"Analytical Comparison Between Wild & Cultivated Variety of *Shankhpushpi* (*Convolvulus Pluricaulis* Choisy.)."

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ABSTRACT

Shankhpushpi (*Convolvulus pluricaulis* Choisy.) is a very important herb in Ayurveda for its various uses in mental health promoting properties. It is the mainly *Medhya* out of four *Medhya Rasayana* according to *Acharya Charka* where it is used as freshly prepared paste. But *Shankhpushpi* available in market is either in powder form or in syrup form which is not freshly prepared. To solve this problem, we must shift our raw drug collection from wild region to agriculture farming. By cultivating the plant everyone can use the herb in fresh form. But a pertinent question arises with cultivation regarding the medicinal quality owing to the changed conditions in cultivation therefore before using the cultivated *Shankhpushpi*, we have to compare the two samples (wild VS cultivated) phytochemically as well as physicochemically. The present research work was planned to compare the physicochemically and phytochemical values of cultivated and collected from wild *Shankhpushpi* (1) Group A- wild grown *Shankhpushpi*, (2) Group B - Cultivated in net house by using farm yard manure, (3) Group C - Cultivated in net house by using inorganic fertilizer and (4) Group D - Cultivated in open are through kitchen garden technique. The study findings suggest- wild Group A of *Shankhpushpi* better in terms of phytochemical parameters and phytochemical parameters.

Keywords: Wild, Cultivated, Organic, inorganic, *Shankhpushpi*, Phytochemical parameters

INTRODUCTION

Medhya Rasayana drugs increase the *Medha* (retention power) and may help a person to increase his performance in modern competitive world. *Acharya Charaka* has mentioned 4 *Medhya Rasyanas* for mental health and memory boosting of a person in *Chikitsa Sthana*(1). The word *Medha* means, intellect or retention power of mind and word *Rasayana* means, a drug or a preparation that on regular use will boost nourishment, health, memory, intellect, immunity, glowing face (*Parbha*) etc. and hence gives a healthy and long lifespan. *Medhya*

Rasayana drugs are good when used as a brain tonic and may also be effective in inhibition of mental illnesses (disorders). These drugs promote the power of grasping, power of retention and recalling capacity (*Dhi, Dhriti, Smriti*) which is beneficial for prevention and cure of mental disorders and can be used as a brain tonic also.

Acharya Charaka has mentioned specially *Shankhpushpi* (*Convolvulus pluricaulis* Choisy.) as *Medhya Rasayana*, and *Kalka* (freshly prepared paste) of *Shankhpushpi* should be used. The main problem is that *Kalka* is made from fresh *Shankhpushpi* plant. But in the market, *Shankhpushpi* is present in form of dry powder mainly or in the form of juices or syrups which is also not freshly prepared.

To solve this problem, to protect the biodiversity of forest region and to minimize the pressure on wild resources of *Shankhpushpi*, we must shift our raw drug collection from wild region to agriculture farming. By cultivating the plant everyone can use the herb in fresh form (*Kalka*) and pharma companies will not be dependent on forest/wild collection. But before using the cultivated *Shankhpushpi*, we have to compare the two samples (wild VS cultivated) phytochemically as well as physicochemically because there are many environmental factors which are responsible for the production of secondary metabolites of a plant such as temperature, rainfall, light hours exposure etc. In Ayurveda, *Acharya Sushruta* emphasized the concept of *Bhumidesa* (habitat), with highlighting the significance of *Bhumipariksha* (examination of the land) in the context of collecting plant products, as in 37th chapter of *Sutra Sathana*[(2)]. Additionally, *Acharya Charaka's* perspective on the impact of ecological conditions on the properties of plants suggests that plants from the *Himalayas* are of superior quality compared to those from the *Vindhyas* [(3)]. There are also some references mentioned in *Nighantus* which shows different properties (*Guna*) of *Vanya* and *Gramya* varieties of plants.The purpose of this study is to compare the physicochemical and phytochemical constituents of wild & cultivated samples of *Shankhpushpi* and find out whether these samples of *Shankhpushpi* are chemically equivalent or not.

