Research Article

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PHYTO-PHARMACOGNOSTICAL STUDY OF *ASTHI SHRINKHALA* (*CISSUS QUADRANGULARIS* LINN.)

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ABSTRACT

Cissus quadrangularis L. (*Asthishrinkhala*) belongs to Vitaceae family is an indigenous medicinal plant of India. It is a succulent plant found in warm tropical areas. It has been prescribed in ancient *Ayurvedic* texts by *Bhavaprakash* and *Chakradutta* as a general tonic especially for the fractured patient and also having high therapeutic potential against several diseases. In present study, all aspects about *Asthishrinkhala* have been taken like name, geographical source, Macroscopic, Microscopic, Physicochemical, Phytochemical (Qualitative and Quantitative) and Chromatographic activities. The macroscopic features of the *Cissus quadrangularis* L. stem were observed under magnifying lens. The powder microscopy showed the presence of starch grain, calcium oxalate, and fragments of vessels, parenchymatous cells and fibers. The physicochemical properties such as foreign matter, moisture content, pH value, aqueous soluble extract, alcohol soluble extract, petroleum ether soluble extract, total ash, acid insoluble ash, water soluble ash values of stem were carried out. In phytochemical study stem of *Asthishrinkhala* showed the presence of many important classes of phytoconstituents like carbohydrates, alkaloids, amino acid, protein, saponin, glycosides, phenolic compound, steroids and tannins. Estimation of carotene, total flavonoid content, steroidal content, Vit C, Vit D, calcium and saponins analysis were carried out for quantitative analysis.

Keywords: *Cissus quadrangularis* Linn., Phytochemical, *Asthishrinkhala*, Physicochemical.

INTRODUCTION

Cissus quadrangularis L. (*Asthishrinkhala*) is the most common species, belonging to the family Vitaceae, commonly known as “*Hadjod*” in Hindi. It is an ancient medicinal plant native to the hotter parts of India and Ceylon. It is said to be also present in some parts of Srilanka, Malaya, Java and West Africa¹. *Cissus quadrangularis* L. distributed throughout India, particularly in the hotter parts; also cultivated in gardens, generally propagated by cuttings. The stem is bitter and it is given internally and applied topically for broken bones, used in complaints of the back and spine. Leaves and young shoots are powerful alternative for bowel affections. Juice of stem is useful in Irregular menstruation². In Southern India, the stem of the plant is eaten as a pickle so as to strengthen the bones and for regaining the damage of epithelial cells in case of any injury³. The juice of plant is said to be beneficial in Scurvy and ash of the plant is used as a substitute for baking powder⁴.

Cissus quadrangularis stem resembles with the shape of bones and joints in the body and really it is very effective in strengthening the bones and joints related disease. *Asthishrinkhala* is not described in *Brihatrayi* of *Ayurveda*, viz: *Charaksamhita*, *Sushrutsamhita* and *Ashtang* but it is mentioned in the *Nighantus*. In *Bhavaprakash Samhita* (16th Cent. A.D.) *Asthishrinkhala* has mentioned in Lakshadhya guggul for Bhagnarog⁵. The roots and stem are most useful for healing of fracture of the bones.

Cissus quadrangularis L. plant is used as union-promoting and also useful in vitiated condition of *Vata*. Its root powders as well as stem paste are very specific for bone fractures. The plant is Thermogenic, Digestive, Haemostatic, Aphrodisiac, Ophthalmic and union-promoting and is useful in vitiated condition of *Vata*⁶. The stem prepared in boiling lime water is a useful stomachic.

The total ash obtained from young shoots and leaves is given in Dyspepsia, Indigestion and Bowel complaints⁷.

Authentication and standardization are prerequisite steps especially for herbal drugs and their formulations in traditional systems of medicine. Standardization contributes to the quality of drug, identity of the raw drug, organoleptic evaluation, pharmacognostic evaluation, quantitative evaluation, phytochemical evaluation, toxicity testing etc. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents which may vary depending on various factors, one amongst is the geographical localities which show quantitative variation in their chemical constituents^{8,9}.

