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(Research Article)

Phytochemical Analysis of *Nardostachys Jatamansi DC*, *Chlorophytum Borivilianum* (Soil based) and *Chlorophytum Borivilianum* (Commercial)

Akhilesh Dubey[1*], Deepak Bhushan[2], Nikhil Kawatra[3]

[1*,2,3]*Department of Biological Sciences and Engineering, Netaji Subhas University of Technology, Sector-3, Dwarka, New Delhi, INDIA*

Abstract

Nardostachys jatamansi DC and Chlorophytum borivilianum are listed as critically endangered under criteria A2cd in the International Union of Conservation of Nature's (IUCN) Red List of Threatened Species. The multi therapeutic and nutritional importance of these species is attributed to the source of phytochemicals. The selected plant species for the study have been reported to be sedative, neurotoxic, cytotoxic, antidepressant, antioxidant, and antimicrobial. The total phenolic, flavonoid, flavonols, tannins, saponins and alkaloids of the selected plant species were analyzed. Nardostachys jatamansi DC had the highest phenolic, flavonoid, flavonols and tannins content in compare to other Chlorphytum borivilianum species. But the saponins and alkaloid content was found more in the Chlorophytum borivilianum (soil based) species than the other species. Nardostachys jatamansi DC had lower saponins and alkaloids content in compare to other Chlorophytum borivilianum species. It is advised that further research work should be carried out to isolate, purify, and characterize the bioactive compounds found in these critically endangered plant species.

Keywords: Nardostachys jatamansi DC, Chlorophytum borivilianum, A2cD, Phenolics, Flavonoids, Flavonols, Tannins, Saponins, Alkaloids.

1. Introduction

Nardostachys jatamansi DC and *Chlorophytum borivilianum* has been assessed for the IUCN red list of threatened species in 2014 and 2020 respectively (Ved et al. 2015). Multi therapeutic has found in these plant species and has nutritional importance due to their high content of medicinally important phytochemicals. *Nardostachys jatamansi* DC species has critically endangered medicinal plant species status which is harvested in the Himalayan ranges of India and its neighboring countries including China, Nepal etc. The roots/rhizomes of these species are very rich source of different phytochemicals and bioactive compounds which are medicinally important. These species are at verge of extinction due to the overexploitation, deforestation and habitat loss and huge demand (Dhiman, N. and Bhattacharya 2020). Approx. 80% decline has been seen in the population of these species in India in last 10 years due to this they are now in the category of critically endangered species (Chauhan, H.K., Oli, S., Bisht, A.K., Meredith, C. and Leaman, D., 2021). In Himachal Pradesh, Uttarakhand and some north eastern states of India are those where these species is harvested (Ved et al. 2015). In the China region provinces of Gansu, Sichuan, and Yunnan provinces and the Xijang region of Tibet are those where these species are found and harvested (Mulliken and Crofton 2008). The rapid decline in the population of these species is due to their extensive harvesting of rhizomes/roots for their higher usage for medicinal purpose. *Nardostachys jatamansi* DC is used for purpose of perfume, ointment, and modern medicine (Anon 1996, Jain 1968, Kirtikar and Basu 1989). The low viability in roots and germination is another reason for the extinction of these species. Different disorders problems including digestive, circulatory, respiratory, urinary, and reproductive systems are cured and prevented by the use of roots and rhizomes of the species (Jain and Defilipps 1991, Joshi and Parle 2006, Kumar et al. 2006, Negi et al. 2018a). Many studies on the storage and conservation of these species are going on in order to conserve this endangered medicinal plant species. Various disorders like cough, fever, headache, food poisoning, cholera, intestinal worms, joint pain, rheumatism, stomach disorders, jaundice, and cardiac disorders and blood purification are the medicinal benefits of

[*]*Corresponding Author: e-mail: Akhilesh.dubey@nsut.ac.in*
Tel-+91-7838886012
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the species (Ghimire and Aumeeruddy-Thomas 2009, Negi et al. 2018a, Kandari et al. 2012). The enhancement of memory can be achieved by consumption of the tonic made by the decoction of roots of these species (Rokaya et al. 2010, Joshi and Parle 2006).