There are many smart farming techniques available now a days in this study we are using net house cultivation technique and kitchen garden technique for cultivation of *Shankhpushpi* in controlled environment and analysis of these varieties is done in research lab result is compared with wild grown *Shankhpushpi.*

NEED OF STUDY

As stated above *Shankhapushpi Kalka* (freshly prepared paste) is specially mentioned by *Acharya Charka* as *Medhya Rasayana*. If someone wants to use *Shankhpushpi* in form of *Kalka,* then it should be fresh as told in *Samhitas*, so it should be grown in nearby place such as kitchen garden or by farmers for those who cannot cultivate for themself and this cultivated *Shankhapushpi* should be chemically similar or equivalent as wild grown *Shankhapushpi* so that medicinal properties of herb remain intact.

Since *Shankhapushpi* is sourced from agriculture, there is a logical possibility of encountering variations in secondary metabolites of herb. Secondly, the purported therapeutic benefits of *Shankhapushpi* as described in *Samhitas* pertain to the *Shankhapushpi* collected from wild. Therefore, it is essential to study the variations between wild and cultivated *Shankhapushpi* to access which source of *Shankhapushpi* is phytochemically the most potent one

To fulfil the above needs, a study of Analytical comparison between wild variety & cultivated variety of *Shankhpushpi* is done here.

AIMS AND OBJECTIVE

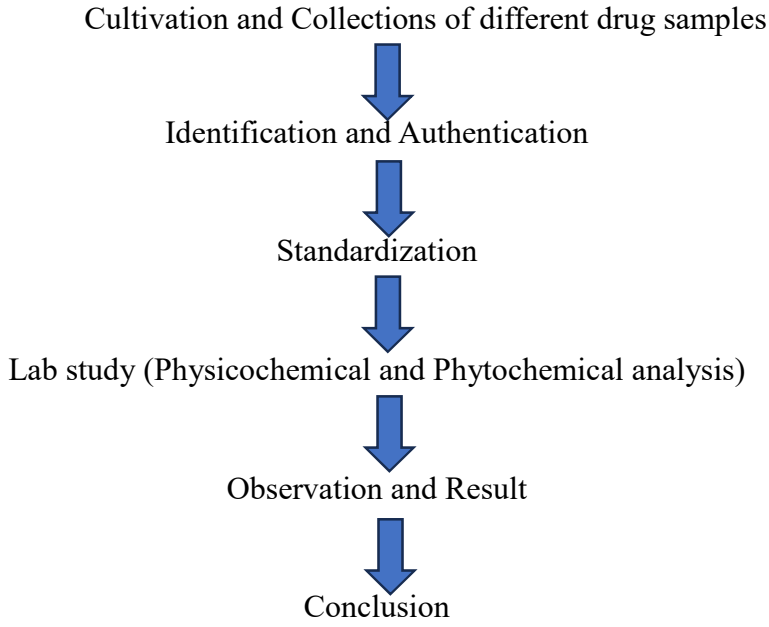
- **AIM:**

To compare physicochemical and phytochemical evaluation of wild and cultivated samples of *Shankhpushpi* (*Convolvulus pluricaulis* Choisy.).

- **OBJECTIVE:**

1. Collection of wild and cultivated *Shankhpushpi*.
2. Analysis of phytochemicals.
3. Comparison of physicochemical and phytochemicals by using available protocols.
4. To find out best potent variety.

STUDY DESIGN



MATERIALS AND METHODS

The present investigation was carried out at the herbal garden of Dravyaguna Vigyan department, Institute for Ayurved Studies and Research, Kurukshetra (Haryana), India during 2023 - 2024. The experiment consisting of four groups. Group A- wild grown *Shankhpushpi*. Group B- Cultivated in net house with farmyard manure (organically) @ 1kg/m^2. Group C- Cultivated in net house with inorganic fertilizer (urea) @ 10gm/m^2. Group D- Cultivated in open ground through kitchen garden technique (organically) @ 1kg/m^2. Seeds of *Shankhpushpi* chosen for cultivation for Group B, Group C and Group D were self-collected from herbal garden of Institute for Ayurved Studies & Research (IASR) and they were free from pests, diseases and foreign matter. Cultivation begins with field preparation, where the soil is carefully prepared to achieve optimal tilth, creating a conducive environment for seed germination and seedling growth. In the greenhouse of the institute, for the concerned study the land is prepared by deep ploughing in June month and left the prepared field for one month. During this time, ploughing is repeated for two times to control weed growth in the prepared field. The land was prepared for cultivating Group B, Group C and Group D of the drug, both in controlled environments and open areas. Additionally, three flower beds of each 70 square feet area were arranged, two were placed under the greenhouse netting for Group B and Group C, while one remained exposed in the open area for Group D. Quantity of inorganic fertilizers and organic manure to be applied to *Shankhpushpi* was calculated as per flower bed. Fertilizers

(Farm yard manure, urea and kitchen garden manure) were applied and well mixed with the soil of respective plots after the sowing of the seeds in 2nd week of September and February i.e. two times after sowing. Seeds were sown on1 July 2023 and 60-70 plants were maintained in each flower bed. The fully matured crop (along with roots and flowers) of each flower bed was harvested in April month of 2024 (between 19 to 23 April 2024). Group A i.e. wild *Shankhpushpi* was collected from open spaces near the Institute to compare with cultivated varieties on 24th April 2024. This approach was taken to ensure that geographical variations in secondary metabolites did not influence the study's outcomes.

