Pol. J. Environ. Stud. Vol. 33, No. 3 (2024), 3367-3379
DOI: 10.15244/pjoes/176049 ONLINE PUBLICATION DATE: 2024-03-26

Original Research

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

Awais Raza [1], Uzma Hanif [1, 2], Shanza Ali [1], Baber Ali [3], Muhammad Nauman Khan[4, 5*], Mahreen Fatima [6], Sadia Rafique [7], Muhammad Rashid [8], Malik Muhammad Sher [9], Riaz Ullah [10]**

[1] Department of Botany, Government College University, Lahore 54000, Pakistan
[2] Department of Drug Design and Pharmacology Faculty of Health and Medical Sciences, University of Copenhagen,Universitetsparken 2, DK-2100, Denmark
[3] Department of Plant Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan
[4] Department of Botany, Islamia College Peshawar, 25120 Peshawar, Pakistan
[5] University Public School, University of Peshawar, 25120 Peshawar, Pakistan
[6] Faculty of Biosciences, Cholistan University of Veterinary and Animal Sciences, Bahawalpur 63100, Pakistan
[7] Department of Botany, University of Gujrat, Gujrat 50700, Pakistan
[8] Plant Breeding and Genetics, Muhammad Nawaz Sharif University of Agriculture, Multan 66000, Pakistan
[9] Department of Agronomy, PMAS-Arid Agriculture University, Rawalpindi 46000, Pakistan
[10] Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia

Received: 20 August 2023
Accepted: 28 November 2023

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 lever value (i.e. 1.05±0.0017). The best reducing power in FRAP was shown by *D. annulatum* followed by *C. pennisetiformis. C. pennisetiformis* showed the highest value

*e-mail: nomiflora@uop.edu.pk
**e-mail: uzmahanif@gcu.edu.pk
Tel.: +92-3325350429

in the chloroform fraction while *D. annulatum* showed a higher significant value in the methanol fraction. These grasses have the potential to control several diseases caused by ROS in animals.

Keywords: anatomical studies, animals, antioxidants, ethnobotany, grasses

Introduction

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 an important component of agricultural crops and animal feed, as well as one of the most important economic sources and sources of income for people in rural areas around the world [15]. Fodder grasses and typical croplands are used by the majority of domesticated animals. Native Poaceae plant species are an in expensive source of animal feed, and help soil conditions, water supplies, and air quality [16, 17]. The low volume and diversity of scavengers available in the dry season, which cannot meet the supplementation needs of ruminants, is a significant barrier to further improvement in the utility of domesticated animals. People in rural areas use grasses as animal fodder and as medicine to cure a variety of diseases in humans and livestock [18]. Members of the Poaceae have been shown to be inundated with alkaloid less species and to have few medicinal properties. To escape herbivores, this group of plants produces only a limited number of defensive compounds and relies on its natural defenses, such as silicates and leaf strength. In this way, only a few plants in the Poaceae family produce substance enhancers that are considered therapeutic. Grasses contain secondary metabolites that are considered organically inert and have low mobility (e.g., tannins and lignin). These mixtures are often characterized by high atomic weight, which reduces edibility and herbivory [19].

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.

$$\text{Antiradical activity} = (A_{control} - (A_{sample} - A_{blank}) / A_{control}) \times 100$$

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 H_2SO_4 prepared by mixing 8 mL H_2SO_4 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 95ºC for 90 minutes. Then the desiccator 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 $FeCl3.6H_2O$ + 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 Na_2CO_3. 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 *Dicanthium 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. The % DPPH was found dependent on the concentration of

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

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

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

Sr. No.	Parameters	Length (mm)	Range of length of cells (mm)	Width (mm)	Range of width of cells (mm)
1.	Epidermal cells	0.19±0.003	0.19-0.20	0.11±0.003	0.11-0.12
2.	Cortical cells (large)	0.66±0.03	0.6-0.7	0.67±0.037	0.6-0.72
3.	Cortical cells (small)	0.50±0.051	0.42-0.6	0.5±0.05	0.4-0.6
4.	Vascular bundle	0.98±0.006	0.98-1.0	0.70±0.052	0.62-0.8
5.	Metaxylem	0.14±0.008	0.13-0.16	0.18±0.005	0.18-0.19
6.	Protoxylem	0.12±0.005	0.09-0.11	0.10±0.003	0.10-0.11
7.	Phloem	0.10±0.003	0.10-0.11	0.1±0.00	0.1-0.1

