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Research Article

PHARMACOGNOSTIC VALIDATION OF WHOLE PLANT OF *CONVOLVULUS PLURICAULIS* CHOISY (*CONVULVULACEAE*)

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ABSTRACT

Convolvulus, a genus of perennial wild herb commonly found on sandy & rocky areas under xerophytic conditions in northern India. *Convolvulus pluricaulis* (CP), a member of family Convulvulaceae & used as traditional folk medicine for variety of ailments. Convolvulus pluricaulis is reputed drug of ayurveda & reported as brain tonic, nervine tonic & laxative. . It has also been found effective in anxiety, neurosis, epilepsy, insomnia, burning sensation, oedema, urinary disorders, & snake bites. This traditionally useful plant was standardized based on the macroscopic, microscopic, Physico-chemical and also estimated for its microbial limits, pesticides and heavy metals. Preliminary phytochemical screening confirms the presence of alkaloids, tannins, Carbohydrates, Proteins & amino acids, steroids and glycosides.

Keywords: *Convolvulus pluricaulis*, *Convulvulaceae*, Phytochemical screening, Standardization, Microbial Load, Pesticide, Heavy Metals.

INTRODUCTION

In India, plants have been traditionally used for human and veterinary health care and medicinal plants and it also play a great role in food supplements for health care as well as in personal care of the mankind. Throughout the world, about 35,000-70,000 species of plant have been used at one time or another for medicinal, neutraceuticals and cosmetic purpose [1]. The drugs of plant origin especially of herbaceous nature are used as whole plant and are identified with their origin, common name, scientific nomenclature, family, geographical source, cultivation, collection, preservation, storage, macroscopy, microscopy, chemical composition, identity, purity, strength and assay, substitute and adulterants etc. The microscopic examination of whole plant of CP includes Transverse section and Longitudinal section are made for identification [2]. Convolvulus, a genus of perennial wild herb commonly found on sandy & rocky areas under xerophytic conditions in Northern India [3]. The leaves and flowers possess hypotensive properties used for treating anxiety neurosis. It is recommended as a brain tonic to promote intellect and memory, eliminate nervous disorders and to treat hypertension [4]. It is also described as anthelmintic, good in dysentery, a brain and hair tonic, cures skin ailments and reduces high blood pressure [5]. The present work is based on the standardization of *Convolvulus pluricaulis* whole plant by pharmacognostically as per WHO guidelines. Although other species of this variety has been reported for its standards, this has been the complete pharmacognostic validation of this variety which may be used for formulation development in future.

MATERIALS AND METHODS

Collection and Identification of Plant material

The Whole plant of *Convolvulus pluricaulis* was collected from Khari-baoli, local market of New Delhi, identified and authenticated by Dr. H. B. Singh (Taxonomist), National Institute of Science Communication And Information Resources (NISCAIR), Pusa Campus, New Delhi, with Specimen No: NHCP/NBPGR 2009-9/900.

Foreign Matter Analysis

Weigh 100-500 g of the drug sample to be examined or the maximum quantity prescribed in the monograph, and spread it out in a thin layer. The foreign matter should be detected by inspection with the naked eye or by the use of a lens (6x). Separate and weigh it and calculate the percentage present [6].

Morphological Characters

The dried whole plant of *Convolvulus pluricaulis* were taken and observed for the various parameters including shape, size, color, taste, surface characteristic, texture, fracture characteristic and appearance of cut surface.

Microscopical Studies

Section cutting

Free hand sections of the plant parts were taken and soaked in water overnight. Transverse sections were cut with razor blade. The clear sections were selected, stained with safranin solution and mounted on a clean glass slide and covered with cover slip using glycerin.

Powder characteristics

The powder of whole plant of *Convolvulus pluricaulis* was passed through sieve no. 60, mounted on glass slide using water, covered with cover slip and viewed under microscope.

Physical Evaluation

The ash values, extractive values and loss on drying were performed according to the official methods prescribed in WHO guidelines [6] on quality control methods for medicinal plants materials.

pH Determination

pH of 1% and 10% solution of powdered drug of *Convolvulus pluricaulis* was determined using standardized glass electrode.

Swelling Index and Foaming Index

Swelling Index and Foaming index were determined by the method prescribed in WHO guidelines [6].

