Original Research

Evaluation of Ethno-Veterinary Grasses from Suburbs through Microscopic Technique: Insights into Antioxidant Defense System and Phenol

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Abstract

This research work was conducted to investigate the anatomical and antioxidant studies of some ethnoveterinary grasses (*Cenchrus pennisetiformis* and *Dichanthium annulatum*). The anatomical characteristics (compact epidermal layer, large cortical cells, thickened sclerenchyma, central and scattered vascular bundles, large metaxylem, small protoxylem, and centrally located pith) were observed. For the antioxidant activity studies, the crude methanol extract was prepared by maceration techniques and different fractions were prepared (n-hexane, chloroform, and aqueous solution). The maximum antioxidant potential in DPPH was shown by *C. pennisetiformis* at a concentration of 250 µL in the chloroform fraction, while *D. annulatum* showed a maximum value of 68.47±0.30 at a concentration of 250 µL in the methanolic extract. In both species, chloroform extract in TAA showed maximum potency at a concentration of 500 µL, but *C. pennisetiformis* showed a higher value (i.e. 1.15±0.0018) and *D. annulatum* showed a lower value (i.e. 1.05±0.0017). The best reducing power in FRAP was shown by *D. annulatum* followed by *C. pennisetiformis*.

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Grasses belong to the Poaceae family, which is the largest family of flowering plants [1-3]. Grasses are divided into 50 tribes, 660 genera, and 10,000 species found throughout the world, making the grass family one of the most diverse and widespread families of plants [1, 3-5]. They are adapted to almost all terrestrial habitats on earth, being found on all continents including Antarctica [6]. Grasses are very important in traditional medical care because they contain natural dynamic substances such as alkaloids, flavonoids, and saponins [7-9]. The presence of alkaloids makes them extremely resistant to external microbes, while flavonoids are associated with anticancer and antiviral properties and help animals overcome oxidative cell damage [10]. Some of the species are used as a cancer remedy, useful remedy for inflammation and also to increase the milk production in animals [11]. Grasses are traditionally the best-known and most reliable forage hotspot for ruminants [12]. The natives want to use grass as fodder, because grasses are considered more acceptable by ruminants than bushy food [13]. Also the grasses have enormous development capacities at different seasons [14].

The botanical taxa of the Poaceae family are divided into 50 tribes, 660 genera, and 10,000 species [1, 2]. Grasses are considered more acceptable by ruminants than bushy food [13]. Also the grasses have enormous development capacities at different seasons [14].

The importance of grasses in traditional medicine and as a source of food for animals cannot be overstated [20, 21]. They’re also used as food crops, as well as pasture, garden and ornamental plants, and are used for decomposition. They are used to make thread, paper, sugar, fragrant oils, glue, starch, and liquor, among other things. Their shaped root structure holds soil in place and prevents soil decomposition on slopes and ridges, along riverbanks, and near the sea. Although grasses are widely regarded as cost-effective plants, research on their use in traditional medicine is limited [22].

ROS are also called free radical oxygen species which include non-radical species such as hydrogen peroxide [23, 24]. They are exceptionally susceptible substance species that are released during typical oxygen consuming cellular digestion in any tissue and damage the various intracellular segments, for example, nucleic acids, lipids and proteins [25-28]. Free radicals generated by endogenous metabolic reactions are associated with various infections such as tumors, shock, irritation, diabetes, cerebrum damage and malignant growth [29, 30]. ROS and free radicals are controlled in nature by certain chemicals with antioxidant activity, for example, superoxide dismutase and peroxidase [31, 32]. An irregularity between ROS and the innate cancer prevention agent suggests the body to make both dietary and therapeutic improvements during disease onset [33]. Polyphenol antioxidants have defensive effects against various infections, including cardiovascular and neurological diseases, as well as malignancies, and possess antioxidant components in rich sources responsible for medicinal properties [33-35]. Moreover, most of the polyphenols known so far, have been shown to be able to scavenge free radicals [36].

Ethnoveterinary medicine is a field of study that deals with animal health values, methods, skills, procedures, and practices [37-40]. Traditional ethnoveterinary knowledge is used in the health care system for domestic animals [41]. Different types of ethno therapeutic medicines are used for the treatment of various animal diseases, which are considered more important compared to synthetic medicines [42]. Ethnoveterinary medicines are not difficult to use, modest, and quickly accessible [43]. Ethnoveterinary medicines are currently gaining popularity in both developed and agricultural countries because they are easily accessible and can be purchased by agronomists at an extremely low cost [44].

