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Pharmacognostical and preliminary phytochemical investigation of *Equisetum debile* Roxb.

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ABSTRACT

Herbal medicines are increasingly gaining popularity as they possess minimal or no side effects therefore, authentication of the crude drugs are necessary to ensure that the herbal drugs so obtained are safe and of optimal quality. In the present study, various pharmacognostical parameters such as organoleptic evaluation, macroscopic evaluation, microscopic evaluation, physico chemical evaluation (moisture content, loss on drying, ash values, extractive values), and phytochemical evaluation were conducted for the plant *E. debile* Roxb. (Equisetaceae). Pharmacognostic evaluation helps to identify the commercial varieties, substitutes, adulterants and any other quality control parameter of the drugs. It is a simple and reliable tool, by which complete information of the crude drugs can be obtained (WHO 1998). Pharmacognostical evaluation helps to establish the authenticity of the drugs, it also helps to differentiate the drugs from other species and it also helps to detect any form of adulteration.

Keywords: *Equisetum debile* Roxb., pharmacognostical, organoleptic evaluation, phytochemical analysis.

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INTRODUCTION

The traditional healers of various tribal communities have made use of the local plants available to treat various disorders. Such plants have proved to be effective in treating an array of disorders however, as these plants lack proper documentation, they are losing their importance. Medicinal plants are the gifts of God to the human beings. The term Ethno medicines are defined as those preparations, prepared by tribal people from local herb or animal products for the treatment of several diseases [1, 2].

In India, plants having medicinal properties have been used to treat numerous disorders since a very long time. Various plants have been used in folk medicine since times immemorable. These traditional healers hardly used to document the plants and their uses, so their significance is getting diminished day by day. This necessitates the importance of documentation of these plants which will help to preserve the rich cultural heritage that has been endowed to us by our ancestors. Therefore, for proper documentation of these plants studies have to be conducted which will ensure the quality and authenticity of the crude drugs. World Health Organization (WHO) estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs for their primary health care. WHO has emphasized the need to ensure the quality of medicinal plants by using modern control techniques and applying suitable standards [3].

Pharmacognostical study helps to identify adulteration. It ensures plant identity, lays down standardization parameters which prevents other forms of adulteration. It also helps in plant authentication and ensures quality of the herbal products and will improve safety and efficacy of natural products.

Equisetum debile Roxb. [Fig. 1] is a pteridophyte belonging to the family Equisetaceae. It is found in parts of temperate Asia. It is a herb which bears spores and has erect, cylindrical and hollow stem. It has long, thin and ribbed branches and are two or three in whorls. The nodes [Fig. 2] are encircled by a tight sheath of connate scale like leaves. It possesses an oblong strobilus at the end of the branches. *Equisetum debile* Roxb. vegetatively propagates by the splitting of rhizome and the spore formation occurs during the months of June-July[4]. Once dispersed, the spores germinate within a few days at favourable climates. Gametophytes are produced by

means of protogynous reproduction which means formation of the female gamete before the male gamete [5]. *Equisetum debile* Roxb. is found in South East Asia, Southern China, India, Nepal, and Sri Lanka. It is mostly seen in humid places at 2,600m.It is known as Simejhar, kukurejhar or Sallibisalli in Nepali [4].

E. debile is used to cure scabies, burns, malarial fever, gonorrhea, dislocated bones, and liver and chest complaints [4]. In the North Eastern region, it is traditionally used to lower blood glucose levels, in the treatment of rheumatism and also to treat joint pain [6]. It is also used as a clotting agent and is used in wounds, coughing up of blood and bleeding of nose. It is also a potent astringent due to which it is used in bleeding urinary tract. *E. debile* contains salicylic acid and silicates in large amount [7].

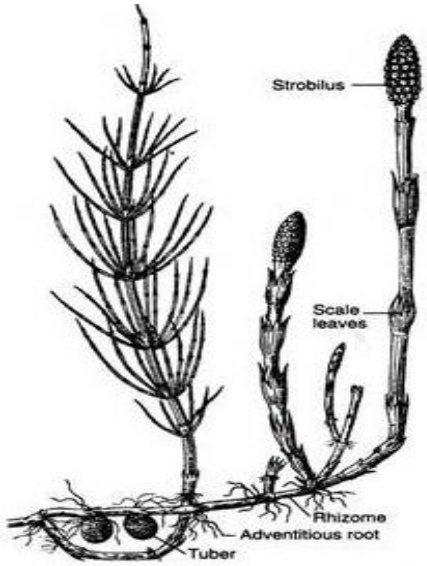

Fig. 1: Whole plant of *Equisetum debile* Roxb.