Cissus quadrangularis L. (Vitaceae) is a common perennial climber, which is distributed throughout India particularly in tropical regions. It is one of the most frequently used indigenous medicinal plants in India. It is commonly known as Hadjod, Asthisamhara, Harjora, Vedhari, Hadbhanga, Vajravalli in Sanskrit, Kandvel in Marathi, Haddjor in Punjabi, Hadbhanga in Oria, Vedhari in Gujrati, Perandai in Tamil, Nalleru in Telugu and Veldgrap in Indian languages and in English it is called as Edible-stemmed Vine. It is also known as Vitis quadrangularis. *Cissus quadrangularis* L. (Vitaceae) is a common perennial climber, which is distributed throughout India particularly in tropical regions. It is one of the most frequently used indigenous medicinal plants in India. It is commonly known as Hadjod, Asthisamhara, Harjora, Vedhari, Hadbhanga, Vajravalli in Sanskrit, Kandvel in Marathi, Haddjor in Punjabi, Hadbhanga in Oria, Vedhari in Gujrati, Perandai in Tamil, Nalleru in Telugu and Veldgrap in Indian languages and in English it is called as Edible-stemmed Vine. It is also known as Vitis quadrangularis. *Cissus quadrangularis* L. (Vitaceae) is a common perennial climber, which is distributed throughout India particularly in tropical regions. It is one of the most frequently used indigenous

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MATERIAL AND METHODS

The plant material which was taken for the study is Genuine sample of *Asthishrinkhala*.

Collection of genuine sample of *Asthishrinkhala*

The genuine sample was collected after identifying the source of plant as per standard description.

RESULTS

A. Pharmacognostical Study

The fresh stem of *Cissus quadrangularis* L. was collected from Durga district of Chhattisgarh in August 2017. The Climate of Chhattisgarh is tropical, it is hot and humid. It is widely growing alongside hedges. Ethno-medicinal information regarding this plant was collected through local people of that region. These people used stem powder of *Asthishrinkhala* in bone related diseases as well as paste of its stem is applied on the fracture site and painful areas. *Asthishrinkhala* grow widely in this region.

The stem was thoroughly washed by tap water and dried in the shade to preserve the active ingredients of plant. After dried it is stored in airtight container.

From the source as mentioned above, sample of *Asthishrinkhala* was collected and herbarium was made. After that identification of plant sample is done by Botanical Survey of India (BSI), Dehradun.

Authentication of collected genuine plant material -

Name of the plant - *Asthishrinkhala*: *Cissus quadrangularis* Linn.

Time of Collection - August 2017

Place of Collection - Durga district of Chhattisgarh.

Authentication of Herbarium - BSI, Dehradun (Acc. no. 118193)

Table 1: Macroscopic study

Features	<i>Asthishrinkhala</i> (<i>Cissus quadrangularis</i> Linn.)
Habit	Annual or perennial herb
Stem	Glabrous, much contracted at the nodes, quadrangular, the angles of the young branches winged
Leaves	2.5-5 cm. long, broadly ovate or reniform, sometimes 3-7-lobed, denticulate, glabrous, cordate, rounded
Flowers	Flowers in shortly peduncle cymes with spreading umbellate branches. Calyx cup-shaped, truncate or very obscurely lobed. Petals 4, ovate-oblong, acute 3 mm. long, hooded at the apex
Berry	Berry obovoid or globose, scarcely 6mm. long, apiculate, red when ripe, 1- very rarely 2- seeded ¹⁰

Table 2: Organoleptic study-Organoleptic characters of *Asthishrinkhala*

Character	<i>Asthishrinkhala</i>
Shape	Quadrangular
Size	Internode 8-10 cm long and 1-1.5 cm in diameter.
Surface	Smooth, Shining and Fleshy
Odor	Odorless
Color	In fresh state Green, After drying light Brown
Taste	Mucilaginous bitter
Fracture	Smooth

Microscopic Study

Microscopic characters of stem of *Asthishrinkhala* (*Cissus quadrangularis* Linn.) in transverse section (T.S)

Epidermis single layer, 6-10 layers of parenchymatous cells, 3-5 Layers of chlorenchyma cells, Cambium: Present, Vascular Bundle: Exarch type, Pith: Large central evident pith.

