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Research Article

Standardisation of *Convolvulus pluricaulis* Choisy Herbs collected from Jalandhar, Punjab

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ABSTRACT

The present study deals with pharmacognostic evaluation including the morphological, microscopical characters. TLC chromatogram and different phsicochemical standard has been developed. Physicochemical constants of *Convolvulus pluricaulis* including determination of loss on drying, ash values and extractive values. The preliminary phytochemical screening of various leaf extracts was also carried out and it is revealed the presence of various phytoconstituents. The results of the standardization may throw immense light on the botanical identity of *Convolvulus pluricaulis* which may furnish a basis of judging the authenticity of the plant and also to differentiate the drug from its allied species and detect adulterants.

Keywords: *Convolvulus pluricaulis*, Standardisation, Microscopy, TLC, Pundraaksha, Shankhpushpi

INTRODUCTION

Standardization of herbal drugs is an essential measurement for ensuring the quality control of the herbal drugs. Standardization of herbal drugs is not an easy task as numerous factors influence the bio efficacy and reproducible therapeutic effect. In order to obtain quality oriented herbal products, care should be taken right from the proper identification of plants. WHO encourages standardization of herbal crude drugs[1]. According to WHO, it is the process involving the physicochemical evaluation of crude drugs covering the aspects such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer[2]. From ancient time Shankhpushpi had been used as brain tonic, memory enhancing drug. The source of plant is controversial but the official source of is believed to be *Convolvulus pluricaulis* also called as *Convolvulus microphyllus.* The leaves and whole plant are used as memory enhancer[3]. Standardisation of medicinal plant is necessary because of the fact that in some cases desirable pharmacological action are not achieved because the biological action of herbal medicine is due to phytoconstituents which can vary batch to batch. The amount of phytoconstituents in a plant can vary according to age of plant, time of collection, environmental condition etc. To overcome this problem standardized medicinal plants, plant extracts and isolated constituents can be used[4]. To insure quality of this valuable medicinal plant so an attempt has been taken to standardize *Convolvulus pluricaulis* Choisy Herbs.

MATERIALS AND METHODS

Plant material

The plant material was collected from Jalandhar, Punjab. Plant materials were authenticated by Dr. H.B. Singh at NISCAIR, Pusa Institute, New Delhi. Reference no is NISCAIR/RHMD/Consult/2010-2011/1569/167.

Morphological / Organoleptic properties

Macroscopic studies of leaves were done visually and evaluated for organoleptic properties.

Microscopic characterisation

Powder of herb was stained with phluoroglucinol and hydrochloric acid and visualize under compound microscope at 10x.

Preliminary phytochemical screening

Petroleum ether, chloroform, Ethyl acetate, Acetone, methanol extract of *herb* was prepared by soxlet extraction. Aquos extract was prepared by refux method.