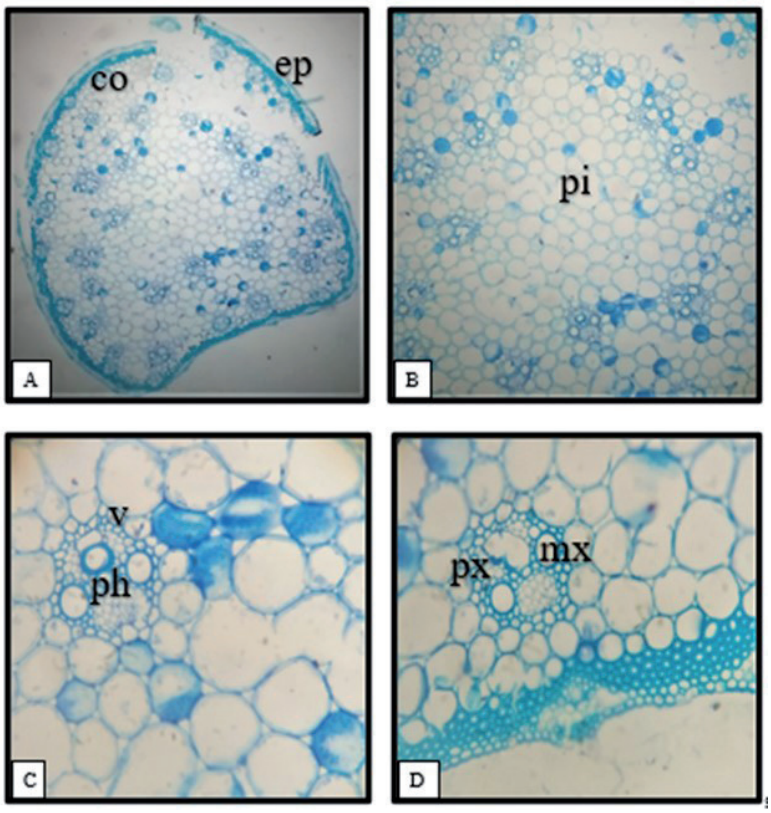

Fig. 1. a) T.S. of stem of Cenchrus pennisetiformis Hochst. showing epidermis and cortical cells; b) central view of pith, c) peripheral view of vascular bundles and phloem; d) metaxylem and protoxylem cells, ep: epidermis, co: cortical cells, v: vascular bundles, ph: phloem, pi: pith, mx: metaxylem, px: protoxylem (10X, 40X).

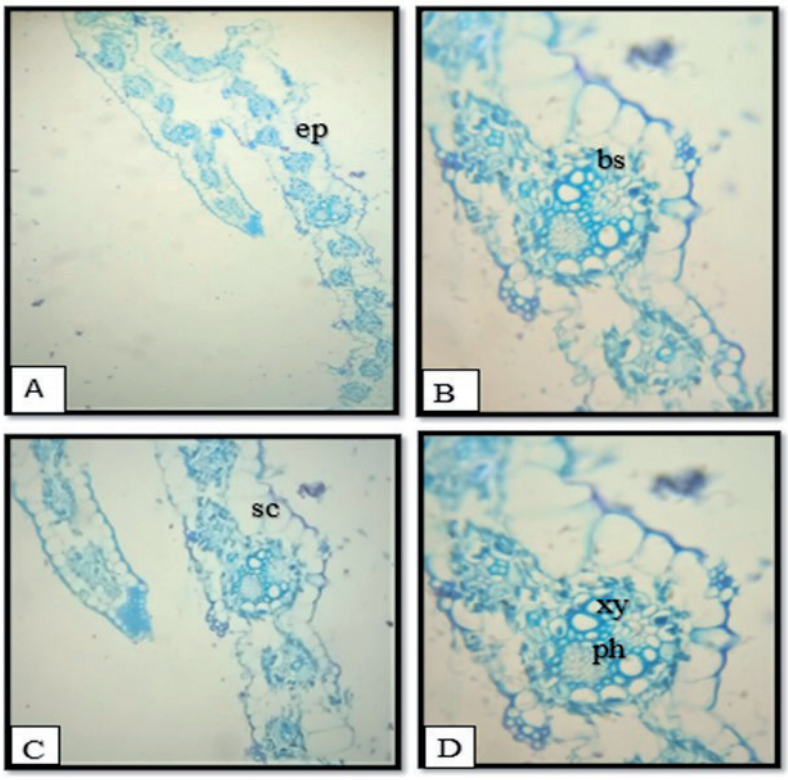

Fig. 2. a) T.S. of leaf section of Cenchrus pennisetiformis Hochst. showing epidermis; b) magnified view of bundle sheath cells; c) sclerenchyma cells; d) xylem and phloem cells, ep: epidermis, bs: bundle sheath cells, sc: sclerenchyma cells, xy: xylem, ph: phloem (10X, 40X).

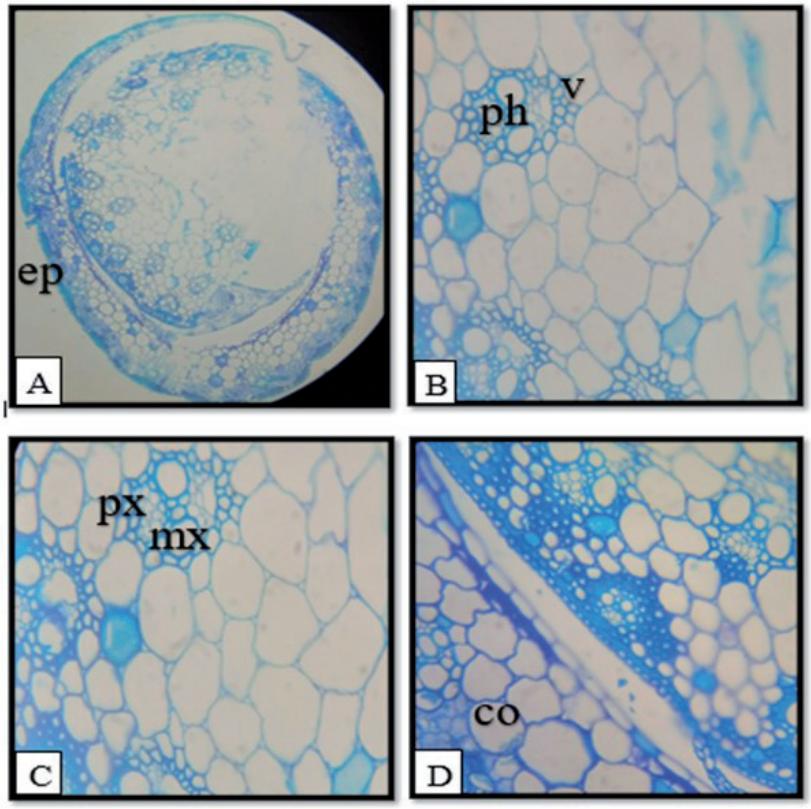

Fig. 3. a) T.S. of stem of Dicanthium annulatum Stapf. showing epidermis; b) peripheral view of section showing vascular bundles and centered phloem; c) central view of protoxylem and metaxylem; d) peripheral view of cortical cells, ep: epidermis, v: vascular bundles, ph: phloem mx: metaxylem, px: protoxylem, co: cortical cells (10X, 40X).

