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Pharmacognostical, physicochemical and phytochemical evaluation of least studied *Cenchrus biflorus* Roxb.

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Abstract

Cenchrus biflorus Roxb. is a member of Poaceae family used in folklore medicine and a staple "famine food" during times of food shortage. Assessments of macroscopic and microscopic characteristics, as well as their physicochemical characteristics of Cenchrus biflorus were evaluated by using standard methods. Phytochemical profile of Cenchrus biflorus leaf and root parts were analyzed in various solvents to determine phenolic, flavonoid and tannin content. Organic solvents like methanol, hydro-ethanol (50:50), aqueous were used for extraction process. Total phenolic content (TPC) and total tannin content (TTC) were analysed using Folin Ciocalteu assay and total flavonoid content (TFC) were measured through $AlCl_3$ calorimetric assay by using UV-Spectrophotometric methods respectively. Microscopic analysis revealed the presence of trichomes, epidermis, vascular bundles, companion cells, and sieve tubes in leaf parts. Root methanolic extract showed the highest amount of TPC (45.18±0.011mg GAE/g) while highest amount of TFC was recorded in leaf methanolic extract (34±0.003mg Quercetin (QE)/g) and TTC was highest in methanol extract of leaf (6.5±0.009mg TA/g). The result presented shows the TPC, TFC and TTC distribution in Cenchrus biflorus leaf and root parts that could be used in the cure of various ailments. The established parameters will be beneficial and appropriate for the creation of a monograph, aid in recognizing this grass in its unadulterated form, preventing its adulteration, and ensuring its therapeutic efficacy.

Keywords: *Cenchrus biflorus*, Pharmacognostical, Physicochemical, Phytochemical analysis.

Introduction

Entire world is affluent with a number of unique medicinal plants. These plants played a tremendous role in human health needs throughout their existence. In pharmacology, medicinal plants are getting more recognition because these plants have innumerable benefits to all mankind. Nearly 80% people of the developing countries are relying on harvested wild plants for their medicinal purposes[1]. India possesses one of the world's richest medicinal plants heritages with about 8000 plant species, which is being used by rural communities[2]. More than 1850 native plant species of India have been used in medicines preparation in different medicinal system like Ayurveda, Siddha and Unani. Traditionally herbal medicines contributed to the identification of natural products for potential development of drugs[3]. Traditional medicines are still consumed by developing countries for primary healthcare as reported by WHO (World Health Organization). Herbal formulations are obtained from medicinal plants, which are important for treatment of various ailments. Many phytocompounds are present in medicinal plants which exert definite pharmacological effects on humans including tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids[4].

Grasses are monocotyledonous plants which are considered as the noval repository due to their medicinal, economical and ecological properties[5]. Poaceae grasses produce chemical compounds that are considered to be of medicinal value[6]. Grasses have several ethanobotanical properties which are used by different tribes present in the different types of forest and they utilize these grasses in their daily life for several purposes[7].

Cenchrus biflorus is an annual grass found in semi arid and drought areas of arid ecosystem[8]. Northern hemisphere, tropical and temperate areas are the native places of this grass[9]. *Cenchrus biflorus* is an annual long and rooted at lower nodes, grasses of *Cenchrus* forms a large ecosystem in the grassland areas. Leaves of *Cenchrus biflorus* are linear and acuminate with smooth surface. Flower are purplish in color, inflorescence is raceme that generally bears 1-3 deciduous involucres. Rachis is not straight and shows angular deviations throughout the length. Roots are fibrous and the nodes are glabrous and dark brown in color. Due to its high nutritional values this grass was introduced as fodder in North America and Australia[10]. Throughout the arid zones of the world *Cenchrus biflorus* was used as human's food[11].

There was no scientific report to give credence to the ethanomedicinal usage of this grass best of our knowledge. Therefore present study was conducted to elucidate and provide information on qualitative and quantitative composition of leaf and root extracts of *Cenchrus biflorus* in order to provide scientific basis to justify its therapeutic usages.

Material and methods

Plant procurement and authentication: *Cenchrus biflorus* were collected from area of Banasthali Vidyapith campus in Tonk district, Rajasthan. Geographical location of sampling site with latitude: 26°22'48"N, longitude: 75°51'19''E and 314m elevation from sea level. The reference specimen was deposited to Department of Bioscience and Biotechnology, Banasthali Vidyapith, Rajasthan (India) herbarium with taxonomical, ethanobotanical details and authentication number is BURI-1393/2021.

Preparation of extracts: Plant parts (leaf and root) were collected freshly and cleaned thoroughly initially with tap water followed by distilled water and then plant parts were separated. Further dried them at room temperature under shady area and pulverized into powder form and kept it into air tight container. A thimble of 10gm dried pulverized powder was prepared for solvent extraction process with 250ml of different solvents (methanol, hydro-ethanol and aqueous) by hot continuous extraction method in Soxhlet apparatus at a temperature of 30°-85°C. This extraction process goes on until the solvent in siphon tube becomes color less[12]. Then the extracts were evaporated to dryness till all the solvent got evaporated and the yield quantity of different solvents was obtained. And this dried extract can be stored in refrigerator at 4°C for further studies.

Organoleptic evaluation: Organoleptic evaluation refers to characterization of medicinal plant extract with the help of sensory organs such as color, shape, size, taste, odor and texture[13].

Macroscopic studies: Macroscopic study of plant parts of *Cenchrus biflorus* were studied according to standard method[14].

Microscopic studies: Microscopic study was accomplished by thin section cutting of stem and leaf parts of *Cenchrus biflorus* Wash them with water and strain them with different reagents like safranin and fast green, mounted them with glycerine for observation under microscope at 10x, 40x lignifications[15].

