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Evaluating the Total Phenolic, Protein Contents, Antioxidant and Pharmacological Effects of *Cynodon dactylon* Extracts Against *Escherichia coli* and *Staphylococcus aureus*

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The study was aimed to characterize the antioxidant and anti-microbial activities of *Cynodon dactylon* with special reference on its precise biochemical analysis. Physiological analysis that total carotenoids content (0.3884 ± 0.0172 mg/g), total chlorophyll content (6.1460 ± 0.2915 mg/g), total phenolic contents (13.4703 ± 0.1494 mg/g), chlorophyll a (3.7708 ± 0.1528 mg/g, catalase (CAT) contents (40.2844 ± 0.1515 units/mg), total anthocyanin contents (5.0166 ± 0.2966 g^{-1} FW) total soluble proteins (2.9916 ± 0.1734 mg/g) and total flavonoids content (TFC) (4.7863 ± 0.0442 μg/g) was found higher in the leaves of the *Cynodon dactylon* whereas, chlorophyll b (2.4881 ± 0.1326 mg/g) was found higher in the stem of *Cynodon dactylon*, while, peroxidase (POD) contents (81.8763 ± 4.6609 units/mg) and superoxidase dismutase (SOD) activity (80.4346 ± 5.9367 units/mg) was investigated higher in roots of *Cynodon dactylon*. The anti-microbial activity of *Cynodon dactylon* extracts was performed using a good diffusion technique against two microbial strains. Among all the plant extracts, the methanolic extracts showed a maximum inhibition zone (26.87 mm) against anti-bacterial strain Escherichia coli whereas n-hexane extract showed a maximum inhibition zone (17.88 mm) against anti-fungal strain Candida albicans. This study reported the antimicrobial activity of *Cynodon dactylon* against some common pathogens such as Staphylococcus aureus, Escherichia coli, and Candida albicans, which are highly associated with nosocomial infection. From the given results it is concluded that *Cynodon dactylon* could be exploited in pharmacology due to its antioxidant and anti-microbial properties.

Keywords: *Cynodon dactylon*, Anti-microbial, Flavonoids, Total Soluble Proteins, Peroxidase, Antioxidant, Super oxide Dismutase.

INTRODUCTION

Since prehistoric days, attempts have been made to develop effective drugs for the treatment of diseases from natural sources, because synthetic medicines entail a variety of unavoidable adverse effects[1, 2]. Plants are used medicinally in many countries and are the foundation of many potential and powerful remedies. There are numerous of evidence exists for the use of plants as medicine in the treatment of diseases in India. Pharmacognosy is the study of medicines derived from natural sources, primarily plants, which may lead to the preparation of new drugs[3, 4]. According to the World Health Organisation, medicinal plants are the foundation of traditional or indigenous health systems used by the majority of the population in many developing nations. Traditional herbal remedies are regarded as cost-effective, easily affordable with no adverse side effects, and safe; as a result, the global demand for herbal drugs is constantly increasing, with India's market expanding at a rate of 20% per year[5, 6]. Phytochemicals are responsible for the medicinal activity of plants[7], which are nonnutritive chemicals that have protected humans from all sorts of diseases[8]. Based on their function in plant metabolism, phytochemicals are divided into two groups: primary and secondary metabolites[7, 9].

Poaceae with almost 780 genera and round about 12,000 species[10] that are categorized into 12 sub-families[11]. Among them, about 40 species are currently being used as turfgrasses[12–14]. The genus *Cynodon* (Bermuda grass), which comprises 10 species and is called couch or star grass, originated from tropical or subtropical areas. In the grass family, *Cynodon* is a genus of plants. The name of genus originated from the Greek word which meant "dog-tooth". Bermuda grass is being used for various purposes such as in the golf and turf grass industries, forage, soil stabilization, and phytoremediation[15, 16]. In Pakistan *Cynodon dactylon* occurs in Punjab, Baluchistan, Sindh, N.W.F.P. and Kashmir. It is categorized as the world's second-worst weed. Mostly, it grows on many soil types, but it is most abundant in sandy and loamy soils and is a substantial meadow and turf grass[17, 18]. It has a lot of medicinal importance. It is also used for decorative purposes and has many other new perspectives. Aqueous fraction of the plant is utilized as a purifying agent, diuretic, anti-inflammatory, and anti-emetic. The phytochemical analysis showed that *Cynodon dactylon* contained fixed oils, flavonoids, volatile oils, alkaloids, proteins, glycosides, carbohydrates, terpenoids, reducing--sugars, triterpenoids steroids, phyto-sterols, saponins, tannins and resins[1, 19–22].

