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A Comparative Study and Assessment of Phytochemicals and Protein in Deferent Species of Chlorophytum Borivilianum from Vindhya Region of Madhya Pradesh

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ABSTRACT
Chlorophytum borivilianum (Safed Musli) is a rare plant species valued for its dried fasciculated roots having aphrodisiac properties and form an important ingredient of herbal tonics prescribed in the ancient System of medicine in India since the Vedic era. In general, the study shows the morphological variation in Chlorophytum species grown in different localities. The methanolic and water extract of leaves and tubers of Chlorophytum Borivilianum (Green type) plants from Ateraila (Rewa), Chitrakoot (Satna) and Churhat (Shidhi) forests in the Vindhya region of Madhya Pradesh is studied for proteins and phytochemicals content. Here we reported the protein content is more in tubers as compared to leaves. We found that numbers of natural organic compounds were present such as alkaloids, Phenol, flavonoids, Resins, Anthocyanins, Terpenoids, saponins, Steroids, Tannin, Starch, Glycoside, Phlobatannins, etc, in leaves and tubers of Chlorophytum borivilianum (Green type) plant and their amount among different localities varies. These organic compounds play an important role in the Growth and development of plants, and during pathogen infection. The phenolic content analysis reveals that these compounds are abundant in Chlorophytum borivilianum plant leaves and tubers which are known to exhibit antioxidant activity in vitro and in vivo and prevent DNA damage from free radicals.

I. INTRODUCTION

Chlorophytum is a rare and well known, medicinal herb for its medicinal properties (Nayar and Sastry 1988). According to Kirtikar and Basu in 1975 a number of Chlorophytum species are used as traditional medicine since the ancient India. The 'Vedas' and more specifically 'Yajur Veda' is the main source of such information. The Chlorophytum borivilianum Or Safed Musli is one of the most commercially exploited and widely growing species to used for lack of libido treatment (Kaushik 2005, Munyao, Dong et al. 2020). The use of plants for treatment of various ailments dates back to over thousands of years. In recent, the market and public demand has been so increased that there is a huge extinction risk to many medicinal plants and obviously the loss of genetic diversity. The WHO estimated that 80% of the world's population depends on traditional medicines for meeting their primary health care needs (Van Loo 2004, Visavadiya, Soni et al. 2010).

Safed musli (Chlorophytum borivilianum) is an herb with sub-erect lanceolate leaves and tuberous root system belonging to the family Liliaceous. It can grow up to a maximum height of 45 cm. Tubers can grow up to a depth of 25 cm. It is a tiny annual herb that grows well in tropical and sub-tropical climates with altitudes up to 1500 meters. There are about 256 species of Chlorophytum and 17 among them are found in India. Out of 17 species 3 species namely as Chlorophytum borivilianum, Chlorophytum arundinaceam and Chlorophytum tuberosum are commercially cultivated by the Indian farmers and Chlorophytum borivilianum is the only species, which is under commercial cultivation. C. borivilianum is a plant well known for its aphrodisiac as well as immunodulatory activity (Munyao, Dong et al. 2020). C.borivilianum is traditionally used for treating oligospermia, pre- and postnatal infections, arthritis, diabetes and dysuria (Visavadiya, Soni et al. 2010, Tripathi, Mishra et al. 2013). Its antiviral, anticancer, immunomodulatory, antidiabetic, antistress, and anti-inflammatory properties have been evaluated(Kothari and Singh 2003, Khanam, Singh et al. 2013) .

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borivilianum in their natural localities and comparative biochemical analysis of phytochemicals of C. borivilianum collected from different habitats, such as Ateraila forest of Rewa (MP), Chitrakoot forest of Satna (MP) and Churhat forest of Shidhi district of Madhya Pradesh (MP). Besides this, attempt has been made to estimate proteins from the in vivo plant leaves and tubers from Satna, Rewa and Shidhi germplasm.