Powder microscopy: Dried powder of root and leaf parts were passed through 355µm IS sieve. Obtained powder were stained with the help of safranin, phloroglucinol, potassium iodide and mounted them with glycerin for temporary slide preparation and observed them under microscope (Metzer) at magnification power 10X, 40X to study various microscopic characters[16].

Physiochemical analysis: Physiochemical analysis of leaf and root extracts were performed which includes parameters-foreign matter, total ash value, acid-insoluble ash, water-soluble ash values, loss on drying/ moisture content by the help of standardized methods[17]. Fluorescence studies are deciding factors for purity and quality of crude drugs. *Cenchrus biflorus* leaf and root parts were examined with non polar to polar solvents inside UV viewer chamber in day light, short (254nm) and long (365nm) ultraviolet radiations. The color of the samples was recorded after reagents applications to plant parts[18].

Phytochemical screening: Qualitative analysis: Plant extracts (leaf and root parts) were evaluated for screening and identification of bioactive constituents that are present. Study was carried out in extracts using standard protocol with few modifications[19,20].

Quantitative analysis: Determination of total phenolic content: Total phenolic content of plant extracts were measured spectrophotometrically by using Folin - Ciocalteu's phenol reagent assay with gallic acid as a standard method with slight modifications[21]. Extract aliquot in diluted form was added to 0.5ml of distilled water and 0.125ml of Folin- Ciocalteu's phenol reagent, then the mixture was shaken and permitted it to stand for 5 min then 1.25ml of 7% Na_2CO_3 was added to this mixture and finally make up the volume up to 3ml. Absorbance was recorded at 765nm using spectrophotometer (LMSP-UV1900, LABMAN, India) and results were expressed as mg of gallic acid equivalent per gram of dry weight (mg GAE/g DW) with gallic acid. All the samples were analyzed in triplicates.

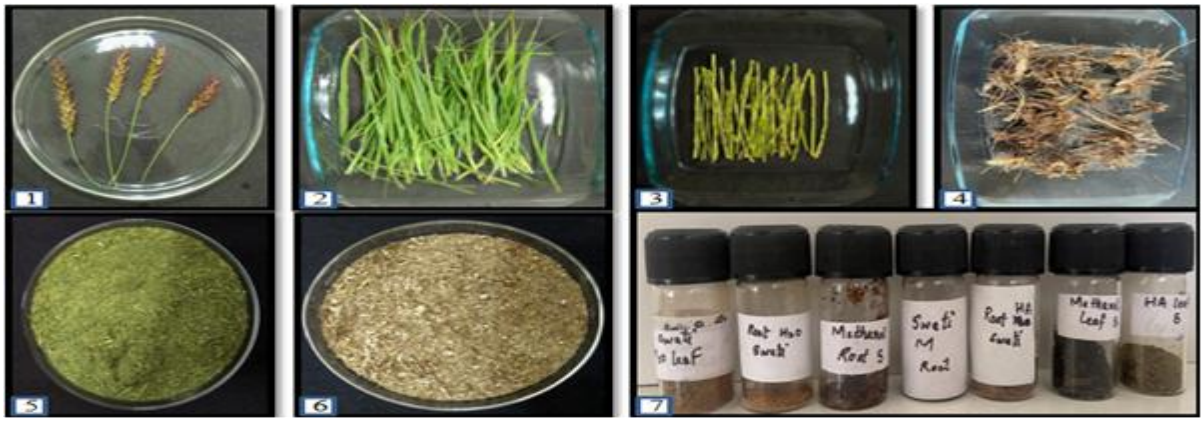

Figure-1: Parts of *Cenchrus biflorus* 1. Inflorescence, 2. Leaves, 3. Stem, 4. Dried root, 5. Leaves powder, 6. Root powder, 7. Dried extracts prepared in various solvents.

Determination of total flavonoid content: Total flavonoid content of plant extracts were investigated using Aluminum chloride ($AlCl_3$) colorimetric method[22]. Calibration curve was established using quercetin (1mg/ml) as standard in methanol. 0.5ml of plant extract and quercetin were separately blended with 1.5ml of methanol, 0.1ml of 10% $AlCl_3$, 0.1ml of 1M potassium acetate and 2.8 ml of distilled water and left this reaction mixture at room temperature for 30 minutes, than the absorbance was recorded at 415nm with a double beam labman UV-VIS spectrophotometer.

The result was expressed as Quercetin in mg QE/g dry weight of sample. All the samples were analyzed in triplicates.

Determination of total tannin content: Total tannin content were evaluated by using Folin-Ciocalteu's phenol reagent method. 0.1ml of plant extract was added to a 10ml volumetric flask containing 7.5ml of distilled water and 0.5ml of Folin-Ciocalteu's phenol reagent, 1ml of 35% sodium carbonate solution and then diluted with distilled water up to 10ml. This mixture was shaken property and kept it at room temperature for 30 min, a set of reference standard solutions of tannic acid (20, 40, 60, 80, 100µg/ml) was prepared in the same manner as described earlier.

Absorbance was recorded at 700nm with UV-Visible spectrophotometer for test and standard solution against the blank. TTC was estimated in triplicate. Tannin content was expressed as mg of tannic acid equivalents per gram of dried sample[23].

Results and discussion

Organoleptic study: Organoleptic study is used for evaluation of morphological and sensory profile of plant powder, which helps in identity and degree of purity of plant material. Organoleptic investigations of *Cenchrus biflorus* were performed, which is an annual grass having leaves with aromatic odor and bitter taste as mentioned in Table-1.

Macroscopic study: Macroscopic study of *Cenchrus biflorus* leaf, stem, seed and root parts are compiled in Table- 2.

Microscopic study: The Cortex is parenchymatous and sclerenchymatous, amount of chlorenchyma is least in stem transverse section of *Cenchrus biflorus* Roxb. as shown in Figure-2. Vascular bundles are embedded distributed in ground tissue that is parenchymatous. Transverse sections of *Cenchrus biflorus* leaf were shown in Figure-2. Epidermis is single layered with unicellular trichomes on both sides. Vascular bundles are distributed throughout. Xylem is more prominent and shows clear differentiation in large vascular bundles. Enormous trichomes were found on both side of epidermal surface. Phloem is present towards lower epidermis and contains companion cells and sieve tubes.