Fluorescence Analysis

Fluorescence analysis of the drug was conducted by the method of Chase and Pratt [7] and Kokoski [8]. The powder was observed as such under ultra violet light. Subsequently, sample of powder were treated with 1N sodium hydroxide in methanol, 1N sodium hydroxide in water, Conc. sulphric acid, Conc. sulphric acid in water, Conc. nitric acid, 50% HCL, Conc. HCL, Methanol and Chloroform. The resultant color was observed under ultra violet light.

Phytochemical Screening

The coarsely powdered drug of *Convolvulus pluricaulis* was extracted with different solvents, viz., petroleum ether, chloroform, acetone, methanol, hydro-alcohol and water. The extracts were then subjected to Preliminary phytochemical screening for alkaloids, glycosides, tannins, Carbohydrates, phenols, sterols, proteins, amino acids, flavonoids, saponins, coumarins and others [9-10].

Determination of Heavy metals

Weighed amount of sample was placed in silica crucible and heated on burner till organic matter was charred. It was then transferred to muffle furnace and heated at 500°C for 5hours. The material was cooled and added 20ml nitric acid. The mixture was boiled on water bath for 1hour and filtered. The residue was washed with distilled water and filtered again. Both filtrate and water were mixed and final volume was made up to 100ml with distilled water. It was then subjected to absorption reading using Atomic Absorption Spectrometer and amount of trace matter was then determined [6].

Determination of Pesticidal Residue

Coarsely powdered sample (10g) was mixed with 120ml of mixture of acetonitrile & water in a beaker. It was kept overnight and filtered using non-absorbent cotton pad premixed with acetonitrile. Filtrate was transferred to a separating funnel and shaken with 120ml of 5% NaCl solution. It was extracted thrice with 50ml of n-hexane; the organic layer was combined and dried over anhydrous sodium sulphate. It was concentrated to 5ml on water bath. The extract was further cleaned by adding 20-25g preactivated florosil (preactivation temperature- 500-555°C) and 5g of anhydrous sodium sulphate previously rinsed with petroleum ether. The mixture was eluted very slowly with 150ml of solvent containing n-hexane (141ml) and diethyl ether (9ml), keeping drop rate of 1drop/second. The extract was concentrated on water bath close to dryness and the volume was made up to 1ml with n-hexane. It was then subjected to GC-MS analysis for estimation of residual organic chlorine pesticides (viz. α and β HCH, δ -HCH, DDT and metabolites) [6].

Determination of Micro-organisms

Determination of micro-organisms was done to ensure that the drug was free from any harmful microbes for its use in herbal formulations and was done as per WHO guidelines [6].

Thin Layer Chromatography Profile

TLC of different extracts was carried out to determine the number and nature of components present in it and was performed as per WHO guidelines [6].

RESULTS AND DISCUSSION

Morphological Parameters

Morphological characteristics of whole plant of *Convolvulus pluricaulis* have been described in Table 1 and fig 1.

Table 1: Observation of Organoleptic Characters of *Convolvulus pluricaulis*

S. No.	Parameters	Whole plant Parts			
		Stem	Root	Leaf	Flower
1.	Color	Light brown	Yellowish brown	Green	White or Pinkish
2.	Odour	Odourless	Indistinct	Indistinct	Indistinct
3.	Taste	Slightly bitter	Slightly bitter	Tasteless	Slightly sweet
4.	Size	Several prostrate stems (30—50 cm)	7- 12 cm long, 0.5- 1.5 cm breadth	15-30 mm length, 5-10 mm width	2-3 cm length
5.	Shape	Cylindrical	straight or tuberous	Linear, upper elliptic	funnel-shaped structure
6.	Touch	Clothed with silky hairs	Fractured surface	Pubescent	Smooth
7.	Fracture	Splintery	Fibrous	Simple	--

