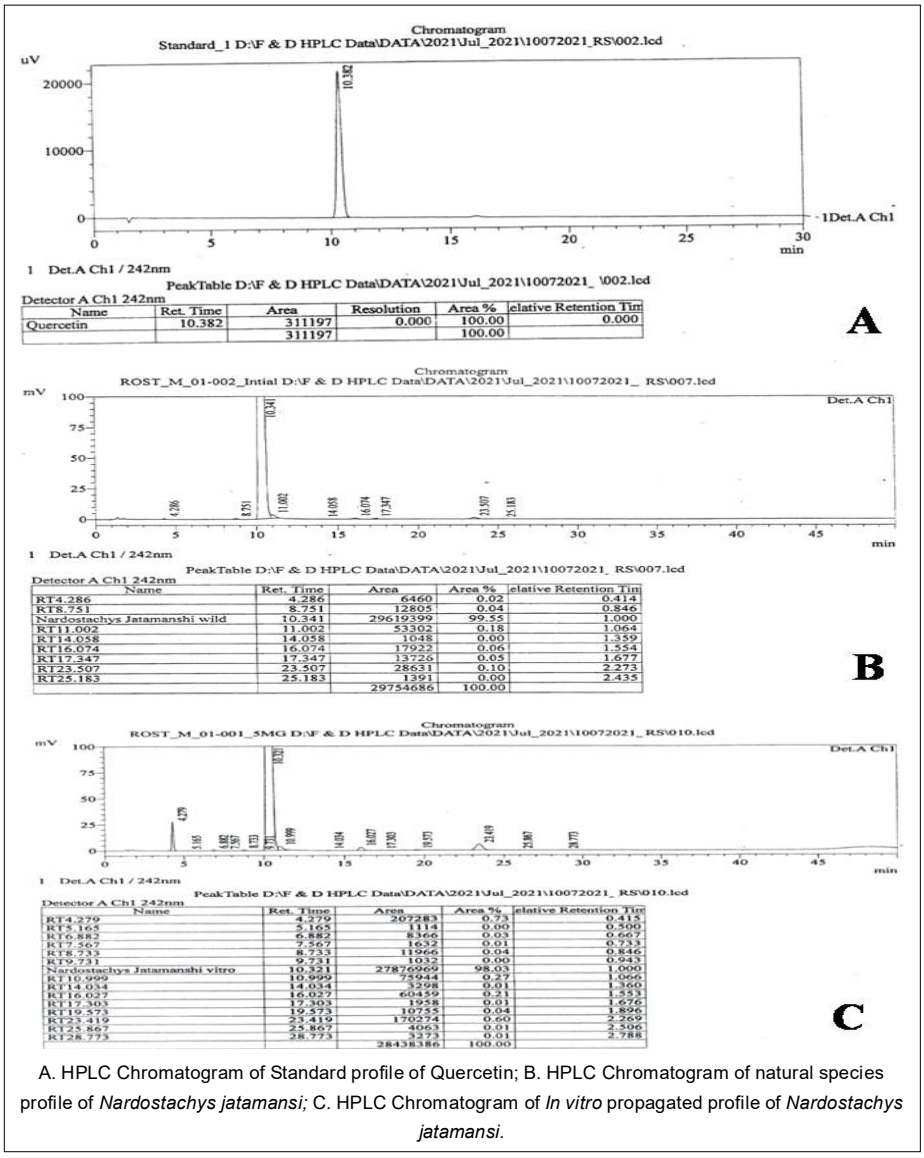
Fig 4: *N. jatamansi* root extract (*in vitro*) with standard ascorbic acid.

Table 1: 517 nm UV visible spectrometer study of *N. jatamansi* (wild) root extract with standard ascorbic acid (DPPH scavenging assay method).