Powder Microscopy: Numerous micro- morphological features was noticed in different parts of plant powder like lignified cells, reticulate xylem vessels, spongy cells, starch granules, calcium oxalate crystals and spiral xylem vessels were observed in root part (Figure-3a) while stone cells, xylem vessel, trichome, cork cells, spiral xylem vessel were noticed in leaf part as shown in Figure-3(b).

Table-1: Organoleptic study of *Cenchrus biflorus.*

Parameters	Leaf	Stem	Root	Seed
Colour	Green	Light green	Brown	Brown
Texture	Powder	Powder	Powder	Powder
Taste	Bitter	Bitter	Bitter	Bitter
Odour	Aromatic	Characterstics	Aromatic	Aromatic

Table-2: Macroscopic examination of various plant parts of *Cenchrus biflorus.*

Parameters	Leaf	Stem	Seed	Root
Condition	Fresh young leaf	Fresh	Dry and smooth	Hard
Odour	Aromatic	Aromatic	Aromatic	Aromatic
Shape	Linear	Cylindrical	Ovoid caryopsis	Fibrous
Taste	Bitter	Bitter	Bitter and aromatic	Bitter
Touch	Scaberulous	Smooth	Smooth	Hard

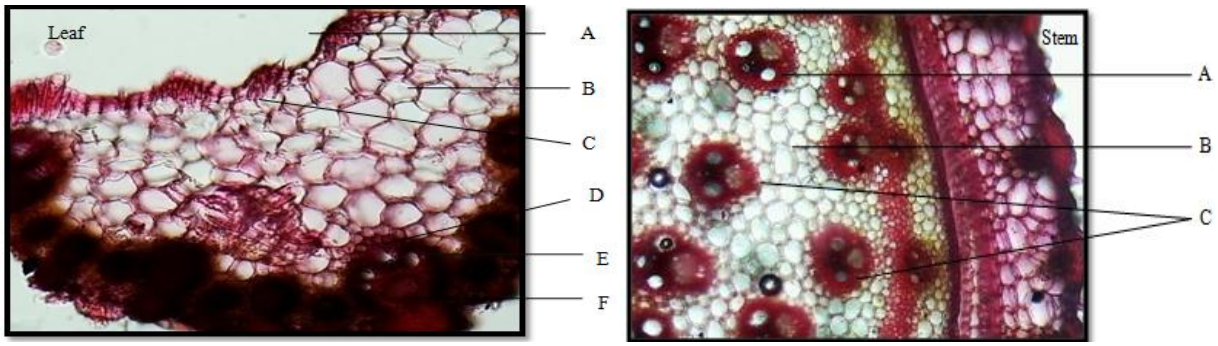

Figure-2: Microscopic study of leaf and stem section of *Cenchrus biflorus*, Leaf : (A) Epidermis, (B) Parenchyma, (C) Sclerenchyma (D) Vascular bundle, (E) Xylem, (F) Phloem, Stem: (A) Phloem (B) Pith, (C) vascular bundles.

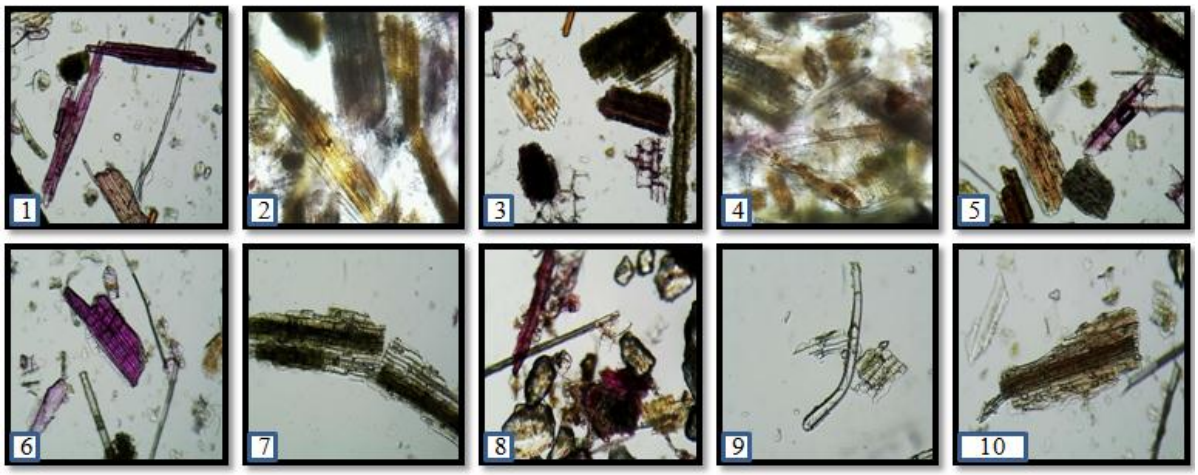

Figure-3: Powder microscopic study of *Cenchrus biflorus* a) Root- (1. Lignified cells, 2. Reticulate xylem vessels, 3. Spongy cells, 4. Starch granules, 5. Calcium oxalate crystals and spiral xylem vessels,); b) Leaf- (6. Spiral xylem vessel, 7. Xylem vessel, 8. Stone cells ,9. Trichome, 10. Cork cells).

Physicochemical analysis: Foreign matter: Foreign organic matters were recorded in leaf and root parts of *Cenchrus biflorus.* Foreign matter study in leaf and root were tabulated in Table-3.