Figure 1: Aerial parts of *Convolovulus pluricaulis*

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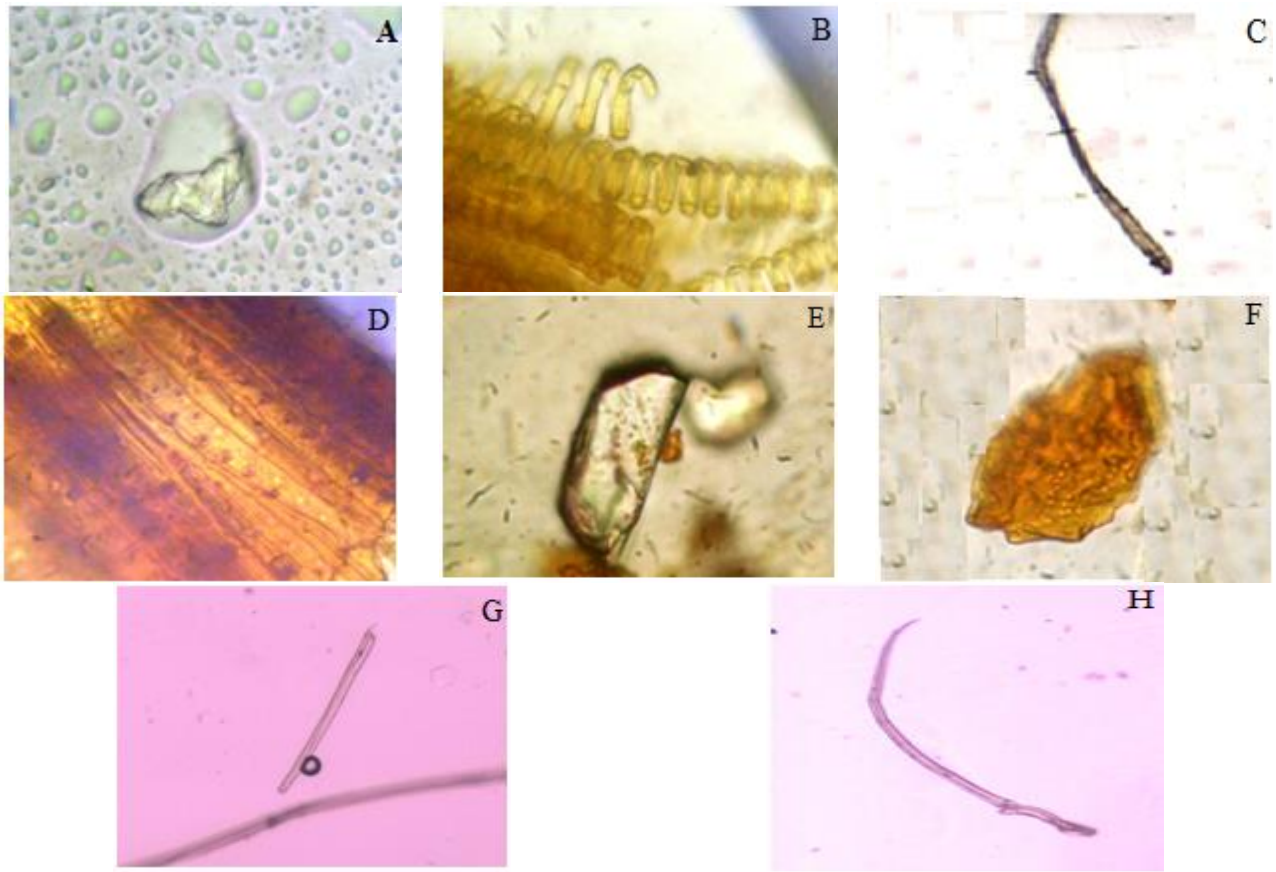

Figure 2: Microscopy of *Convolvulus pluricaulis* A. Calcium oxalate crystal, B. Vessels (Spiral), C. Fiber, D. Trachieds, E. Starch grain, F. Calcium oxalate crystal, G. Single Needle Crystal, H. Uniseriate trichome.

Table 1: Qualitative chemical examination of extracts

S.No	Compound	Reagent	Pet .Ethr	Chloroform	Eth. Acetate	Acetone	Methanol	Water
1.	Alkaloids	Hager's	-	+	-	-	+	-
		Dragondorff's	-	-	-	+	+	-
2.	Carbohydr	Benedict's	-	-	-	-	-	+
	ates	Fehling's	-	-	-	-	-	-
3.	Glycosides	Borntrager's	-	-	-	-	-	-
		Modified born trager's	-	-	-	-	-	-
		Legal test	-	-	-	-	-	-
4.	Saponin	Froth test	-	-	-	-	-	+
5.	Fixed oils	Stain test	+	-	-	-	-	-
6.	Phenols	$Fecl_3$ test	-	-	+	-	+	+
7.	Tannins	Gelatin test	-	-	-	-	-	-
8.	Flvonoids	Alkali test	-	-	+	+	-	+
		Lead acetate	-	-	-	-	+	+
9.	Resin	Acetone+water	-	-	-	-	+	-

All the extracts were subjected to phytochemical screening for qualitative analysis for presence and absence of secondary metabolite[5].

