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PHARMACOGNOSTIC, PHYSICOCHEMICAL AND PHYTOCHEMICAL STUDY OF *CENCHRUS BIFLORUS* ROXB. STEM

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ABSTRACT

Cenchrus biflorus Roxb. popularly called "Bhurat," is a member of the Poaceae family were used as "famine food" during periods of food scarcity and is also employed in folk medicine. Because of its growing importance in ethnomedicine, accurate identification and assessment are essential to preventing adulteration. Macroscopic, microscopic and chemo-microscopic characteristics of the fresh stem were assessed. The physicochemical characteristics and phytochemical screening were conducted in accordance with the standard protocols and this will be utilized for the identification and ensuing standardization of species. Macroscopic results showed that the stem were green in colour, a bitter taste, aromatic odour and cylindrical shape. Chemo-microscopic analyses showed the presence of calcium oxalate, lignin, tannins, a small number of proteins, calcium carbonate, starch and fibres in stem part. The contents of the total ash, water soluble ash, sulphated ash, and acid insoluble ash were in order: 14.1±0.51, 1.83±0.15, 6.13±0.2, 4.36±0.51. The results showed that the moisture content was 7.4±0.37. The presence of steroids, alkaloids, saponins, phenols and glycosides is revealed by a preliminary phytochemical examination. *Cenchrus biflorus* Roxb. stem pharmacognostic standards were established and function as quality control measures for their identity, purity, and uniformity.

KEYWORDS: Macroscopic, Microscopic, Physicochemical, Phytochemical.

Abbreviations

MeOH – Methanol

HE- Hydroethanol

AQ- Aqueous

1. INTRODUCTION

Since the beginning of time, varied formulations containing plants, plant parts, and extracted phytochemicals have been utilized to prevent and treat a variety of illnesses, particularly in traditional medical systems like Ayurveda, Siddha and Unani. In rural parts of developing countries, where modern medicine is the norm, about 60% of the global population receives their main medical treatment from traditional practitioners.[1] Herbal medicine is a major component of traditional medicine.[2] As a result, people in developing countries prefer traditional medicine due to its cheapness and accessibility than Orthodox Medicine.[3] Plants have been an important source for the discovery of novel pharmacologically active compounds, with many blockbuster drugs being derived from plants directly or indirectly. There are several phytocompounds found in medicinal plants, such as tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids, that have specific pharmacological effects on humans.[4] Throughout history, a wide range of individuals have reported using medicinal plants as a source of biologically active substances having therapeutic capabilities to cure a variety of maladies. A plant's entire collection of primary and secondary metabolites, or metabolome, is made up of over 4,000 different chemicals. There are over 2,000 000 chemicals in the kingdom of plants. All of these substances are found in plants and serve a variety of functions, including defence, reproduction, nourishment, upkeep, and attack.[5] Grasses are reported to be relatively rich in bioactive secondary compounds that have medicinal properties[6] but the phytochemical investigations of many grasses are least investigated, they are full sources of the phytochemicals of novel origin. This work attempts to assess the pharmacognostic standards in depth with the goal of producing standardization parameters for *Cenchrus biflorus Roxb.* stem.

Taxonomical status

Kingdom: Plantae

Division: Spermatophyta

Class: Angiospermae

Subclass: Monocotyledonae

Series: Glumiflorae

Order: Poales

Family: Poaceae

Subfamily: Panicoideae

Genus: *Cenchrus*

Species: *biflorus* Roxb.

Vernacular names

Argana, Basla, Bharbhunt, Bhurat, Bhurut, Dhaman, Kukar.

2. MATERIAL AND METHOD

2.1. Collection and identification of plant

Cenchrus biflorus Roxb. was gathered from the Tonk, Rajasthan, campus of Banasthali Vidyapith. A sample of the plant material was deposited with voucher specimen number BURI-1393/2021 to the herbarium of the Department of Bioscience and Biotechnology, Banasthali Vidyapith, Rajasthan (India), for future reference after cleaning and a shade-dried.