Cynodon dactylon has been utilized as an anti-diabetic agent in the conventional system of medicine used in India[23–26]. The articulated juice of the plant is used as an astringent. It is also applied to wounds to stop the bleeding from cuts and sores. Its extract is applied to the lower part of the abdomen to reduce extreme bleeding in the vagina. The decoction of this plant is amalgamated with sugar and used in the disease of urine retention. Its paste is mixed with honey and used in the epitaxis problem. Its juice is mixed with the honey and used as an oral dosage 2–3 times a day for several days for the treatment of menorrhagia. *Cynodon dactylon* is used as a folk remedy for dandruff, diarrhea, weak vision, and bronchitis. In epitaxis, a plant paste mixed with honey is used[9, 27–29]. In this research, five various extracts of the *Cynodon dactylon* (ethyl acetate, n-hexane, methanol, and chloroform extract) were investigated for their anti-microbial activity against the gram-negative bacteria (*Escherichia coli*) gram-positive bacteria (*Staphylococcus aureus*) and *Candida albicans*. However the drug industries have developed a number of novel antibiotics in recent decades, microorganism resistance to these drugs has also increased. Due to the increasing resistance of microbes and the side effects of synthetic antibiotics, such medicinal plants are becoming increasingly important for the treatment of fungal and bacterial infections. Based on the above context, the goals of the current research were to investigate the antimicrobial activity of various extracts of *Cynodon dactylon* against pathogenic fungi and bacteria and to compare the extract against both gram-positive and gram-negative bacteria using a standard antibiotic.

MATERIALS AND METHODS

Collection of Plant Material

The complete plant of *Cynodon dactylon* with roots was taken from the local area of District Okara in January 2018, identified by Dr. Iqbal Hussain Associate Professor, Department of Botany, Government College University Faisalabad. Analytical and HPLC grade chemicals like methanol, acetone, ascorbic acid, nitro blue tetrazolium (NBT), methionine, riboflavin, Bradford reagent, aluminum trichloride ($AlCl_3$), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), potassium phosphate buffer (KH_2PO_4), guaiacol, sodium nitrate ($NaNO_2$), hydrogen peroxide (H_2O_2) was obtained from reliable sources.

Anti-oxidant Assays

Sample Extraction

A fresh plant (0.25 g) was thoroughly cleaned with tap water and cut into small pieces and extracted in 2mL of potassium dihydrogen phosphate (KH_2PO_4) (50 mM) buffer at pH 7.0 at 20°C. A fresh plant part was centrifuged at 14,000 × g for 5 minutes at 4°C. Supernatant was isolated for several biological activities.

Photosynthetic Pigments

To assess the photosynthetic pigments such as total Chlorophyll, Chlorophyll a, b, and carotenoid concentration, Yoshida (1976) approach was used[30]. The plant material was mixed with 80% acetone in 10 ml, and the supernatant was isolated for measurement of wavelengths at 480 nm, 645 nm, and 663 nm using a UV/VIS Spectrophotometer. Moreover, carotenoids were determined using the Davies and Taylor (1976) method[31].

$$Chl.a\left(\frac{mg}{g}FW\right) = \left\{12.7\left(OD_{663} - 2.69\,(OD_{645}) \times \frac{V}{1000} \times W\right)\right\}$$

$$Chl.b\frac{mg}{g}FW) = \left\{12.9\,\left(OD_{645}4.68\,(OD_{663}) \times \frac{V}{1000}\,\times W\right)\right\}$$

$$Total\ Chl.\left(\frac{mg}{g}FW\right) = \left\{20.2\,\left(OD_{645} - 8.02\,(OD_{663}) \times \frac{V}{1000} \times W\right)\right\}$$

$$arotenoids\left(\frac{mg}{g}FW\right) = \{OD_{480} + (0.114 \times OD_{663}) - (0.638 \times OD_{645})\}$$