Loss on drying (LOD): Plant material with low moisture content is of high quality and stability, and can be considered for future studies or applications[24]. Moisture content of drug should be as low as possible while storage to prevent the development of bacteria, yeast, or fungi. Inefficient drying may result in the degradation of phytoconstituents during storage. Having high water content may make it easier for fungi to grow and interfere with drug quality[25].

Percentage of weight loss or moisture content of plant parts after drying at 105°C of leaf and root part were performed as mentioned in Table-3.

Ash Value: Ash value of leaf and root parts of *Cenchrus biflorus* were studied and ash value were evaluated by three different forms total ash, water soluble ash, acid insoluble ash and the results were compiled in Table-3 and Figure-4.

Table-3: Ash value of powder of leaf and root parts of *Cenchrus biflorus.*

Parameters	Values on dry weight basis	
	Leaf (%w/w)	Root (%w/w)
Foreign matter	0.5±0.2	0.8±0.1
Loss on drying / Moisture content	7.5±0.26	6.3±0.52
Total ash	13.5±0.2	15± 0.28
Acid Insoluble Ash	4±0.58	4.4 ±0.45
Water soluble Ash	2.23± 0.26	1.8 ±0.36

Moreover, the present study reinforces the earlier *Cynodon dactylon* (L.) report. According to a report, *Cynodon dactylon* (L.) have total ash value 9.9% w/w, water soluble ash value 2.4 w/w while acid insoluble was 5.8 w/w [26]. Another study on *Echinochloa colonum* (L.) having total ash value (8.66% w/w),

acid-insoluble ash (0.133% w/w), water soluble ash (8.2% w/w) were reported[27]. Ash values are also helpful in evaluating the nature of material, adulteration, impurities, authenticity of the sample, and its quality and purity. Ash value shows impurities like carbonate, oxalate, and silicate[13].

Solvent extraction and yield: Phytochemical estimation of leaf and root crude extracts of *Cenchrus biflorus* was done by Soxhlet extraction method using solvents – methanol, hydro-ethanol and aqueous with ascending order of polarity. Percentage yield, color and consistency of all extracts for leaf and root part was studied and tabulated in Table-4. For root extract in different solvent color varies from brown to dark brown while in leaf extracts color varies from greenish to green and consistency varies from sticky to non sticky and minimum % yield were observed in aqueous extract of leaf i.e. 0.066±0.27, while max % yield were calculated in root methanol extract 0.169±0.02. Similarly study conducted on *Cenchrus ciliaris* and *Cenchrus setigerus* and report states that the highest % yield was found in root extract of *Cenchrus ciliaris* in ethyl acetate extract (52.7mg/g) and in root glacial acetic acid extracts (48mg/g) of *Cenchrus setigerus.* As compared to extracts with lower polarity, high polar solvents have a sticky consistency[28].

Qualitative phytochemical screening: Since the dawn of civilization herbal medicines are considered as one of the most valuable field of traditional medicine. Now a day's treatment using natural medicines gaining attention among people due to rare side effects and low cost value as compaired to synthetic drugs. Presence of secondary metabolites such as tannins, flavonoids, alkaloids and some other aromatic compounds in plants have significant therapeutic potential. Qualitative phytochemical screening of *Cenchrus biflorus* leaf and root extracts in different solvents was execute to discover the existing phytochemicals, using standard procedure. Leaf and root extract of *Cenchrus biflorus* were tested for primary metabolites and observed that proteins were moderately present in MeOH (methanol) and HE (hydro-ethanol) extract while trace amount in (aqueous) root extract. Moderate carbohydrates amount in MeOH and HE extract of leaf, while AQ extract of leaf and root extracts (MeOH, AQ and HE) were found in trace amount. Secondary metabolites in leaf and root extract of *Cenchrus biflorus*. were qualitatively analysed. Highest quantity of phenols was observed in methanolic extract of leaf, while HE, AQ leaf extract and root methanol, HE and AQ extract were in moderate amount as tabulate in Table-5.

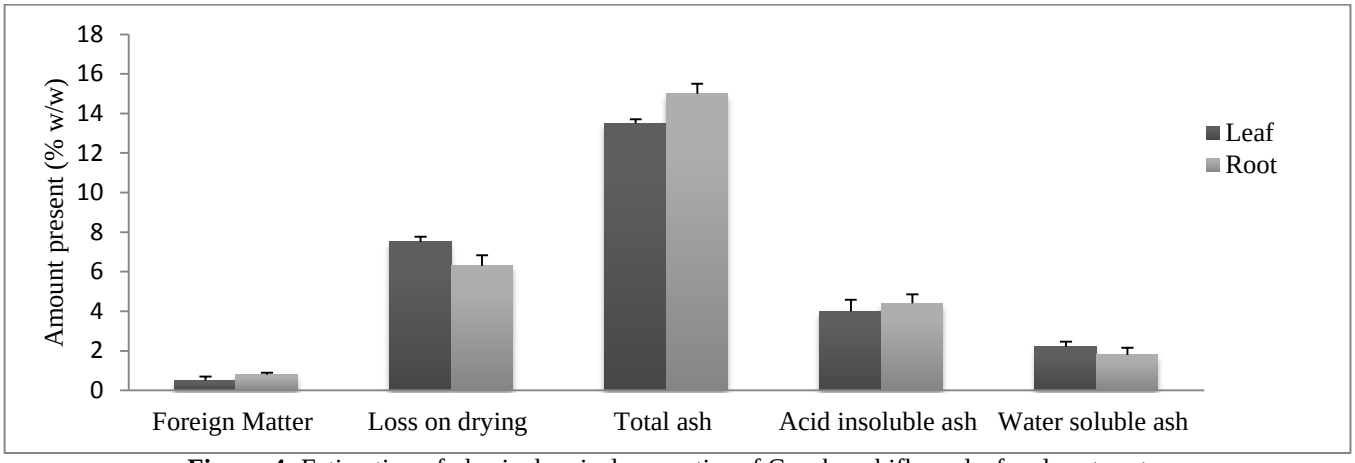

Figure-4: Estimation of physiochemical properties of Cenchrus biflorus leaf and root parts.