Drug Identification and Standardization

The identification and authentication of the all four Groups of *Shankhpushpi* i.e. Group A, B, C and D is carried out in the Pharmacognosy Department of NIPER (National Institute of Pharmaceutical Education and Research); Mohali, Punjab having Reference no: NIP-H-3375. For this, 50 gm of sample of *Shankhpushpi* along with herbarium sheet and an application of the same is sent to NIPER Chandigarh. Further standardization of all four groups of *Shankhpushpi* i.e. Group A, B, C and D were analysed by the State Drug Testing Laboratory in Kurukshetra with COA NO. - GDTL/RM/260624/012, 013, 014 and 015for Group A, B, C and D respectively and were found to meet standard quality criteria.

Lab study (Physicochemical and Phytochemical analysis)

Further Phytochemical, physicochemical analyses and thin layer chromatography of both the cultivated and wild *Shankhpushpi* are conducted in the Research and Innovation Lab of Shri Krishna AYUSH University with sample registration number- SKAU/R&I/2024/829. Observations of all above studies are enlisted in Observations and Results section of the article.

OBSERVATION AND RESULT

Table No. 1 - Shows Progressive Report of all four groups *Shankhpushpi* Along with observations and results

Sr. no.					
1	**Identification of *Shankhpushpi* plant**				
	Title	**Group B (organically cultivated in net house)**	**Group C (inorganically cultivated in net house)**	**Group D (organically cultivated in open through kitchen garden technique)**	**Group A (wild grown)**
1.1	Scientific name	*Convolvulus pluricaulis* Choisy.	*Convolvulus pluricaulis* Choisy.	*Convolvulus pluricaulis* Choisy.	*Convolvulus pluricaulis* Choisy.
1.2	Local name (language for)	*Shankhpushpi*	*Shankhpushpi*	*Shankhpushpi*	*Shankhpushpi*
1.3	Botanical name	*Convolvulus pluricaulis* Choisy.	*Convolvulus pluricaulis* Choisy.	*Convolvulus pluricaulis* Choisy.	*Convolvulus pluricaulis* Choisy.
1.4	Plant part for medicinal use & harvested	Whole plant	Whole plant	Whole plant	Whole plant
1.5	Cultivation site/ collection site	Greenhouse, Herbal Garden, IASR	Greenhouse, Herbal Garden, IASR	Open area Herbal Garden, IASR	Free open space nearby college campus, IASR, Kurukshetra.
2	**Method of propagation: direct seed sowing.**				
2.1	Month of sowing	1 July 2023	1 July 2023	1 July 2023	-
2.2	Area of cultivation	70 square feet	70 square feet	70 square feet	-
2.3	Number of plants	60 - 65	62 - 65	60 - 64	65 - 68
3	**Fertilizers and chemicals applied after planting organic / inorganic**				

3.1	Name	Farmyard manure	Inorganic manure (urea)	Kitchen Garden manure	Not any fertilizer is used
3.2	Quantity	1kg/m^2	10gm/m^2	1kg/m^2	Not any
3.3	Method	Spraying and mixing	Spraying and mixing	Spraying and mixing	Not any
4	**Water Irrigation of cultivated groups**				
4.1	Summer season (July to mid-October)	Initially at interval of 5-7 days and later at interval of 10-15 days	Initially at interval of 5-7 days and later at interval of 10-15 days	Initially at interval of 5-7 days and later at interval of 10-15 days	Natural water through rain
4.2	Winter season (mid of October to mid of March)	At regular interval of 10- 17 days	At regular interval of 10- 17 days	At regular interval of 10- 17 days	Natural water through rain
4.3	Method of irrigation	Tub well water through spraying	Tub well water through spraying	Tub well water through spraying	Natural water through rain
5	**Growth in *Shankhpushpi***				
5.1	During rainy season	Early flowering as compared to wild variety	Early flowering as compared to B and D group	Early flowering as compared to wild variety	Late flowering as compared to cultivated varieties
5.2	During winter season	Plants tend to dry out significantly during the winter season.	Plants tend to dry out significantly during the winter season.	Plants tend to dry out significantly during the winter season.	Plants tend to dry out significantly during the winter season.
5.3	During spring season	Plants regain their lush green color in spring	Plants regain their lush green color in spring	Plants regain their lush green color in spring	Plants regain their lush green color in spring
6	**Harvest / Collection of *Shankhpushpi***				
6.1	Date of harvest	19th April 2024	23rd April 2024	20 April 2024	24 April 2024