Total Soluble Proteins

using Bradford (1976) method, Total Soluble Proteins (TSP) was determined[32]. In this method, 0.25 g of the plant was ground properly, 5 ml of the potassium phosphate buffer (KH2PO4) having pH of 7.3 was preserved in a test tube and then centrifuged for ten minutes at 12,000 rpm. Further added 1ml of extract, 2 ml of Bradford reagent for 30 minutes it was kept at r.t to measure absorbance at the wavelength of 595 nm, and then 1ml of buffer, 2 ml of Bradford reagent were used as a blank solution.

Total Anthocyanin Content

With the aid of Hodges & Nozzolillo (1995) method the total anthocyanin contents were observed[33]. In this method, 0.25 g of fresh plant material was ground; 5 ml acidic acetone was added and then centrifuged for 12 minutes at 12000 rpm. The absorbance of the supernatant was measured at 540 nm and 600 nm, respectively. Methanol was utilized as a blank solvent.

$A = (A_{540} - A_{600})$

By using the given formula, the quantity of Anthocyanins was measured:

$$Anthocyanin\ contents\ \left(\frac{mg}{L}\right) = \frac{A \times Mol.W \times DF \times 1000}{\varepsilon \times l}$$

Where, MW (molecular weight), DF (dilution factor), and ε (molecular absorptivity). The molecular weight of cyanide-3-glycoside pigment MW = 449.2 and

Non-Enzymatic Anti-Oxidant Activity

Total Phenolic Contents

Total phenolic contents (TPC) were obtained as described by the Julkunen-Tiitto (1985). 0.5 g of fresh plant material was extracted in 1 ml of 80% acetone solution. The reaction mixture was centrifuged at 12000 rpm for 15 minutes. The supernatant was then diluted to 1 ml in the test tubes with 1000 mL of supernatant. After shaking, 0.5 ml of Folin- Ciocalteu's was added. After that, 2.5 ml of 20% sodium carbonate was added, and the volume was increased to 5 ml by adding distilled water. After 20 minutes, the absorbance of the reaction mixture was measured at 750 nm with a spectrophotometer[34].

Enzymatic Anti-oxidant Assays

Superoxide Dismutase Content (SOD)

The activity of superoxide dismutase was evaluated by a minute modification proposed by Gong (2005)[35]. The enzyme extract (20–50 μL) was reacted in a mixture which has 13 mM of methionine, 50 μM of nitro-blue tetrazolium (NBT), 75 mM of EDTA, 1.3 μM of riboflavin, and 750 mM of potassium phosphate buffer at pH 7.0. Under fluorescent light (15 fluorescent lamps), an irradiated reaction mixture (3ml) was kept at 78 mol m^{-2} s^{-1} for 15 minutes. The solution's absorbance was measured at 560 nm. One unit of SOD activity is the amount of enzyme that prevented half of the NBT photo-reduction.

Catalase (CAT) and Peroxidase Activity (POD)

CAT and POD activities were measured using the Cakmak method (1993)[36]. 3 ml of the reaction mixture was composed of 50 mM potassium phosphate (KH2PO4) buffer at pH 7.0, 20 mM of guaiacol, 5.9 mM of hydrogen peroxide (H2O2), and 0.1 ml of enzyme extract. After the time interval of every 20 seconds, deviations in the absorbance of the reaction mixture were observed at 470 nm and 240 nm.

Anti-microbial activity

Preparation of plant extract

The whole plant of *Cynodon dactylon* was washed thoroughly, dried in the shaded area, ground into moderately coarse powder (7 kg) and then soaked in 25 L methanol for 20–25 days, along with constant agitation from time to time. The extract was filtered and concentrated by using a rotary evaporator under reduced pressure. Then the methanolic extract was diluted with distilled water and left overnight. The filtrate was subjected to extraction by different solvents with increasing polarity (n-hexane, chloroform, ethyl acetate) to ensure complete and efficient extraction of all phytochemical active compounds. Solvent extraction was performed by using a separating funnel and the evaporation of the solvent was carried out by a rotary evaporator under reduced pressure and resulting extracts were discharged in three different petri plates and allowed to dry.