Materials and Methods

Grasses were collected from different locations of Lahore and were made shade dry for some days. Some
part of the stem and leaves of grasses were preserved in fixative i.e. 95% Ethanol (500 mL) + Acetic Acid (50 mL) + Formalin 14% (100 mL) + Distilled Water (350 mL) to study the anatomical features by section cutting through microtome. After that the permanent slides were made, sections were observed under microscope and photographs were taken with the help of a mobile camera at different resolutions such as 4X, 10X, and 40X.

Preparation of Crude Methanol Extracts by Maceration Technique

Extraction method used according to [45] with slight modifications. Each species’ dried and chopped grass material was steeped in methanol for at least one week at room temperature. After 12 days the samples were filtered and subjected to evaporation to get a dark green viscous oily mass under vacuum pressure using a rotary evaporator.

Fractionation of Different Solvents

For antioxidant activity evaluation, methanol extract was mixed with different organic solvents according to the polarity for fractionation in separating funnel. The extract suspended with water and hexane (1:1) being nonpolar was mixed with the suspension in double. It was shaken vigorously; hexane and water layer were separated. In the same way chloroform and aqueous solution (water) was separated through the standard protocol of [46].

Evaluation of Antioxidant Activity

DPPH Radical Scavenging Activity

According to the procedure of [47], different concentrations (60 µL, 80 µL, 100 µL, 150 µL and 250 µL) of distinct fractions were prepared and placed in six different test tubes, with the volume elevated to 3 mL using a methanolic solution of 0.1 mM DPPH. The entire arrangement was wrapped in aluminum foil and kept in the dark for one hour at room temperature after being shaken. The absorbance was measured at 517 nm wavelength against methanol as a blank. Three duplicates of each sample were run. The following formula was used to determine the percentage of DPPH discoloration.

\[
\text{Antiradical activity} = \frac{(A_{\text{control}} - (A_{\text{sample}} - A_{\text{blank}}))}{A_{\text{control}}} \times 100
\]

The absorbance of the control is represented by A control, the absorbance of the sample is represented by A sample and the absorbance of the sample blank is represented by A blank.

Total Antioxidant Activity by Phosphomolybdenum Method

According to the standard method of [48] with the slight modification of [49], the reaction mixture was prepared by taking 0.6 M H₂SO₄ prepared by mixing 8 mL H₂SO₄ in 250 mL of distilled water. For the preparation of 4 mM ammonium molybdate, 1.24 g was dissolved in 250 mL of distilled water. To prepare 28 mM of sodium phosphate, 2.66 g was dissolved in 250 mL of distilled water. All of them were mixed together to prepare the phosphomolybdenum complex. Different concentrations i.e., 125, 250 and 500 µg/mL of each extract were made and mixed with 3 mL of the above prepared solution and total volume was raised up to 4 mL in test tubes and heated in a water bath at a temperature of 95°C for 90 minutes. Then the desicator was used to cool them. After that the absorbance was measured at 695 nm wavelength [50].

Ferric Reducing Antioxidant Power (FRAP) Assay

According to [51] approach, 2.5 mL of 2,4,6-Tripyridyl-S-triazine (TPTZ) solution + 2.5 mL of FeCl₃.6H₂O + 25 mL of acetate buffer was mixed together to prepare a fresh solution. It was then gently warmed at 37°C. 2990 µL of FRAP solution was discharged in test tubes containing 10 µL of sample and Standard Butylated hydroxytoluene (BHT) solution to bring the total amount up to 3 mL. The mixture was then put in the dark for 30 minutes. The absorbance was measured at 593 nm wavelength [52].

Total Phenolic Content

According to [53] and [54], 1 mL of each grass extract was combined with 9 mL of distilled water, 1 mL of Folin-Ciocalteu reagent, and 10 mL of Na₂CO₃. After that the volume was raised up to 25 mL by adding distilled water and placing it for 90 minutes. The absorbance was then measured at 725 nm wavelength [55].