Fig. 2: Nodes and strobilus of *E. debile* Roxb.

MATERIALS AND METHODS

The whole plant of *Equisetum debile* were collected from Mungpoo, West Bengal, India in the month of August 2018. The plant was identified as per the standard literature [8, 9].

The collected plant material was shade dried. The dried plant material (whole plant) was coarsely powdered by means of a mechanical grinder. Then the resulting plant material was used for further studies.

2.1. Chemical and reagents

All the reagents used were of analytical grade purchased from Sigma-Aldrich (USA) and S. D. Fine Chemical Ltd., Mumbai.

2.2. Instruments and equipments

Standard glassware of Borosil were used for experimental purpose. Hot air Oven, Muffle furnace, Water bath (Remi Instrument Ltd., Mumbai), Digital balance (CP225 D, Sartorious AG, Germany), Microscope (Almicro, Micromeasures and Instruments, Mumbai), Mixer grinder (Jaipan Instrument Ltd., Mumbai) Vacuum pump, (Riviera Glass Pvt. Ltd., Mumbai), U.V. cabinets (Technology Instruments, Mumbai) were used for the study.

2.3. Pharmacognostical Evaluation

a. Macroscopical Evaluation:

The macroscopical evaluation was carried out as per the standard methods to determine the shape, size, taste, colour, odour, and texture of the crude drug.

b. Powder microscopy:

A small amount of powdered plant material was taken and boiled with chloral hydrate to remove chlorophyll. Then it was stained with phloroglucinol solution for few minutes followed by conc. HCL (1:1) in a watch glass. It was mixed well and allowed to stand for about 3 mins. It was then mounted in a glass slide and a drop of glycerine was added. Then it was observed under a microscope. The powder was again stained with iodine solution in order to identify starch grains. It was then treated with 60% H_2SO_4 to identify CaC_8O_4 crystals. The microscopic characters were then noted [10].

2.4. Physico-chemical Analysis

Physicochemical values such as the percentage of ash values and extractive values were performed according to official methods prescribed in Indian Pharmacopeia 1996 and WHO guidelines on quality control methods for medicinal plant material.

2.4.1. Extractive Values

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values are determined to indicate the nature of the constituents present in a crude drug.

a. Determination of Alcohol Soluble Extractive Value

About 5 gm. of coarsely powdered, air dried whole plant of *Equisetum debile* was macerated with 100 ml of 90% ethanol in a closed flask for 24 hours shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly. Then 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish.

It was then dried at 105°C and weighed. The percentage w/w of alcohol soluble extractive value was calculated with reference to the air-dried drug.

b. Determination of Water Soluble Extractive Value

This method is applied to those drugs which contain water soluble active constituents such as tannins, sugars, plant acid, mucilage, glycoside etc.

About 5gm of the air dried, coarsely powdered plant material was taken and macerated with 100 ml of chloroform water (2.5ml in 1000ml of water) in a stoppered flask for 24 hours. It was shaken frequently during the first 6 hours and allowed to stand. After 18 hours it was filtered rapidly. Then 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish, dried at 105°C and weighed. The percentage w/w of water-soluble extractive value was calculated with reference to the air-dried drug.

c. Determination of Moisture Content

Moisture is an inevitable component of crude drugs which must be eliminated as far as possible. Drying plays an important role in the quality as well as purity of the material. Moisture will lead to the activation of enzymes and also gives shelter to micro-organisms.

About 5gm of the air dried, coarsely powdered plant material was accurately weighed in a tarred watch glass. The drug was kept in a hot air oven at 105^0 C and dried for a period until a constant weight was obtained. The difference in weight gives the moisture content of the drug.

2.4.2. Ash Values

Ash values are helpful in determining the quality and purity of crude drug, especially in the powdered form. It usually represents the inorganic salts naturally occurring in the drug and adhering to it. Hence, an ash determination furnishes a basis for judging the identity and cleanliness of a drug. Procedure given in Indian Pharmacopoeia was used to determine the different ash values such as total ash, acid insoluble ash, and water soluble ash etc.

a. Determination of total ash value

2- 3 grams of air dried powdered drug was taken in a tarred silica crucible. It was incinerated at the temperature not exceeding 450^0 C until free from carbon. The crucible was cooled and weighed. Then the percentage of total ash was calculated with reference to the air-dried drug.

b. Determination of acid insoluble ash value

The ash obtained as directed under total ash value was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, ignited and weighed, then the percentage of acid insoluble ash was calculated with reference to the air dried drug.