Microorganisms

Gram-Negative bacteria *Escherichia coli* ATCC# 8739 and Gram-Positive bacteria *Staphylococcus aureus* ATCC# 6538 were used for anti-bacterial activity. Anti-fungal activity was determined against *Candida albicans* ATCC# 10231. Antibiotic reference standard Gentamicin, Amikacin and Nystatin was used respectively.

Well diffusion Assay

Anti-microbial potential of *Cynodon dactylon* extracts was observed with minor modification in the method adopted by[37, 38]. Mueller Hinton Agar and Sabouraud Dextrose Agar were used and prepared separately for anti-bacterial and anti-fungal activities respectively. Media bottles were kept at a constant temperature of about 45°C to 50°C.

Preparation of Inoculum

An inoculating loop was sterilized by enlightening the spirit lamp. Loopful growth was taken from 24–48 hours of freshly grown culture of bacteria. Also, loopful growth was taken from 4–5 days of freshly grown culture of bacteria. Then, this loopful growth was suspended in 50 ml of sterile saline solution in a culture bottle and shaken vigorously.

Inoculation

Inoclume of *Escherichia coli*, *Staphylococcus aureus,* and *Candida albicans* of NMT 100 cfu was mixed into Mueller Hinton Agar from 100 mL bottle stored at 50°C. Four sterile glass Petri dishes were taken and (20–25 mL) inoculated Mueller Hinton Agar was poured and allowed to solidify into a smooth and uniform media layer. After solidifying, Petri dishes were divided into two equal halves and marked as sample and standard. Each petri dish was divided into three equal halves by an indication as "RS" for reference standard. The reference standard solution was aseptically transferred into "RS" well and prepared sample solution 1 into **"TS1"** location and **"TS2"** on the inoculated surface of the plates and covered the plates to avoid contamination.

Incubation

Plates were incubated at 30 to 35°C for 24 to 48 hours and at 20 to 55°C for 5–7 days for bacterial and fungal cultures respectively.

Statistical Analysis

Descriptive statistics was applied for the analysis of experimental results. Two-way ANOVA with replications was used for the analysis of data.

RESULTS AND DISCUSSION

Antioxidant Assays

The mean values of different parameters of *Cynodon dactylon* were shown in Table 1. The chlorophyll *a* (3.7708 ± 0.1528), chlorophyll *b* (2.4881 ± 0.1326), total chlorophyll contents (6.1460 ± 0.2915) and total carotenoid contents (0.3884 ± 0.0172 mg/g) found to be highest in the leaves of *Cynodon dactylon* as compared to other parts of the plant that was shown in Table 1 and Figure 1A, 1B, 1C, 1D. Our results agreed with[39].

Chlorophyll is recognized due to its anti-oxidant potential. Chlorophyll is also helpful in the purging of the liver. In the human body, chlorophyll is responsible for the regulation of blood sugar levels which is valuable for common health and well-being[40]. Chlorophyll also offsets the toxins and obstructs the activities of cancer-causing components[41]. Nutritional carotenoids are considered to contribute health relieves in reducing the chance of disease, predominantly specific tumors and eye disease. In this view, the most considered carotenoids are β-carotene, lutein, lycopene, and zeaxanthin[42].

β-Carotene is beneficial because it can be converted into vitamin A[42]. Furthermore, other carotenoids like lutein and zeaxanthin are protective in eyes ailments since they captivate harmful blue light which enters the eye. A great variety of fruits and vegetables are the food