Statistical Analysis

The data was compiled in terms of three replicates as means ± of standard error. One way analysis (ANOVA) was employed to check the differences among the means via the method used [56].

Results

Anatomical Characters

The transverse section of the stem of Cenchrus pennisetiformis showed an outer layer of epidermal cells, rounded in shape having a cuticle and cutinized walls (Fig. 1a) while in Dicanthus annulatum, epidermis was
also the outermost layer of the stem that had completely rounded the structure (Fig. 3a). The epidermis of the stem consists of only a single layer of cells arranged in a compact arrangement. In Cenchrus pennisetiformis, next to the epidermal layer cortical cells were seen much larger in size and scattered on different positions (Fig. 1a), while cortical cells were smaller in size and appearing on different locations in Dicanthium annulatum (Fig. 3d). On the other hand, the presence of large metaxylem and smaller sized protoxylem was seen in stem structure of both species with the centrally located pith. Comparison of length and width of parameters of stem is shown in Table 1 and 2. While observing the sections of the leaf of both species, the arrangement of the outer layer of cells was observed under the light microscope which was considered to be layer of epidermal cells (Fig. 2a and Fig. 4a) containing no trichomes (hairs). In Cenchrus pennisetiformis sclerenchyma cells were broader and shorter in size pointed from both ends (Fig. 2c) while in Dicanthium annulatum these cells were long and narrow, tapering from both ends, and this is due to the deposition of lignin (Fig. 4b). Bundle sheath cells were clearly seen in the magnified view of Dicanthium annulatum (Fig. 4c) covering the major portion with the presence of centered phloem cells surrounded by the xylem cells as compared to the bundle sheath cells in Cenchrus pennisetiformis that were present in compact arrangement along with xylem and phloem cells but covering less space (Fig. 2d). Comparison of the length and width of the parameters of the leaf is shown in Table 3 and 4.

Antioxidant Potential

**DPPH Free Radical Scavenging Activity**

Plants have a special defense system of antioxidant enzymes to counteract the harmful effects of ROS and oxidative damages [57]. DPPH is a stable nitrogen-centered free radical molecule that is commonly used to evaluate the potential of various substances including single compounds, food and different extracts to scavenge free radicals. DPPH potential obtained by different fractions was compared with the standard BHT (Table 5 and 6). At a concentration of 250 µL, the chloroform extracts of Cenchrus pennisetiformis had a maximum value of 76.33±0.12. At varied concentrations, the DPPH potential of the different fractions of the extracts was found to be between 76.33±0.12 and 40.6±0.20. While on the other hand, the methanol extract of the Dicanthium annulatum displayed a maximum value of 68.47±0.30 at a concentration of 250 µL. The DPPH potential of the different fractions of the extracts at various concentrations was found to be in between the range of 68.47±0.30 to 24.58±0.13.

### Table 1. Length and width of different parameters of stem of Cenchrus pennisetiformis Hochst.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Parameters</th>
<th>Length (mm)</th>
<th>Range of length of cells (mm)</th>
<th>Width (mm)</th>
<th>Range of width of cells (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Epidermal cells</td>
<td>0.09±0.01</td>
<td>0.09-0.12</td>
<td>0.15±0.03</td>
<td>0.15-0.25</td>
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<td>2.</td>
<td>Cortical cells (large)</td>
<td>1.2±0.05</td>
<td>1.0-1.3</td>
<td>0.94±0.12</td>
<td>0.7-0.9</td>
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<td>3.</td>
<td>Cortical cells (small)</td>
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<td>0.6±0.07</td>
<td>0.65-0.85</td>
</tr>
<tr>
<td>4.</td>
<td>Vascular bundles</td>
<td>1.15±0.16</td>
<td>1.15-1.25</td>
<td>1.14±0.03</td>
<td>1.0-1.2</td>
</tr>
<tr>
<td>5.</td>
<td>Metaxylem</td>
<td>0.64±0.16</td>
<td>0.4-0.68</td>
<td>0.5±0.02</td>
<td>0.4-0.5</td>
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<tr>
<td>6.</td>
<td>Protoxylem</td>
<td>0.17±0.02</td>
<td>0.15-0.25</td>
<td>0.15±0.01</td>
<td>0.15-0.19</td>
</tr>
<tr>
<td>7.</td>
<td>Phloem</td>
<td>0.10±0.02</td>
<td>0.09-0.11</td>
<td>0.12±0.006</td>
<td>0.08-0.14</td>
</tr>
</tbody>
</table>