Table 3: Powder Microscopy Study

Features	<i>Asthishrinkhala</i>
Starch grains	+
Calcium oxalate	+
Fragments of vessels	+
Parenchymatous cells	+
Fibres	+

A. Physicochemical Study**Table 4: Physicochemical Study**

S. No.	Test	<i>Asthishrinkhala</i>	In API
1.	Foreign Matter (%)	0 %	NMT 1 %
2.	Moisture content (%)	7.32	-
3.	pH value	6.1	-
4.	Aqueous soluble extract (%)	25.35	NLT 2%
5.	Alcohol soluble extract (%)	12.36	NLT 7%
6.	Petroleum ether soluble extract (%)	5.24	-
7.	Total ash (%)	16.65	NMT 20%
8.	Acid insoluble ash (%)	1.12	NMT 3%
9.	Water soluble ash (%)	11.36	-

B. Phytochemical Study**Table 5: Carbohydrate test**

S. No.	Name of test	<i>Asthishrinkhala</i>	
		Aqueous extract	Alcohol extract
A.	Molisch test	+ ve	- ve
B.	Benedict test	+ ve	- ve
C.	Barfoed's test	- ve	- ve
D.	Fehling test	+ ve	- ve

Table 6: Analysis of Alkaloids

A.	Dragendorff test	+ ve	- ve
B.	Wagner's test	- ve	+ ve
C.	Hager's test	+ ve	- ve

Table 7: Analysis of Amino Acids

A.	Ninhydrin test	+ ve	+ ve
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Table 8: Analysis of Protein

A.	Biuret test	+ ve	- ve
B.	Xanthoprotic test	- ve	- ve
C.	Millon's test	- ve	+ ve

Table 9: Analysis of Saponin

A.	Foam test	+ ve	- ve
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Table 10: Analysis of Glycosides

A.	Borntrager test	+ ve	- ve
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Table 11: Analysis of Phenolic Compound

A.	Phenolic test	+ ve	+ ve
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Table 12: Analysis of Steroids

A.	Salkowski reaction	+ ve	+ ve
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Table 13: Analysis of Tannin

A.	FeCl ₃ test	+ ve	- ve
B.	Lead acetate test	+ ve	+ ve
C.	Potassium dichromate test	- ve	- ve

Quantitative analysis**Table 14: Estimation of Carotene**

S. No.	Sample	Concentration	Absorbance	Concentration
1	Quercetin solutions	10 µg/ml	0.025	
2		20 µg/ml	0.045	
3		30 µg/ml	0.075	
4		40 µg/ml	0.098	
5		50 µg/ml	0.125	
6	Extract of <i>Asthishrinkhala</i>	1000 µ/ml	0.162	9.724 µg/g

Table 15: Estimation of Total Flavonoid Content

S. No.	Sample	Concentration	Absorbance	Concentration
1	Quercetin solutions	10 µg/ml	0.107	
2		20 µg/ml	0.325	
3		30 µg/ml	0.519	
4		40 µg/ml	0.831	
5		50 µg/ml	1.120	
6	Extract of <i>Asthishrinkhala</i>	1000 µ/ml	0.612	31.225