Physicochemical parameter

Triplicate reading of each physicochemical parameter were taken and value are presented as Mean ± Standard error mean (SEM)

Determination of Swelling Index

The swelling index is the volume in ml taken up by the swelling of 1g of plant material under specified conditions. The plant material was reduced to fineness passing from sieve no. 22 and was accurately weighed 1g into a 25 ml glass-stoppered measuring cylinder. Water (25 ml) was added and shaken thoroughly after every 10 min for 1 hr. Then the mixture was allowed to stand for 3 hr at room temperature. The volume was measured in ml occupied by the plant materials. The mean value of the individual determinations was calculated related to 1g of plant material.

Swelling index = (Final volume – Initial volume / Final

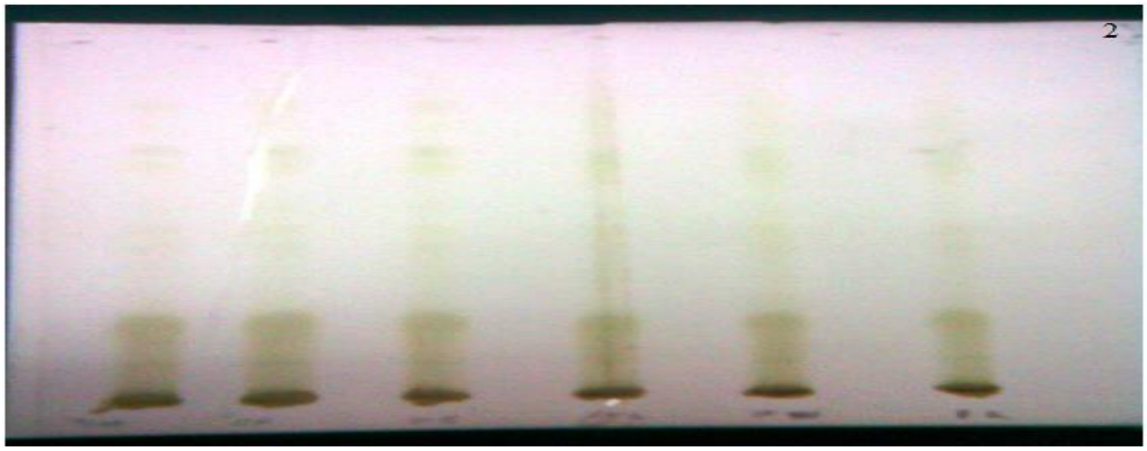

Figure 3: TLC profile of methanolic extract of *Convolvulus pluricaulis* in Daylight