### Table 2. Length and width of different parameters of the stem of Dicanthium annulatum Stapf.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Parameters</th>
<th>Length (mm)</th>
<th>Range of length of cells (mm)</th>
<th>Width (mm)</th>
<th>Range of width of cells (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Epidermal cells</td>
<td>0.19±0.003</td>
<td>0.19-0.20</td>
<td>0.11±0.003</td>
<td>0.11-0.12</td>
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<tr>
<td>2.</td>
<td>Cortical cells (large)</td>
<td>0.66±0.03</td>
<td>0.6-0.7</td>
<td>0.67±0.037</td>
<td>0.6-0.72</td>
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<tr>
<td>3.</td>
<td>Cortical cells (small)</td>
<td>0.50±0.051</td>
<td>0.42-0.6</td>
<td>0.5±0.05</td>
<td>0.4-0.6</td>
</tr>
<tr>
<td>4.</td>
<td>Vascular bundle</td>
<td>0.98±0.006</td>
<td>0.98-1.0</td>
<td>0.70±0.052</td>
<td>0.62-0.8</td>
</tr>
<tr>
<td>5.</td>
<td>Metaxylem</td>
<td>0.14±0.008</td>
<td>0.13-0.16</td>
<td>0.18±0.005</td>
<td>0.18-0.19</td>
</tr>
<tr>
<td>6.</td>
<td>Protoxylem</td>
<td>0.12±0.005</td>
<td>0.09-0.11</td>
<td>0.10±0.003</td>
<td>0.10-0.11</td>
</tr>
<tr>
<td>7.</td>
<td>Phloem</td>
<td>0.10±0.003</td>
<td>0.10-0.11</td>
<td>0.1±0.00</td>
<td>0.1-0.1</td>
</tr>
</tbody>
</table>
Fig. 1. a) T.S. of stem of Cenchrus penissetiformis Hochst. showing epidermis and cortical cells; b) central view of pith, c) peripheral view of vascular bundles and phloem; d) metaxylem and protoxylem cells, ep: epidermis, co: cortical cells, v: vascular bundles, ph: phloem, pi: pith, mx: metaxylem, px: protoxylem (10X, 40X).

Fig. 2. a) T.S. of leaf section of Cenchrus penissetiformis Hochst. showing epidermis; b) magnified view of bundle sheath cells; c) sclerenchyma cells; d) xylem and phloem cells, ep: epidermis, bs: bundle sheath cells, sc: sclerenchyma cells, xy: xylem, ph: phloem (10X, 40X).
Fig. 3. a) T.S. of stem of Dicanthium annulatum Stapf. showing epidermis; b) peripheral view of section showing vascular bundles and centered phloem; c) central view of protoxylem and metaxylem; d) peripheral view of cortical cells, ep: epidermis, v: vascular bundles, ph: phloem mx: metaxylem, px: protoxylem, co: cortical cells (10X, 40X).

Fig. 4. a) T.S. of leaf section of Dicanthium annulatum Stapf. showing epidermis b). magnified view of sclerenchyma cells; c) bundle sheath cells b; d) xylem and phloem cells, ep: epidermis, sc: sclerenchyma cells, bs: bundle sheath cells, xy: xylem, ph: phloem (10X, 40X).
sample i.e. more the concentration of sample more the % inhibition (Fig. 5a).

**Total Antioxidant Activity**

In the present study, phosphomolybdenum assay was used to determine the total antioxidant activity of various fractions of *Cenchrus pennisetiformis* (Fig. 5b). Chloroform extract had the highest antioxidant efficacy (1.15±0.0018 at a concentration of 500 µL), whereas methanol extract had the lowest antioxidant potency (0.74±0.0020 at a concentration of 250 µL) while in *Dicanthium annulatum* chloroform extract had the highest antioxidant efficacy, 1.05±0.0017 at a concentration of 500 µL, while aqueous solution extract had the lowest antioxidant potency, 0.22±0.0016 at a concentration of 125 µL. BHT has a total antioxidant activity of 1.22±0.09 (Table 7). All of these absorbances at 695 nm wavelength readings were the total of three parallel replicates, with the stated standard error differing significantly at p≤0.05.