c. Determination of water soluble ash value:-

The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water-soluble ash. The percentage of water soluble ash was calculated with reference to the air-dried drug [11, 12, 13, 14].

$$Ash\ value = \frac{Initial\ weight - Final\ weight}{Initial\ weight} X\ 100$$

2.4.3. Fluorescent analysis

The fluorescent analysis of shade dried and powdered, methanolic extract of *Equisetum debile* was studied under UV light and daylight. Fluorescent analysis of plant powder was carried out according to the methods [15, 16]. Powder was subjected to different chemicals like, 1N NaOH, Acetic acid, 1N HCl, 1N HNO_3, 5% Iodine, 5 % $FeCl_3$, 1N NaOH in methanol.

2.4.4. Extraction of Phytoconstituents:

About 500gms of coarsely powdered plant material of *Equisetum debile* was taken in a 3000ml round bottomed flask. It was extracted successively first with petroleum ether and then it was refluxed first with chloroform, ethyl acetate, and then methanol for about 7-8 hrs. The solutions were filtered through a Buchner funnel to remove the impurities present. The filtrates were then concentrated by rotary evaporator and finally placed in a desiccator to remove the excessive moisture [17].

2.4.5. Preliminary Phytochemical Analysis:

The concentrated extracts were subjected to chemical tests as per the methods mentioned below for the identification of various constituents.

a. Detection of Carbohydrates:

The extract was dissolved first in HCL (except for the methanolic extract) and then water and filtered. The filtrate was subjected to the following tests:

Molish's test:

To 2-3 ml extract, few drops of α-naphthol solution in alcohol were added and concentrated H_2SO_4 was added from the sides of the test tube. A violet ring at the junction of two liquids indicate the presence of sugar.

Fehling's test:

1ml Fehling's A and 1ml Fehling's B Solution was mixed and boiled for one minute. To this equal volume of test solution was added. On heating in boiling water bath for 5-10 min., initially it forms a yellow and then brick red precipitate.

Benedict's test:

Equal volume of Benedict's reagent and test solution were mixed in test tube and heated on boiling water bath for 5 min. Solution appears green, yellow or red color depending on the amount of reducing sugar present in test solution.

Barfoed's test:

Equal volume of Barfoed's reagent and test solution were added and heated on boiling water bath and cooled. Red precipitate was observed indicating the presence of monosaccharide.

b. Detection of Saponins:

Foam test:

The drug extract or dry powder was shaken vigorously with water. It was then observed for persistent foam.

c. Detection of Alkaloids:

Dragendorff's test:

To 2-3 ml filtrate, few drops of Dragendorff's reagent was added. Then it was observed for the formation of orange brown precipitate.

Mayer's test:

2-3 ml of filtrate was mixed with few drops of Mayer's reagent then it was observed for the formation of precipitate.

Wagner's Reagent:

2-3 ml filtrate was treated with Wagner's reagent then it was observed for the formation of reddish brown precipitate.

d. Detection of Tannins and Phenolic Compounds:

To 2-3 ml of the filtrate, few drops of following reagents were added and the changes in color were observed:

- 5 % $FeCl_3$ solution: - deep blue- black color.
- Lead acetate solution: - white precipitate.

e. Detection of Flavonoids:

Shinoda test:

To the filtrate, 5 ml of 95% ethanol, few drops concentrated HCl and 0.5 g magnesium turnings were added. Pink color was observed. This indicates the presence of flavonoids.

f. Detection of Proteins and Amino acids:

Biuret test:

To 3 ml test solution, 4 % NaOH and few drops of 1 % $CaSO_4$ solution was added and observed for violet or pink color.

Million's test:

3 ml of test solution was mixed with 5 ml of Million's reagent to obtain white precipitate. The precipitate was further warmed which turned to brick red precipitate which then dissolved giving red color.

Ninhydrin test:

To 3 ml test solution 3 drops of 5 % Ninhydrin solution was added and heated in boiling water bath for 10 min. It was then observed for purple to bluish color.

g. Detection of Phytosterols:

Libermann-Burchard's test: Few ml of the extract was taken and glacial acetic acid was added to it. To this solution 1-2 drops of conc. H_2SO_4 was added along the sides of the test tube. An array of colour changes show the presence of phytosterols

h. Detection of Glycosides:

Legal's test:

To the filtrate, 1 ml Pyridine and 1 ml sodium nitroprusside were added and then observed for pink to red color.