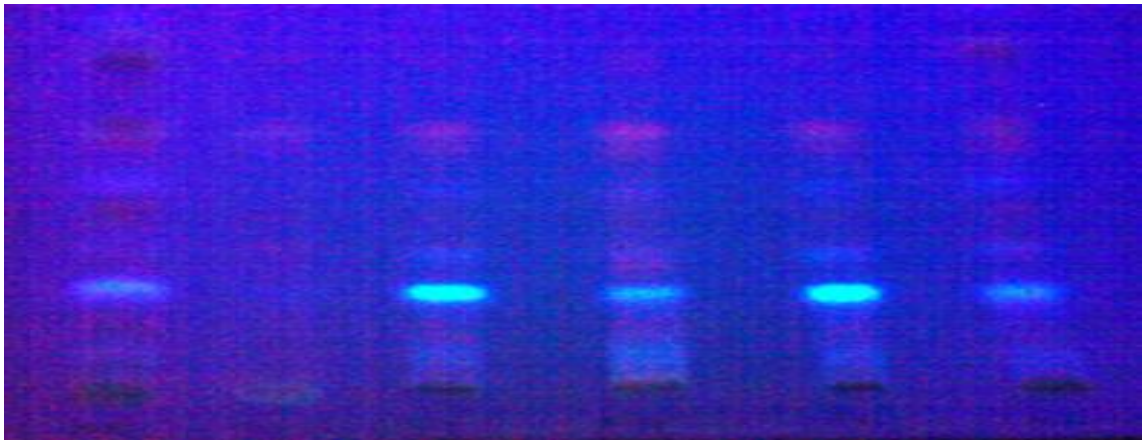

Figure 4: TLC profile of methanolic extract of *Convolvulus pluricaulis* in 366nm

Table 2: Physicochemical parameter

Parameters		Results (%)
Total Ash		9.1 ± 0.221
Water soluble ash		8.2 ± 0.112
Acid insoluble ash		0.6 ± 0.011
Determination of Swelling Index		4.3 ± 0.238
Determination of Extractive value		
Ethanolic	Hot	2.4 ± 0.036
	Cold	2.1± 0.018
Aqueous	Hot	4.43± 0.021
	Cold	3.21± 0.016
Determination of foreign matter		0
Moisture content		2.03 ± 0.024

Values are presented as mean ± SEM (n=3)

volume) X 100

Determination of extractive value

Cold and hot extractive value was determined using ethyl acetate, dichloromethane, alcoholic and water.

Hot extractive value

Coarsely powdered air-dried material 4 g was accurately weighed and placed in glass-stoppered conical flask. 100 ml of solvent was added and total weight including the flask was noted. It was shaken well and allowed to stand for 1 hr. Reflux condenser was attached to the flask and gently boiled for 1 hr, cooled and weighed. Original total weight was readjusted with the solvent specified in the test procedure. It was then shaken well and filtered rapidly through a dry filter. The 25 ml of filterate was transferred to a tared flat-bottomed dish and evaporated to dryness on a water bath. Then dried at 105°C untills constant weight, cooled in a dessicator for 30 min and then weighed without delay. Percentage hot extractive value was calculated using following formula.

% hot extractive value = (extract obtained / plant material taken) X100

Cold extractive value: Coarsely powdered air-dried material 4 g was accurately weighed and placed in glass-stoppered conical flask. It was macerated with 100 ml the solvent specified for the plant material concerned for 6 hr, shaken frequently and then allowed to stand for 18 hr. Filtered rapidly taking care not to lose any solvent, 25ml of filterate was transferred to tared flat-bottomed dish and evaporated to dryness on a water bath. Dried at 105°C untills constant weight, cooled in a dessicator for 30 min and weighed without delay. Percentage cold extractive value was calculated using following formula.

% cold extractive value = (extract obtained / plant material taken) X100

Determination of Ash value

The ash remaining after ignition of medicinal plant materials is determined by three different methods which measure total ash, acid insoluble ash and water soluble ash.

Total ash

Weigh accurately 1g of finely powdered leaves and placed in a previously ignited and tarred crucible. The material was spread in an even layer and was ignited by gradually increasing the heat to 550^0C until it turns white, indicating the absence of carbon. It was cooled in desiccators for 30 min and weighed without delay. The content of total ash was calculated using following formula.

% Total ash = (Ash obtained after calcination / Plant material taken) X 100

Acid insoluble ash: To the crucible containing total ash, hydrochloric acid (25 ml) was added. It was covered with watch glass and allowed to boil for 5 min. The watch glass was rinsed with hot water (5 ml) and this liquid was added to the crucible. The insoluble matter was collected on ash less filter paper and washed with hot water until the filtrate was neutral. The filter paper containing insoluble matter was transferred to the original crucible. It was dried on a hot plate and ignited to constant weight. The residue was cooled in a dessicator for 30 min and weighed without delay. The content of acid insoluble ash was calculated using following formula.