II. MATERIAL AND METHODS

Plant collection and authentication The plants of C. borivilianum was collected from different forests, e.g. Rewa (MP), Satna (MP) and Shidhi (MP) and authenticated by botanist. Plants were collect from their natural habitat during last week of September to first week of October months. The leaves and tubers of C. borivilianum was washed thoroughly under running tap water dried on paper towel then aerial parts of it blender, it was extracted in petroleum ether and methanol by macerating at room temperature (30 °C) for 72 hours respectively. The product was filtered through the filter paper. The percentage yields of extracts from leaves and Tubers were estimated (10.5 % w/v and 12.3% respectively).

Preliminary phytochemicals screening Air- dried and powdered plant materials were screened for the presence of different Phytochemicals such as alkaloids, glycosides, saponins, steroids, tannins, etc using the methods described below (PÃ¢ques, Bercetche et al. 1992, Chu, Knight et al. 1993, Dwivedi, Patel et al. 2017). All experiments were performed in triplicate and compared with control sample from Jayanti Kunj as described in previous study (Guru et al 2022).

Test for Flavonoids:

NaOH test: A fraction of the methanolic extract was taken and treated with NaOH drop wise and observed for an intense yellow color which disappeared after adding Dilute HCl Indicate the presence of Flavonoids.

Shinoda test (HCl test): Few fragments of Mg ribbon and drop wise concentrated HCl were added to 1 ml plant methanolic extract, which gives pink reddish/ brownish pink color in few minutes indicate Flavonoids presence.

Test for Proteins:

Biuret test: 1% of NaOH was added to 1 ml of extract and few drops of 1% $CuSO_4$ were then added. Blue/ purple or violet/ pinkish color indicates the presence of proteins.

Millon's test: 1 ml of test extract was mixed with H_2SO_4 then Millon's reagent was added drop wise. White/ yellow precipitate appears which turns into red color precipitate, after heating the mixture. This indicates the presence of proteins.

SDS PAGE and Protein Concentration estimation from extract

Soluble proteins were extracted from the fresh leaves by grinding in a mortar with a pestle with Na-phosphate buffer 0.5 M (pH 7.0) and determined with BCA Protein Assay (Pierce™), using BSA as standard. 50 mg dried extract powder of both leaves and tubers wash resuspended in 200 µl of PBS and separately. Then 20 µl of resuspended samples were mixed with 20 µl of 2X SDS loading buffer and heat the sample at 95^0C for 10 min. Each Sample was centrifuged at13000 rpm for 5 min. Then 20 µl samples were loaded on SDS PAGE gel. After electrophoresis the gel was stained with coomassie stain and destained with destaining buffer.

Protein concentration estimation of extracts from both tuber and leaves were performed using Pierce™ BCA Protein Assay Kit as described manually in the kit protocol.

Test for Rasins: 1ml of methanolic extract was dissolved in acetone and then 1 ml of distilled water is added. Turbidity indicates the presence of rasins.

Test for Tannins: To 1 ml of the extract, 2ml of 5% $FeCl_3$ is added which gives dark blue or greenish black color indicates a positive tannin test.

Salvoski test for Steroids: 1 ml of test sample was dissolved in 1 ml of chloroform and equal amount of concentrated H_2SO_4. Formation of Bluish red to cherry color in chloroform layer shows the presence of steroids.

Foam test for Saponins: A small amount of extract was shaken with water and observed for the presence of foam indicates saponins. Sodium Bicarbonate test: Few drops of Sodium bicarbonate were added to 1 ml of plant extract. If honey comb like structure forms, it confirms saponins.

Test for Anthocyanins and Betacyanins: 1 ml of plant extract was treated with 1 ml of 2N NaOH then heated. Formation of bluish-green color indicated the presence of Anthocyanin while yellow color indicated the presence of betacyanin.

Starch-Iodine test: 1 ml of I_2 (Iodine) and KI (Potassium Iodide) solution is mixed in 1ml of extract, formation of deep blue color indicated the presence of starch in the extract.