Kellar- killiani test:

To 2 ml extract, glacial acetic acid, one drop of 5 % $FeCl_3$ and conc. $H_2SO_4$was added and observed for reddish brown color at the junction of the two liquids and the upper layer appears bluish green.

i. Detection of Gums and Mucilages:

To the filtrate, 10ml of distilled water was added and to this 25ml of absolute ethanol was added with constant stirring. White or cloudy ppt. indicates the presence of gums and mucilages [18, 19].

RESULTS

Table 1.Macroscopic evaluation

Parameter	Observation
Size	15-20 cm in length
Colour	Green
Odour	Characteristics
Taste	Slightly bitter

Powder Microscopy

Coarsely powdered whole plant of *Equisetum debile* showed the presence of Xylem vessels, Phloem fibers, epidermal cells and Stomata when treated with Phloroglucinol-HCL. Upon treating with Iodine solution it showed the presence of Starch grains and upon treating with H_2SO_4, it showed the presence of Calcium Oxalate Crystals.

Table 2.Powder microscopy of *Equisetum debile*

SL. NO.	REAGENTS	COLOUR	OBSERVATION
1	Phloroglucinol-HCL	Pink	Xylem, Phloem fibers, Epidermal cells, Stomata.
2	Iodine solution	Bluish black	Starch grains
3	H_2SO_4	Colourless	Calcium Oxalate Crystals

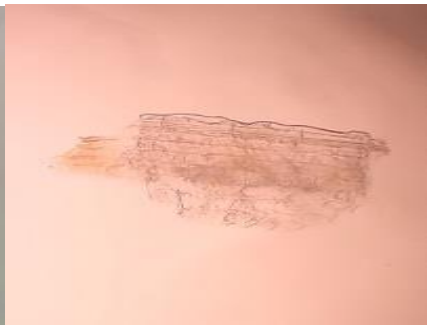

Fig. 3 **Fig. 4** **Fig. 5**

Powder microscopy depicting Spiral Xylem vessels (fig.3), Phloem fibers (fig.4) and Epidermal Cells (fig. 5) respectively.

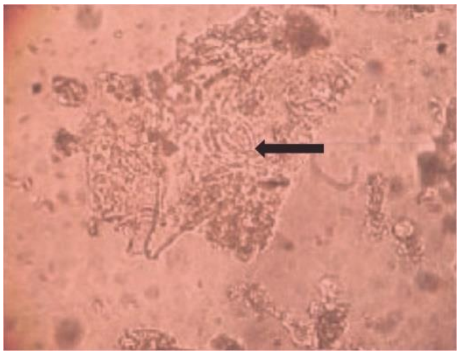

Fig. 6: Powder microscopy depicting Stomata.

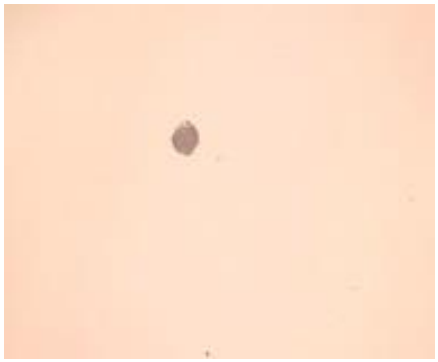

Fig. 7: Powder microscopy depicting Starch Grains

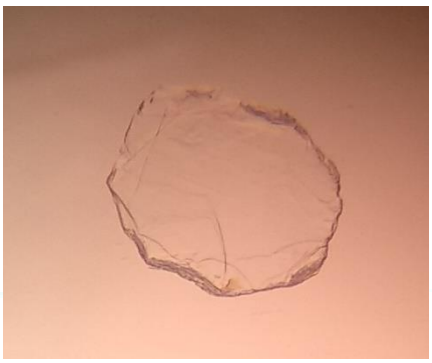

Fig. 8: Powder microscopy depicting Calcium oxalate crystals

Physico-chemical analysis:

The results of extractive value, moisture content and ash values are shown in the tables below:

Extractive value:

Table 3: Extractive value of *Equisetum debile*

SL. NO.	TYPE OF EXTRACT	PERCENTAGE(W/W)
1.	Alcohol	4
2.	Water	14.32

Moisture content:

Table 4: Moisture content of *Equisetum debile*

Wt. of drug taken	Percentage Loss in weight (w/w)	Mean value
1gm	1.4	1.4
1gm	1.1	
1gm	1.6	

Ash Value:

Table 5: Total ash value of *Equisetum debile*

Wt. of drug taken (gm)	Weight of ash obtained	Percentage w/w total ash	Mean value
3	0.269	9.83	10.16
3	0.271	10.66	
3	0.277	10.01	