2.2. Preparation of extracts

Freshly harvested stems were completely cleaned, first with tap water and then with distilled water, and then they were separated. Then further dried at ambient temperature in a shaded spot, ground into a powder, and stored in an airtight container. Using a hot continuous extraction method by soxhlet apparatus at a range of 30° to 85°C, powder was produced for the solvent extraction process, including methanol, hydro-ethanol, and aqueous.[7] Once the solvent in the siphon tube becomes colorless, the extraction process is carried out. The extracts were then dried out until all of the solvent get evaporated then yield amount of the various solvents were determined. Additionally, this dehydrated extract can be kept for later use in a refrigerator at 4°C.

2.3. Chemicals, reagents and solvents

Analytical grade reagents, solvents, and chemicals were utilized.

2.4. Pharmacognostic evaluation

Organoleptic qualities, such as colour, flavour, and odour were observed and recorded.[8] Visual inspection was conducted on *Cenchrus biflorus Roxb.* stem part for macroscopic study. *Cenchrus biflorus* stem was thinly sectioned in order to conduct a microscopic investigation. After giving them a thorough cleaning with water and straining them through various reagents such as safranin and fast green, they were mounted with glycerine and observed under a microscope at 10x to 40x magnifications.[9]

2.5. Chemo-microscopic characteristics of *Cenchrus biflorus Roxb.* stem

A variety of reagents and stains, including iodine, sulfuric acid (80%), concentrated hydrochloric acid, ferric chloride, ruthenium red, and picric acid solution were used in chemo-microscopic studies of the dried stem sample to examine the presence of various metabolites.[10]

2.6. Physicochemical parameters

The presence of any foreign material was manually determined from the dried stem using a magnifying lens. Physicochemical parameters such as total ash value, water soluble ash value, acid insoluble ash value, sulphated ash and loss on drying loss were calculated.[11] Every parameter was measured three times, and the output was displayed as mean± standard deviation.

Fluorescence analysis

An essential qualitative diagnostic method for determining whether a chromophore is present in crude powdered medication is fluorescence analysis. A tiny amount of stem powder were applied to a clean watch glass, and one or two drops of freshly made reagent solution are added. The mixture is then gently tilted, and after a few minutes, the watch glass is inserted inside the UV chamber where the colour in visible light, short (254 nm) and long (366 nm) ultraviolet radiations is observed. It was noted what colour was seen when various chemicals were applied in various radiation levels.[12]

2.7. Powdered microscopy

Stem powder that had been dried was filtered through a 355 µm IS sieve. The obtained powder was dyed using safranin, potassium iodide, and phloroglucinol. After temporarily preparing the slide with glycerin, it was inspected under a Metzer microscope at magnifications of 10X and 40X to look at various microscopic features.[13]

2.8. Qualitative phytochemical screening

Using the standard procedures, the presence or lack of the primary and secondary phytoconstituents was determined.[14] The presence of distinct phytoconstituents in the powdered drugs that had been extracted using different solvents was examined. Typically, conventional protocols are employed to detect the presence of alkaloids, flavonoids, tannins, phenols, steroids, and saponins in stem part of *Cenchrus biflorus Roxb*.

3. RESULT AND DISCUSSION

3.1. Pharmacognostic evaluation

Fresh *Cenchrus biflorus* stem were examined for their organoleptic characteristics. Sense organs can be used for organoleptic evaluation, which offers the quickest and easiest way to determine the identification and purity of a medicine in order to guarantee its potency. Organoleptic characteristics like size, colour, aroma, taste, and fragmentation of stem bark, are assessed. Macroscopic study reveals that stem is green in color, smooth, glabrous and cylindrical in shape Fig1b. The organoleptic results showed that the stem extract were green in colour with bitter taste and aromatic odour. It is essential to keep in mind that the macroscopic evaluation of plants is arbitrary and that adulterants or replacements could have striking similarities to the original specimen. Therefore, microscopic and physicochemical tests are required to validate and authenticate the macroscopic findings. The identification of grounded or powdered components in crude pharmaceuticals requires microscopic examination. Several unusual and helpful anatomical features were identified by microscopic examination. In the compilation of contemporary monographs, it is also a crucial pharmacognostic criterion. The stem of *Cenchrus biflorus* displayed vascular bundles, xylem, phloem, hypodermis, ground tissue, and chlorenchyma in a transverse section as represented in Fig 1 a. Vascular bundles are dispersed throughout the parenchymatous ground tissue.