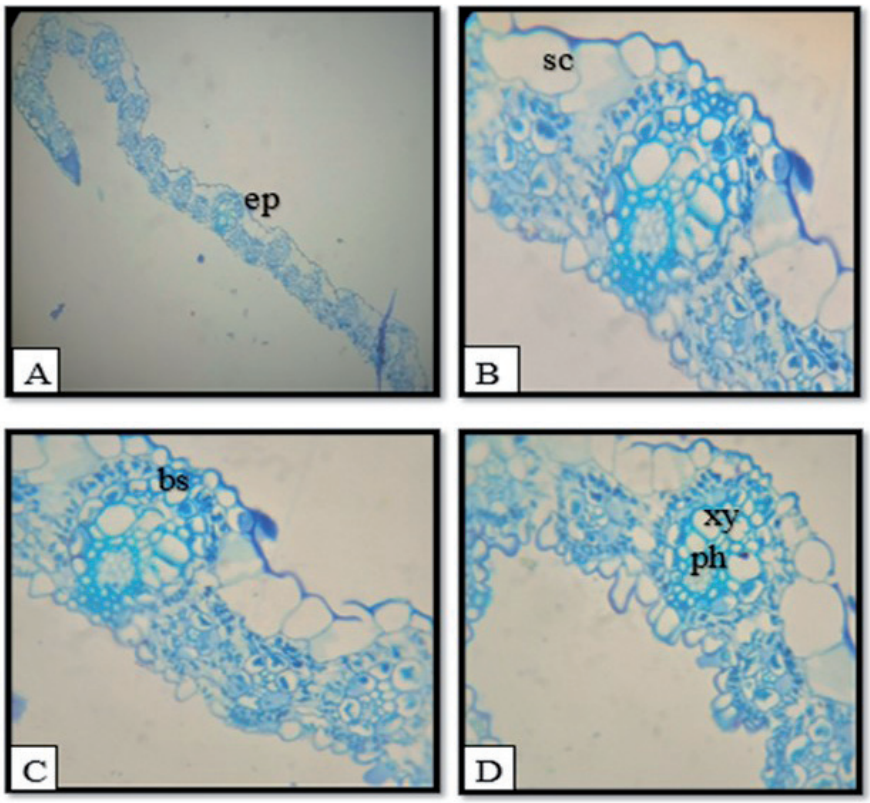

Fig. 4. a) T.S. of leaf section of Dicanthium annulatum Stapf. showing epidermis b). magnified view of sclerenchyma cells; c) bundle sheath cells b; d) xylem and phloem cells, ep: epidermis, sc: sclerenchyma cells, bs: bundle sheath cells, xy: xylem, ph: phloem (10X, 40X).

Table 3. Length and width of different parameters of leaf section of Cenchrus pennisetiformis Hochst.

Sr. No	Parameters	Length (mm)	Range of length of cells (mm)	Width (mm)	Range of width of cells (mm)
1.	Epidermal cells	0.55±0.21	0.5-0.7	0.57±0.10	0.45-0.6
2.	Sclerenchyma cells	0.33±0.07	0.3-0.4	0.17±0.01	0.15-0.25
3.	Bundle sheath cells	0.73±0.05	0.6-0.8	0.55±0.03	0.5-0.6
4.	Mesophyll cells	1.25±0.38	1.2-1.4	1.7±0.11	1.6-1.8
5.	Bulliform cells	1.19±0.28	1.1-1.2	0.78±0.03	0.7-0.8
6.	Xylem	0.37±0.02	0.3-0.4	0.35±0.01	0.35-0.45
7.	Phloem	0.06±0.005	0.6-0.8	0.12±0.02	0.09-0.15

Table 4. Length and width of different parameters of the leaf of Dicanthium annulatum Stapf.

Sr. No.	Parameters	Length (mm)	Range of length of cells (mm)	Width (mm)	Range of width of cells (mm)
1.	Epidermal cells	0.32±0.01	0.30-0.35	0.21±0.005	0.20-0.22
2.	Sclerenchyma cells	0.20±0.00	0.20-0.20	0.13±0.008	0.12-0.15
3.	Bundle sheath cells	1.26±0.03	1.20-1.30	0.61±0.04	0.55-0.70
4.	Mesophyll cells	0.33±0.01	0.30-0.35	0.35±0.02	0.30-0.40
5.	Bulliform cells	0.90±0.01	0.80-1.00	1.10±0.05	1.00-1.20
6.	Xylem cells	0.26±0.008	0.25-0.28	0.22±0.01	0.20-0.24
7.	Phloem cells	0.10±0.006	0.10-0.12	0.13±0.008	0.12-0.15

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) wfhile 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±0.0026 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 $p \leq 0.05$.

FRAP Assay

In *Cenchrus pennisetiformis* chloroform, methanol and n-hexane fractions exhibited higher FRAP values, i.e. 1.32, 1.21 and 1.08 TE µM/ mL as compared to the aqueous solution that showed value 0.97 TE µM/ mL respectively (Fig. 5c); Table 8). In *Dicanthium annulatum* chloroform, 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.

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 10). *Dicanthium annulatum* showed higher significant value in methanol fraction 73.15±0.020 mg/ mL GAE followed by chloroform (43.6±0.152 mg/ mL), aqueous solution (23.05±0.008 mg/mL) and n-hexane fraction (4.16±0.011 mg/ mL) respectively (Table 11). The results were computed using the Gallic acid standard curve and expressed in milligrams per milliliter of GAE.

Table 5. % DPPH free radical scavenging activity of different solvent extracts of Cenchrus pennisetiformis Hochst.