Roots and tubers of *Chlorophytum borivilianum* are harvested due to their medicinal benefits. The tubers of *Chlorophytum borivilianum* contain very rich amount of medicinally important phytochemicals. Dried roots and tubers of the *Nardostachys jatamansi DC* and *Chlorophytum borivilianum* are available on many online shopping sites. Many people in the urban cities like Delhi, Mumbai, and Kolkata buy the dried powder due to its medicinal benefits. 'White gold' or 'Divya aushadi' in Indian systems of medicine is the name of these species. The roots and tubers of *Chlorophytum borivilianum* are traded in the global market by the name of 'Safed Muesli'. The leaves of this plant are also used for making extracts due to high phytochemical content in it. This species is harvested found only in some parts of Rajasthan, Maharashtra and Gujarat. The tubers of these species also used to prevent the low sperm count in men. It is also beneficial for curing the sexual problems. This species has been used to increase the strength, stamina and satisfaction. The foreign demand of this species is also very high which is recorded as 300-700 tons annually.

These both species contains the considerable amount of phenolic compounds and through which their antioxidant activity can be easily related and compared. Their application is very important for the use in the medicinal activities and food industry as well. There is very growing demand of natural preservatives in the food industry. So, these species can be utilized as the natural preservatives in the form of antioxidants in the food industry and can also be used for the food fortification of different food products.

In this current study we have compared the antioxidant activities of these species which cultivated in India. For this, *Nardostachys jatamansi DC*, *Chlorophytum borivilianum* (soil based) and *Chlorophytum borivilianum* (commercial) has been collected in order to compare the total phenolic, flavonoid, and flavonol contents. The tannins content and alkaloids content has also been estimated for these species.

2. Materials and Methods

2.1. Sample preparation: The species of *Nadrostachys jatamansi* DC and *Chlorophytum borivilianum* (soil based) were collected from the CSIR-IHBT (Institute of Himalayan Bio-resource Technology), Palampur, Himachal Pradesh, India. The *Chlorohytum borivilianum* (commercial) was bought from Amazon, India. The roots, rhizomes and tubers of the species were cut and washed with distilled water manually. These were dried in the hot-air oven at 60 °C. Dried samples were grinded by grinder, and further stored at room temperature in incubator until extraction.

2.2. Aqueous extract preparation by the Decoction Method: The roots, rhizomes and tubers of the plant species were washed thoroughly with distilled water and then kept inside the oven to dry at 60°C for 12 hours. The 2 g of dried roots powder were boiled in the 50 ml of distilled water for five minutes. The temperature of the boiled sample is reduced to the room temperature. After that centrifugation of the sample was done at 5000 rpm for 15 minutes and then it was filtered by the help of nylon membrane of pore size of 0.22 µm. The aqueous extract is collected after the filtration. Then the aqueous extracts of the species were stored at 4-5 °C until the further use

2.3. Determination of total phenolic content: Folin-Ciocalteu's phenol method was used for the determination of total phenolic content. The standard calibration curve was plotted using Gallic acid at different concentrations. To 100 µl of each diluted roots, rhizomes and tubers extracts of the species 500 µl of Folin-Ciocalteu's phenol reagent was added (Merck, Germany). After 5 minutes, 7.5% sodium carbonate solution of volume 400 µl was added to the assay and then incubated for 30 min. The Gallic acid equivalents in (mg/g) in discrete concentrations used for generation of standard calibration curve. At 765 nm wavelength, the absorbance of the sample was measured and the total phenolic content was calculated using the regression equation obtained from the standard curve (Parsaei et al., 2013).

2.4. Determination of total flavonoids: In this present study, Aluminum chloride colorimetric technique is used for determination of total flavonoids in the given samples. To 0.5 ml of each root, rhizome and tuber extract 3 ml potassium acetate (5%) was mixed. 0.5 ml Aluminum chloride (2%) (Merck, Germany) was added to it. The mixture was incubated around 40 minutes at 25-28 °C and at 415 nm the absorbance of the final solution was read by spectrophotometer and the total flavonoids was calculated using the regression equation obtained from the standard curve.