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Test for Terpenoids: To 5 ml of plant extract, 2ml of chloroform and 3ml of concentrated H_2SO_4 was added to form a layer. A reddish brown interface, confirmed the presence of terpenoids.

Test for Glycosides: To 1 ml of plant extract, 1 ml Ferric Chloride $FeCl_3$ (5%), and equal amount of acetic acid is added, then few drops of concentrated H_2SO_4 is added to the mixture. Greenish blue color indicates the presence of glycosides.

Test for phenols: 1ml of plant extract, when treated with few drops of $FeCl_3$ solution; it gives blue, green, red or purple, color and confirms the presence of phenols.

Test for Phlobatannins: 1ml of plant extract was treated with 1 ml of 1% HCl and heated. Red color precipitate indicates the presence of Phlobatannins in the test sample.

Determination of Total Polyphenolic content

Total polyphenolic content of plant leaves and tubers extract was measured by using Folin-Ciocalteu reagent (Dwivedi, Tiwari et al. 2018). The 25 µl of plant extract diluted with 125 µl water followed by addition of 150 µl of Folin-Ciocalteu reagent (1N) & 25 µl of Na_2CO_3 (20%w/v) and incubated at 45^0C for 60 min then absorbance was measured in UV-Vis spectrophotometer at 765nm. Quantification was performed with respect to the standard curve of Catechol (Y= 0.006x+0.0966; R^2 =0.975). The phenolic content was expressed as microgram of Catechol equivalent per ml of extract (Dwivedi, Tiwari et al. 2018).. All determinations were carried out in triplicate.

III. RESULT AND DISCUSSION

Comparison of C. Borivilianum plants morphology from different Region

Maximum number of shoots were reported from Churhat forest of Shidhi District whereas Chitrakoot forest of Satna and Ateraila forest of Rewa District have similar number of shoot per plants.(Figure1). Plants shoots from each region differ in their shape and size. The shoot width is more in Chitrakoot plant followed by Ateraila and Churhat Plants respectively whereas shoot length is more in Churhat plant and followed by Ateraila and Chitrakoot plants respectively. It was also observed the number of tubers per plant also differ in different localities. Churhat plants have maximum no. of tubers in comparison to Chitrakoot and Ateraila plants.

Figure 1 - Chlorophytum borivilianum plants collected from different districts of Vindhya region of Madhya Pradesh in their Natural habitats; A (i) Plant after collection from Ateraila forest of Rewa district and (ii) Plant in Natural habitat; B (i) Plant after collection from Chitrakoot forest of Satna district and (ii) Plant in Natural habitat; C (i) Plant after collection from Churhat forest of Shidhi district and (ii) Plant in Natural habitat.

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SDS PAGE and Protein Concentration estimation

Protein Profiling of the in vivo plants will be useful in characterizing the biochemical and Molecular aspects of growth and differentiation in both in vivo and in vitro plantlets. SDS PAGE of Chlorophytum borivilianum leaves (Fig.2B) and tubers (Fig. 2A) extract samples showing multiple Bands which indicate that both leaves and tubers of Chlorophytum borivilianum plant are rich in protein content. The estimated protein concentrations through BCA method are listed in Table-1. Our finding indicates that C. borivilianum plants have more protein content in tubers in comparison to leaves. Leaves and tuber extracts from Churhat forest of Shidhi district contains maximum amount of protein content in comparison to Chitrakoot and Ateraila forest.

S. No.	Chlorophytum borivilianum plant	Leaves (Protein mg w/W of dried weight of plant extract)	Tubers (Protein mg w/W of dried weight of plant extract)
1.	Chitrakoot, Satna	1.76 ± 0.002	2.3 4± 0.003
2.	Ateraila, Rewa	1.83 ± 0.003	2.66 ± 0.004
3.	Churhat, Shidhi	2.08 ± 0.008	2.70 ± 0.008
4.	Jayanti Kunj control	1.92 ± 0.003	2.45 ± 0.006

Table-1 Protein concentration estimation through BCA method in Chlorophytum borivilianum plant leaves and tubers from different region.

