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Research Article

Pharmacognostical & Tlc Fingerprinting of Kiratatikta

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Abstract: Standardization plays crucial role for the selection proper raw materials in the production of herbal formulation. Swertia chirata has been used in traditional and folklore medicine for the treatment of several critical diseases and disorders. The present study was focused on preliminary pharmacognostic and phytochemical investigations for determining and establishing the identity, purity and quality of the plants. This will endow with an appropriate guidance for future exploration. The plant was subjected to determination of various physicochemical parameters including ash values (total ash, water soluble ash) and extractive values (alcohol soluble extractive, water soluble extractive). The powdered crude drug was extracted successively with various solvents with increasing polarity and further the extracts were subjected to phytochemical screening for the identification of various phytoconstituents. More over the fingerprint profile of Swertia chirata was established using thin layer chromatography(TLC) methods. TLC was carried out Toluene, Ethyl Acetate: Formic acid [5:4.5:0.5] as a solvent systems, which showed different R_f value. The results of the TLC profiling of the extract confirm about the presence of various phytochemicals.

Keywords: Kiratatikta(*Swertia chirata*),Microscopy, Phytochemical investigations & TLC profiling.

INTRODUCTION

Nature has been a resource of medicinal agents for thousands of year and a remarkable number of modern drug have been isolated from natural sources, many based on their use in traditional medicine (Soni, H., & Singhai, A. K. 2012). One of the prerequisites for the triumph of primary health care is the availability and use of appropriate drugs. Traditional medicine is still the most inexpensive and easily accessible source of treatment in the primary healthcare system. Swertia, a genus in the family Gentianaceae comprise a large group of annual and perennial herbs having approximately135 species. Swertia species are general ingredients in a number of herbal remedies. S. chirayita, common name: "Chiretta" is a significantly endangered medicinal herb that grows at high altitudes in the sub-temperate regions of the Himalayas between 1200 and 2100 m altitudes from Kashmir to Bhutan (Kumar, V., & Van Staden, J. 2016). In India S. chirata is known as Chirayata. In Hindi the herb is called Chiretta and in Sanskrit it is called Bhunimba or Kirata tikata (Naveen, K. *et al*., 2017).S.chirayita is considered the most significant for its medicinal properties like bitterness, antihelmintic, hypoglycemic and antipyretic properties (Joshi, P., & Dhawan, V. 2005).

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Synonym (Khare, C.P. 2007; Bapalal ji, V., & Nighantu, A., 2005)	Botanical description (www.gbif.org)[5]	
Hindi Chirayata English Chiretta Bengali Chirata Tamil Nilavembu Punjabi Charaita Marathi Kiraita Gujarati Kariyatum Kannada Nilavebu Malayalam Nilavippa	Kingdom: Plantae Phylum: Tracheophyta Class: Magnoliopsida Order: Gentianales Family: Gentianaceae Genus: Swertia Species: chirata Binomial Name: *Swertia chirata Buch* Ham	 ***Swertia chirata Buch***

Morphological Description

Morphological character[8]	
Leaves: **Broadly lanceolate- acute, 10 × 3.75 cm.**	
Flowers**: In Corymbosely paniculate cymes; calyx & corolla 4 lobed.Corolla- greenish yellow tinged with purple with two glands on each lobe, fringed with long hairs.Stamens 4-5, inserted at the base of corolla; capsule sessile, oblong.**	

Ayuvedic Formulation[9]
Properties and action
Rasa: Tikta
Guna: Laghu,Ruksa
Vipaka: Katu
Virya: Sheeta
Karma: Jwaragna, Kalpha-pittahara, Raktshodaka, varanshodka.

Important Formulation:- Sudarshanchurna, Chinodbhunibayadi kwatha, churna
Therapeautic Usage: *Soth, daah, kushta, trishna, Varan.*

MATERIALS AND METHODS

Collection

The samples of Kiratatikta were collected from high hills of Dhanolti and Kalamegha from Haridwar, State – Uttrakhand. The sample of Kiratatikta was collected by scholar under the guidance of Supervisor after identifying the source of plant as per standard description.