Table-4: Phytochemical estimation of leaf and root crude extracts of *Cenchrus biflorus*.

Plant part	Solvent	Total Yield mg/10gm±S.D.	Color	Consistency
Leaf	Methanol	0.151±0.01	Greenish	Sticky
	Hydro ethanol	0.112±0.03	Greenish	Non-sticky
	Aqueous	0.066±0.27	Greenish	Non-sticky
Root	Methanol	0.169±0.02	Dark brown	Sticky
	Hydro ethanol	0.139±0.04	Brown	Non-sticky
	Aqueous	0.072±0.03	Brown	Non-sticky

Table-5: Qualitative estimation of primary and secondary metabolites in *Cenchrus biflorus* leaf and root extracts in various solvents.

Phytochemical	Tests	Leaf extract			Root extract		
		Methanol	Hydro ethanol	Aqueous	Methanol	Hydro ethanol	Aqueous
Saponins	Foam test	++	+++	+++	+++	+++	+++
Alkaloid	Mayer's test	++	++	+	++	+	+
Phenolics	Wagner's test	+++	++	++	++	+	+
	Ferric chloride test	+++	++	++	++	++	++
	Lead acetate test	++	+++	+++	+ +	++	++
Steroid	Salkowski test	++	+	++	+	+	+
Terpenoids	Liebermann burchard	+	+	+	+	+	+
Cardiac glycosides	Killer Killani test	+	+	++	+	++	+
Reducing Sugars	Fehling's test	+	+	-	+	-	-
	Benedict test	+	+	-	+	-	-
Flavonoids	Alkaline test	++	++	+	++	+	+
	Shinoda test	+	+	+	+	+	+
Triterpenes	Salkowski test	++	+	++	++	+	+
Glycoside	Keller- Killiani test	+	+	+	+	+	+
	Libermann-burchard test	+	+	+	+	+	+
Tannins	Braymer's test	+	+	+	+	+	+
	Gelatin test	+	+	+	+	+	+
Carbohydrates	Molisch's test	++	++	+	+	+	+
	Fehling's test	+	+	-	+	-	-
	Benedict's test	+	+	-	+	-	-
Resins	Acetic anhydride test	-	-	-	-	-	-
Proteins	Biuret test	++	++	+	+	+	+
	Ninhydrin test	+	+	+	+	-	-
Anthraquinone	Borntrager's test	+	-	-	+	-	-

Key: Highly present: +++, Moderately present: ++, Weakly present: +, Absent -

Roots and leaves contain primary metabolites in different solvents. Moderate carbohydrates amount in methanolic and hydro-ethanolic extract of leaf and trace carbohydrates amount root extract in methanol were found. Secondary metabolites in root extracts of *Cenchrus biflorus* have appreciable saponins in methanolic and aqueous extract, moderate saponins in hydro-ethanol extract, moderate alkaloid in methanolic extract of root, appreciable alkaloid in methanolic extract of leaf, appreciable phenolics in methanol leaf extract, moderate phenolics in root methanol extract, trace terpenoids in leaf and root extracts, moderate steroids in methanolic and aqueous extract of leaf were found.

Fluorescence Analysis: In order to evaluate crude drug pharmacognostic properties, fluorescence studies are an efficient, most useful and fastest method. Powder form of leaf and root extract of *Cenchrus biflorus* shows different behavior recorded on the basis of treatment with numerous chemical reagents. When leaf powder extract react with 50% H_2SO_4 then green color turns into dark green (254nm) and reddish brown (365nm) under UV light, when treated with 10% 1N NaOH then dark yellow turns into dark brown (254nm) and reddish brown color (365nm). Root powder under normal light was brownish in color which turned into dark brown under UV light (254nm) and light brown under UV light (365nm) when dissolved with methanol, when treated with 1M HCL, light brown color turns into greenish (254nm) and light brown (365nm) color. Reddish brown color turns into dark green (265nm) and brown (365nm) when treated with 1N NaOH as tabulated in Table-6.

Quantitative analysis: TPC: A number of bioactivities are associated with phenolic compounds along with antioxidant, anti-aging, anti-inflammatory, and anti-proliferative properties[29]. TPC content was found higher in methanolic extract of root (45.18±0.011mg GAE/g) as compaired to leaf methanolic (44.27±0.007mg GAE/g) extract followed by root HE extract. Order of TPC in *Cenchrus biflorus* leaf and root extract in various solvents was as follows: RM (root methanol) > LM (leaf methanol) > RHE (root hydro ethanol) > LHE (leaf hydro ethanol) > RAQ (root aqueous) > LAQ (leaf aqueous). TPC levels were reported to be greater in barley grass (82.56±0.59mg GAE/g) as compared to present study[30]. Root methanol extract shows highest phenolic content among all the extract (45.18±0.011mg GAE/g) while minimum TPC in aqueous extract of leaf (19.5±0.001mg GAE/g) as shown in Figure-5 and Table-7.

TFC: As chelators of metal ions, flavonoids contain antioxidant activity because they scavenge free radicals and inhibit the activity of enzymes that produce them[31]. Order of TFC for *Cenchrus biflorus* leaf and root parts in different extract was as follows: LM>RM>LHE>RHE>LAQ>RAQ. TFC content were higher in leaf methanol extract (34±0.003mg QE/g) as tabulated in Table-7 and Figure-5.