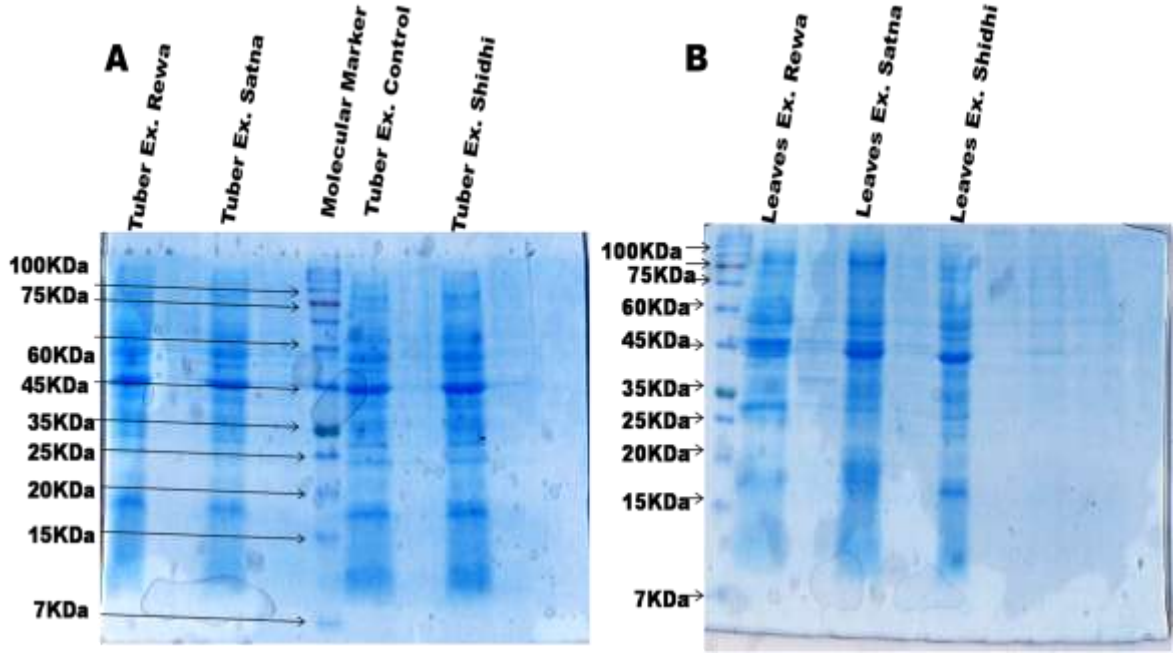

Figure 2- SDS PAGE illustrating proteins of C. Borivilianum [A]. Tuber Extract from different districts on of Vindhya region (control was taken from Jayanti Kunj facility; Ex.- Extract) [B] Leaves Extract from different districts on of Vindhya region

Phytochemicals Screening

Phytochemicals screening in Chlorophytum borivilianum results indicated that all the leaves and tubers are rich in various organic compounds, like terpenoids, phenols, flavonoids, alkaloids, Anthocyanins rasins, saponins, steroids, tannins, starch, glycosides, Phlobatannins, terpenoids as well as carbohydrates.

The plant alkaloids are the large and most diverse class of secondary metabolites, involved in several biosynthesis pathways and possess an array of structure, and pharmacological activities. These are being used as drugs, teas, poisons, etc for 4000 years. Alkaloids are present in water and absent in methanolic extract from Chitrakoot and Ateraila region whereas these compounds are present in

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both water and methanolic extract from Churhat region in both leaves (Table-2) and tubers (Table-3).

Terpenoids or isoprenoids are the largest class of secondary metabolites, derived from the 5-carbon compound isoprene with additional functional groups. Terpenoids are employed for growth and development of plants and for the protection from biotic and abiotic stress. Here we reported that terpenoids were present in the extracts Chlorophytum borivilianum leaves and tubers from each region.