Date of Collection:
Swertia chirayita-3/08/2017

Authentication
The plant was authentication was done Botanical Survey of India (BSI), Dehradun.
Acc.No: 118082

Specimen of *Swertia chirayita*

Microscopic Study

Microscopic study of crude drugs is another aid of Pharmacognosy which can be helpful in the process of standardization of medicinal plants. This study can be helpful in identifying genuine drug by their known histological characters through Transverse section (T.S.) or Longitudinal Section

(L.S.) or Radial Longitudinal Section (R.L.S.) or Tangential Longitudinal Section (T.L.S.) and Powder microscopy which can help in evaluation of different constituents by using different staining reagents.Specimens were soaked in water or other sovents depending upon the hardness of the sample and transverse sections were taken using sharp razor blades. Few microscopic sections were cut by Microtome sectioning. Numerous temporary and permanent mounts of the microscopical sections of the specimen were made and examined microscopically. Different staining reagents were applied on transverse sections so as to differentiate between different cell wall components.

Preliminary Physiochemical Screening of Plant Extracts (Mukharjee, P.K. 2002; Khandelwal, K.R. 1996; Himesh, S. *Et al.*, 2011 Soni, H. *Et al.*, 2011; Sapkota, S., *Et al.*, 2019)

Determination of Solvent Extractive Values:

Determination of Water Soluble Extractive Value:

5 g of the air-dried drug, coarsely powdered were macerated with 100 ml of water in closed flask for 24 hours, shaking frequently during the first 6 hours and allow standing for 18 hours. It was filtered rapidly taking precaution against loss of water, then the filtrate was evaporated 25 ml of the filtrate to dryness in a tared flat-bottomed shallow dish, and dried at 105^0C then weighed. The percentage of water-soluble extractive with reference to the air dried was calculated.

Determination of alcohol soluble extractive value:

5 gm of the air dried and coarsely powdered drug was macerated with 100 ml of ethanol of the specific strength in a closed flask for 24 hours, shaking frequently during the first 6 hours and allow standing for 18 hours. There after filter rapidly taking precaution against loss of ethanol. Evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, dry at 105^0c and weigh. The percentage of ethanol soluble extractive with reference to the air dried drug has to be calculated.

Determination Of Moisture Content:

Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. Method of determination of moisture content include the loss on drying, the test for loss on drying determines both water and volatile matter in the crude drug. It can be carried out either by heating at 100^0C-105^0C or in a dessicator over phosphorous pentoxide under atmospheric or reduced pressure at room temperature for specific period of time.

Ash value:

Ash value is helpful in determining the quality and purity of a crude drug, especially in the powdered form. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The total ash of a crude drug reflects the care taken in its preparation. A higher limit of acid-insoluble ash is imposed, especially in case where silica may be present or when the calcium oxalate content of the drug is very high.

Total ash value

Weighed accurately about 2 to 3 g of the powdered drug in a tared silica crucible. Incinerated at a temperature not exceeding 450 ^{0}C for 4 hr, until free from carbon, cooled and weighed. The percentage of ash with reference to air-dried was calculated following formula.

$$\% \text{ Total ash value} = \frac{\text{Wt. of total ash}}{\text{Wt. of crude drugs}} \times 100$$

Water Soluble Ash Value:

Boiled the ash with 25 ml of water. Filtered and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited in a tared crucible at a temperature not exceeding 450 ^{0}C for 4 hr. Cooled in a desiccator and weighed. Substrate the weight of insoluble matter from the total weight of ash. The difference in weight represented weight of water soluble ash. Calculated the percentage of water soluble ash with reference to the air- dried drug by using the following formula.

$$\% \text{ Water soluble ash value} = \frac{\text{Wt. of total ash-Wt of water insoluble ash}}{\text{Wt. of crude drug taken}} \times 100$$

Acid Insoluble Ash Value:

Boiled the ash for 5 min with 25 ml of 2 M HCL. Filtered and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited in a tared crucible at a temperature not exceeding 450 ^{0} C for 4 h. cooled in a desiccator and weighed. Calculated the percentage of acid insoluble ash with reference to the air -dried drug was calculated by using following formula,

$$\% \text{ Acid insoluble ash value} = \frac{\text{Wt. of acid insoluble ash}}{\text{Wt. of crude drug taken}} \times 100$$

Preliminary Qualitative Test

The various extract of *Swertia chirayita* was subjected to preliminary qualitative phytochemical investigation. The various tests and reagent used are given below.

Alkaloids

Preparation of Test Solution:

The test solution was prepared by dissolving extracts in the dilute hydrochloric acid.

