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PHARMACOGNOSTICAL AND PRELIMINARY PHYTOCHEMICAL SCREENING OF ROOTS OF *SECHIUM EDULE (JACQ.)SW*

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1. INTRODUCTION

Plants have been an exemplary source of medicine since ancient age. Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments. India has about 45000 plant species and among them, several thousands have been claimed to possess medicinal properties. From the ancient time and in the absence of reliable liver protective drugs in allopathic medical practices, the plants from different families have been used in herbal formulations for the treatment of liver disorder.[2] The selected plant *Sechium edule* belonging to family Cucurbitaceae , grown up in many regions of the world preferably between 800 and 1800 m altitude. In many regions there are variants adapted to cultivation at sea leveland traditionally used to treat various diseases.[5] Though the plant is native to South Africa but, in India, it is widely cultivated in the Meghalaya and Sikkim at elevation of 850m-1700 m and in some parts of West Bengal and Assam. Traditionally it is reported that juice from the leaves of the plant from *Sechium edule* from Cucurbitaceae family used for the treatment of various kind of liver disorder. So in the present study been made to evaluate the pharmacognostical and phytochemical parameters of roots of *Sechium edule*. The identification and authentification of the plant material taxonomically and pharmacognostically is important to standardized and also to avoid spurious or adulterated drugs and phytochemical analysis is also important to find out the presence of active constituents present in the roots of *Sechium edule*.

2. MATERIALS AND METHODS

Collection of Plant - The entire plant and the plant parts were collected from West Bengal during the month of June-July and washed in running water, segregated from the grass and

other extraneous material and the field data of the plant like its height, flower color and soil condition were noted in the note book.

Authentication of Plant

The selected plant was collected in flowering and fruiting condition and deposited in the form of herbarium and submitted to the Botany Department of Calcutta University and authentication of submitted plant was carried out by the authorised person of Department of Botany, University of Calcutta, Kolkata, W.B., India.

Macroscopical Observation

The macroscopical observation were carried out as per performed by the standard methods to determine the shape, size, taste, colour, odour of powdered drug etc.

Microscopical Examination

Transverse section of root

The roots of the plant was sectioned by using a new blade, a clean glass slide taken and placed a drop of glycerine water in the centre of slide into this the section was placed then into this one drop of phluroglucinol and HCL (1:1) given. Placed the cover slip by using the finger and thumb of the left hand and let the edge of the cover slip rest on the slide at the left hand edge of the drop. Insert a dissecting needle under the right hand edge of the cover slip and let the latter rest on the needle. Lower the cover slip slowly on to the drop of the liquid exactly fills the space between the slide and the cover slip without any air bubbles being trapped inside. Then placed the slide in position on the stage of microscope and observed the T.S of the leaf by using 10X and 45X lens.

Powder microscopy

For powder analysis the whole plant was collected and washed thoroughly with water to remove any unwanted matter. This was further dried in the shade. After complete drying, it was powdered and passed through sieve no. 60. This was further subjected with different reagents like chloralhydrate, phloroglucinol and conc. HCl (1:1), iodine solution for the presence of the constituents like lignin, starch and calcium oxalate crystals.

Physicochemical constants

(i) Determination of Ash Value: Ash values are helpful in determining the quality and purity of a crude drug especially in a powdered form. The following parameters were performed like Total ash, Acid insoluble ash, Water soluble ash as per standard procedure.

(ii) Determination of Extractive Values

Extractive values of a crude drug determine the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which no suitable chemical or biological assay exists. Under this parameter Alcohol soluble extractive and Water soluble extractive value were performed as per standard procedure.

(iii) Determination of moisture content: Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. Drying plays a very important role in the quality as well as purity of the material. Moisture will lead to the activation of enzymes and gives suitable condition, to the proliferation of living micro-organisms.

(iv) Fluorescence analysis: The organic molecules absorbs light usually over a specific range of wavelength, get excited to a high energy level and many of them emit such radiations while coming back to the original state. Such a phenomenon of re-emission of absorbed light that occurs only when the substance is receiving the exciting rays is known as "Fluorescence". The powdered drug was examined under U.V. and ordinary light with different reagents. The powdered drug was taken in a petridish and treated with different reagents. These were observed under different wavelengths i.e., visible rays and ultraviolet rays (254 nm and 365 nm). Various colour radiations emitted were observed and noted.