**FRAP Assay**

In *Cenchrus pennisetiformis* chloroform, methanol and n-hexane fractions exhibited higher FRAP values, i.e. 1.62, 1.50 and 1.14 TE µM/mL as compared to the aqueous solution that showed value 0.75E µM/mL respectively (Table 9). The maximum FRAP value was found in the chloroform extract, indicating a synergistic effect of antioxidant components, while the lowest FRAP value was found in the aqueous solution fraction. The FRAP experiment was used to calculate the reduction potential of several solvents at different concentrations. It was essentially the sum of antioxidants and the sample’s lowering capability. The test was completed, and the findings were given in TE µM/mL. To express the data in agreement, a Trolox standard curve was created.

**Total Phenolic Content**

*Cenchrus pennisetiformis* showed highest value in chloroform fraction 78.36±0.020 mg/mL GAE followed by methanol (67.56±0.176 mg/ mL), aqueous solution (10.65±0.017 mg/ mL) and n-hexane fraction (7.34±0.018 mg/ mL) respectively (Fig. 5d; Table 8). In *Dicanthium annulatum* chloroform, methanol and n-hexane fraction, methanol and n-hexane exhibited higher FRAP values, i.e. 1.62, 1.50 and 1.14 TE µM/mL as compared to the aqueous solution that showed value 0.75E µM/mL respectively (Table 9). The maximum FRAP value was found in the chloroform extract, indicating a synergistic effect of antioxidant components, while the lowest FRAP value was found in the aqueous solution fraction. The FRAP experiment was used to calculate the reduction potential of several solvents at different concentrations. It was essentially the sum of antioxidants and the sample’s lowering capability. The test was completed, and the findings were given in TE µM/mL. To express the data in agreement, a Trolox standard curve was created.
Discussion

Anatomical studies have been used successfully to demonstrate the taxonomic status and also played an important role in the identification of grass species. These studies demonstrated that different species showed varieties in different anatomical features which are considered to be very useful in the identification and differentiation of the species. But on the other hand, there are some of the characteristics which are morphologically or anatomically alike in all species of the tribe, such as the presence of compact epidermal layers, arrangement of cortical and mesophyll cells and properly arranged bundle sheath cells [58]. Our results agree with the work of [59], they also reported the micromorphology of stem and leaf of *C. sinensis* and determined anatomy of epidermal layer cells with the absence of hairs (trichomes). These anatomical characteristics create such an environment to place the species in the same tribe [60]. Similar studies also done by many workers: [61] determined the anatomical characteristics of *Setaria* genus, [62] worked on the taxonomy of wetland grasses from Azad Kashmir, Pakistan and determined different anatomical and morphological features. The effective protecting tool against ROS and their derivatives produced inside the living cells during biochemical processes are considered as antioxidants. For the last three decades natural antioxidants are considered of very keen interest due to the health and toxicity issues collaborating with synthetic antioxidants found in the market such as Butylated hydroxyanisole (BHA) and