S. No.	Phytochemicals	Chlorophytum borivilianum leaves Control from Jayanti Kunj		Chlorophytum borivilianum leaves from Ateraila (Rewa)		Chlorophytum borivilianum leaves from Chitrakoot (Satna)		Chlorophytum borivilianum leaves from Churhat (Shidhi)	
		Water	Methanol	Water	Methanol	water	Methanol	Water	Methanol
1.	Carbohydrate	-	+	+	+	+	+	+	+
2.	Alkaloid	+	-	+	-	+	-	+	+
3.	Flavonoid	+	++	+	++	+	++	+	+
4.	Protein	+	+	+	+	+	+	+	+
5.	Resin	+	+	+	+	+	+	+	+
6.	Anthocyanin	-	-	-	-	-	-	-	-
7.	Saponins	+	++	+	++	+	++	+	+
8.	Steroid	-	-	+	-	+	-	-	-
9.	Tannin	-	-	-	-	-	-	-	-
10.	Starch	-	-	-	-	-	-	-	-
11.	Glycoside	+	+	+	+	+	++	+	++
12.	Phlobatannins	+	+	+	+	+	+	-	+

Table- 2 Comparison of qualitative phytochemicals analysis of C. borivilianum plant leaves extracts collected from Ateraila forest of Rewa district, Chitrakoot forest of Satna district and Churhat forest of Shidhi district of Vindhya region of Madhya Pradesh.

Steroids are the essential for normal growth and development, However, some adverse effects are also associated with their prolong use such as osteoporosis, immunosuppression, hypertension and metabolic disturbance. Here we found that steroids are present in water extract of both leaves and tubers from Chitrakoot and Ateraila region whereas absent in methanolic extract. Steroids are absent were absent in both methanolic and water extracts of Chlorophytum borivilianum from Churhat, Shidhi region.

S. No.	Phytochemicals	*Chlorophytum borivilianum* Tubers Control from Jayanti Kunj		*Chlorophytum borivilianum* Tubers from Ateraila (Rewa)		*Chlorophytum borivilianum* Tubers from Chitrakoot (Satna)		*Chlorophytum borivilianum* tubers from Churhat (Shidhi)	
		Water	Methanol	Water	Methanol	water	Methanol	Water	Methanol
1.	Carbohydrate	-	+	+	+	+	+	+	+
2.	Alkaloid	+	-	+	-	+	-	+	+
3.	Flavonoid	+	++	+	++	+	++	+	+
4.	Protein	+	+	+	+	+	+	+	+
5.	Resin	+	+	+	+	+	+	+	+
6.	Anthocyanin	-	-	-	-	-	-	-	-
7.	Saponins	+	++	+	++	+	++	+	+
8.	Steroid	-	-	+	-	+	-	-	-
9.	Tannin	-	-	-	-	-	-	-	-
10.	Starch	-	-	-	-	-	-	+	-
11.	Glycoside	+	+	+	+	+	+	+	+
12.	Phlobatannins	+	+	+	+	+	+	-	+

Table- 3 Comparison of qualitative phytochemicals analysis of C. borivilianum plant tubers extracts collected from Ateraila forest of Rewa district, Chitrakoot forest of Satna district and Churhat forest of Shidhi district of Vindhya region of Madhya Pradesh.

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Saponins are naturally occurring high molecular weight and amphiphilic compounds in which a sugar molecule is combined with triterpene or steroid aglycon. There are two major groups of saponins; triterpene saponins and steroid saponins. These chemicals protect healthy plants from insect, fungal and bacterial pathogen. These are therapeutically important because they have antiseptic and antioxidant activity. Here, we observed that saponins are present throughout the plant body (Leaves and tubers) in both aqueous and methanolic extract from each region. Here we also observed that quantity of saponins are more in methanolic extract in comparison to aqueous extract from Chitrakoot and Ateraila region whereas, it is equal in both extracts from Churhat region.