6.2	Timing of harvest	Morning hours	Morning hours	Morning hours	Morning hours
6.3	Conditions	Clear and dry weather	Clear and dry weather	Clear and dry weather	Clear and dry weather
6.4	Method	Manual collection	Manual collection	Manual collection	Manual collection
6.5	Yield of wet drug	505 gm	601 gm	525 gm	554 gm
7	**Records of drying of *Shankhpushpi***				
7.1	Drying method	Shade drying	Shade drying	Shade drying	Shade drying
7.2	Duration of drying (days)	One month	One month	One month	One month
7.3	Initial weight of sample	505 gm	601 gm	525 gm	554 gm
7.4	Final weight after drying	258 gm	295 gm	274 gm	300 gm
7.5	Percentage of weight loss due to Moisture content (after drying) (%)	48.9%	50.9%	47.8%	45.8%

Figure No. 1: Shows pictures of macroscopic characters of *Shankhpushpi*.

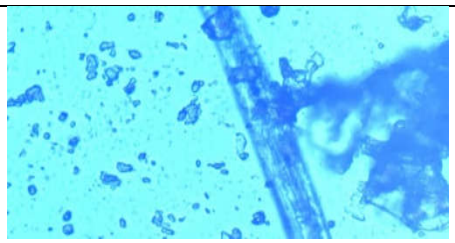
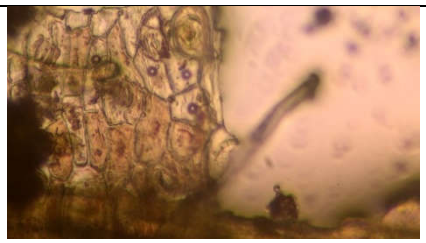
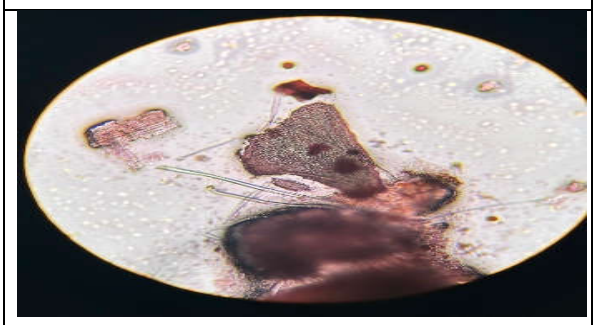
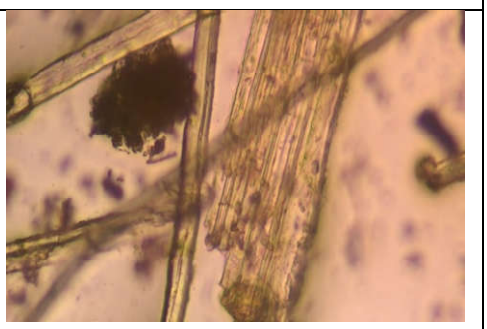
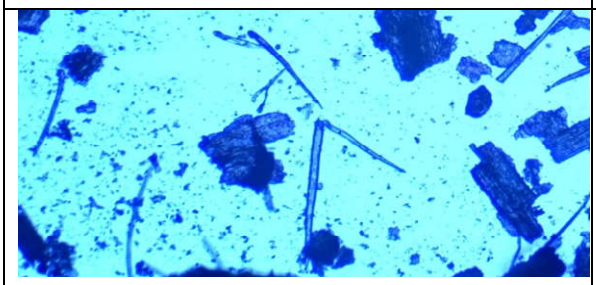
	
Stem and flower	Fruit and seed
	
Root	Flower

Table No. 2 - Shows Macroscopic Study of whole plant of *Shankhpushpi*.