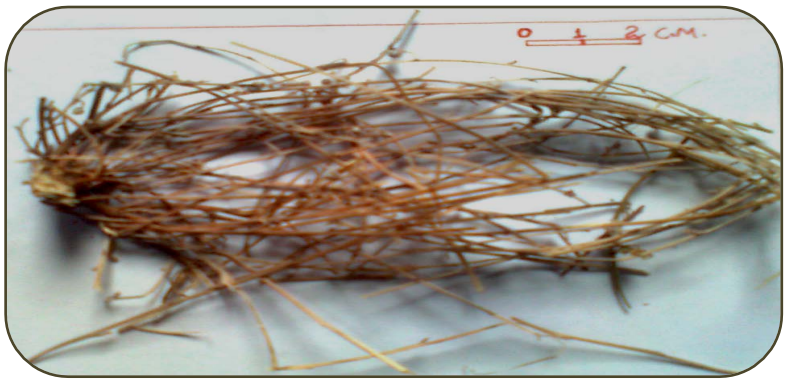

Fig. 1: Dried Herb of *Convolvulus pluricaulis*

Microscopical Characteristics

The TS of stem, leaf and root were observed and different histological parameters determined under microscope.

A: Transverse Section of Stem (Fig 2)

TS of stem shows a layer of epidermis covered with striated cuticle, bearing long unicellular thick- walled, simple trichomes & stomata; cortex narrow composed of 3 to 4 layered outermost chlorenchyma followed by a layer or two of cholenchyma. Pericycle characterized by a discontinuous layer of lignified fibres.

B: Transverse Section of Root (Fig 3)

TS of root shows outer cork composed of 10 to 15 layers of tangentially elongated, thick- walled suberized cells; cortex many layered parenchymatous, transverse by yellowish-brown taniniferrous secretory cells and starch grains, phloem composed of sieve elements, phloem, parenchyma and phloem rays. Xylem vessel solitary or in groups of 2 to 5; tracheids and parenchyma pitted, medullary rays 1 to 3 seriate; starch grains, round to oval in shape, simple or compound, measuring 3 to 8 µ in diameter.

C: Transverse Section of Leaf (Fig 4 & 5)

TS of leaf show convex midrib on the lower side & flat but with a small centrally placed notch at the upper side. Epidermis single layered and covered with thick striated cuticle and long unicellular trichomes. A layer of spongy parenchyma is seen in between the upper and lower palisade tissue. The lower epidermis show cruciferous type of stomata. Stomatal index being 17 to 20 for lower epidermis and 14 to 17 for upper epidermis, palisade ratio of upper epidermis ranges from 6 to 9.

Powder microscopy (Fig 6-10)

Powder of whole plant of *Convolvulus pluricaulis* was light to dark brown in color.The powder drug analysis showed the presence of unicellular trichomes, fragments of spiral annular pitted vessels, anther cells, parenchymatous cells, fibers and pitted xylem parenchyma.

