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Phytochemical Study and Physicochemical Evaluation Whole Plant of *Equisetum diffusum* D. Don

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ABSTRACT

Pteridophytes plants are neglected groups of plant communities. Now there is a lot of scopes to study in this plant group. *E. diffusum* plant is a popular plant of this group whose tribble and the local community was used in bone fracture and fish feed in all over the country. Our main objective of this research was collecting data for use in medicinal, economical, commercial importance and proposed to detailed study in further. Material and methods adopted for this study were according to Ayurvedic pharmacopeia of India (API) and standard procedure prescribed by various earlier researchers. The experimental findings of this study were helpful in the development of monographs of this plant because no such type of previous work was done on this plant.

Keywords: *Equisetum diffusum*, API, Physicochemical, Phytochemical, Fluorescence, Monograph.

INTRODUCTION

Equisetum diffusum D. Don main stem is annual, horizontal, hollow, underground, monomorphic, dark brown and branched shiny brown rhizome. These found along a road in thickets or semi-shaded places and near the river in sandy soil, occurring from 450-3500 m. altitude (Mir S.A. *et al*., 2015; Page C.N. 1974; Singh B. *et al*., 2012). It belonged to Equisetaceae family and it is widely distributed in Sehore, Satpura Hills Hoshangabad, Amarkantak Anuppur, Tamia Chhindwara, Jammu and Kashmir, Himalayan Mountains from Shimla to Tibet, Sikkim, Assam, Meghalaya and South India along with also found in other countries namely Bhutan, Myanmar, Nepal, Pakistan, Japan and Vietnam (Shakoor *et al*., 2015; Singh & Upadhyay, 2014; Bawistale, 2010; Singh *et al*., 2012; Singh & Sinku 2015). It is commonly known as 'Horsetail' or 'Scouring rushes'. The horsetail name arises due to the stalk matched to a horse's tail, the name Equisetum being from the Latin equus means "horse", and seta, means "bristle. The main chlorophyllous stem of the plant is differentiated into nodes and internodes. In stem, fertile and sterile branches in the plant are almost identical and each branch can produce cones (Bir 1978; Hauke 1974). The number of ridges is equal to the number of leaves present on the node and each ridge is found in line with the leaf on the top node. 6 ridges, 6 grooves and were found in the stem of *E. diffusum* plant.

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No past study was conducted on the whole plant (Wh. P.) of *E. diffusum*. So, the present study was performed on the phytochemical study and physicochemical evaluation of the whole plant of *E. diffusum* D. Don which were helpful in the development of monographs of this plant and identification and authentication of this plant.

MATERIALS AND METHODS

2.1 Collection of plant and sample preparation

The whole plant of Equisetum diffusum was collected from the 'Khanda bad' forest range of Budani Tehsil in the Sihor district of Madhya Pradesh, India. The sample was identified by Dr. Ravi Upadhyay, Head & Professor of Botany, Department of Botany Govt. P.G. College Pipariya, Hoshangabad (M.P). The collected sample was washed, dried in tray dryer at 35oC and grinded with the help of electric grinder. The powder was sieved (No. 43) and stored in airtight containers.

2.2 Organoleptic characteristics

An organoleptic character of powder of the whole plant of *Equisetum diffusum* such as colour, odour and taste was recorded.

2.3 Physicochemical evaluation

The percentage yield of physicochemical values like loss on drying at 105^{o}C, total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive, water soluble extractive and chloroform soluble extractive values were carried out in triplicate as per the method described in the Ayurvedic Pharmacopoeia India, API (Anonymous, 1990 & 2008 and Tripathi, 2015).

2.4 Fluorescence study

To record the color properties of powder of the whole plant of *Equisetum diffusum* concerning various chemical and reagents was observed in daylight and ultraviolet light at 366 nm (Chase and Pratt, 1949; Kokoski *et al.*, 1958; Sujan *et al.*, 2009; Parwaiz *et al.*, 2011).