Sr. No	Solvent	Conc. (µg/ml)	% Scavenging
1.	Methanol	20	58.49±0.13
		60	70.44±0.12
		80	72.42±0.15
		100	74.36±0.21
		150	67.39±0.16
		250	76.15±0.10
2.	N-hexane	20	48.7±0.15
		60	58.44±0.16
		80	51.51±0.10
		100	53.76±0.05
		150	46.71±0.16
		250	50.60±0.15
3.	Chloroform	20	50.53±0.11
		60	63.63±0.07
		80	76.30±0.12
		100	67.56±0.15
		150	65.39±0.15
		250	76.33±0.10
4.	Aqueous Solution	20	44.77±0.06
		60	40.6±0.20
		80	43.71±0.24
		100	52.47±0.15
		150	49.51±0.13
		250	49.57±0.05
BHT	Standard		77.3±0.7

Table 6. % DPPH free radical scavenging activity of different solvent extracts of Dicanthium annulatum Stapf.

Sr. No	Solvent	Conc. (µg/ml)	% Scavenging
1.	Methanol	20	44.85±0.20
		60	49.44±0.23
		80	54.4±0.37
		100	62.40±0.18
		150	64.61±0.19
		250	68.47±0.30
2.	N-hexane	20	44.28±0.16
		60	39.53±0.12
		80	38.36±0.18
		100	36.59±0.14
		150	48.65±0.06
		250	47.65±0.19
3.	Chloroform	20	48.46±0.19
		60	59.39±0.16
		80	65.69±0.15
		100	65.12±0.37
		150	66.54±0.12
		250	67.47±0.19
4.	Aqueous Solution	20	28.58±0.25
		60	24.58±0.13
		80	35.71±0.09
		100	27.53±0.11
		150	31.51±0.15
		250	57.67±0.14
BHT	Standard		77.3±0.7

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 characteristicss 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

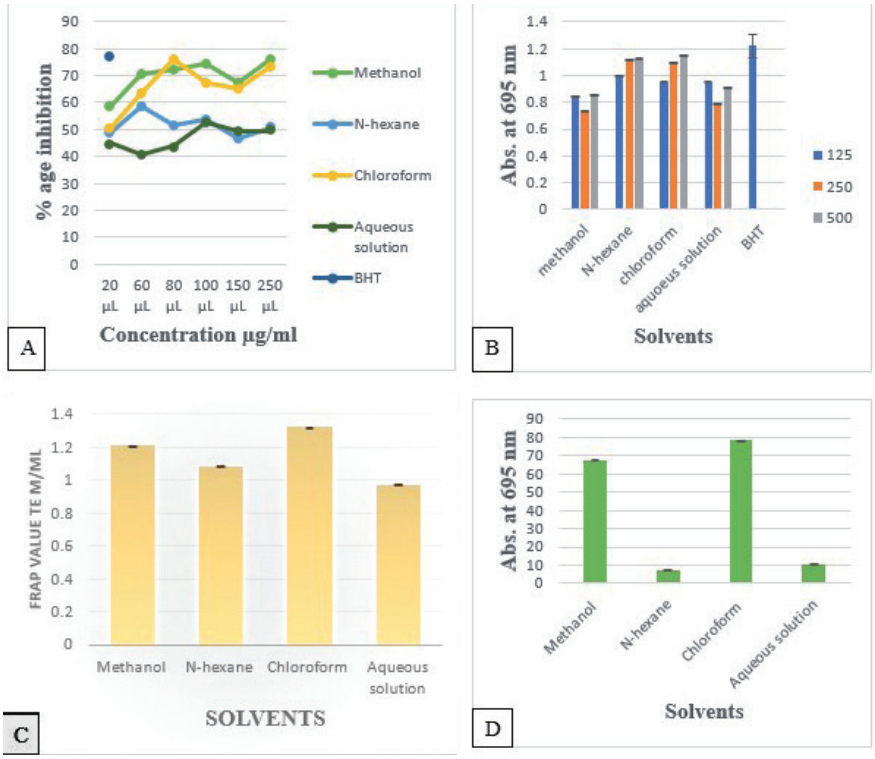

Fig. 5. a) % DPPH free radical scavenging activity of different solvent extracts at various concentrations in Cenchrus pennisetiformis Hochst; b) Total Antioxidant activity of different solvent extracts at various concentrations in Cenchrus pennisetiformus Hochst; c) Different solvent extracts used for the estimation of the FRAP value in Cenchrus pennisetiformis Hochst; d) TPC evaluation of various solvent extracts of Cenchrus pennisetiformis Hochst.

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

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

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

Sr. No	Solvent	Abs. at 593 nm
1.	Methanol	1.21±0.0011b
2.	N-hexane	1.08±0.0023c
3.	Chloroform	1.32±0.0018a
4.	Aqueous solution	0.97±0.0021d

Table 9. FRAP assay of different solvent extracts of Dicanthium annulatum Stapf.

Sr. No	Solvent	Abs. at 593 nm
1.	Methanol	1.50±0.0023b
2.	N-hexane	1.14±0.0008c
3.	Chloroform	1.62±0.0017a
4.	Aqueous solution	0.75±0.0020d

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

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

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

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

reduction measures, and it has the potential to grow quickly. Meat, milk, eggs, dung, fibers, hides, and horns are all produced by livestock, and demand for these items is fast increasing as a result of population growth, urbanization in developing countries, and higher revenue [66].

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].

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

Conceptualization: A.R & U.H; Methodology: A.R, U.H, & S.A.; Data Curation: U.H, S.A & M.N.K; Writing original draft preparation: S.A, A.R, B.A M.F, M.R, S.R, M.N.K.; Writing-Review and Editing: M.F, M.R, S.R, R.U, B.A & M.N.K; Supervision: U.H.

Acknowledgments

The Authors wish to thank Researchers Supporting Project number (RSP2024R110) at King Saud University, Riyadh, Saudi Arabia for financial support.

Conflict of Interest

The authors declare no conflict of interest.