TTC: TTC were found to be higher in methanol extract of leaf (8.14±0.006mg TA/g) while aqueous extract of root (2.57±0.002mg TA/g) shows minimal TTC among all the extracts of leaf and root. Order of TTC in *Cenchrus biflorus* was LM>RM>LHE>RHE>LAQ>RAQ as shown in Table-7 and Figure-5. Qualitative analysis revealed that 6.3% of tannin content was reported in *Cynodon dactylon*[32]. In addition to possessing antibacterial and antioxidant properties, tannins possess ability to form complexes along metal ions and macromolecules. We found that methanol extracted phenolic, flavonoid and tannin compounds from *Cenchrus biflorus* Roxb. significantly better than hydro-ethanol and aqueous extracts.

In present study from qualitative and quantitative estimation it is revealed that *Cenchrus biflorus* Roxb. contains various secondary metabolites. Phenols, flavonoids and Tannins exhibited inhibitory effects against bacteria, viruses and posses free radical scavenging activity and anticancerous and anti-inflammatory activity.

Statistical Analysis: The outcome are given as mean (n=3) of triplicates. All data are offered as mean ±standard deviation. All obtained data were evaluated IBM SPSS Statistics 20 software. For each and every output variable multiple-comparison Turkey's *post-hoc* ($p \leq 0.05$) tests was performed to compare the variance (ANOVA) of data between groups to verify correlation coefficient among TTC, TPC and TFC.

Table-6: Fluorescence characteristics of powdered leaf and root of *Cenchrus biflorus*.

Reagent	Leaf			Root		
Powder + Reagent	Day light	Low-light (254nm)	High-light (365nm)	Day light	Low-light (254nm)	High-light (365nm)
Only Powder	Light grey	Dark grey	Light grey	Brown	Dark brown	Light brown
Powder + Methanol	Light yellow	Dark yellow	Dark brown	Brown	Dark brown	Light brown
Powder + Ethanol	Light yellow	Dark brown	Brown	Brown	Dark brown	Light brown
Powder + Petroleum Ether	Colorless	Green	Brown	Yellowish brown	Brown	Dark Brown

Powder + Acetone	Light yellow	Light green	Yellowish brown	Light yellow	Dark green	Reddish brown
Powder + chloroform	Lemon yellow	Light green	Dark yellow	Brown	Dark brown	Brown
Powder + 50% H_2SO_4	Light yellow	Dark yellow	Reddish brown	Reddish brown	Light green	Light brown
Powder + 50% HCl	Green	Dark green	Reddish brown	Light brown	Greenish	Light brown
Powder +10% NaOH	Dark yellow	Dark brown	Reddish brown	Reddish brown	Dark green	Brown
Powder + Ammonia	Yellowish brown	Dark green	Dark brown	Light yellow	Light green	Light brown
Powder + Acetic Acid	Grey	Dark grey	Dark brown	Light yellow	Dark yellow	Dark brown
Powder + distilled water	Grey	Light green	Light brown	Light brown	Dark brown	Light brown
Powder + Iodine	Yellow	Dark green	Brown	Light Yellow	Dark green	Dark brown
Powder + $FeCl_3$	Grey	Light green	Light brown	Light yellow	Dark green	Brown
Powder + Picric Acid	Brownish yellow	Dark green	Reddish brown	Brownish yellow	Dark green	Yellow
Powder +$K_2Cr_2O_7$	Brownish Yellow	Light green	Light brown	Light Yellow	Light green	Light brown
Powder + Toluene	Light grey	Dark green	Reddish brown	Reddish brown	Dark green	Grey
Powder + Benzene	Colorless	Brown	Brown	Colorless	Blue	Dark brown
Powder + n-butanol	Grey	Dark grey	Brown	Dark yellow	Dark green	Dark Brown
Powder + Hexane	Light yellow	Dark yellow	Brown	Colorless	Blue	Black
Powder +Ethyl acetate	Colorless	Dark green	Reddish brown	Colorless	Brown	Dark brown

Table-7: Quantative analysis of *Cenchrus biflorus* leaf and root in three different solvents.

Plant parts	Solvents	TPC[A]	TFC[B]	TTC[C]
Leaf	Methanol	44.27 ± 0.007^{c}	34 ± 0.003^{b}	8.14 ± 0.006^{c}
	Hydro ethanol	34.5 ± 0.019^{b}	23 ± 0.005^{a}	6.14 ± 0.005^{b}
	Aqueous	19.5 ± 0.001^{a}	16 ± 0.004^{a}	3.28 ± 0.001^{a}
Root	Methanol	45.18 ± 0.011^{c}	28 ± 0.012^{a}	6.57 ± 0.302^{c}
	Hydro ethanol	40.9 ± 0.008^{b}	19 ± 0.007^{a}	4 ± 0.002^{b}
	Aqueous	25.86 ± 0.002^{a}	11 ± 0.015^{a}	2.57 ± 0.002^{a}

Values are represented as mean of three replicates ± SD. [A]TPC is expressed mg Gallic acid (GAE)/g, [B]TFC is expressed as mg Quercetin (QE)/g, [C]TTC is expressed as mg Tannic Acid (TA)/g.