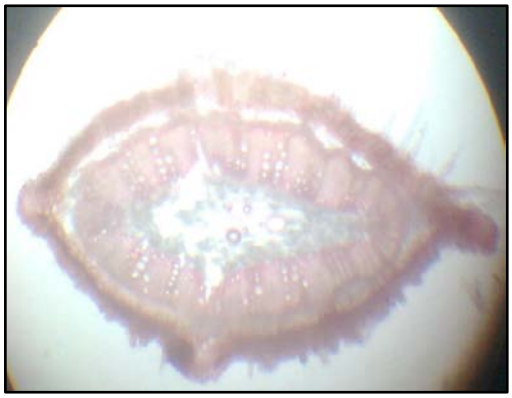

Fig. 2: T. S. of Stem of *C. pluricaulis*

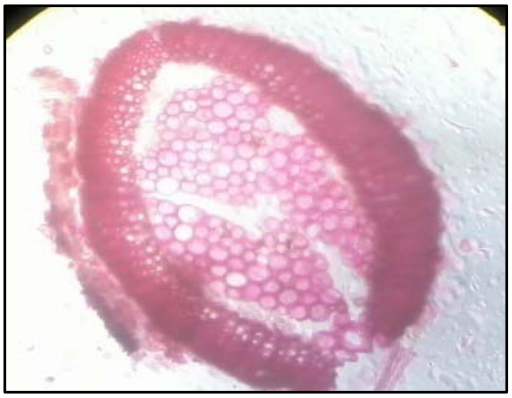

Fig. 3: T.S. of Root of *C. pluricaulis*

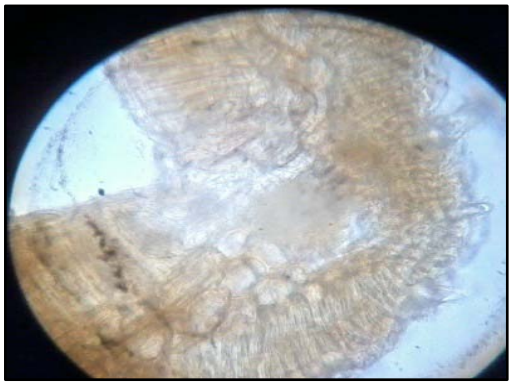

Fig. 4: T.S. of Leaf through midrib

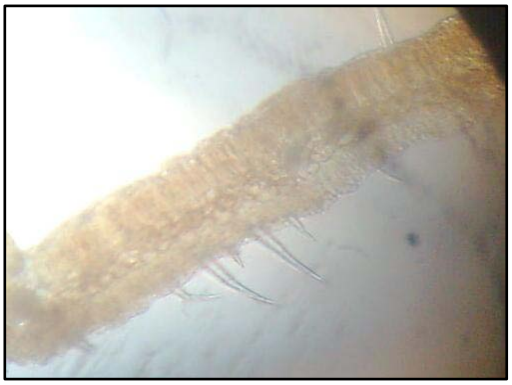

Fig. 5: T.S. of Leaf through lamina

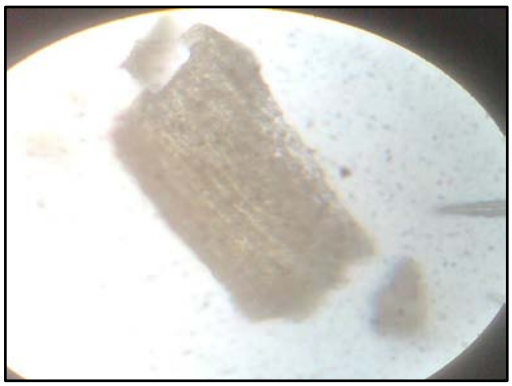

Fig. 6: Fragment of spiral, annual pitted vessels

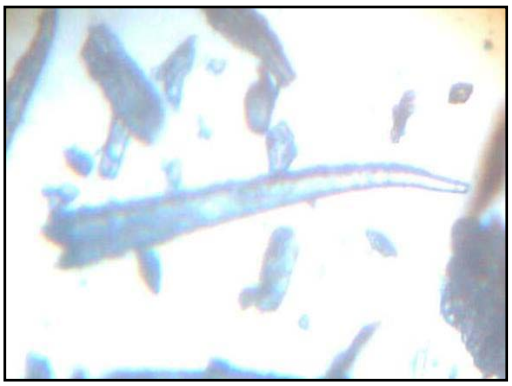

Fig. 7: Unicellular Trichome

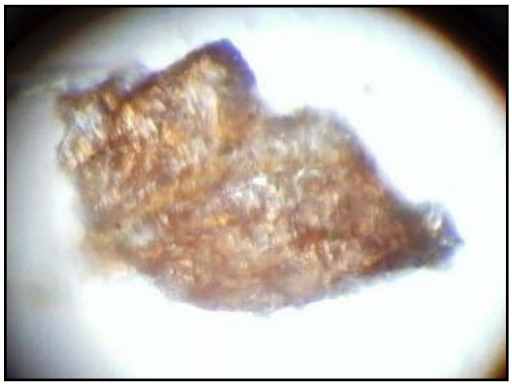

Fig. 8: Parenchymatous cells with Starch grains

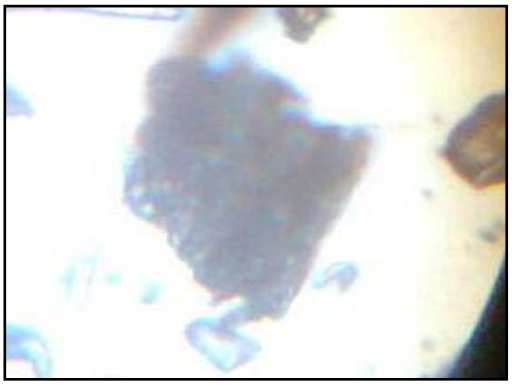

Fig. 9: Anther cells

Fig. 10: Fibbers

Physical Parameters

Results of foreign matter analysis, loss on drying, ash values, extractive values in different solvents, pH determination, swelling index and foaming index are given in Table 2.