3.2. Chemo- microscopic characteristics of the stem of *Cenchrus biflorus Roxb.*

The results of chemo-microscopic analyses showed the presence of calcium oxalate, lignin, tannins, small number of proteins, calcium carbonate, starch and fibres in stem part (Table 1).

3.3. Physicochemical parameters

Ash values are crucial quantitative benchmarks for assessing the identification, quality, and purity of crude pharmaceuticals, especially when they are in powdered form.[15] Four distinct procedures were used to determine the ash values: total ash, acid insoluble ash, water soluble ash, and sulphated ash. 14.1±0.51 w/w of the total ash was recovered, which indicates how much ash was left over after ignition. The plant material had 0.7±0.2w/w foreign matter and displayed 7.4±0.37 w/w loss during drying. The results of each approach were shown in Table 3. The moisture content measurement (7.4±0.37) indicates that the active ingredients may undergo enzymatic hydrolysis and deterioration upon contact with air. Since the volume of water present determines how easily plant material can degrade, high moisture content is a sign of easy bacterial or fungal growth.[16] The efficacy and purity of crude medication are

assessed using ash values. It shows an existence of several contaminants, including silicate, oxalate, and carbonate. Drug manufacturers utilize the water soluble ash to assess the quantity of inorganic component they contain. The mostly silica-based acid insoluble ash is indicative of earthy material contamination. Drugs should have very low moisture content in order to prevent the formation of fungi, yeast, or bacteria while being stored. The amount of active ingredients in a given amount of plant material when extracted with a certain solvent is determined by estimating extractive values.[17]

Acid-insoluble ash, which is a component of total ash, quantifies the quantity of silica found in siliceous earth and sand. The distinction between mineral pollutants and variations from the natural ash is shown by the values of total ash and acid insoluble ash.[16] As 4.36±0.51 of acid-insoluble ash was recovered. Water soluble ash is the term used to describe the water soluble fraction of the total ash. The amount of water-soluble ash that was collected was as low as 1.83± 0.15, suggesting that only a small percentage of the total ash elements were soluble in water. The sulphated ash measurement, which came out at 6.133±0.2, shows that nonvolatilized residual material is present.

Fluorescence Analysis

The extracts were made using a hot, consecutive extraction process according to their polarity. They were then treated with chemicals, and the colour changes were seen under an ultra violet light source (short light (254nm) and long light (365nm)) and day light. The outcomes were tabulated in Table 4. In daylight, certain components exhibit visible fluorescence. Many natural compounds that do not glow noticeably in daylight are made to fluoresce by ultra violet light. When substances lack fluorescence, they can be transformed into fluorescent derivatives or breakdown products by the use of distinct reagents.[11]

3.4. Powder Microscopy

Different fragments of tissues showing rounded to elongated parenchymatous cells with starch grains, reddish tannin content, Xylem Vessel with pitted thickening and tracheids.

The stem's coarse powder had a green hue. Under microscopic examination, the stem powder revealed prism-like crystals, unicellular trichomes, annular spiral vessels, various tissue fragments displaying elongated to spherical parenchymatous cells with starch grains, Tracheid, and a pitted xylem (Fig 2).

Different fragments of tissues showing rounded to elongated parenchymatous cells with starch grains, reddish tannin content, Xylem Vessel with pitted thickening and tracheids.