S. No.	Macroscopic study	Group A	Group B	Group C	Group D
1.	Colour	Light green	Light green	Light green	Light green
2.	Odour	Odour less	Odour less	Odour less	Odour less
3.	Taste	Bitter	Bitter	Bitter	Bitter
4.	Texture	Hairy	Hairy	Hairy	Hairy

- **Microscopic Study (Powder Microscopy)**

Figure No. 2 - Show the result of Powder Microscopy

	
Deep blue colour of mucilage (Methylene blue)	**Parenchymatous cell**
	
Cellulose (Iodine solution)	**Bundle of xylem**
	
Crystals	**Fibre and parenchymatous cells**

	
Trichomes	**Red colour of cell nuclei (Safranin)**

Table No. 3 - Shows observations of quantitative examination of physicochemicals of *Shankhpushpi*

Test name	Group A	Group B	Group C	Group D	API Standards
Moisture Content	5.90%	5.81%	5.38%	5.62%	N.M.T. 12%
Alcohol Soluble Extractive Determination	7.54%	6.10%	6.0%	6.44%	N.L.T. 6.0%
Water Soluble Extractive Determination	12.66%	11.84%	10.95%	11.23%	N.L.T.10%
Calculation of total ash	4.62%	6.62%	8.82%	6.01%	N.M.T. 17%
Acid insoluble ash determination	0.51%	0.84%	1.03%	0.89%	N.M.T. 8%

- **Phytochemical Analysis of *Shankhpushpi* Drug**

1. Carbohydrate test

Table No. 4 - Shows Result of Carbohydrate Test

S. No.	Sample of *Shankhpushpi* (Alcohol extract)	Molisch test	Benedict test	Barfoed's test	Fehling test
1.	Group A	-ve	+ve	-ve	+ve
2.	Group B	-ve	+ve	-ve	+ve
3.	Group C	-ve	+ve	-ve	+ve
4.	Group D	-ve	+ve	-ve	+ve

2. Alkaloid test

Table No. 5 - Shows Result of Alkaloid Test

S. No.	Sample of *Shankhpushpi* (Alcohol extract)	Dragondrof test	Mayer's test	Wagner's test	Hager's test
1.	Group A	+ve	-ve	-ve	+ve
2.	Group B	+ve	-ve	-ve	+ve
3.	Group C	+ve	-ve	-ve	+ve
4.	Group D	+ve	-ve	-ve	+ve

3. Amimo Acids

Table No. 6 - Show Result of Amino Acids Test

S. No.	Test name	Group A (Alcohol extract)	Group B (Alcohol extract)	Group C (Alcohol extract)	Group D (Alcohol extract)
1.	Ninhydrine test	+ve	+ve	+ve	+ve

4. Saponin test

Table No. 7 - Shows Result of Saponin Test

S. No.	Test name	Group A (powder)	Group B (powder)	Group C (powder)	Group D (powder)
1.	Foam test	+ve	+ve	+ve	+ve

5. Glycosides

Table No. 8 - Shows Result of Glycosides Test

S. No.	Test name	Group A (Alcohol extract)	Group B (Alcohol extract)	Group C (Alcohol extract)	Group D (Alcohol extract)
1.	Borntragor's test	-ve	-ve	-ve	-ve

6. Steroids

Table No. 9 - Show the Result of Steroids Test

S. No.	Test name	Group A (Alcohol extract)	Group B (Alcohol extract)	Group C (Alcohol extract)	Group D (Alcohol extract)
1.	Salkowaski reaction	-ve	-ve	-ve	-ve

➢ **Chromatography**

Figure No. 3: Shows TLC of all four groups of *Shankhpushpi* under different wavelength of light.

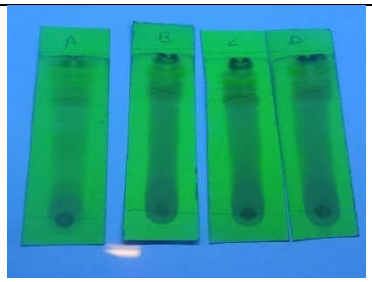
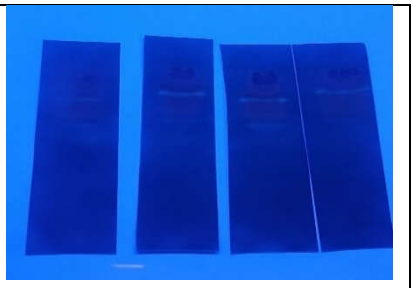
		
Visible light	**U.V.A 254**	**U.V.C 365**

Figure No. 4: Shows TLC of different groups of *Shankhpushpi*.