2.5 Phytochemical screening

Sample preparation: Take 10g of prepared powder and 200ml different solvents like water, methanol, chloroform, acetone, hexane and petroleum ether were used for Soxhlet extraction for 72 hours at their boiling points. The obtained extract was filtered through Whatman filter paper no. 1 separately. These extracts were used for the screening of different phytoconstituents by using standard methods (Agrawal *et al.*, 2017; Audu S.A. *et al.*, 2007; Roopashree T.S. *et al.*, 2008; Obasi N.L. *et al.*, 2010, Anonymous, 2010; Thimmaiah S.K. 1999).

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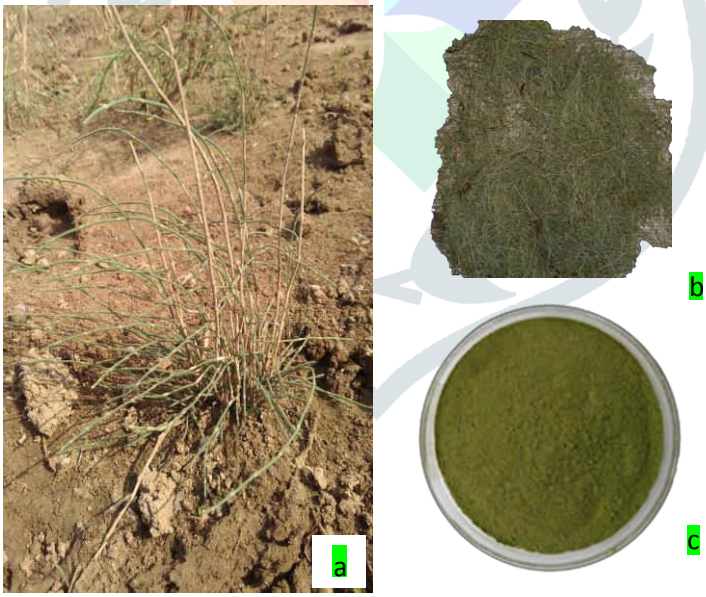


Fig-1 Sample preparation on the Soxhlet extraction method

RESULT AND DISCUSSION

3.1 Organoleptic characteristics

An organoleptic character of powder of the whole plant of *E. diffusum* was like as dark green in color, shriller in odour and taste was tasteless.



a. Plants of *E. diffusum*, b. Dry Wh. P. of *E. diffusum* & c. Powder of Wh. P. of *E. diffusum*

Fig-2: External morphology of *E. diffusum* (a,b,c)

3.2 Physicochemical analysis

Physicochemical parameters were used to check the identity, purity and strength of the plant material. The percentage yields (w/w) of different physicochemical parameters were carried out in triplicate.

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Table-1: The percentage yield (w/w) of various physicochemical analysis parameters

S. No.	Name of parameters	*E. diffusum* (Wh. P.); %w/w (n = 3 ± SD)
1.	Loss on drying (LOD)	2.77633333 ± 1.63097933
2.	Water soluble extractive (WSE)	57.3316667 ± 1.08777678
3.	Methanol soluble extractive (MSE)	42.5983333 ± 1.01907229
4.	Chloroform soluble extractive (ChSE)	10.1716667 ± 0.56476396
5.	Acetone soluble extractive (ASE)	15.35 ± 0.38603756
6.	Hexane soluble extractive (HSE)	6.20333333 ± 0.18583146
7.	Petroleum ether soluble extractive (PESE)	8.83666667 ± 0.53926648
5.	Total ash (TA)	19.7005533 ± 0.03023572
6.	Acid insoluble ash (AIA)	10.3206667 ± 0.36135347
7.	Water soluble ash (WSA)	15.316 ± 0.6338598

The highest extractive value was found in water extract and lowest in hexane extract. The descending order of extractive values was in the water (57.33 ± 1.63), methanol (42.60 ± 1.02), acetone (15.35 ± 0.39), chloroform (10.17 ± 0.56), petroleum ether (8.84 ± 0.54) and hexane extract (6.20 ± 0.19) were recorded in percentage triplicate.