Tannins are polyhydroxy phenols of high molecular weight soluble in water and alcohol and acetone and can coagulate proteins. Tannins based formulations are used to treat diarrhea, leucorrhoea and rhinnorhoea. Here, we found that Tannins were absent in Chlorophytum borivilianum leaves and tubers from each region.

Anthocyanins or anthocyans are water soluble pigments that, depending on their pH, give blue, purple, red, or black colors. These are the members of the flavonoid class synthesized via polypropanoid pathway and are the most recognized visible members of bioflavonoid phytochemicals. Anthocyanins have shown antioxidant effect so they can protect from DNA damage. Several studies have reported that Anthocyanins can also boost the immune system and help fight from heart disease, inflammation, viral infections, etc. Here, Anthocyanins were absent in Chlorophytum borivilianum leaves and tubers from each region.

Glycosides are Na+/K+ pump inhibitors used to treat congestive heart failure and cardiac arrhythmia (Lohar and Singh 2008, Tiwari, Tripathi et al. 2014, Dwivedi, Tiwari et al. 2018). Here we reported that glycosides were present in the extracts of Chlorophytum borivilianum leaves and tubers from each region.

Resins were, present in both methanolic and water extract from each region. Starch were absent in Chlorophytum borivilianum in both extract of leaves and tubers from Chitrakoot and Ateraila whereas it is present in small quantity in water extract of tubers from Churhat region. Phlobatannins were present in, Chlorophytum borivilianum in Chitrakoot and Ateraila region whereas it is absent from Churhat region in water extract of both leaves and tubers.

Quantitative Analysis of Total Polyphenolic Contents

Polyphenolic compounds are commonly found in both edible and inedible plants, and reported for multiple biological effects, including antioxidant activity. The antioxidant activity of phenolic compounds is due to their redox properties (Rice-Evans, Miller et al. 1996, Shahidi 1997, Ramalakshmi, Kubra et al. 2008). It has been reported that phenolic compounds show antioxidant activity and play an important role in stabilizing lipid peroxidation (Dwivedi, Tiwari et al. 2018) and inhibiting various types of oxidizing enzymes(Laughton, Evans et al. 1991, Cos, Ying et al. 1998). Total polyphenolic content of selected part of the plant were shown in table-4.

S. No.	Chlorophytum borivilianum plant	Leaves (TPC in µg/ml equivalent to Catechol)	Tubers (TPC in µg/ml equivalent to Catechol)
1.	Chitrakoot, Satna	4.30 ± 0.002	5.36 ± 0.007
2.	Ateraila, Rewa	4.0 ± 0.003	6.05 ± 0.004
3.	Churhat, Shidhi	3.80 ± 0.008	5.80 ± 0.008
4.	Jayanti Kunj control	4.50 ± 0.003	5.50 ± 0.003

Table- 4 Concentration of polyphenolic contents in leaves and tubers of Chlorophytum borivilianum plants from different region.

IV. CONCLUSION

In conclusion, the current study reveals that Chlorophytum borivilianum collected from different localities varies in shape and size of their leaves and tubers. They also differ in the number of leaves and tubers from region to region. Chlorophytum borivilianum plants are rich in protein content and thus offer a healthy food diet. Phytochemicals analysis of plants from different regions reveals that Chlorophytum borivilianum leaves and tubers are rich in the active compo, terpenoids, phenols, flavonoids, alkaloids, Anthocyanins resins, saponins, steroids, tannins, glycosides, terpenoids, carbohydrates, etc. Many of

these are of medicinal importance. These organic compounds are reported to have multiple biological effects, including antioxidant, anti-fungal, anti-viral, and anti-bacterial anti-aging activities. The antioxidant activity of these compounds prevents DNA damage from free radicals. Further experiments need to be performed to evaluate the relationship between total phenolic and flavonoid content and antioxidant activity using DPPH assay and cellular oxidant assay respectively.

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