Sr no.	Concentration µg/ml	Ascorbic acid (Abs) control Mean±SEM	Methanol (Abs) *in vitro* Mean±SEM	% free radical inhibition Mean±SEM
1	10	0.210±0.003	0.175±0.033	26.72±6.07
2	15	0.240±0.016	0.206±0.029	25.35±5.70
3	20	0.232±0.040	0.181±0.031	43.01±6.17
4	25	0.163±0.089	0.153±0.067	43.33±10.03
5	30	0.234±0.018	0.123±0.001	46.81±4.64
6	35	0.179±0.029	0.174±0.075	56.25±11.34
One sample t test	P value=0.04		IC50 value = 24.18	

Table 2: 517 nm UV visible spectrometer study of *N. jatamansi* root extract (*in vitro*) with standard ascorbic acid (DPPH scavenging assay method).

Sr. no.	Concentration µg/ml	Ascorbic acid (Abs) control	Methanol (Abs) wild Mean±SEM	% free radical inhibition Mean±SEM
1	10	0.256±0.043	0.226±0.010	18.15±4.03
2	15	0.206±0.029	0.212±0.021	26.85±3.52
3	20	0.173±0.070	0.132±0.043	32.96±3.41
4	25	0.095±0.058	0.070±0.033	37.53±1.23
5	30	0.166±0.062	0.117±0.026	44.48±6.55
6	35	0.129±0.055	0.063±0.022	66.83±6.81
One sample test	P Value=0.0188		IC50 value = 29.55	



A. HPLC Chromatogram of Standard profile of Quercetin; B. HPLC Chromatogram of natural species profile of *Nardostachys jatamansi;* C. HPLC Chromatogram of *In vitro* propagated profile of *Nardostachys jatamansi.*

Fig 5: HPLC detection of phytochemical of *N. jatamansi.*

Table 3: Flavonoid (Quercetin) concentration of natural and *in vitro* propagated *Nardostachys jatamansi* (mg/ml).

Name	Retention time	Area	Relative retention time	Concentration of quercetin (mg/ml)
Quercetin	10.38	311197	0.00	2.12
Natural plant species	10.34	29619399	1.00	1.95
In vitro grown	10.32	27876969	1.00	1.83

assurance. To enhance quality, clarity and identification of the upcoming herb *Nardostachys jatamansi*, this study will offer preliminary information about it. The Quercetin chemical was found in *Nardostachys jatamansi* and measured the amount of Quercetin in both naturally occurring and *in vitro* cultivated plant species (Garg *et al*., 2021). Le *et al.* (2017) explain that the chromatography method gives us a chromatogram of the several substances found in the methanolic extract of the plant's roots.

CONCLUSION

Firstly, the *Nardostachys jatamansi* plant was grown utilizing the *in vitro* micropropagation technique. The best potential and activity of both naturally occurring and *in vitro* cultivated plants are compared utilizing antioxidant tests and HPLC analysis. According to the results of the current study's DPPH test, antioxidant levels were found to be higher in *in vitro* propagated plants than in naturally

growing plants. The analytical techniques described here can easily separate the natural and *in vitro* cultured *Nardostachys jatamansi* species when it comes to Quercetin identification and quantification. According to this compression study, plants cultivated *in vitro* can also be employed as a source of medicine.

ACKNOWLEDGEMENT

The authors are thankful to the Management and Staff members of Shri Guru Ram Rai University Patel Nagar, Dehradun for their support and providing necessary facilities.

Add-on information authors' contribution

Develop thought work, Conducted the research, experimentation and data analyses, Prepared and written the initial draft.

Research content

The research content of manuscript is original and has not been published elsewhere.

Ethical approval

Not applicable.

Data from other sources

Not applicable.

Consent to publish

All authors agree to publish the paper in Agricultural Science Digest.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

Airi, S., Dhar, U., Rawal, R. and Purohit, A.N. (2000). Assessment of Availability and Habitat Preference of *jatamansi*- A Critically Endangered Medicinal Plant of West Himalaya. Current Science. 79(10): 1467-1471.

Berghe, W.M. (2012). Epigenetic impact of dietary polyphenols in cancer chemo prevention: Lifelong remodeling of our epigenomes. Pharmacol Res. 65(6): 565-576.