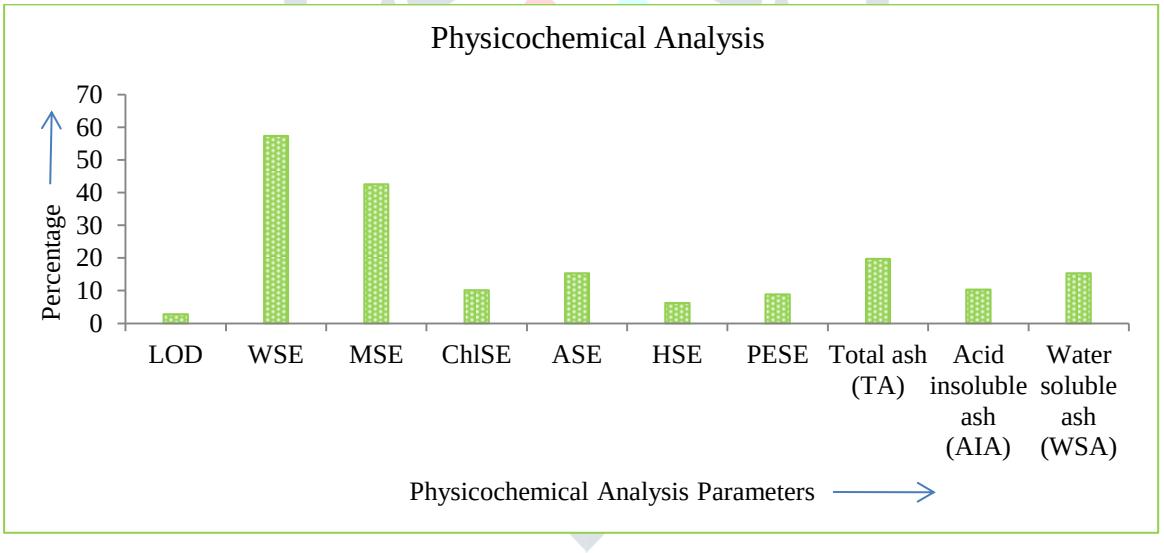


Fig.-3: Physicochemical analysis of wh. p. of *E. diffusum*

3.3 Determination of yield

This is an important study that is used to find out the number of phytoconstituents in various solvents.

Table-2: Yield in different extracts of wh. p. of *E. diffusum*

S. No.	Extract	Yields (in gm.)
1	Water	2.29326
2	Methanol	1.70393
3	Acetone	0.614
4	Chloroform	0.40686
5	Hexane	0.24813
6	Pet. ether	0.35346

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2.29326 gm maximum yield was found in water extract and 0.24813gm minimum yield was found in hexane extract because the phytoconstituents come out on the base of polarity. Water is a more polar solvent in comparison to other solvents.

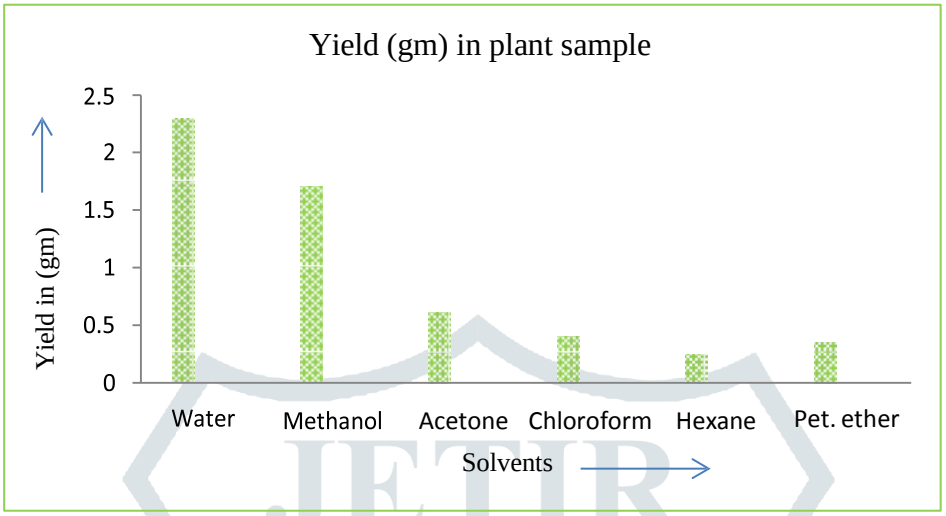


Fig.-4: Yield in wh. p. of *E. diffusum*

3.4 Fluorescence study

Fluorescence studies of *E. diffusum* stem were carried out in daylight and UV light at 366 nm which represent the chemical nature of plant material with specific chemicals and reagents. It is a qualitative test used to know the different properties of powder drugs in a specific condition.