Sample	Distance of Solvent	Distance of Spot	R_f Value	Image
Group A	**5.8cm**	**3.9, 4, 4.2, 4.4, 4.6 and 5.2cm**	**0.67, 0.68, 0.72, 0.75, 0.79 and 0.89.**	

Group B	**5.8cm**	**3.9, 4, 4.2, 4.4, 4.6 and 5.2cm**	**0.67, 0.68, 0.72, 0.75, 0.79 and 0.89.**	
Group C	**5.8cm**	**3.9, 4, 4.2, 4.4, 4.6 and 5.2cm**	**0.67, 0.68, 0.72, 0.75, 0.79 and 0.89.**	
Group D	**5.8cm**	**3.9, 4, 4.2, 4.4, 4.6 and 5.2cm**	**0.67, 0.68, 0.72, 0.75, 0.79 and 0.89.**	

Table No. 10 - Shows total Alkaloid Content in four Sample of *Shankhpushpi*.

Test name	Group A	Group B	Group C	Group D
Quantitative estimation of alkaloids	**16.18%**	**8.36%**	**7.62%**	**8.28%**

DISCUSSION

When comparison drawn between cultivated and wild sample of *Shankhpushpi* for yield parameter revealed that the samples grown inorganically (Group C) is yielded high as compared to other cultivated groups (Group B and D). So, it was concluded that inorganic cultivation increases the final yield of plants as compared to organic cultivation. The data presented in Table 1, also reflect that the percentage of weight loss due to moisture content after drying was highest in Group C (inorganically cultivated) at 50.9%, while Group A (wild variety) exhibited the lowest weight loss at 45.8% which shows wild variety grown in extreme conditions has low moisture content than cultivated variety. The average total Ash value of the wild sample was lowest in Group A (wild) i.e. 4.62% and highest in Group C (inorganically cultivated) i.e. 8.82%. the acid insoluble ash of Group C was also highest among all the groups. The extractive values show the fact that all four samples contained more aqueous soluble substances as compared to alcohol soluble extractives. The Group A possessed highest extractability while Group C has lowest. Table 10 reveals that the total alkaloid content was found to be the highest in Group A (Wild variety) that is 16.18%, while Group C (inorganically cultivated) has the lowest that is 7.62%. Group B (inorganically cultivated in net house) and Group D (organically cultivated in open area) contained 8.36% and 8.28%.

The T.LC analysis represented in Figure 4 revealed that total six no, of spots were observed in the Alcoholic Extract in Chloroform: Methanol: Toluene (7:2:1) as mobile solution, Visualization done under U.V.A(365), U.V.C(254), Visible Light. All four Group showed same R_f, value i.e. 0.67, 0.68, 0.72, 0.75, 0.79 and 0.89. with different intensity in all chromatogram, which shows quantitative phytochemical variations between the samples. As per Ayurvedic Pharmacopoeia of India (API) main chemical constituents of *Shankhpushpi* is alkaloid so total quantitative estimation of alkaloids was done for all four Groups of *Shankhpushpi*. The result shown in table no. 10, clarify that total alkaloid content was highest in Group A (Wild variety) that is 16.18%, while Group C (inorganically cultivated) has the lowest that is 7.62%. Group B (inorganically cultivated in net house) and Group D (organically cultivated in open area) contained 8.36% and 8.28%, respectively. Thus, logically it was proved that phytochemically the wild *Shankhpushpi* enjoyed qualitative and quantitative (low moisture content, low total ash content, high extractive values, high alkaloid %) superiority over cultivated samples of *Shankhpushpi*.

CONCLUSION

By observing all the findings and their critical analysis the following conclusion was drawn the cultivated variety of *Shankhpushpi* (*Convolvulus pluricaulis* Choisy.) is not much potent or equivalent to the wild variety. The total alkaloid content was found to be the highest in Group A (Wild variety) that is 16.18%, while Group C (inorganically cultivated) has the lowest that is 7.62%. Group B (inorganically cultivated in net house) and Group D (organically cultivated in open area) contained 8.36% and 8.28%, respectively. The data indicates that the cultivated varieties did not match the wild variety quantitatively; specifically, the cultivated varieties exhibited nearly 50% less alkaloid content compared to the wild *Shankhpushpi*. As the cultivated *Shankhpushpi* also meets the Standards mentioned in API, so it can also be used as *Medhya Rasayana* clinically but may be used for longer period of time to get desired results.

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