2.5. Determination of total flavonols: In the present study, the total flavonols content in three different samples were also determined by the Aluminum chloride technique, as described above. The same procedure is used for the total flavonols. The incubation time of the sample was 150 minute. After that the absorbance was read at 440 nm (Miliauskas et al., 2004).

2.6. Determination of total tannins content: Bouaziz et al. (2008) method used for the estimation of the total tannins content in the aqueous extract of roots were estimated. 3 ml of methanol (4%) was added to 50 µl (approx.) of the extract. The mixture of stirred by stirrer. After that, 1.5 ml concentrated HCl was added. The absorbance was measured at 500 nm after 15 minutes. The total tannins were calculated using the regression equation obtained from the standard curve of catechin equivalents (mg/g).

2.7. Determination of total alkaloids content: 5 g of dried and grinded powder of each sample was taken in 250ml beaker and followed by addition of 200 ml of 10 % acetic acid in ethanol. The mixtures of each sample were allowed to stand for a time period of 4 hours at room temperature. After 4 hours, filtration of different samples were done and the extracts were taken out from the mixture. The extracts of each sample were concentrated by using water bath continuously until one-quarter of the original volume is reached. The complete precipitation of the extracts were done by using the concentrated ammonium hydroxide which was added drop wise to the extracts of the different sample. It was then allowed to stand and settle. After the settlement, the precipitated content was collected and dilute ammonium hydroxide was used to wash it thoroughly and then followed by filtration. The residue was the alkaloid, which was dried, weighed and percentage was calculated.

2.8. Determination of total saponins content: The method used in the determination of total saponins in the present study was described by the Anhawange et al. (2004). The dried grinded powder of the root, rhizomes and tubers of the species were weighed around 5 g each and 75% ethanol (100 ml) was added to it. Agitation of the mixture was accomplished on the hot-plate magnetic-stirrer device at a temperature of 55 °C for 12 hour interval. 40ml was the reduced volume of the mixture at vaccum. It was taken into a 250 ml separatory funnel and the diethyl ether (20 ml) was used to separate the saponins. The purification of the mixture was done until the clear aqueous extract was obtained. The extraction of saponins was done 30 and 60 ml of n-butanol. It was washed twice by the 10 ml of 5% Sodium Chloride solution, followed by evaporation to dryness. The remained residue was weighed and saponins were calculated as mg/g dry weight.

3. Results and Discussions

3.1. Total phenolic, flavonoid and flavonol content: According to the recent studies, health benefits of phenolics, flavonoids and flavonols were observed. In Table 1, the values of total phenolics, flavonoids and flavonols of the three different species are represented. It is observed that *Nardostachys jatamansi DC* contained the highest level of total phenolics, total flavonoids and total flavonols in comparison to the remaining species of the *Chlorophytum borivilianum* (safed museli). According to our results, the total phenolic, flavonoid and flavonols content of *Nardostachys jatamansi DC* are 8.631 mg GAE/g, 30.267 mg rutin/g and 72.971 mg rutin/g respectively. The total phenolic content of *Nardostachys jatamansi DC* was 2.5 and 15.80 fold higher than those *Chlorophytum borivilianum* (soil based) and *Chlorophytum borivilianum* (commercial). In case of total flavonoids and total flavonols content, again the *Nardostachys jatamansi DC* showed higher content in comparison of other two species of *Chlorophytum borivilianum*. The total flavonoids and total flavonols content of *Nardostachys jatamansi DC* was 30.267 mg rutin/g and 72.971 mg rutin/g respectively. The total flavonoids of *Nardostachys jatamansi DC* was 5 and 18 folds higher than *Chlorophytum borivilianum* (soil based) and *Chlorophytum borivilianum* (commercial) and in case of total flavonols, it was 1.5 and 2.2 fold higher than *Chlorophytum borivilianum* (soil based) and *Chlorophytum borivilianum* (soil based). These species are particularly interesting for estimation of their antioxidant effects on the body. So these species can be used as antioxidant in food industry because they are natural and having no hazard on the body. According to many findings, the antioxidant activity and phenolic, flavonoids and flavonols content are correlated to each other.