3.5. Estimation of crude extracts of *Cenchrus biflorus* stem

Using methanol, hydro-ethanol, and aqueous solvents in ascending polarity order, Soxhlet extraction method was used to perform a phytochemical estimate of the stem crude extracts of *Cenchrus biflorus*. Table 2 presents a tabulation of the yield of crude extract, colour, and consistency of all extracts for stem part. An analogous investigation was carried out on the stem sections of *Cenchrus biflorus*, and the results indicate that the stem extract of aqueous extract yielded the highest value 0.186±0.01 mg/g. Similar research on the leaf and stem sections of *Madhuca indica* was carried out revealed that methanol had the highest extractive value.[18]

3.6. Qualitative phytochemical screening

Natural medicine is becoming more and more popular as a kind of treatment since it has fewer side effects and is less expensive than manufactured medications. Plants that contain secondary metabolites including flavonoids, tannins, alkaloids, and certain other aromatic chemicals have a great deal of therapeutic promise. Using a conventional approach, phytochemical screening was performed on the MeOH, HE and AQ extracts of stem part of *Cenchrus biflorus* to determine whether the contents were present or absent. When *Cenchrus biflorus* stem extracts were examined for primary metabolites, it was found that proteins were present in trace amounts in the MeOH extract and trace in the aqueous and HE (hydro-ethanol) extracts. MeOH stem extract had a moderate amount of flavonoids and phenolics while HE and AQ extract of stem contains only moderately amount. The results were summarized in Table 5. The methanol extract of *Cenchrus biflorus* stem contained alkaloids, tannins, flavonoids, steroids, saponins, and terpenoids, according to the preliminary phytochemical qualitative analysis. Out of all the phytochemicals present, the flavonoids had the highest concentration. The secondary metabolites and bioactive substances that are typically in charge of a plant drug's pharmacological actions are found through phytochemical investigation.

Analytical statistics

Every experiment was carried out in three duplicates. The experimental outcomes, with n equal to 3, are shown as Mean± SD.

Table 1: Chemo- microscopic characteristics of *Cenchrus biflorus Roxb.* stem part.

S.no.	Parameters	Test reagents	Observation	Stem
1.	Lignin	Sample + Iodinated zinc chloride solution	Yellow coloration observed in the xylem vessels	+
2.	Cellulose	Sample + Iodinated zinc chloride solution	Blue color observed on epidermal cells	-
3.	Oxalate crystals	Sample + 80% H_2SO_4	Crystals of calcium oxalate dissolved	+
4.	Tannin	Sample + Ferric chloride solution	No greenish colour.	-
5.	Starch	Sample + Iodinated zinc chloride solution	Yellow coloration observed in the xylem vessels	+
6.	Mucilage	Sample + Ruthenium red	Light grayish color	-
7.	Protein	Sample + 1% Picric acid and million's reagent	Red colour observed	+
8.	Fibres	Picric acid solution	Yellow coloration observed	+
9.	Fatty oils	Sample+ Sudan IV reagent	Light pinkish color observed	+

Key: + = present, - = absent

Table 2: Physicochemical evaluation of the crude drug of *Cenchrus biflorus* stem parts.

S. No.	Parameters	Values on dry weight basis (%w/w)
1.	Foreign matter	0.7±0.2
2.	Loss on drying / Moisture content	7.4±0.37
3.	Total ash	14.1±0.51
4.	Acid Insoluble Ash	4.36±0.51
5.	Water soluble Ash	1.83± 0.15
6.	Sulphated Ash	6.133±0.2

The percentage composition values displayed are Mean±SD for n = 3.

Table 3: Fluorescence analysis of powdered stem powder drug of *Cenchrus biflorus*.