(iv) Behaviour of powder of roots of *Sechium edule* with different reagents / solvents

Powdered drug was examined by mixing with different solvents or reagents as per the procedure and the colour changes was observed in naked eyes under sufficient light.

Preliminary Phytochemical Evaluation

Preparation of Plant Extract: The roots of the plant were collected and washed thoroughly with water to remove any unwanted matter. This was further dried in shade. After complete drying it was powdered and passed through sieve no 60 and stored in an air tight container. Then using this air dried powder Successive Solvent Extraction was done using Soxhlet apparatus. The extraction was carried out, by using solvents of increasing polarity starting from Petroleum Ether, Benzene, Chloroform and Acetone, Ethyl acetate, methanol, Ethanol and water respectively. The concentrated extracts were re-dissolved in respective solvents & subjected to various chemical tests as per the standard methods for the identification of the various constituents.

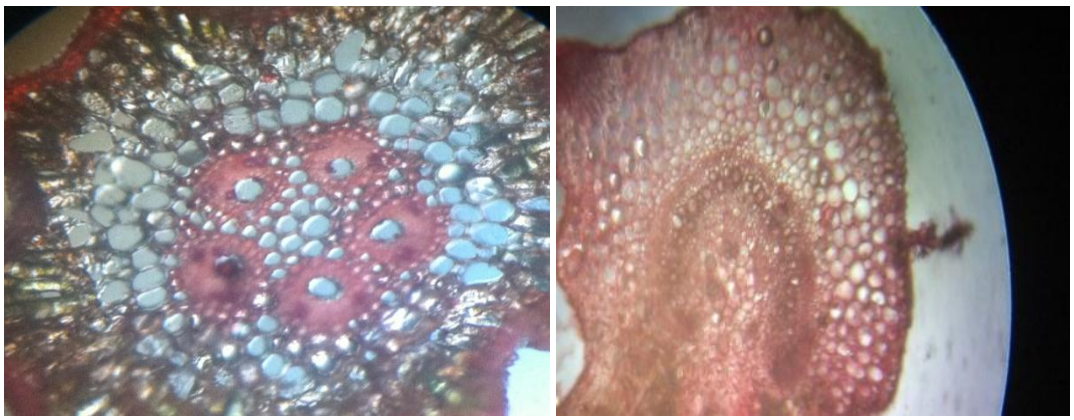
3. RESULT AND DISCUSSION

Table No. 1. Macroscopical Evaluation

Colour	Brown to deep brown (Fresh form) Light brown to Yellowish-brown (After drying)
Odour	Characteristic
Taste	Slightly bitter
Size	Various size in length, diameter of root is 1cm to 4 cm
Shape	Cylindrical, irregular
Extra features	Longitudinal wrinkles and small trichomes