Mayer' tests:

The acidic test solution with Mayer's reagent (Potassium Mercuric iodide) gave cream colored precipitate.

Hager's test:

The acidic test solution with Hager's reagent (Saturated picric acid solution) gave yellow precipitate.

Dragendorff's test:

The acidic solution with Dragendorff's reagent (Potassium bismuth iodide) showed reddish brown precipitate.

Wagner's test:

The acidic test solution treated with Wagner's reagent (Iodine in potassium iodide) gave brown precipitate.

Tannic acid test:

The acidic test solution treated with Tannic acid gave buff colour precipitate.

Picrolonic acid test:

Alkaloids gave yellow colour precipitate with picrolonic acid.

Amino ACID:

Millon'test:

To the test solution add about 2 ml of millon's reagent white precipitate indicates presence of amino acid.

Ninhydrine test:

To the test solution add Ninhydrine solution, boil, violet colour indicates presence of amino acid.

Carbohydrates

Preparation of test solution:

The test solution was prepared by dissolving the test extracts with water. Then it was hydrolyzed with 1 volume of 1 N-HCL and subjected to following chemical test.

Molisch's test:

Test solution with few drops of Molisch's reagent and 2 ml of conc.H_2So_4 added slowly from the sides of the test tubes. It showed a purple ring at the junction of two liquids.

Barfoed's test:

1 ml of test solution is heated with 1 ml of Barfoed, s reagent on water bath, if red cupric oxide is formed, monosaccharide is present. Disaccharides on prolong heating (about 10 min.) may also cause reduction, owing to partial hydrolysis to monosaccharide.

Benedict's Test:

Test solution treated with Benedict' reagent and after boiling on water bath, it showed reddish brown precipitate.

Fehling's Test:

The test solution when heated with equal volume of Fehling's A and B solution, gave orange red precipitate, indicating the presence of reducing sugars

Flavonoids

The flavonoids are all structurally derived from the parent substance called flavones. The flavonoids occur in the free from as well as bound to sugars as glycosides. For this reason, when analyzing flavonoids it is usually better to examine the flavonoids in hydrolyzed plant extracts.

Preparation of Test Solution:

To a small amount of extract added equal volume of 2 M HCL and heated in a test tube for 30 to 40 min at 100^0C.The cooled extract was filtered, and extracted with ethyl acetate The ethyl acetate was concentrated to dryness, and used to test for flavonoids.

Shinoda Test:

Test solution with few fragments of magnesium ribbon and conc. HCL showed pink to magenta red colour.To a small quantity of test solution when lead acetate solution was added, it formed yellow colored precipitate.

Alkaline Reagent Test:

Test solution when treated with sodium hydroxide solution showed increase in the intensity of yellow colour, which becomes colorless on addition of few drops of dilute acid.

Glycosides

Preparation of test solution:

The test solution was prepared by dissolving extract in the alcohol or hydro- alcoholic solution.

Test for Cardiac Glycosides:

Kedde' test:

Add one drop of 90% alcohol and 2 drops of 2 % 3, 5- dinitro benzoic acid in 90% alcohol. Make alkaline with 20 % sodium hydroxide solution, purple colour is produced. The colour reaction with 3, 5-dinitro benzoic acid depends on the presence of α, β-unsaturated lactones in the aglycone.

Baljet's test:

The test solution treated with sodium picrate gave yellow to orange colour.

Raymond's test:

Test solution treated with hot methanolic alkali, violet colour is produced.

Bromine water test:
Test solution dissolve in bromine water give yellow precipitate.

Keller-killani test for digitoxose:
The test solution treated with few drops of Fecl3 solution and mixed, then H_2So_4 containing Fecl3 solution was added, it formed two layers. Lower layer reddish brown, upper layer turns bluish green.

Legal's test:
Test solution when treated with pyridine (made alkaline by adding sodium nitroprusside solution) gave pink to red colour.

Test for Anthraquinone Glycosides:
Borntrager's test:
Boiled powdered drug with 5 ml of 10 % sulphuric acid for five minutes. Filtered while hot, cooled the filtrate shaken gently with equal volume of benzene. Benzene layer was separated and then treated with half of its volume solution ammonia (10%). Allowed to separate it. The ammonical layer acquired rose pink colour due to presence of anthraquinones.

Proteins
Preparation of test solution:
The test solution was prepared by dissolving the extract in water.