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Solvent</th>
<th>Conc. (µg/ml)</th>
<th>% Scavenging</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Methanol</td>
<td>20</td>
<td>58.49±0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>70.44±0.12</td>
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<td></td>
<td></td>
<td>80</td>
<td>72.42±0.15</td>
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<td></td>
<td></td>
<td>100</td>
<td>74.36±0.21</td>
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<tr>
<td></td>
<td></td>
<td>150</td>
<td>67.39±0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>76.15±0.10</td>
</tr>
<tr>
<td>2.</td>
<td>N-hexane</td>
<td>20</td>
<td>48.7±0.15</td>
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<td></td>
<td>60</td>
<td>58.44±0.16</td>
</tr>
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<td></td>
<td></td>
<td>80</td>
<td>51.51±0.10</td>
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<td></td>
<td>100</td>
<td>53.76±0.05</td>
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<td></td>
<td>150</td>
<td>46.71±0.16</td>
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<td>250</td>
<td>50.60±0.15</td>
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<td>80</td>
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<td>100</td>
<td>67.56±0.15</td>
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<td>65.39±0.15</td>
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<td>250</td>
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<td>4.</td>
<td>Aqueous Solution</td>
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<td>49.57±0.05</td>
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<tr>
<td>BHT</td>
<td>Standard</td>
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<td>77.3±0.7</td>
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Table 5. % DPPH free radical scavenging activity of different solvent extracts of *Cenchrus pennisetiformis* Hochst.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Solvent</th>
<th>Conc. (µg/ml)</th>
<th>% Scavenging</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Methanol</td>
<td>20</td>
<td>44.85±0.20</td>
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<td>49.44±0.23</td>
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<td></td>
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<td>80</td>
<td>54.4±0.37</td>
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<td></td>
<td></td>
<td>100</td>
<td>62.40±0.18</td>
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<td>150</td>
<td>64.61±0.19</td>
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<td></td>
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<td>250</td>
<td>68.47±0.30</td>
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<tr>
<td>2.</td>
<td>N-hexane</td>
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<td>44.28±0.16</td>
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</tr>
<tr>
<td>3.</td>
<td>Chloroform</td>
<td>20</td>
<td>48.46±0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>59.39±0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>65.69±0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>65.12±0.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150</td>
<td>66.54±0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>67.47±0.19</td>
</tr>
<tr>
<td>4.</td>
<td>Aqueous Solution</td>
<td>20</td>
<td>28.58±0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>24.58±0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>35.71±0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>27.53±0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150</td>
<td>31.51±0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>57.67±0.14</td>
</tr>
<tr>
<td>BHT</td>
<td>Standard</td>
<td></td>
<td>77.3±0.7</td>
</tr>
</tbody>
</table>

Table 6. % DPPH free radical scavenging activity of different solvent extracts of *Dicanthium annulatum* Stapf.
BHT. They are linked with the metabolism of lipids and are an automatic source of antioxidants [63]. A plan of various solvents has been utilized in the measurement of cancer prevention agents in grasses in the current examination. DPPH examination is a widely utilized convention to decide the forage capacities of the free radicals by the normal cancer prevention agents incorporated inside the living cells [64]. In Pakistan, livestock is considered a subsector of agriculture [65]. This industry is critical to poverty

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Solvent</th>
<th>Concentration (µg/ml)</th>
<th>Abs. at 695 nm ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Methanol</td>
<td>125</td>
<td>0.45±0.0021c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>0.62±0.0020b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>0.99±0.0017a</td>
</tr>
<tr>
<td>2.</td>
<td>N-hexane</td>
<td>125</td>
<td>0.39±0.0020c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>0.43±0.0017b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>0.81±0.0012a</td>
</tr>
<tr>
<td>3.</td>
<td>Chloroform</td>
<td>125</td>
<td>0.55±0.0021c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>0.58±0.0017b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>1.05±0.0017a</td>
</tr>
<tr>
<td>4.</td>
<td>Aqueous solution</td>
<td>125</td>
<td>0.22±0.0026d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>0.29±0.0015c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>0.32±0.0020b</td>
</tr>
<tr>
<td>BHT</td>
<td>Standard</td>
<td></td>
<td>1.22±0.09</td>
</tr>
</tbody>
</table>

Table 7. Total antioxidant activity of different solvent extracts of Dicanthium annulatum Stapf.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Solvent</th>
<th>Abs. at 593 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Methanol</td>
<td>1.21±0.0011b</td>
</tr>
<tr>
<td>2.</td>
<td>N-hexane</td>
<td>1.08±0.0023c</td>
</tr>
<tr>
<td>3.</td>
<td>Chloroform</td>
<td>1.32±0.0018a</td>
</tr>
<tr>
<td>4.</td>
<td>Aqueous solution</td>
<td>0.97±0.0021d</td>
</tr>
</tbody>
</table>

Table 8. FRAP assay of different solvent extracts of Cenchrus pennisetiformis Hochst.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Solvent</th>
<th>Abs. at 593 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Methanol</td>
<td>1.30±0.0023b</td>
</tr>
<tr>
<td>2.</td>
<td>N-hexane</td>
<td>1.14±0.0008c</td>
</tr>
<tr>
<td>3.</td>
<td>Chloroform</td>
<td>1.62±0.0017a</td>
</tr>
<tr>
<td>4.</td>
<td>Aqueous solution</td>
<td>0.75±0.0020d</td>
</tr>
</tbody>
</table>