Microscopical Examination

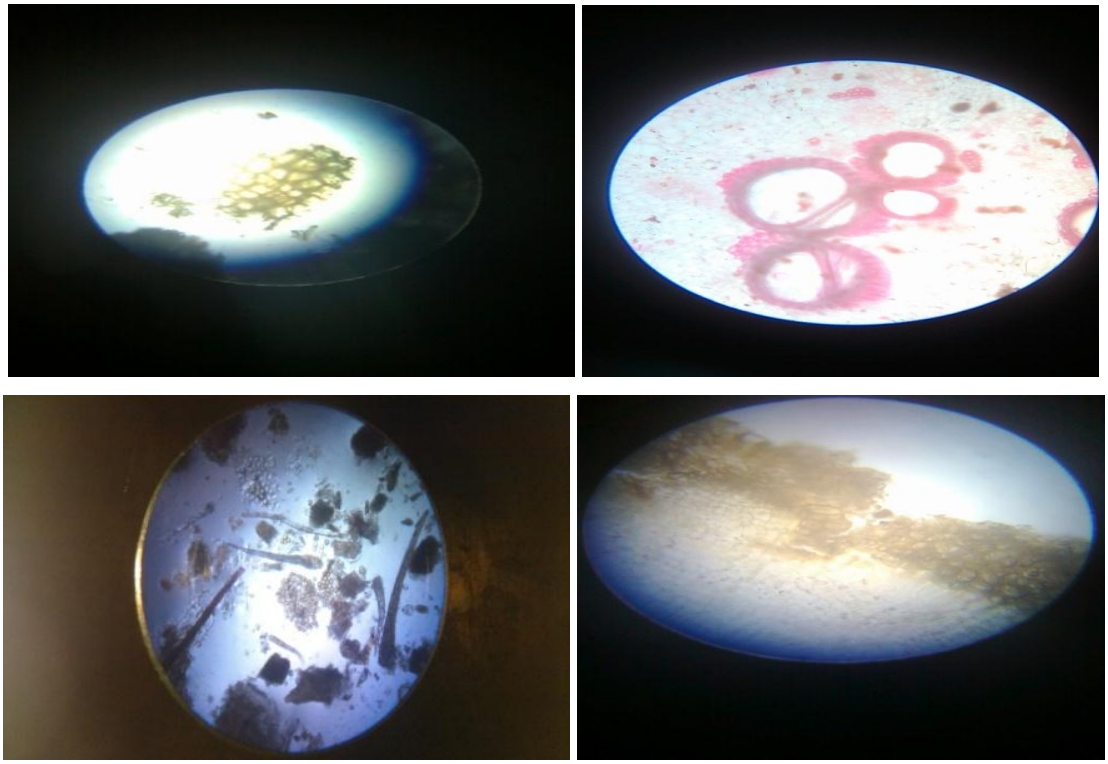
T.S. of root: The fresh root of *Sechium edule was* taken for transverse section (T.S.),When T.S. of the root was mounted with chloral hydrate, phloroglucinol and dil. HCl and stained with saffranin, iodine solution following elements were observed- Cork, Starch grains, Fragment of vessels, Fibres, Calcium oxalate crystal, Xylem, Phloem.



Powder microscopy of root

The powdered of roots of *Sechium edule* is light brown in color, slight bitter in taste and has characteristic odour. When powder was mounted with chloral hydrate, phloroglucinol and dil. HCl and stained with saffranin, iodine solution following elements were observed-

Flatted Starch grains, Fragment of vessels, Fibres, Calcium oxalate crystal, Xylem, Phloem.



Ash Value: Ash values are helpful in determining the quality and purity of a crude drug especially in a powdered form. The object of ashing the vegetable drug is to remove all traces of organic matter that may otherwise interfere in an analytical determination. On incineration, a crude drug normally leaves an ash consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium.

Table No. 2. Ash Value

Sl. No.		
1.	Total ash	7.03% (w/w)
2	Acid insoluble ash	2.70% (w/w)
3	Water soluble ash	3.60% (w/w)

Extractive Values: Extractive values of a crude drug determine the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which no suitable chemical or biological assay exists.

Table No. 3. Extractive Value

Sl. No.	Extracts	Extractive value (% w/w)
1	Alcohol soluble extracts	16.89 %
2	Water soluble extracts	12.16 %

Moisture content

Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. Drying plays a very important role in the quality as well as purity of the material. Moisture will lead to the activation of enzymes and gives suitable condition, to the proliferation of living micro-organisms.

Table No. 4. Moisture Content

1st observation	2nd observation	3rd observation	Avg. Value
9.71%(w/w)	9.70% (w/w)	9.69% (w/w)	9.70% (w/w)

Fluorescence Analysis- The organic molecules absorbs light usually over a specific range of wavelength, get excited to a high energy level and many of them emit such radiations while coming back to the original state. Such a phenomenon of re-emission of absorbed light that occurs only when the substance is receiving the exciting rays is known as "Fluorescence". These were observed under different wavelengths i.e., visible rays and ultraviolet rays (254 nm and 365 nm). Various colour radiations emitted were observed and noted.

Table No. 5. Fluorescence Analysis

Treatment of powder of *Sechium edule* roots	Visible rays	Ultra -violet light	
		short wave (254 nm)	long wave (365 nm)
Powder as such	Light brown	Deep brown	Black
Powder+50% H_2SO_4	Brown	Dark brown	Black
Powder+50% HNO_3	Brown	Dark brown	Black
Powder+5% KOH	Brown	Deep brown	Black
Powder+Methanol	Brown	Deep brown	Black
Powder+1N HCl	Light brown	Deep brown	Black
Powder+1N Methanolic NaOH	Deep brown	Dark brown	Black
Powder+Cold water	Light brown	Brown	Black
Powder+Hot water	Light brown	Brown	Black
Powder+Picric acid	Deep brown	Dark brown	Black
Powder+Ammonia solution	Brown	Deep brown	Black
Powder+Chloroform	Deep brown	Dark brown	Black
Powder+Glacial acetic acid	Deep brown	Dark brown	Black
Powder+5% Iodine solution	Deep brown	Dark brown	Black
Powder+$FeCl_3$	Brown	Dark brown	Black

Behavior of powder of *Sechium edule roots* with different reagents / solvents- Powdered drug was examined by mixing with different solvents or reagents as per the procedure and the colour changes was observed in naked eyes under sufficient light.