Millon's test:
Test solution was treated with millon's reagent and heated on a water bath. The proteins were stained red

Biuret test:
Test solution was treated with 40% sodium hydroxide and dilute copper sulphate solution gave blue colour.

Xanthoproteic test:
Test solution was treated with conc. HNO_3 and boiled which gave yellow precipitate.

Modified Borntrager's test:
C-glycosides of anthraquinones require more drastic conditions for hydrolysis. Hydrolysis of the drug was carried out with 5 ml of dilute of HCL and 5 ml of 5 % solution of $Fecl_3$. For hydrolyzed extract procedure was carried out as described under Borntrager's test.

Test for Steroids
Preparation of test extract solution:
The extract was refluxed separately with alcoholic solution of potassium hydroxide till complete saponification. The saponified extract was diluted with water and unsaponificable matter was extracted with diethyl ether. The ethereal extract was evaporated and the residue (saponificable matter) was subjected to the following test by dissolving the residue in the chloroform.

Salkowski test:
To the test extract solution add few drops of conc. H_2SO_4 shaken and allowed to stand, lower layer turned red indicating the presence of steroids.

Libermann - Burchard test:
The test solution treated with few drops of acetic anhydride and mixed, when conc**.** H_2SO_4 was added from the sides of the test tubes, it showed a brown ring at the junction of the two layers and the upper layers turned green. Added few drops of concentrated H_2SO_4. Blue colour appeared.

Sulphur test:
Sulphur test when added in to the test solution, it sank it.

Tannins and Phenol Compound
To 2-3 ml of alcoholic or aqueous extract, added few drops of following reagents.

5% $Fecl_3$ solution:
Deep blue- black colour**.**

Lead acetate solution:
White precipitate.

Bromine water:
Discoloration of bromine water.

Acetic acid solution:
Red colour solution.

Dilute iodine solution:
Transient red colour**.**

One drop of NH_4OH, excess 10% $AGNO_3$ solution. Heated for 20 min in boiling water bath. White precipitate was observed, then dark silver mirror deposited on wall of test tube.

Triterpenoids
Preparation of test extract solution:
The test extract solution was prepared by dissolving extract in the chloroform.

Salkowski test:
Few drops of concentrated sulphuric acid were added to the test solution, shaken and on standing lower layer turned golden yellow (Mukharjee, P.K. 2002; Khandelwal, K.R. 1996; Himesh, S. *et al*., 2011 Soni, H. *et al*., 2011; Sapkota, S., *et al*., 2019).

TLC analysis for different Phytochemicals
T.L.C. plate coated with 0.25 mm layer of silica gel GF 254 with fluorescent indicator,

(Mercks) were used. (Each plate dimension is 10 cm long and 2 cm width).

Activation of pre-coated Silica gel G60F$_{254}$
Dry in hot oven at 105^0 C for one to two hour.

Sample: Alcoholic Extract of test sample.
Solvent system:
Toluene, Ethyl Acetate:
Formic acid [5:4.5:0.5]

Visualization:
Iodine Vapor **&** UV Short Wave Length.

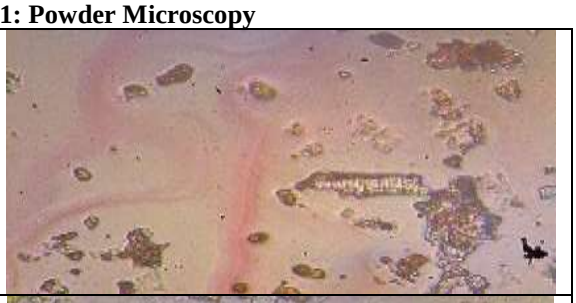
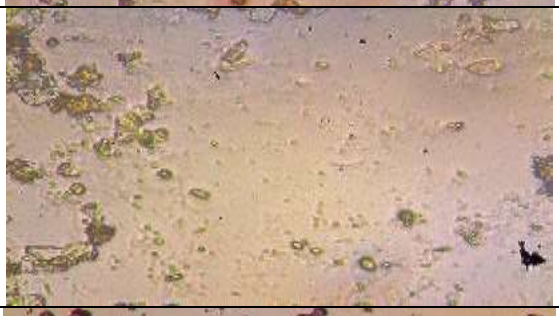
RESULT AND DISCUSSION