3.2. Total tannins content: The water soluble polyphenols which are called tannins are present in the *Nardostachys jatamansi DC* and *Chlorophytum borivialinum species*. The tannins have anti-carcinogenic and anti-mutagenic activity which is very beneficial for health. The tannin content in *Nardostachys jatamansi DC* is very high in compare to other species. *Nardostachys Jatamansi DC* contains around 8 fold higher tannin content in compare to both *Chlorophytum borivilianum* species. As *Nardostachys jatamansi* DC contained high tannin content therefore it is called high inflammatory and wound healing medicinal plant.

3.3. Total saponins and alkaloids: Saponins and alkaloids content in *Nardostachys jatamansi* DC was less than in compare to the *Chlorophytum borivilianum* species. The total saponins and alkaloids content in the *Chlorophytum borivilianum* (soil based) is higher than the other species. The *Chlorophytum borivilianum* (soil based) contains the saponins and alkaloid content around 6.82 mg/g and 16.87 mg/g respectively. *Nardostachys jatamansi DC* contains very less amount of saponins content.

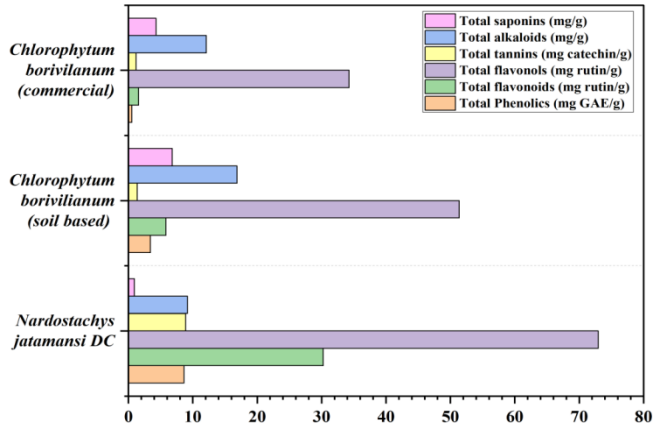

Figure 1. Graphical representation of phytochemicals content in *Nardostachys jatamansi* DC, *Chlorophytum borivilianum* (soil based) and *Chlorophytum borivilanum* (commercial)

Table 1. Amount of phytochemicals content in *Nardostachys jatamansi* DC, *Chlorophytum borivilianum* (soil based) and *Chlorophytum borivilanum* (commercial)

Sample	*Nardostachys jatamansi* DC	*Chlorophytum borivilianum* (soil based)	*Chlorophytum borivilanum* (commercial)
Total Phenolics (mg GAE/g)	8.631	3.418	0.546
Total flavonoids (mg rutin/g)	30.267	5.822	1.6
Total flavonols (mg rutin/g)	72.971	51.377	34.275
Total tannins (mg catechin/g)	8.875	1.375	1.211
Total alkaloids (mg/g)	9.19	16.87	12.11
Total saponins (mg/g)	0.927	6.82	4.31

4. Conclusions

Therefore, the above results show that the species of *Nardostachys jatamansi DC* and *Chlorophytum borivilianum* have the significant amounts of the medicinally important compounds in roots, tubers and rhizome. The extract of these plants have good potential of being a source of useful drugs. These species can also utilized for making the super foods or foods for special dietary purposes. As these species are critically endangered, we have to conserve these species using different techniques like Hydroponics technology. The result of the current study provided evidence that decocted aqueous extracts of these critically endangered plant species contain medicinally beneficial and therapeutic bioactive compounds. It is advised that further research work should be done in order to isolate and characterize the bioactive.

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Biographical notes

Dr. Akhilesh Dubey is working as an Assistant professor in the Department of Biological Sciences and Engineering, Netaji Subhas University of Technology. His research interests are hydroponics technology, plant biotechnology and environmental toxicology**.**

Deepak Bhushan is pursuing M. Tech in Biochemical Engineering from the Department of Biological Sciences and Engineering, Netaji Subhas University of Technology. His research interest is plant biotechnology.

Nikhil Kawatra is a Ph.D. Research Scholar in the Department of Biological Sciences and Engineering, Netaji Subhas University of Technology. His research interests are hydroponics technology, plant biotechnology and biochemical engineering.