Table 2: Physical Parameters

S. No.	Parameters	Values		
1.	**Foreign matter analysis**	1.135±0.1407%w/w		
2.	**Loss on drying**	11.85±0.7188% w/w		
3.	**Ash Values**			
	A. Total ash	7.13±0.3371%w/w		
	B. Acid insoluble ash	3.95±0.3905%w/w		
	C. Water soluble ash	1.95±0.1528%w/w		
4.	**Extractive Values**	**Cold Maceration (w/w)**	**Hot Extraction (w/w)**	**Successive Extraction (w/w)**
	A. Petroleum ether	0.167±0.0417%	0.800±0.0288%	0.363±0.0995%
	B. Chloroform	0.442±0.0363%	0.925±0.0866%	0.312±0.0669%
	C. Acetone	0.508±0.1833%	1.075±0.1507%	0.428±0.1065%
	D. Methanol	1.900±0.4964%	2.000±0.0803%	1.447±0.2419%
	E. Hydro-alcohol	3.500±0.5050%	4.975±0.3744%	1.615±0.1213%
	F. Aqueous	4.208±0.1387%	16.930±1.0760%	1.462±0.1005%
5.	**pH Determination**			
	A. 1% Solution	7.22		
	B. 10% Solution	7.53		
6.	**Swelling Index**	Absent		
7.	**Foaming Index**	Less than 100		

Note: Datas are represented in Mean±SEM.

Fluorescence analysis

Table 3: Observations of Fluorescence Analysis of *C. pluricaulis*

S. No.	Materials /Treatments	Observations Under UV Cabinet		
		Day Light	**At Short Wavelength(254nm)**	**At Long Wavelength(366nm)**
1.	Powdered drug	Yellowish Brown	Greenish brown	Green
2.	Drug + Conc. H_2SO_4	Dark Brown	Greenish black	Brownish black
3	Drug + Conc. H_2SO_4 + Distilled Water	Greenish brown	Green	Greenish black
4.	Drug + Conc. HCl	Yellowish green	Green	Brown
5.	Drug + 1N NaOH + Distilled Water	Yellowish Green	Yellowish Green	Dark Green
6.	Drug + 1N NaOH + Methanol	Light Brown	Light Green	Greenish Black
7.	Drug + Conc. HNO_3	Brown	Brown	Black
8.	Drug + 50 % HCl	Light Brown	Light Green	Greenish Black
9.	Drug + Methanol	Greenish Brown	Green	Greenish Black
10.	Drug + Chloroform	Greenish brown	Brown	Dark Brown

Heavy Metal Analysis

Heavy metal analysis was carried out for determination of Arsenic, Lead, Mercury and Cadmium by atomic absorption spectrometry method as mentioned in WHO guidelines, are found to be within the prescribed limit and are reported in Table 5.

Table 5: Quantitative Estimation of Heavy Metals

S. No.	Type of Heavy Metal	Max. Residual Limits (ppm)	*C. pluricaulis* (ppm)
1.	Arsenic (As)	3.00	0.287
2.	Lead (Pb)	10.00	1.754
3.	Mercury (hg)	1.00	0.0434
4.	Cadmium (Cd)	3.00	0.0202