Bhatt, M. and Kothiyal, P. (2015). A review article on phytochemistry and pharmacological profiles of *Nardostachys jatamansi* DC-medicinal herb. Journal of Pharmacognosy and Phytochemistry. 3(5): 102-106.

Bhattarai, N. and Tandon, M.K. (2002). Report of the Conservation Assessment and Management Plan workshop in Nepal. Med. Plant Conservation. 8: 28-30.

Blosis, M.S. (1958). Antioxidant determination by the use of a stable free radical. Nature. 181: 1199-1200.

Choudhary, G.P. (2007). *In vitro* antioxidant studies of the ethanolic extract of *Tephrosia purpurea* L. Ancient Science of Life. 27(1): 26-30.

Dhiman, N., Devi, K. and Bhattacharya, A. (2021). Development of low cost micropropagation protocol for *Nardostachys jatamansi*-A critically endangered medicinal herb of Himalayas. South African Journal of Botany. 140(6): 468-477.

Garg, P. (2021). HPLC Estimation of Flavanoid (quercetin) of leaves and stem extracts of *Ocimum sanctum* and *Tinospora cordifolia*. The Journal of Phytopharmacology. 10(4): 220-224.

Ghimire, S.K., Gimenez, O., Pradel, R., McKey, D. and Thomas, D.Y. (2008). Demographic variation and population viability in a threatened Himalayan medicinal and aromatic herb *Nardostachys grandiflora*- matrix modeling of harvesting effects in two contrasting habitats. Journal of Applied Ecology. 45: 41-51.

Ghimire, S.K., McKey, D. and Thomas, D.Y. (2005). Conservation of Himalayan medicinal plants harvesting patterns and ecology of two threatened species, *Nardostachys grandiflora* DC and *Neopicrorhiza scrophulariiflora* (Penel) Hong. Biological Conservation. 124(4): 463-475.

Hasan, S.M., Hossain, M.M., Faruque, M.M.A., Mazumder, M.E.H., Rana, M.S., Akter, R. and Alam, M.A. (2008). Comparison of antioxidant potential of different fractions of *Commelina benghalensis* Linn. Bang. J. Life. Sci. 20: 9-16.

Kinsella, J.E., Frankel, B., German B. and Kanner, J. (1993). Possible mechanisms for the protective role of antioxidant in wine and plant foods. Food Technol. 47(4): 85-89.

Kumar, S.S. and Pal, S.A. (2011). Standardization and preliminary phytochemical screening of *Nardostachys jatamansi* Dc Rhizome. International Journal of Research in Ayurveda and Pharmacy. 2(3): 978-982.

Le, V.N.H., Khong, T.Q., Kimand, K.T., Kang, J.S. (2017). An optimized HPLC-UV method for quantitatively determining sesquiterpenes in *Nardostachyos* Radix ET Rhizome. Journal of Pharmaceutical and Biomedical Analysis.145: 406-413.

Mahindra, J.K., Susy, K.A. and Hari, A.M. (2011). Standardization of Feronialimonial leaves by HPLC, HPTLC, physicochemical and histological parameters. Boletín Latinoamericano y del Caribe de PlantasMedicinales y Aromáticas. 10(6): 525-535.

Malik, R., Firdose, K.F. and Bhat, M.D.A. (2018). Efficacy of *Nardostachys jatamansi* DC. In the management of premenstrual syndrome-A randomized controlled study. Journal of Herbal Medicine. 14: 17-21.

Márquez, A.J., Stougaard, J., Udvardi, J.M., Parniske, M.M., Spaink, H., Saalbach, G., Webb, G.J., Chiurazzi, J.M. and Márquez, A.J. (2005). Lotusjaponicus Handbook. Springer. 978: 349-355.

Mishra, D., Chaturvedi, R.V. and Tripathi, S.C. (1995). The fungi toxic effect of the essential oil of the herb *N. jatamansi* DC. Trop Agric. 72(1): 48-52.