S. No.	(Powder + Reagent)	Day light	Short-light (254nm)	Long-light (365nm)
1.	Only Powder	Light grey	Grey	Light grey
2.	Powder + Methanol	Light yellow	Yellow	Dark brown
3.	Powder + Ethanol	Light yellow	Light brown	Dark brown
4.	Powder + Petroleum Ether	Colorless	Light green	Light brown
5.	Powder + Acetone	Light yellow	Green	Yellowish brown
6.	Powder + chloroform	Yellow	Light green	Dark yellow
7.	Powder + 50% H_2SO_4	Light yellow	Dark yellow	Reddish brown
8.	Powder + 50%HCL	Light green	Green	Reddish brown
9.	Powder +10% NaOH	Light yellow	Light brown	Reddish brown
10.	Powder + Ammonia	Corn silk	Yellow green	Olive drab
11.	Powder + Acetic Acid	Grey	Light grey	Dark brown

12.	Powder + distilled water	Grey	Light green	Brown
13.	Powder + iodine	Yellow	Dark green	No Fluorescence
14.	Powder + $Fecl_3$	Grey	Green	Light brown
15.	Powder + Picric Acid	Light yellow	Dark green	Reddish brown
16.	Powder +$K_2Cr_2O_7$	Yellow	Green	Brown
17.	Powder + Toluene	Grey	Dark green	Reddish brown
18.	Powder + Benzene	Colorless	Light brown	Brown
19.	Powder + n-butanol	Light grey	Dark grey	Light brown
20.	Powder + Hexane	Light yellow	Dark yellow	Brown
21.	Powder +Ethyl acetate	Light green	Dark green	Brown

Table 4: Estimation of stem crude extracts of *Cenchrus biflorus*.

Plant part	Solvent	Total Yield mg/10gm±S.D.	Color	Consistency
Stem	MeOH	0.154±0.005	Dark green	Stickiness
	HE	0.127±0.011	Greenish	No stickiness
	AQ	0.186±0.01	Light green	No stickiness

MeOH: Methanol, HE: Hydro ethanol, AQ: Aqueous

Table 5: Measurement of primary and secondary metabolites qualitatively in different solvents using stem extracts from *Cenchrus biflorus.*

S. No.	Phytochemical	Tests	Stem extract		
			MeOH	HE	AQ
1.	Saponins	Foam test	+	+	++
2.	Alkaloid	Mayer's test	+	+	+
3.	Phenolics	Wagner's test	++	+	+
		Ferric chloride test	++	+	+
		Lead acetate test	++	+	+
4.	Steroid	Salkowski test	+	+	+
5.	Terpenoids	Liebermann burchard	+	+	+
6.	Cardiac glycosides	Killer Killani test	+	+	+
7.	Reducing Sugars	Fehling's test	+	+	-
		Benedict test	-	-	-
8.	Flavonoids	Alkaline test	++	+	+
		Shinoda test	++	+	+
9.	Triterpenes	Salkowski test	+	+	+
10.	Glycoside	Keller- Killiani test	+	+	+
		Libermann-burchard test	+	+	+
11.	Tannins	Braymer's test	+	-	-
		Gelatin test	+	+	-
12.	Carbohydrates	Molisch's test	++	+	+
		Fehling's test	+	+	-
		Benedict's test	+	+	-
13.	Resins	Acetic anhydride test	-	-	-
14.	Proteins	Biuret test	+	-	-

		Ninhydrin test	+	+	+
15.	Anthraquinone	Borntrager's test	-	-	-

Key: Moderately present: ++, Weakly present: +, Absent -, MeOH: Methanol, HE: Hydro ethanol, AQ: Aqueous.