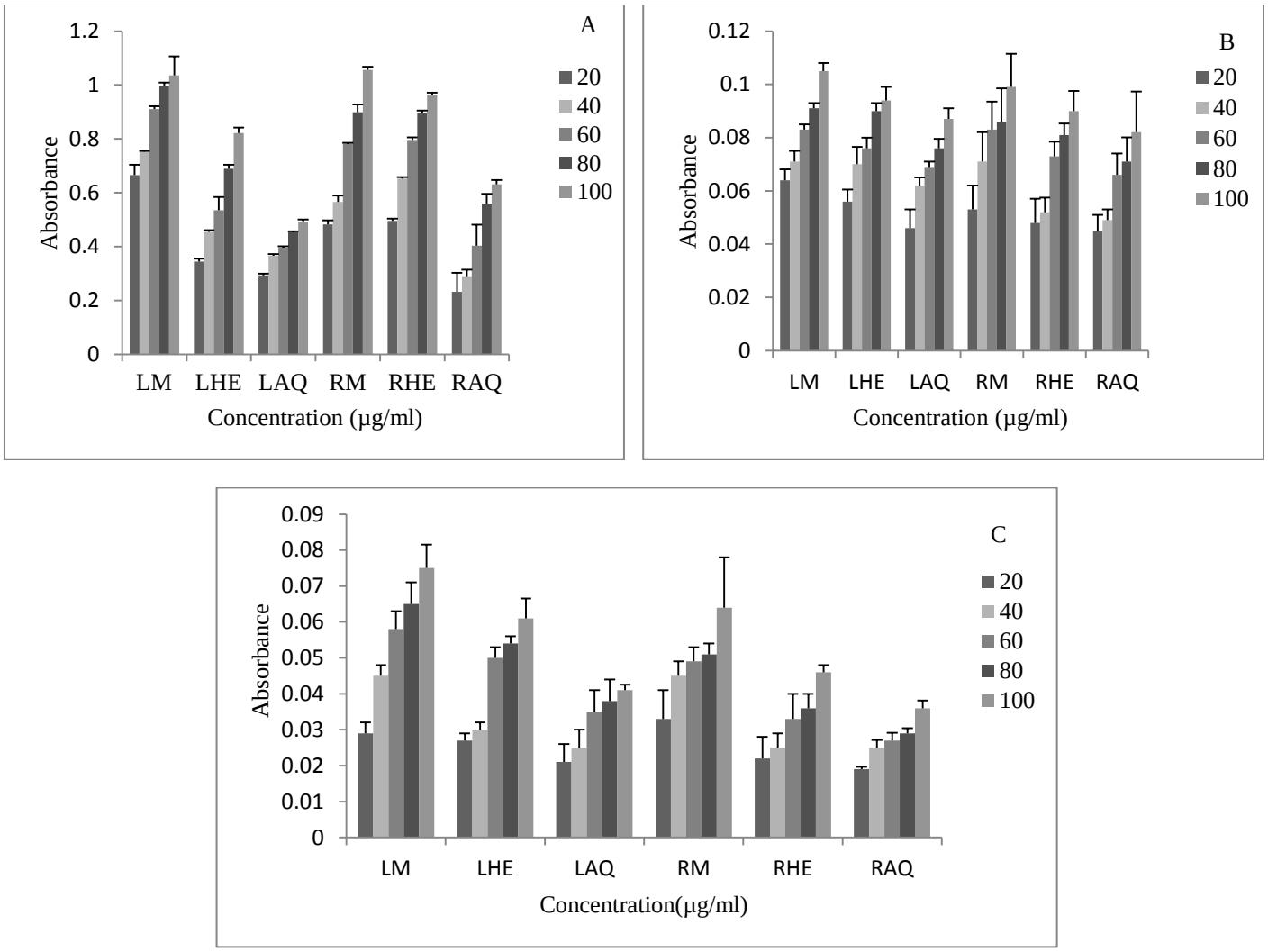

Figure-5: TPC (A), TFC (B) and TTC (C) in leaf and root extract of Cenchrus biflorus (LM= leaf methanol extract, LHA= leaf hydro-ethanol extract, LAQ= leaf aqueous extract, RM = root methanol extract, RHA= root hydro-ethanol extract, RAQ= root aqueous extract). Data represents the mean of three replicates ±SD and indicates significance at $P < 0.05$.

Conclusion

There is no detailed and comparative physicochemical, pharmacognostical and phytochemical analysis of *Cenchrus biflorus* Roxb. leaf and root parts has been reported till date. From all the analysis it could be concluded that *Cenchrus biflorus* leaf and root extracts possess phytochemical like phenols, flavonoids, tannins, alkaloids etc. Flavonoids, including a variety of classes that have been found to have numerous biological activities including anticancer, antibacterial activity, antifungal, anti-diabetic, anti-malarial action, neuroprotective, cardio-protective, and anti-inflammatory effects[33]. Methanolic extract of leaf and root shows higher content of TPC, TFC and TTC among all extract. Therefore extract of *Cenchrus biflorus* could be seen as a good source for synthesis of new drugs in pharmaceutical industries and macroscopic and microscopic analysis of plant will aid in the discovery of new drugs. To justify their safe use, it is mandatory that these herbal drugs must be fractionated, screened for toxicity and their active constituents must be determined.

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Abbreviations: TPC: total phenolic content, TFC: total flavonoid content, TTC: total tannin content, MeOH: methanol, HE: hydro-ethanol, AQ: aqueous.

References

1. Arora, S., & Kumar, G. (2018). Phytochemical screening of root, stem and leaves of *Cenchrus biflorus* Roxb. *Journal of Pharmacognosy and Phytochemistry*, 7(1), 1445-1450.

2. Kumar, M. R., & Janagam, D. (2011). Export and import pattern of medicinal plants in India. *Indian Journal of Science and Technology*, 4(3), 245-248.

3. Saxena, M., Saxena, J., Nema, R., Singh, D., & Gupta, A. (2013). Phytochemistry of medicinal plants. *Journal of pharmacognosy and phytochemistry*, 1(6), 168-182.

4. Edeoga, H. O., Okwu, D. E., & Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African journal of biotechnology*, 4(7), 685-688. https://doi.org/10.5897/AJB2005.000-3127

5. Khyade, V. B., Pawar, S. S., & Sarwade, J. P. (2018). Novel Sacrificial Medicinal Repositories: Halfa grass, *Desmostachya bipinnata* (L.) and Cogon grass, *Imperata cylindrica* (L.). *World Scientific News*, 100, 35-50.

6. Gebashe, F., Aremu, A. O., Gruz, J., Finnie, J. F., & Van Staden, J. (2020). Phytochemical profiles and antioxidant activity of grasses used in South African traditional medicine. *Plants*, 9(3), 371.