Table No. 6. Behavior of powder of *Sechium edule roots* with different reagents / solvents

Treatment of powder of *Sechium edule* roots	Visible rays
Powder as such	Light brown
Powder+50% H_2SO_4	Brown
Powder+50% HNO_3	Brown
Powder+5% KOH	Brown
Powder+Methanol	Brown
Powder+1N HCl	Light brown
Powder+1N Methanolic NaOH	Deep brown
Powder+Cold water	Light brown
Powder+Hot water	Light brown
Powder+Picric acid	Deep brown
Powder+Ammonia solution	Brown
Powder+Chloroform	Deep brown
Powder+Glacial acetic acid	Deep brown
Powder+5% Iodine solution	Deep brown
Powder+$FeCl_3$	Brown

Preparation of Extracts- The shade-dried roots were powdered to get a coarse granule. About 500 grams of dried powder was extracted first with Petroleum Ether at 60 to 65^{o}C by continuous hot percolation, using Soxhlet apparatus. The extraction was carried out, by using solvents of increasing polarity starting from Petroleum Ether, Benzene, Chloroform and Acetone, Ethyl acetate, methanol, Ethanol and water respectively. The extraction was continued for 72 hours. The Petroleum Ether extract was filtered and concentrated to dry mass by using vacuum distillation. The marc left after petroleum ether extraction was taken and then subsequently extracted with Chloroform, acetone, ethyl acetate, methanol, ethanol & water for 72 hrs. These extracts were filtered & concentrated to a dry mass.

Table No. 7. Successive solvent extraction & Nature of extracts

Sl. No.	Solvent	Color	Consistency	% of Yield(%w/w)
1.	Petroleum Ether	Black	Sticky	2.69
2.	Benzene	Black	Sticky	1.73
3.	Chloroform	Black	Sticky	1.22
4.	Acetone	Black	Sticky	1.27
5.	Ethyl acetate	Black	Sticky	1.05

6.	Methanol	Reddish Black	Sticky	2.94
7.	Ethanol	Reddish Black	Sticky	3.84
8.	Water	Dark Brown	Sticky	1.52

Phytochemical Screeing- The concentrated extracts were re-dissolved in respective solvents & subjected to various chemical tests as per the standard methods for the identification of the various constituents.

Table No. 8. Phytochemical Evaluation of *Sechium edule* extracts

Test	**P**	**B**	**C**	**A**	**EA**	**M**	**E**	**W**
Alkaloids	_	_	_	_	_	+	+	_
Carbohydrates	_	_	_	+	+	+	+	+
Glycosides	_	_	_	_	_	+	+	_
Phytosterols	_	_	_	_	_	+	+	_
Fixed oil and fats	+	+	+	+	+	_	_	_
Phenolic compound and Tannins	_	_	_	_	_	+	+	+
Saponins	_	_	_	+	+	+	+	+
Proteins& Aminoacids	_	_	_	_	_	+	+	_
Gums and Mucilage	_	_	_	_	_	_	_	_

P = Petroleum Ether, B = Benzene, C = Chloroform, A = Acetone,

EA = Ethyl acetate, M = Methanol, E = Ethanol, W = Water

4. CONCLUSION

An attempt has been made to evaluate the pharmacognostical and preliminary phytochemical parameters of roots of *Sechium edule.* The identification of the plant material taxonomically and pharmacognostically is important to provide pharmacognostical standards and also to avoid spurious or adulterated drugs. The physicochemical constants like moisture constant, ash values such as total ash, acid insoluble ash, and water soluble ash, extractive values such as water soluble extractive value and alcohol soluble extractive value were determined. These help in formulating pharmacopoeial standards for the drug. The extracts obtained by successive solvent extraction were subjected to preliminary phytochemical analysis to find out the presence of compounds.

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