Nautiyal, B.P., Chauhan, R.S., Prakash, V., Purohit, H. and Nautiyal, M.C. (2003). Population studies for the evaluation of germplasm and threat status of the alpine medicinal herb, *Nardostachys jatamansi*. PGR Newsletter. 136: 34-39.

Pant, H.C., Tyagi, M., Sharma, M.D., Pant, H.V., Kumar, A. and Gaurav, N. (2021). Collection practices of Himalayan medicinal plants (*Nardostachys jatamansi*): Ancient, ayurvedic, traditional and present application. International Journal of Botany Studies. 6(2): 18-21.

Pant H.C., Pant, H.V., Kumar, A., Tomar, H., Sharma, M.D. and Gaurav, N. (2021). *In vitro* clonal propagation of *Nardostachys jatamansi*, A Traditional Himalayan medicinal plant. Journal of Mountain Research. 16(3): 87-98.

Pradhan, R. and Paudel, K. (2014). Seasonal variation of the essential oil of *Nardostachys grandiflora DC*. Bull. Dept. Pl. Res. 36: 76-78.

Purohit, V.K., Chauhan, R.S. andola, H.C., Prasad, P., Nautiyal, M.C. and Nautiyal, A.R. (2012). *Nardostachys jatamansi* DC- Conservation, multiplication and policy issues. Medicinal Plants. 4(3): 162-166.

Rahman, A. and Amin, K.M.Y. (2013). Physicochemical assay along with HPLC profile of a pharmacopeial preparation contain Colchicum, Ginger and Aloe. International J. of Pharm Research. 5(4): 51-55.

Rahman, H., Shaik, H.A., Madhavi, P. and Eswaraiah, M.C. (2011). A review pharmacognostics and pharmacological profiles of *Nardastachys jatamansi* DC. Elixir Pharmacy. 39: 5017-5020.

Rahman, M.A.A. and Moon, S.S. (2007). Antioxidant polyphenol glycosides from the Plant *Drabanemorosa* Bull. Korean Chem. Soc. 28(5): 827-831.

Rashid, M., Rahman, A., Ahmad, Q.Z. and Mian, S.S. (2018). Physicochemical analysis of Sumbul-al-Teeb (*Nardostachys jatamansi* D.C.) rhizome along with its HPLC profile. Pharmacogn J. 10(2): 278-284.

Sarbhoy, A.K. and Saxena, D.B. (1978). Isolation and pharmacodynamic activity of the sesquiterpene valeranone from *N. jatamansi* DC. Arzeimitteforschunmg. 28(1): 7-13.

Sharma, G.K., Sharma, S., Chasta, P., Joshi, R.K., Tiwari, A. and Kaushal, K. (2020). Chandrul assessment of pharmacognostic parameters and antioxidant potential of Jatamansi (*Nardostachys jatamansi* DC.) roots and rhizomes by DPPH method. DerPharmacia Lettre. 12(7): 43-50.

Sharma, N., Sharma, A.R., Patel, B.D. and Shrestha, K. (2016). Investigation on phytochemical, antimicrobial activity and essential oil constituents of *Nardostachys jatamansi* DC in different regions of Nepal. Journal of Coastal Life Medicine. 4(1): 56-60.

Sharma, S.K. and Gupta, V.K. (2008). *In vitro* antioxidant study of *Ficus racemosa* Linn. Root. Pharmacognosy Magazine. 4(13): 70-74.

Singh, A., Kumar, A. and Duggal, S. (2009). *Nardostachys jatamansi* DC potential herb with CNS effects. Asian Journal of Pharmaceutical Research and Health Care. 1(2): 276-290.

Stefova, M. and Stafilov, T. (2001). Assay of Flavonols and Quantification of Quercetin in medicinal plants by HPLC with UV-diode array detection. J. Liq. Chrom. and Rel. Technol. 24(15): 2283-2292.

Yue-ling, M., Chen, Y.J., Wan, D., Chen, P. and Xu, R. (2017). HPLC determination of quercetin in three plant drugs from Genus *Sedum* and conjecture of the best harvest time. Pharmacogn. J. 9(6): 725-728.