Phytochemical Investigation

Table 4: Observations of Phytochemical Screening of *C. pluricaulis*

Extract constituents			P E	Ch	Ac	Me	AqMe	Aq
Alkaloids			+++	+++	++	+++	++	+++
	Molisch Test		-	-	-	++	+++	+++
Carbohydrates	Fehlings Test		-	+	-	++	++	++
	Benedicts Test		-	-	-	+++	+++	+++
		Keller killiani Test	-	-	-	++	+++	+++
Glycosides	Cardiac Glycoside	Liebermann Test	-	-	-	+	+	-
		Baljet Test	-	-	-	-	-	-
	Anthraquinone Glycoside	Brontragers Test	-	-	-	++	++	-
		Mod. Brontrager	-	-	-	++	++	-
	Saponin Glycoside		-	-	-	-	-	-
	Coumarine glycosides		+++	+++	++	+++	+++	+++
	5% $FeCl_3$		-	-	++	+++	+++	+++
Tannins	Lead Test		-	-	++	+++	+++	+++
&	Pot. dichromate Test		-	-	++	+++	+++	+++
Phenols		Lead acetate	-	-	++	+++	+++	+++
	PPT. Test	Acetic acid	-	-	++	+++	+++	+++
		Dil.HNO_3	-	-	++	+++	+++	+++
		Pot. dichromate	-	-	++	+++	+++	+++
Flavonoids	Ammonia Test		-	-	+	+++	++	-
	Biuret Test		-	-	-	-	-	-
Proteins	Millons Test		-	-	-	+++	+++	+++
		Absolute alcohol	-	-	-	+++	++	+++
	PPT. Test	5% Copper sulphate	-	-	++	+++	+++	+++
		5% Lead acetate	-	-	++	+++	+++	+++
	Ninhydrine Test		-	-	-	-	-	-
Amino acids	Cysteine Test		-	-	++	+++	+++	+++
	Salkowaski Test		-	-	++	-	+++	++
Steroids	Liberman-Burchad Test		+	-	++	+++	-	+
	Liberman Test		+	+	-	-	-	-

(+++) Instant Result; (++) Result after heating; (+) Change appear on a leaving for a while; (-) Absent

Pesticide Residue

Pesticide residue was determined by the method of GC-MS and all observations were found to be within the prescribed limits. Results are reported in Table 6.

Table 6: Quantitative Estimation of Pesticide Residue of *C. pluricaulis*

Observations of Pesticide Residues in *C. pluricaulis*		
S. No.	**Types of Pesticides**	**Comment(ppb)**
1.	α HCH	0.025
2.	β HCH	0.65
3.	γ HCH	0.36
4.	P-P' DDE	0.07
5.	P-P' DDT	6.01

Microbial Determination

Microbial determination was carried out for Total viable aerobic count and Total aerobic count by serial dilution method. Results are reported in Table 7.

Table 7: Determination of Aerobic Count and Microbial Load of *C. pluricaulis*

S. No.	Microbiological examination	UOM	Result	Specifications
1.	Total aerobic count	CFU per g	700000	1250000
2.	*Enterobacteriaceae*	per g	100	1000
3.	*E. coli*	per g	NAD	10
4.	*Salmonella sp.*	per 25g	Absent	Absent
5.	*Staphylococcus aureus*	per g	NAD	100
6.	Yeasts	per g	NAD	100
7.	Moulds	per g	2000	10000
8.	*Bacillus cereus*	per g	NAD	1000
9.	*Pseudomonas aeruginosa*	per g	NAD	100

NAD = Not Adequately Detected

Thin Layer Chromatography Profile

Petroleum ether extract, chloroform extract, Acetone extract and Methanolic extract were subjected to TLC analysis using solvents of different polarity. The R_f values of the resolved components were determined and detailed results of number of components present are given in Table 7.

TLC Profile of different extracts of *C. pluricaulis*

S. No.	Extracts	Solvent System	No. of Spots Observed		R_f Values
			In U. V.	In I_2	
1.	Pet. Ether	2(M):1(C):1 drop(A)	2	3	0.788, 0.211, 0.138
2.	Chloroform	1(EA):1(M)	3	3	0.849, 0.357, 0.269
3.	Acetone	5(EA):4(T):1(A)	2	3	0.832,0.318,0.126
4.	Methanol	9.5(C) : 0.5(M)	4	5	0.815, 0.676, 0.400, 0.253, 0.138

P: Pet-ether**; T:** Toluene**; C:** Chloroform**; M:** Methanol**; A:** Ammonia**; EA:** Ethyl acetate**; AA:** Acetic acid

CONCLUSION

The Pharmacognostical and Phytochemical analysis carried out with a focus on bringing out diagnostic characters will be of immense help in the proper identification and standardization of botanical species of the memory enhancing drug *Convolvulus pluricaulis* mentioned in ayurveda. It will also help in carrying out further research and revalidation of its use in ayurveda.

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