% Acid insoluble ash = (Acid insoluble material / Plant material taken) X 100

Water soluble ash: To the crucible containing total ash, water (25 ml) was added and boiled for 5 min. The insoluble matter was collected on ash less filter paper and washed with hot water. It was ignited in a crucible for 15 min at a temperature not exceeding 450°C. The weight of this residue in mg was subtracted from the weight of total ash. The content of water soluble ash was calculated using following formula.

% water soluble ash = (Water soluble material / Plant material taken) X 100

Thin layer chromatography

Preparation of test sample

The coarsely powdered dried plant materials (1 g) were successively extracted on small scale with methanol (10 ml) at 90°C 1 hrs using Reflux condenser.

Method

Stationary phase

Silica gel precoated aluminium sheets (5x5cm) (Silica gel 60F-254) of thickness 0.2 mm were used.

Mobile Phase: Toulene: Diethyl ether: Acetic acid in ratios of 5:5:1

Application of spot: Applied manually with a Capillary Tube.

Development of Chromatogram: The development chamber was washed, dried and then saturated with mobile phase. The plate was then introduced in developing solvent. The chamber was made air tight and allowed to stand until 80% front was developed. The chromatogram was taken out and heated in oven at 105^0C for 5 minutes.

Calculation of Rf value Rf values of the spots were calculated using following formula

Rf = Distance travelled by solute/ Distance travelled by solvent front

RESULTS AND DISCUSSION

Macroscopic characteristics

The colour of dried herb was of dark brown colour. Surface was characteristic (Hairy and Brached. Taste was bitter and astringent however the odor was characteristic.

Microscopy of Convolvulus pluricaulis plant

The powder microscopy of *Convolvulus pluricaulis* revealed Calcium oxalate crystals, Spiral vessels, Fibers, Trachieds, Starch grains, Single needle crystals and Uniseriated trichomes.

Phytochemical screening: The presence or absence of phytoconstituents in different extract is shown in table 1.

Physicochemical parameter: Result obtained for different physicochemical parameter is reported in table 2

Thin layer chromatography: Chromatogram of TLC in visual light and under UV light is shown in fig.3 and 4. Rf value of separated constituent for methanol extract are 0.325, 0.575, 0.6, 0.8, 0.825 etc

DISCUSSION

Morphological assessment of crude drug helps in identification of plant as well as detection of adulteration. In some cases, quality of crude drug can be checked on the basis of morphology only. The powder microscopy of crude drug allows more detail examination of a drug and it can be used to identify the unorganized drugs by their known histological character[6]. The powder microscopy can be used as standard for authentication of this valuable powder form of crude drug. Phytochemical screening help in evaluating for presence and absence of phytoconstituents. Standardisation of medicinal plant is a complicated process so different physicochemical parameter together could help in better understanding. Physicochemical parameter can be used as standard to ensure the quality of crude drug. High ash value of shows the presence of very high inorganic content. Lower value of the acid insoluble ash suggests the greater physiological availability of drug. Extractive value gives information about availability of soluble phytoconstituents in particular solvent[7]. Alcohol soluble extractive is more as compared to aqueous extractive value suggesting alcoholic extract would be more beneficial as compared to aqueous extract for therapeutic aspect. Low value of moisture content does not promote microbial contamination as the general requirement of moisture content in crude drug is not more than 14 % (W/W). The swelling index of leaf is 4.3 % it may be due to presence of gum. Thin layer chromatography can be a valuable tool in standardisation of medicinal plant. The TLC chromatogram and Rf value of phytoconstituent is preliminary tool of evaluation of crude drug[8].

CONCLUSION

The present study on pharmacognostic standardisation, physicochemical evaluation of *Convolvulus pluricaulis*

leaf can be used as standard in regard to its identification parameters and quality control assuming significantly in the way of acceptability of medicinal plant in present scenario.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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