Table 9. FRAP assay of different solvent extracts of Dicanthium annulatum Stapf.
but also their livestock since ancient times [75]. Humans have been employing plant resources for medical purposes for not only themselves [68, 72-74]. Ethnoveterinary field studies, particularly those involving traditional herbal remedies for treating animal diseases, are critical in many rural areas around the world for several reasons [67, 68], (a) to propose effective and less expensive treatments as alternatives to and complements to pharmaceuticals, and especially to reduce antibiotic abuse in animal breeding, which is detrimental to the quality of animal food products [69], (b) to encourage the long-term use of local medicinal plant resources in animal care and thus contribute to rural development policies [70], (c) to promote local bio-cultural heritage [71], and (d) to investigate the link between human and veterinary plant uses in order to possibly assess the origin of herbal practices [68, 72-74]. Humans have been employing plant resources for medical purposes for not only themselves but also their livestock since ancient times [75]. Animal caretakers were able to reduce the prohibitive cost of certain veterinary medicines by using these indigenous botanicals to manage different health conditions in their cattle [76]. This traditional ethnoveterinary approach is playing an important role in preserving animal production in rural areas around the world where livestock is the primary source of income [77].

In Pakistan, many restorative plants and grasses are utilized for treating animals in distant regions where admittance to current medications is restricted and individuals have adequate information about customary treatments. Pakistani laypeople, shepherds, ranchers, traveling nibblers, and customary healers utilized these restorative plants to treat animal sicknesses. The current research documented 2 different ethnoveterinary grasses that have significant importance in treating different types of ailments by producing natural antioxidants in their bodies [68]. The aerial parts of *Dicanthium annulatum* acquire good antioxidant and antiparasitic activity due to which it has significant anticancer effects. It is known as “marvel grass” and is used as a fodder for animals and from seed it can be developed easily and is used for the treatment of diarrhea problems [78].

### Table 10. TPC evaluation of different solvent extracts of Cenchrus pennisetiformis Hochst.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Solvent</th>
<th>GAE mg/ml ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Methanol</td>
<td>67.56±0.176b</td>
</tr>
<tr>
<td>2.</td>
<td>N-hexane</td>
<td>7.34±0.018d</td>
</tr>
<tr>
<td>3.</td>
<td>Chloroform</td>
<td>78.36±0.020a</td>
</tr>
<tr>
<td>4.</td>
<td>Aqueous solution</td>
<td>10.65±0.017c</td>
</tr>
</tbody>
</table>

### Table 11. TPC evaluation of different solvent extracts of Dicanthium annulatum Stapf.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Solvent</th>
<th>GAE mg/ml ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Methanol</td>
<td>73.15±0.020a</td>
</tr>
<tr>
<td>2.</td>
<td>N-hexane</td>
<td>4.16±0.011d</td>
</tr>
<tr>
<td>3.</td>
<td>Chloroform</td>
<td>43.6±0.152b</td>
</tr>
<tr>
<td>4.</td>
<td>Aqueous solution</td>
<td>23.05±0.008c</td>
</tr>
</tbody>
</table>

### Conclusions

Anatomical results are accommodating in studying the internal features of grasses and how nature has enclosed such species alluringly. Taxonomic disputes can easily be resolved using anatomical parameters. Different anatomical parameters were studied and considered to play an important role in the identification and classification of the grass species. The data analysis revealed a wide range of palatability for major ruminant species (cattle, buffalo, goat, and sheep), abundant availability in the study area, and a variety of feeding strategies. It is important not only for the preservation of ethnoveterinary knowledge, but it may also aid in the development of sustainable livestock nutrition for ruminants. So, it is concluded that the present investigation has given experimental evidence to study more and more different grasses to demonstrate their anatomical features along with the phytochemical analysis and antioxidant potential so they can be used as a vital medication source for the treatment of various ailments and as fodder for different animals. As this was the first time in Pakistan that the anatomy and phytochemical analysis of ethnoveterinary grasses was studied collectively, it will benefit the scientists and their future research work in upcoming days to perpetuate the quality standards.

### Author’s Contributions


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Conflict of Interest

The authors declare no conflict of interest.

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