References

1. CHAUDHARI S.K., ARSHAD M., AMJAD M.S., FATIMA S. Taxonomic inventory of grasses on the basis of morphological attributes from Thal desert, Pakistan. International Journal of Scientific & Engineering Research. **4** (11), 509, **2013**.
2. PETERSON P.M., SORENG R.J. Systematics of California grasses (Poaceae). California grasslands: ecology and management. 7, **2007**.
3. KHAN M.N., ALI S., YASEEN T., ULLAH S., ZAMAN A., IQBAL M., SHAH S. Eco-taxonomic study of family Poaceae (Gramineae). RADS Journal of Biological Research & Applied Sciences. **10** (2), 63, **2019**.
4. FATIMA I., KANWAL S., MAHMOOD T. Evaluation of biological potential of selected species of family Poaceae from Bahawalpur, Pakistan. BMC complementary and alternative medicine. **18** (1), 1, **2018**.
5. ISHTIAQ M., MAQBOOL M., HUSSAIN T., BHATTI K.H., MUSHTAQ W., NADEEM A. Application of leaf epidermal anatomical technique for identification of some grass species from district Bhimber of Azad Jammu and Kashmir, Pakistan. Pakistan Journal of Botany. **50** (5), 1853, **2018**.
6. BLAIR J., NIPPERT J., BRIGGS J. Grassland ecology 14. Ecology and the Environment. **389**, 389, **2014**.
7. BABU R.H., SAVITHRAMMA N. Phytochemical screening of underutilized species of Poaceae. An International Journal, **1** (10), 947, **2013**.
8. JABEEN S., ALI M.F., MOHI UD DIN A., JAVED T., MOHAMMED N.S., CHAUDHARI S.K., JAVED M.A., ALI B., ZHANG L., RAHIMI M. Phytochemical screening and allelopathic potential of phytoextracts of three invasive grass species. Scientific Reports. **13** (1), 8080, **2023**.
9. KIANI H.S., ALI B., AL-SADOON M.K., AL-OTAIBI H.S., ALI A. LC-MS/MS and GC-MS Identification of Metabolites from the Selected Herbs and Spices, Their Antioxidant, Anti-Diabetic Potential, and Chemometric Analysis. Processes. **11** (9), 2721, **2023**.
10. ANYASOR G.N., OGUNWENMO O., OYELANA O.A., AKPOFUNURE B.E. Phytochemical constituents and antioxidant activities of aqueous and methanol stem extracts of Costus afer Ker Gawl.(Costaceae). African Journal of Biotechnology. **9** (31), 4880, **2010**.
11. MARZETZ V., KOUSSOROPLIS A.-M., MARTIN-CREUZBURG D., STRIEBEL M., WACKER A. Linking primary producer diversity and food quality effects on herbivores: a biochemical perspective. Scientific Reports. **7** (1), 11035, **2017**.
12. ROMA R., CORRADO S., DE BONI A., FORLEO M.B., FANTIN V., MORETTI M., PALMIERI N., VITALI A., CAMILLO D.C. Life cycle assessment in the livestock and derived edible products sector. In Life cycle assessment in the agri-food sector, Notarnicola B., Salomone R., Petti L., Renzulli P., Roma R., Cerutti A., Springer, Cham. pp. 251, **2015**.
13. HARUN N., CHAUDHRY A.S., SHAHEEN S., ULLAH K., KHAN F. Ethnobotanical studies of fodder grass resources for ruminant animals, based on the traditional knowledge of indigenous communities in Central Punjab Pakistan. Journal of ethnobiology and ethnomedicine. **13**, 1, **2017**.
14. SCHOLES R.J. The future of semi-arid regions: A weak fabric unravels. Climate. **8** (3), 43, **2020**.
15. GENG Y., HU G., RANJITKAR S., WANG Y., BU D., PEI S., OU X., LU Y., MA X., XU J. Prioritizing fodder species based on traditional knowledge: a case study of mithun (Bos frontalis) in Dulongjiang area, Yunnan Province, Southwest China. Journal of ethnobiology and ethnomedicine. **13** (1), 1, **2017**.
16. NUNES A.T., PAIVADE LUCENA R.F., FERREIRA DOS SANTOS M.V., ALBUQUERQUE U.P. Local knowledge about fodder plants in the semi-arid region of Northeastern Brazil. Journal of ethnobiology and ethnomedicine. **11** (1), 1, **2015**.
17. IQBAL R., USMAN M., MURTAZA G., VALIPOUR M., MAJEED A., MUNIR A., ALI B., ERCISLI S., AL-GHAMDI A.A., AL-HEMAID F.M. Exploration of Medicinal Plants by Local Communities of Khanewal, Pakistan for Sustainable Development: A Quantitative Analysis. ACS Agricultural Science & Technology. **3** (10), 855, **2023**.
18. KAZEMI M., MOKHTARPOUR A. Chemical, mineral composition, in vitro ruminal fermentation and buffering capacity of some rangeland-medicinal plants. Acta Scientiarum. Animal Sciences. **44**, **2022**.
19. EKWEALOR K., ECHEREME C., OFOBEZE T., OKEREKE C. Economic importance of weeds: A review. Asian Plant Research Journal. **3** (2), 1, **2019**.
20. KHAN R., KHAN M.N., ULLAH H., BASIT A., RAZZAQ A., AHMAD M., OZDEMIR F. A comparative assessment of proximate and elemental composition six weedy grasses for their potential use as fodder. Progress in Nutrition. **20** (1-S), 182, **2018**.
21. KHAN M.N., ALI S., YASEEN T., ADNAN M., ULLAH S., ZAMAN A., IQBAL M., SHAH N., ALI A., RAZZAQ A. Assessment of proximate and nutritional contents in selected weedy grasses for potential use as fodder in district charsadda, KP. Proceedings of the Pakistan Academy of Sciences: B. Life and Environmental Sciences. **57** (2), 83, **2020**.
22. AHMAD F., KHAN M.A., AHMAD M., ZAFAR M., MAHMOOD T., JABEEN A., MARWAT S.K. Ethnomedicinal uses of grasses in salt range region of northern Pakistan. Journal of medicinal plants research. **4** (5), 362, **2010**.
23. ABEED A.H., SALEEM M.H., ASGHAR M.A., MUMTAZ S., AMEER A., ALI B., ALWAHIBI M.S., ELSHIKH M.S., ERCISLI S., ELSHARKAWY M.M. Ameliorative Effects of Exogenous Potassium Nitrate on Antioxidant Defense System and Mineral Nutrient Uptake in Radish (*Raphanus sativus* L.) under Salinity Stress. ACS Omega. **8** (25), 22575, **2023**.
24. ABEED A.H., AL-HUQAIL A.A., ALBALAWI S., ALGHAMDI S.A., ALI B., ALGHANEM S.M., AL-HAITHLOUL H.A.S., AMRO A., TAMMAM S.A., EL-MAHDY M. Calcium nanoparticles mitigate severe salt stress in Solanum lycopersicon by instigating the antioxidant defense system and renovating the protein profile. South African Journal of Botany. **161**, 36, **2023**.
25. FOSS K., PRZYBYŁOWICZ K.E., SAWICKI T. Antioxidant activity and profile of phenolic compounds in selected herbal plants. Plant Foods for Human Nutrition. **77** (3), 383, **2022**.
26. ALI B., HAFEEZ A., AFRIDI M.S., JAVED M.A., SUMAIRA, SULEMAN F., NADEEM M., ALI S.,