Table-4: Fluorescence studies of Wh. P. of *E. diffusum*

Powder + Reagents	***E. diffusum***	
	Observation in day light	**Observation at 366 nm**
Powder such as	Green colour	Light green colour
Powder + 1N HCl	Greenish brown colour	Green colour
Powder + Conc. H_2SO_4	Black colour	Brick-red colour
Powder + Iodine solution	Green colour	Green colour
Powder + 50% KOH	Dark green colour	Brick-red colour
Powder + 1N NaOH in methanol	Dark green colour	Turmeric colour
Powder + Conc. HNO_3	Dark brown colour	Greenish yellow colour
Powder + 1N NaOH in water	Dark green colour	Greenish brown colour
Powder + 50% HNO_3	Brown colour	Black colour
Powder + 50% H_2SO_4	Dark green colour	Greenish yellow colour
Powder + Distilled water	Dark green colour	Greenish yellow colour

3.5. Preliminary phytochemical screening

Phytochemical screening was performed for the identification of phyto-constituents which present in the plant extracts. It is carried out in six different extracts namely water, methanol, chloroform, acetone, hexane and petroleum ether of the whole plant of *E. diffusum*.

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Table-3: Preliminary phytochemical screening of different extracts of Wh. P. of *E. diffusum*

Name of phytoconstituents	Water extract	Methanolic extract	Acetone extract	Chloroform extract	Petroleum ether extract	Hexane extract
Alkaloids test						
• Mayer's test	Present	Present	Absent	Absent	Absent	Present
• Wagner's test	Present	Present	Absent	Absent	Absent	Present
Carbohydrate test						
• Anthrone's test	Absent	Absent	Absent	Present	Absent	Absent
• Fehling's test	Absent	Absent	Absent	Present	Absent	Absent
Proteins test						
• Bieuret's test	Present	Present	Absent	Present	Absent	Present
• Xanthoproteic test	Present	Present	Absent	Present	Absent	Present
Resins test	Present	Present	Present	Present	Absent	Present
Saponin test						
• Foam test	Present	Absent	Absent	Absent	Absent	Absent
Starch test	Absent	Absent	Absent	Absent	Absent	Absent
Flavonoid test						
• Shinoda's test	Present	Present	Absent	Present	Absent	Present
• Alkaline reagent test	Present	Present	Absent	Present	Absent	Present
Phenolic compound	Absent	Present	Present	Absent	Absent	Absent
Steroid test						
• Salkowski's test	Present	Present	Absent	Absent	Absent	Present
Glycoside test						
• Borntrager's test	Absent	Present	Present	Present	Absent	Absent
• Killer kiliani test	Absent	Present	Present	Present	Absent	Absent
Tannins test						
• Lead acetate test	Present	Present	Present	Present	Absent	Absent
• Ferric chloride test	Present	Present	Present	Present	Absent	Absent
Quinones test	Present	Absent	Absent	Absent	Absent	Absent

The maximum number of phyto-constituents was present in both water & methanolic solvent and the minimum number of phyto-constituents was present in petroleum either solvent.

CONCLUSION

Phytochemical study and physicochemical evaluation of the whole plant of *E. diffusum* D. Don were carried out and their experimental findings determine the good quality, purity, strength, identity and authenticity of plant materials along with also showed the presence of phytoconstituents for medicinal and other commercial purposes. Present literature and documents are indicated very few studies have been done in this plant so far, the data available from this research in the future will help in the detailed study of this plant.

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