Table 16: Estimation of Total Steroids Content

S. No.	Name of Sample	% of Steroids
1	<i>Asthishrinkhala</i>	0.12

Table 17: Estimation of Vitamin C

S. No.	Name of Sample	Mg/100 gm dry weight
1	<i>Asthishrinkhala</i>	3.64 µg/gm dry weight

Table 18: Estimation of Calcium

S. No	Name of Sample	Result
1	<i>Asthishrinkhala</i>	0.096 % w/w

Table 19: Estimation of Saponins

S. No	Name of Sample	Result
1	<i>Asthishrinkhala</i>	1.25 % w/w

Table 20: Estimation of Vitamin D

S. No	Name of Sample	Result
1	<i>Asthishrinkhala</i>	0

D. Chromatographic Study

TLC (Thin Layer Chromatography) analysis for *Asthishrinkhala*

Mobile solution

Toluene: Ethyl Acetate (9: 1) for *Asthishrinkhala* sample.

Sample

Ethanol Extract

Visualization

Iodine Vapour, Vanillin-Sulphuric Acid for *Asthishrinkhala* sample.

Table 21: TLC Profile of samples on Silica Gel 60 F254

Samples	Iodine vapour		Vanillin-Sulphuric acid reagent	
	No. of spots	R _f Value	No. of spots	R _f Value
<i>Asthishrinkhala</i>	6	0.23, 0.45, 0.56, 0.58, 0.66, 0.92	7	0.23, 0.45, 0.47, 0.56, 0.58, 0.66, 0.92

DISCUSSION

Standardization is the essential preliminary part for establish identity, purity and strength of any drug. In powder microscopy *Asthishrinkhala* had found Calcium oxalate, Starch grains, Fragments of vessels, Parenchymatous cells and Fibres. Moisture content of *Asthishrinkhala* sample was found 7.32%, pH value 6.1, Water soluble extract value 25.35 %, Alcoholic soluble extractive value 12.36 %, Petroleum ether extractive value 5.24 %, Total ash in sample 16.65 %, The acid insoluble ash 1.12 % and Water-soluble ash 11.36 % in *Asthishrinkhala* sample. Most of the major plants' metabolites such as Carbohydrate, alkaloid, amino acid,

protein, saponin, glycosides, phenolic compounds, steroids, tannin were positive in aqueous extract. While Alkaloid, Amino acid, protein, phenolic compound, steroid and tannin were positive in alcoholic extract too. In quantitative analysis *Asthishrinkhala* had found 31.225 µ/ml flavonoid content, 9.724 µg/g Carotene content, 3.64 µg/gm vitamin C, 0.12% steroid content, and 0.096 % calcium content. In Chromatography Study, T.L.C. of *Asthishrinkhala* shows under Iodine vapors, 6 spots were found, R_f values of spots are 0.23, 0.45, 0.56, 0.58, 0.66, 0.92 and in Vanillin-Sulphuric acid reagent 7 spots were found, R_f value of spots are 0.23, 0.45, 0.47, 0.56, 0.58, 0.66, 0.92.

Study photographs

Figure 1: Macroscopic Characters of *Asthishrinkhala* (*Cissus quadrangularis* Linn.)

Figure 2: T.S. of Stem *Asthishrinkhala* (*Cissus quadrangularis* Linn.)

Cissus quadrangularis

Figure 3: Powder microscopy of *Asthishrinkhala* (*Cissus quadrangularis* Linn.)

CONCLUSION

Cissus quadrangularis a traditionally important medicinal plant used to treat various ailments; in the present study, the phytochemical and physicochemical characteristics of *Cissus quadrangularis* Linn. Stem were studied. The presence of high number of phytochemical compounds suggests that the *Cissus quadrangularis* plant has higher medicinal values. It can be concluded that

the Physico-phytochemical analysis of *Cissus quadrangularis* have shown the presence of various versatile constituents such as glycoside, alkaloids, flavonoids, tannin, saponin, vit C, steroid contents and calcium. The present investigation adds to the existing knowledge of *Cissus quadrangularis* Linn. and will be quite useful to pharmaceutical industries for quality control, pharmacological evaluation and development of a formulation for treating various ailments.

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