ALWAHIBI M.S., ELSHIKH M.S. Bacterial-Mediated Salinity Stress Tolerance in Maize (*Zea mays* L.): A Fortunate Way toward Sustainable Agriculture. ACS omega. **8**, 20471, **2023**.
27. ULLAH R., JAN S.A., KHAN M.N., NAZISH M., KAMAL A., KAPLAN A., YEHIA H.M., ALARJANI K.M., ALKASIR R., ZAMAN W. Euphorbia royleana Boiss Derived Silver Nanoparticles and Their Applications as a Nanotherapeutic Agent to Control Microbial and Oxidative Stress-Originated Diseases. Pharmaceuticals. **16** (10), 1413, **2023**.
28. ULLAH R., BIBI S., KHAN M.N., AL MOHAIMEED A.M., NAZ Q., KAMAL A. Application of Bio-Inspired Gold Nanoparticles as Advanced Nanomaterial in Halt Nociceptive Pathway and Hepatotoxicity via Triggering Antioxidation System. Catalysts. **13** (4), 786, **2023**.
29. FARYAL S., ULLAH R., KHAN M.N., ALI B., HAFEEZ A., JAREMKO M., QURESHI K.A. Thiourea-capped nanoapatites amplify osmotic stress tolerance in *Zea mays* L. by conserving photosynthetic pigments, osmolytes biosynthesis and antioxidant biosystems. Molecules. **27** (18), 5744, **2022**.
30. ZAMAN S.A.R., OZDEMIR F.A. Effect of Thermopriming and Alpha-Tocopherol Spray in Triticum Aestivum L. under Induced Drought Stress: A Future Perspective of Climate Change in the Region. Sains Malaysiana. **51** (4), 977, **2022**.
31. ALI Q., SHABAAN M., ASHRAF S., KAMRAN M., ZULFIQAR U., AHMAD M., ZAHIR Z.A., SARWAR M.J., IQBAL R., ALI B. Comparative efficacy of different salt tolerant rhizobial inoculants in improving growth and productivity of Vigna radiata L. under salt stress. Scientific Reports. **13** (1), 17442, **2023**.
32. ALSHEGAIHI R.M., MFARREJ M.F.B., SALEEM M.H., PARVEEN A., AHMAD K.S., ALI B., ABEED A.H., ALSHEHRI D., ALGHAMDI S.A., ALGHANEM S.M. Effective citric acid and EDTA treatments in cadmium stress tolerance in pepper (Capsicum annuum L.) seedlings by regulating specific gene expression. South African Journal of Botany. **159**, 367, **2023**.
33. BAGDAS D., GUL Z., MEADE J.A., CAM B., CINKILIC N., GURUN M.S. Pharmacologic overview of chlorogenic acid and its metabolites in chronic pain and inflammation. Current Neuropharmacology. **18** (3), 216, **2020**.
34. WIJEKOON M.J.O., BHAT R., KARIM A.A. Effect of extraction solvents on the phenolic compounds and antioxidant activities of bunga kantan (Etlingera elatior Jack.) inflorescence. Journal of food composition and analysis. **24** (4-5), 615, **2011**.
35. SINGH A.P., WILSON T., LUTHRIA D., FREEMAN M.R., SCOTT R.M., BILENKER D., SHAH S., SOMASUNDARAM S., VORSA N. LC-MS–MS characterisation of curry leaf flavonols and antioxidant activity. Food Chemistry. **127** (1), 80, **2011**.
36. SOUSA R., FIGUEIRINHA A., BATISTA M.T., PINA M.E. Formulation effects in the antioxidant activity of extract from the leaves of cymbopogon citratus (Dc) stapf. Molecules. **26** (15), 4518, **2021**.
37. MCGAW L.J., FAMUYIDE I.M., KHUNOANA E.T., AREMU A.O. Ethnoveterinary botanical medicine in South Africa: A review of research from the last decade (2009 to 2019). Journal of ethnopharmacology. **257**, 112864, **2020**.
38. GREENE A.M., PANYADEE P., INTA A., HUFFMAN M.A. Asian elephant self-medication as a source of ethnoveterinary knowledge among Karen mahouts in northern Thailand. Journal of ethnopharmacology. **259**, 112823, **2020**.