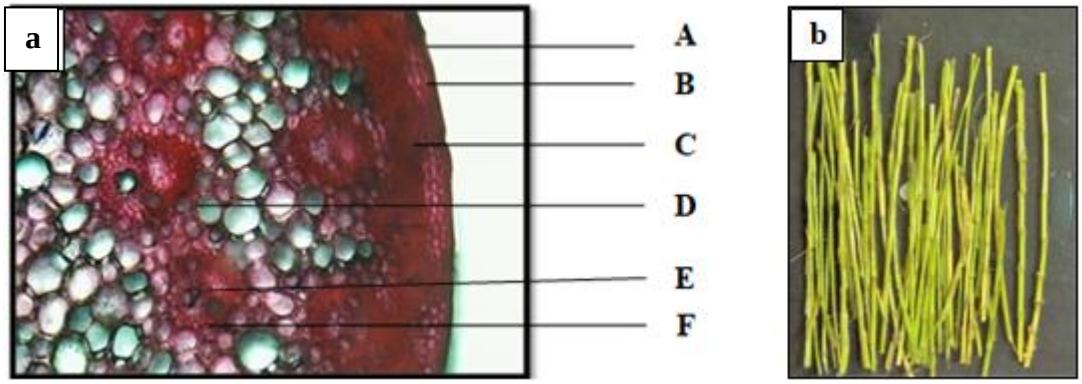

Fig. 1: a) Microscopic study of *Cenchrus biflorus* stem (A) Epidermis, (B) Hypodermis, (C) Chlorenchyma, (D) Ground tissue, (E) Xylem, (F) Vascular bundle, b) Macroscopic study of *Cenchrus biflorus* stem.

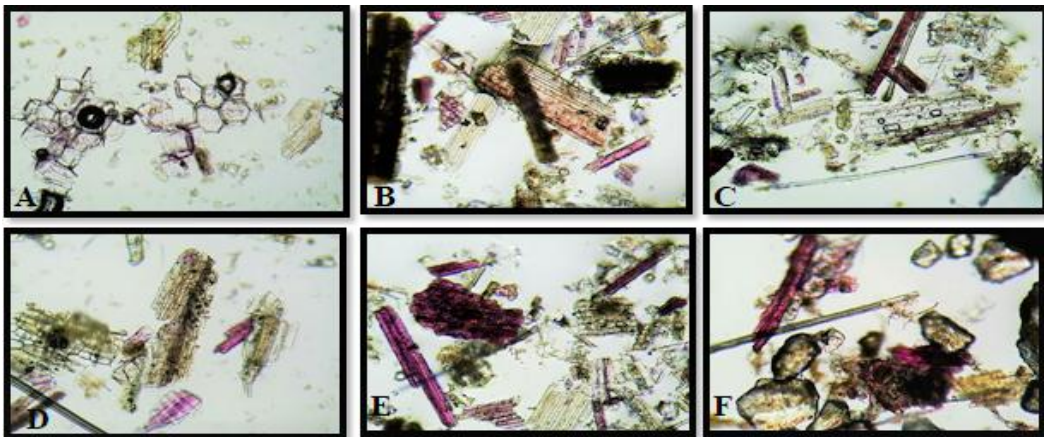

Fig. 2: Powder microscopic assessment of *Cenchrus biflorus* stem parts : A) Polygonal Parenchymatous cells, B) Parenchymatous cells showing group of starch grains, C) Different fragment of tissue, D) Pitted xylem vessel in groups E) Tracheid, F) Crystals of calcium oxalate.

CONCLUSION

Medicinal plants have emerged as one of the most important sources for drug development in recent times. They have proven invaluable in the treatment of a wide range of ailments. *Cenchrus biflorus Roxb.* has undergone pharmacognostic, physicochemical, and phytochemical examinations that have been assessed. Official monographs and the standardization of *Cenchrus biflorus Roxb.* can be prepared using the phytochemical and

pharmacognostic data obtained from the plant. Important information regarding the phytoconstituents contained in the plant material is provided by the quantitative estimation of the principal secondary metabolites and the preliminary phytochemical screening. The current study's parameters may prove beneficial in the future for the identification, verification, and maintenance of the drug's quality, purity, and efficacy. It was evident from the data that the phytochemicals in the plant may have physiological or therapeutic effects on humans.

Conflicts of Interest

We declare that we have no conflict of interest in the publication.

Author's Contribution

We affirm that each author listed in the article has made an equal effort to both the research and the submitted work.

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