Table 6: Acid insoluble ash value of *Equisetum debile*

Weight of the drug taken (gm)	Weight of ash obtained	Percentagew/w acid insoluble ash	Mean value
3	0.284	6.1	6.033
3	0.280	5.5	
3	0.279	6.5	

Table 7: Water soluble ash value of *Equisetum debile*

Weight of the drug taken (gm)	Weight of ash obtained	Percentage w/w water soluble ash	Mean value
3	0.235	3.6	4.06
3	0.233	4.1	
3	0.240	4.5	

Table 8: Fluorescence analysis of *Equisetum debile*

Reagents	Visible	Short UV	Fluorescence
Powder + 1% glacial acetic acid	Light Brown	Yellowish brown	Brownish black
Powder +10% NaOH	Brown	Dark brown	Dark brown
Powder + dil. NH_3	Yellowish brown	Dark yellowish brown	Bluish brown
Powder + Conc. HNO3	Light brown	Light brown	Brown
Powder+dil.NH_3+Conc.HNO_3	Yellowish brown	Blackish brown	Dark brown
Powder +1M HCl	Brownish yellow	Light brown	Blackish brown
Powder +1M $H2SO_4$	Dark brown	Dark brown	Yellowish black
Powder + 10% $FeCl_3$	Brownish yellow	Brown	Dark brown
Powder +Acetone + Methanol	Reddish brown	Light brown	Brownish yellow
Powder +10% Iodine	Light brown	Brown	Black

Table 9: Phytochemical analysis of *Equisetum debile*

TEST	PETROLEUM ETHER	CHLOROFORM	ETHYLACETATE	METHANOL
Carbohydrates	+	+	+	+
Saponins	-	-	-	-
Alkaloids	-	-	-	-
Tannins and phenolic compounds	-	-	-	-
Flavonoids	-	-	-	-
Proteins and amino acids	-	-	-	-
Phytosterols	-	-	-	+
Glycosides	-	-	-	+
Gums and mucilage	-	-	-	-

DISCUSSION

To identify a crude drug, its evaluation parameters play an important role. Therefore, by taking the whole plant of *E. debile* various pharmacognostical parameters were carried out. The identity and degree of purity of crude drugs can be affirmed through macroscopical and microscopical studies. Morphological study is a qualitative study and it involves the study of morphological parameters. Macroscopic evaluation was expressed in Table no 1. The results of powder microscopy (Table no. 2) showed the presence of spiral xylem vessels (Fig. 3), phloem fibers (Fig. 4), epidermal cells (Fig. 5), stomata (Fig. 6), starch grains (Fig. 7) and calcium oxalate crystals (Fig. 8). Alcohol and water soluble extractive study was also carried out and the results obtained were 4 and 14.32% w/w respectively (Table no. 3). This shows that more water soluble compounds are present in the chosen plant material. Extractive value helps to identify exhausted drugs and it also highlights the type of chemical constituents the drug contains. The moisture content (1.4%w/w) has been shown in Table no. 4. The moisture content should be minimum, as high moisture content leads to the degradation of the active constituent present in the plant material. The amount of total ash (Table no. 5) was found to be 10.16%w/w; acid insoluble ash (Table no. 6) was 6.033%w/w and water soluble (Table no. 7) ash was 4.06%w/w. Ash values help us to determine the purity and quality of crude drugs. Total ash is the residue which remains after incineration, acid insoluble ash is that part of total ash which is insoluble in dil. HCL and water soluble ash is that part of total ash which is soluble in water. The result of fluorescent analysis of the whole plant of *E. debile* has been shown in Table no. 8. Fluorescence is a phenomenon shown by certain compounds present in the plant material. Some compounds show fluorescence in daylight, some produce fluorescence when seen under ultraviolet light and some can be converted into fluorescent derivatives upon treatment with various chemical reagents. Therefore, this parameter can be used to qualitatively assess crude drugs. The plant drug was subjected to preliminary phytochemical analysis, which revealed the presence of Carbohydrates, Phytosterols and Glycosides (Table no. 9).

CONCLUSION

*E. debile*is a medicinal plant that is widely used by the people of the Himalayan region. It was observed that this plant was secluded and much work had not been done in this regard. Therefore, pharmacognostical study was carried out in order to identify, validate and authenticate the crude drug which in turn can provide useful information for preparing various formulations from the crude drug. These formulations will then help in treating various disorders and ailments. The secondary metabolites present in the plant material are responsible for the therapeutic effects. These secondary metabolites are important both in the field of research as well as in industries. Further study is needed to isolate and identify the compounds present in different extracts of this plant. Pharmacological activity will also be carried out for its medicinal value.

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