7. Katewa, S. S., Guria, B. D., & Jain, A. (2001). Ethnomedicinal and obnoxious grasses of Rajasthan, India. *Journal of* ethnopharmacology, 76(3), 293-297. https://doi.org/10.1016/S0378-8741(01)00233-1

8. Peerzada, A. M., & Naeem, M. (2018). Germination ecology of *Cenchrus biflorus* Roxb. : Effects of environmental factors on seed germination. *Rangeland ecology & management*, 71(4), 424-432. https://doi.org/10.1016/j.rama.2018.04.002

9. Hussey, B. M. J., Keighery, G. J., Dodd, J., Lloyd, S. G., & Cousens, R. D. (2007). Western weeds. A guide to the weeds of Western Australia.

10. Chase, A. (1920). The north american species of Cenchrus. Contributions from the United States National Herbarium, 22(1), 45-77.

11. Burkill, H. M. (1994). The useful plants of west tropical Africa. Volume 2: Families EI (No. Edn 2). Royal Botanic Gardens.

12. Yadav, R. N. S., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of phytology*, 3(12).

13. Prakash, A., Janmeda, P., Pathak, P., Bhatt, S., & Sharma, V. (2019). Development and standardization of quality control parameters of different parts of *Trianthema portulacastrum* L. *SN Applied Sciences*, 1(9), 1-14. https://doi.org/10.1007/s42452-019-1074-3

14. Brain, K. R., & Turner, T. D. (1975). The practical evaluation of phytopharmaceuticals. Vol. 1. Bristol: Wright-Scientechnica.

15. Pande, J., Padalia H., Rokad N., Chanda S. (2018). *Cyperus conglomeratus* (Cyperaceae) a halophyte from Gujarat: Physicochemical, Phytochemical and Pharmacognostic studies.

16. Bijeshmon, P. P., & George, S. (2014). Antimicrobial activity and powder microscopy of the flowers of *Tabernaemontana divaricata* R. BR. *Br. Indo Am. J. Pharm. Res*, 4(3).

17. Zahid, H., Rizwani, G. H., & Shareef, H. (2014). Standardization of *Hibiscus schizopetalus* (Mast) Hook according to World Health Organization (WHO) guidelines. *Journal of Medicinal Plants Research*, 8(22), 802-810.

18. Kumar, M., Mondal, P., Borah, S., & Mahato, K. (2013). Physico-chemical evaluation, preliminary phytochemical investigation, fluorescence and TLC analysis of leaves of the plant *Lasia spinosa* (Lour) Thwaites. *Int J Pharm Pharm Sci*, 5(2), 306-310.

19. Soni, A., & Sosa, S. (2013). Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. *Journal of Pharmacognosy and Phytochemistry*, *2*(4), 22-29.

20. Tadhani, M., & Subhash, R. (2006). Preliminary studies on Stevia rebaudiana leaves: proximal composition, mineral analysis and phytochemical screening. *J. Med. Sci*, 6(3), 321-326.

21. Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants*, 8(4), 96. https://doi.org/10.3390/plants8040096

22. Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of food and drug analysis*, 22(3), 296-302. https://doi.org/10.1088/1757-899X/736/2/022063

23. Haile, M., & Kang, W. H. (2019). Antioxidant activity, total polyphenol, flavonoid and tannin contents of fermented green coffee beans with selected yeasts. *Fermentation*, 5(1), 29. https://doi.org/10.3390/fermentation5010029

24. Evans, W.C. (2005) Trease and Evans' Pharmacognosy, 16th edn. Rajkamal Electric press, Delhi, pp 516–536

25. Loganathan, V., Devi, K. M., & Selvakumar, P. (2017). A study of the physico-chemical and phytochemical parameters of leaves of *Mallotus*

rhamnifolius. *International journal of pharmacognosy and phytochemical research*, 9(6), 858-863.

26. Singh, L. R. (2019). Pharmacognostic Studies of Organically Cultivated Panacea Herb *Cynodon Dactylon* (l.) Pers. *International Journal of Ayurveda and Pharma Research*, 65-68.

27. Singh, L. R. (2019). Pharmacognostic evaluation of organically cultivated herb *Echinochloa colonum* (L.) Link.

28. Singariya, P., Mourya, K. K., & Kumar, P. (2011). Preliminary phyto-profile and pharmacological evaluation of some extracts of Cenchrus grass against selected pathogens. *J Pharm Sci Res*, 3(8), 1387-1393.

29. Koirala, N., Pandey, R. P., Thuan, N. H., Ghimire, G. P., Jung, H. J., Oh, T. J., & Sohng, J. K. (2019). Metabolic engineering of Escherichia coli for the production of isoflavonoid-4′-O-methoxides and their biological activities. *Biotechnology and Applied Biochemistry*, 66(4), 484-493. https://doi.org/10.1002/bab. 1452

30. Panthi, M., Subba, R. K., Raut, B., Khanal, D. P., & Koirala, N. (2020). Bioactivity evaluations of leaf extract fractions from young barley grass and correlation with their phytochemical profiles. *BMC complementary medicine and therapies*, 20(1), 1-9. https://doi.org/10.1186/s12906-020-2862-4

31. Mondal, M., Hossain, M. S., Das, N., Khalipha, A. B. R., Sarkar, A. P., Islam, M. T., ... & Kundu, S. K. (2019). Phytochemical screening and evaluation of pharmacological activity of leaf Methanolic extract of Colocasia affinis Schott. *Clinical Phytoscience*, 5(1), 1-11.

32. Al-Snafi, A. E. (2016). Chemical constituents and pharmacological effects of *Cynodon dactylon*-A review. *IOSR Journal of Pharmacy*, 6(7), 17-31.

33. Ullah, A., Munir, S., Badshah, S. L., Khan, N., Ghani, L., Poulson, B. G., ... & Jaremko, M. (2020). Important flavonoids and their role as a therapeutic agent. *Molecules*, *25*(22), 5243.