39. OLIVEIRA M., HOSTE H., CUSTÓDIO L. A systematic review on the ethnoveterinary uses of mediterranean salt-tolerant plants: Exploring its potential use as fodder, nutraceuticals or phytotherapeutics in ruminant production. Journal of Ethnopharmacology. **267**, 113464, **2021**.
40. LIPKOVÁ N., MEDO J., ARTIMOVÁ R., MAKOVÁ J., PETROVÁ J., JAVOREKOVÁ S., MICHALKO J. Growth promotion of rapeseed (Brassica napus L.) and blackleg disease (Leptosphaeria maculans) suppression mediated by endophytic bacteria. Agronomy. **11** (10), 1966, **2021**.
41. GONZÁLEZ J.A., VALLEJO J.R. The use of domestic animals and their derivative products in contemporary Spanish ethnoveterinary medicine. Journal of Ethnopharmacology. **271**, 113900, **2021**.
42. BULLITTA S., RE G.A., MANUNTA M.D.I., PILUZZA G. Traditional knowledge about plant, animal, and mineral-based remedies to treat cattle, pigs, horses, and other domestic animals in the Mediterranean island of Sardinia. Journal of ethnobiology and ethnomedicine. **14** (1), 1, **2018**.
43. EIKI N., MAAKE M., LEBELO S., SAKONG B., SEBOLA N., MABELEBELE M. Survey of ethnoveterinary medicines used to treat livestock diseases in omusati and kunene regions of Namibia. Frontiers in Veterinary Science. **9**, 762771, **2022**.
44. REHMAN S., IQBAL Z., QURESHI R., RAHMAN I.U., SAKHI S., KHAN I., HASHEM A., AL-ARJANI A.-B.F., ALMUTAIRI K.F., ABD_ALLAH E.F. Ethnoveterinary Practices of Medicinal Plants Among Tribes of Tribal District of North Waziristan, Khyber Pakhtunkhwa, Pakistan. Frontiers in Veterinary Science. **9**, 815294, **2022**.
45. ISHTIAQ S., HANIF U., SHAHEEN S., BAHADUR S., LIAQAT I., AWAN U.F., SHAHID M.G., SHUAIB M., ZAMAN W., MEO M. Antioxidant potential and chemical characterization of bioactive compounds from a medicinal plant Colebrokea oppositifolia Sm. Anais da Academia Brasileira de Ciências. **92**, **2020**.
46. AHMED D., FATIMA K., SAEED R. Analysis of phenolic and flavonoid contents, and the anti-oxidative potential and lipid peroxidation inhibitory activity of methanolic extract of Carissa opaca roots and its fractions in different solvents. Antioxidants. **3** (4), 671, **2014**.
47. FUSO A., DEJONGHE W., CAUWENBERGHS L., ROSSO G., ROSSO F., MANERA I., CALIGIANI A. DPPH radical scavenging activity of xylo-oligosaccharides mixtures of controlled composition: A step forward in understanding structure–activity relationship. Journal of Functional Foods. **101**, 105417, **2023**.
48. JAFRI L., SALEEM S., ULLAH N., MIRZA B. In vitro assessment of antioxidant potential and determination of polyphenolic compounds of Hedera nepalensis K. Koch. Arabian Journal of Chemistry. **10**, S3699, **2017**.
49. QURESHI N.A. In vitro Anticoccidial, Antioxidant Activities and Biochemical Screening of Methanolic and Aqueous Leaves Extracts of Selected Plants. Pakistan Veterinary Journal. **41** (1), **2021**.
50. HOSSAIN M.A., SHAH M.D. A study on the total phenols content and antioxidant activity of essential oil and different solvent extracts of endemic plant Merremia borneensis. Arabian Journal of Chemistry. **8** (1), 66, **2015**.
51. BENZIE I.F., DEVAKI M. The ferric reducing/ antioxidant power (FRAP) assay for non-enzymatic antioxidant capacity: concepts, procedures, limitations and applications. Measurement of antioxidant activity & capacity: Recent trends and applications. 77, **2018**.