Ayurveda is holistic system of medicine which utilizes broad range of herbs occurring in nature to cure various diseases. Among them chirayita (*S. chirayita*) is one precious herb which is used as single drug and in compound formulations for treatment of various diseases. It is also used as nutritive supplement. Ayurveda describes use of chirayita in extensive range of ailments like jwar, kustha, kandu, pramehaswaasa, kaasa, aruchi, trishna,daaha, krimi,vrana.etc. Modern researches have also highlighted those uses which have been described in ayurvedic classics. Some of them are fever, malaria, anaemia, bronchial asthma, liver disorders, hepatitis, gastritis, constipation,dyspepsia & skin diseases[15].The Microscopy of root showed presence of Parenchymatic cells, Lignified fibres, Mucilaginous cells & Starch (table 1).The standardization parameters were loss on drying , total ash value, acid insoluble ash value, water soluble ash value. Water soluble extractive value and alcohol soluble extractive value were tabulated in table 2. The preliminary phytochemical screening of aqueous, alcoholic and Pet.ether extract revealed the presence of the Carbohydrate, Amino acids, Protein, Alkaloids, Glycosides, while Tannin and phenolic compound were present in alcoholic extract but absent in both the extracts i.e. aqueous and petroleum ether extract. The result of phytochemical screening was tabulated in table 3. TLC fingerprinting revealed the presence of phytoceuticals showed the therapeutic utility of the plant (table 4).

CONCLUSION

The present study may be useful to appendage information in regard to its characterization and identification of plant. This study is significant and lays down parameters for standardization and authentication of medicinal plants with the assist of which adulteration and substitution can be prevented.

Table 1: Powder Microscopy

Annular ring Lignified cells Safranine	
Mucilaginous cells Methylene blue	
Parenchyma Eosin	

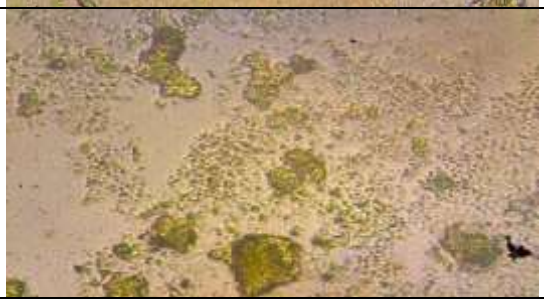
Fibres Iodine	
Starch Grain Iodine	

Table 2: Physiochemical Parameters

S.No	Tests	*Swertia chirayita* (S2)
1	Moisture content	9.52% w/w
2	pH	4.64
3	Alcohol Extractive Value	15.15% w/w
4	Aqueous Extractive Value	15.98% w/w
5	Foreign matter	0.95% w/w
6	Total Ash	5.19% w/w
7	Acid Insoluble Ash	0.98% w/w
8	Water Soluble Ash	1.24% w/w

Table 3: Results of preliminary phytochemical analysis

Sr. No.	Name of the Test	Observation		
		Aqueous Extract	Alcoholic Extract	Pet.ether Extract
1	**Tests for sterols**			
	Salkowski's Test	+	+	+
	Libermann Burchard's Test	+	+	+
2	**Test for glycosides**			
	Baljet's Test	+	-	-
	Brontrager Test	+	-	-
3	**Tests for saponins**			
	Foam Test	+	+	-
4	**Test for carbohydrates**			
	Molish's Test	+	-	-
	Barfoed's Test	+	-	+
	Benedict's Test	+	-	-
5	**Tests for alkaloids**			
	Mayer's Test.	+	+	+
	Wagner's Test.	+	+	+
	Dragendorff's Test	+	+	+
6	**Tests for flavonoids**			
	Ferric chloride Test.	+	+	+
	Shinoda Test.	+	+	+
	Alkaline Reagent Test.	+	+	+
	Lead Acetate Test.	+	+	+
7	**Tests for tannins**			
	Ferric chloride Test.	-	+	-
	Gelatin Test	-	+	-
8	**Test for amino acid and protein**			
	Biurete test	+	-	-

Table 4: TLC Fingerprinting

Solvent system: Toluene, Ethyl Acetate: Formic acid [5:4.5:0.5]	 TLC IN DAY LIGHT	 TLC at 366 nm	 TLC at 254 nm
R_f value	0.50,0.53,0.68	0.25,0.27,0.32,0.50,0.53, **0.59, 0.64,0.69,0.77**	**0.21,0.36,0.46,0.52,0.60,0.68**

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