52. RAJURKAR N.S., HANDE S. Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. Indian journal of pharmaceutical sciences. **73** (2), 146, **2011**.
53. MOHAMMED E.A., ABDALLA I.G., ALFAWAZ M.A., MOHAMMED M.A., AL MAIMAN S.A., OSMAN M.A., YAGOUB A.E.A., HASSAN A.B. Effects of extraction solvents on the total phenolic content, total flavonoid content, and antioxidant activity in the aerial part of root vegetables. Agriculture. **12** (11), 1820, **2022**.
54. BOUSLAMTI M., EL BARNOSSI A., KARA M., ALOTAIBI B.S., AL KAMALY O., ASSOUGUEM A., LYOUSSI B., BENJELLOUN A.S. Total polyphenols content, antioxidant and antimicrobial activities of leaves of Solanum elaeagnifolium Cav. from Morocco. Molecules. **27** (13), 4322, **2022**.
55. KURZAWA M., WILCZYŃSKA E., BRUDZYŃSKA P., SIONKOWSKA A. Total Phenolic Content, Antioxidant Capacity and UV Radiation Protection Properties of Marigold (Calendula officinalis), Carrot (Daucus carota), Tomato (Solanum lycopersicum) and Hop (Humulus lupulus) Extracts. Cosmetics. **9** (6), 134, **2022**.
56. SCHIAVI D., RONCHETTI R., DI LORENZO V., VIVANI R., GIOVAGNOLI S., CAMAIONI E., BALESTRA G.M. Sustainable Protocols for Cellulose Nanocrystals Synthesis from Tomato Waste and Their Antimicrobial Properties against Pseudomonas syringae pv. tomato. Plants. **12** (4), 939, **2023**.
57. SHAH N.A., ULLAH S., NAFEES M., KHAN M.N. Exogenous Effect of Sugar Beet Extract On Physio-biochemical Traits of Hordeum vulagre L. Under Induced Salinity Stress. Gesunde Pflanzen. 1, **2023**.
58. BIBI H., AFZAL M., KAMAL M., SOHAIL I., KHAN S., SHER A., ZIAULHAQ I., ALI A., KHAN S., UR RAHMAN I. Morphological and anatomical characteristics of selected dicot xerophytes of district Karak, Khyber Pakhtunkhwa, Pakistan. Middle-East J. Sci. Res. **23** (4), 545, **2015**.
59. DAS P., CHETTRI V., GHOSH S., GHOSH C. Micromorphological studies of the leaf and stem of Camellia sinensis (L.) Kuntze with reference to their taxonomic significance. Microscopy Research and Technique. **86** (4), 465, **2023**.
60. DESCOLA P. Beyond nature and culture. Routledge, **2014**.
61. SHAHEEN S., AHMAD M., KKAN F., ZAFAR M., KHAN M.A., SULTANA S., ABBAS S., JAMIL M., KHAN S. Systematic application of palyno-anatomical characterization of Setaria species based in scanning electron microscopy (SEM) and light microscope (LM) analysis. Journal of medicinal plants research. **5** (24), 5803, **2011**.
62. BUTT M.A., ZAFAR M., AHMAD M., KAYANI S., BAHADUR S., ULLAH F., KHATOON S. The use of taxonomic studies to the identification of wetlands weeds. Advances in Weed Science. **39**, **2021**.
63. MORALES P., FERREIRA I.C., CARVALHO A.M., FERNÁNDEZ-RUIZ V., SÁNCHEZ-MATA M.C., CÁMARA M., MORALES R., TARDÍO J. Wild edible fruits as a potential source of phytochemicals with capacity to inhibit lipid peroxidation. European Journal of Lipid Science and Technology. **115** (2), 176, **2013**.
64. GANESAN K., KUMAR K.S., RAO P.S. Comparative assessment of antioxidant activity in three edible species of green seaweed, Enteromorpha from Okha, Northwest coast of India. Innovative food science & emerging technologies. **12** (1), 73, **2011**.
65. REHMAN A., JINGDONG L., CHANDIO A.A., HUSSAIN I. Livestock production and population census in Pakistan: Determining their relationship with agricultural GDP using econometric analysis. Information Processing in Agriculture. **4** (2), 168, **2017**.
66. OTTER C. Diet for a large planet: industrial Britain, food systems, and world ecology. University of Chicago Press, **2023**.
67. MIARA M.D., BENDIF H., OUABED A., REBBAS K., HAMMOU M.A., AMIRAT M., GREENE A., TEIXIDOR-TONEU I. Ethnoveterinary remedies used in the Algerian steppe: Exploring the relationship with traditional human herbal medicine. Journal of ethnopharmacology. **244**, 112164, **2019**.
68. AZIZ M.A., KHAN A.H., PIERONI A. Ethnoveterinary plants of Pakistan: a review. Journal of ethnobiology and ethnomedicine. **16** (1), 1, **2020**.
69. WANZALA W., ZESSIN K., KYULE N., BAUMANN M., MATHIA E., HASSANALI A. Ethnoveterinary medicine: a critical review of its evolution, perception, understanding and the way forward. Livestock Research for Rural Development. **17** (11), **2005**.
70. UPADHYAY B., ROY S., KUMAR A. Traditional uses of medicinal plants among the rural communities of Churu district in the Thar Desert, India. Journal of ethnopharmacology. **113** (3), 387, **2007**.
71. MCGAW L.J., ELOFF J.N. Ethnoveterinary use of southern African plants and scientific evaluation of their medicinal properties. Journal of Ethnopharmacology. **119** (3), 559, **2008**.
72. KHAN M.N., BADSHAH L. Floristic diversity and utility of flora of district Charsadda, Khyber Pakhtunkhwa. Acta Ecologica Sinica. **39** (4), 306, **2019**.
73. HASSAN M., ABDULLAH A., HAQ S.M., YAQOOB U., BUSSMANN R.W., WAHEED M. Cross-ethnic use of ethnoveterinary medicine in the Kashmir Himalaya-A Northwestern Himalayan region. Acta Ecologica Sinica. **43** (4), 617, **2023**.
74. KHAN K., JAN G., IRFAN M., JAN F.G., HAMAYUN M., ULLAH F., BUSSMANN R.W. Ethnoveterinary use of medicinal plants among the tribal populations of District Malakand, Khyber Pakhtunkhwa, Pakistan. Ethnobotany Research and Applications. **25**, 1, **2023**.
75. KHAN M.A., KHAN S.A., QURESHI M.A., AHMED G., KHAN M.A., HUSSAIN M., GHULAM G.M. Ethnobotany of some useful plants of Poonch Valley Azad Kashmir. Journal of Medicinal Plants Research. **5** (26), 6140, **2011**.
76. BAKARE A.G., SHAH S., BAUTISTA-JIMENEZ V., BHAT J.A., DAYAL S.R., MADZIMURE J. Potential of ethno-veterinary medicine in animal health care practices in the South Pacific Island countries: a review. Tropical animal health and production. **52**, 2193, **2020**.
77. PIERONI A., REXHEPI B., NEDELCHEVA A., HAJDARI A., MUSTAFA B., KOLOSOVA V., CIANFAGLIONE K., QUAVE C.L. One century later: the folk botanical knowledge of the last remaining Albanians of the upper Reka Valley, Mount Korab, Western Macedonia. Journal of Ethnobiology and Ethnomedicine. **9** (1), 1, **2013**.
78. MUHAMMAD F. Ethnomedicinal uses of plants from district Bahawalpur. Current research journal of biological sciences: Maxwell scientific organization. **6** (5), 183, **2014**.