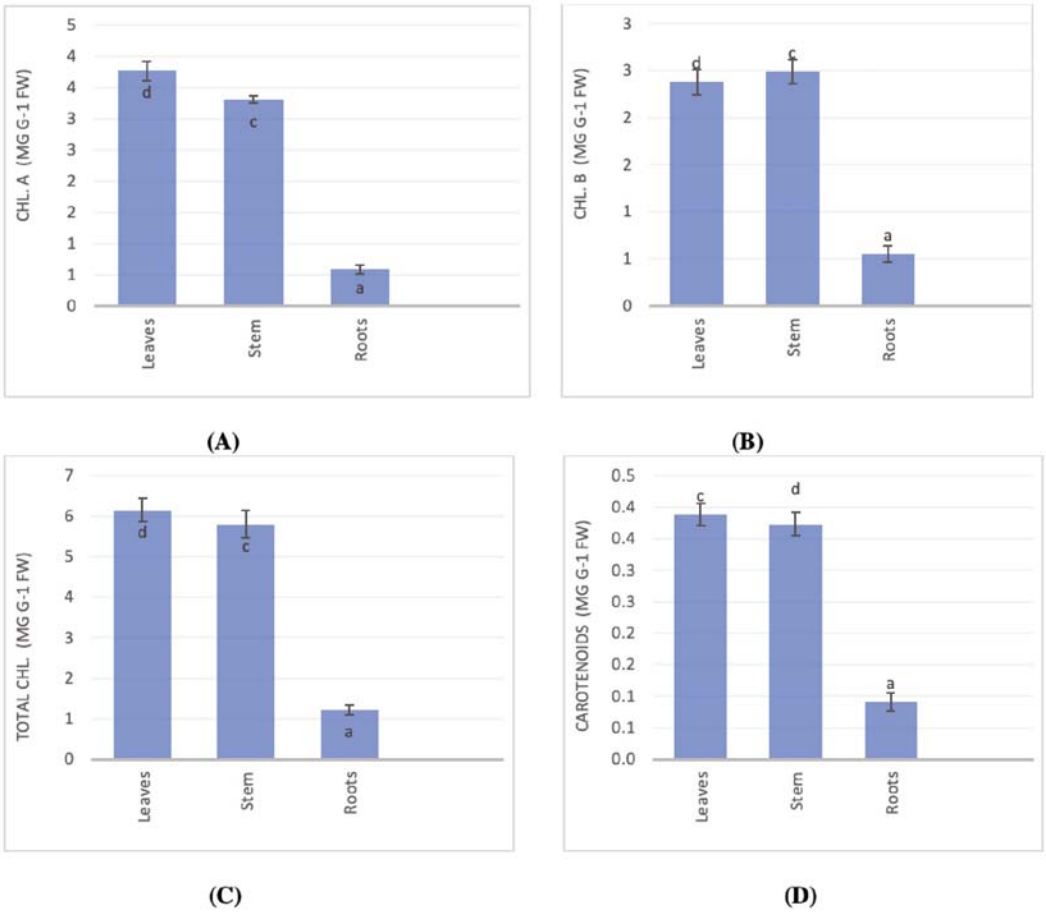

Figure 1. (A) Comparative analysis of Chlorophyll in various organs of *Cynodon dactylon*. (B) Comparative analysis of Chlorophyll b in various organs of *Cynodon dactylon*. (C) Comparative analysis of Total Chlorophyll in various organs of *Cynodon dactylon*. (D) Comparative analysis of Carotenoids in various organs of *Cynodon dactylon*

supplement of these amalgams. Though, tomatoes and the products obtained from tomatoes are the basic source of lycopene. Also, the yolk of the egg is a rich biological origin of lutein and zeaxanthin. These carotenoids are obtainable in the supplement form[43].

Phenolic compounds are the most abundant constituents of plants. Phenolics are the largest group of phytochemicals that account for most of the anti-oxidant activity in plants or plant products[44]. Major sources of phenolic compounds in human food are different fruits, vegetables, and a variety of beverages[45]. Catalases are antioxidant enzymes that are responsible for the catalyzation of hydrogen peroxide (H_2O_2). In numerous industrial applications, catalases are used for the processing of food or textile to eliminate H_2O_2 that is used for bleaching or sterilization process[46]. As an anti-oxidant enzyme, it has been used in many biotechnological disciplines including bioremediation[47]. Anthocyanins are the blue, purple, and red pigments usually found in the fruits, flowers, and tubers of most plants. Commonly red, purple, and blue-colored flowers have anthocyanins. From these, red flowers (Red hibiscus, red rose, red clover, pink blossom, and red pineapple sage) are edible and occur in acidic conditions. Anthocyanins possess anti-diabetic, anti-cancer, anti-microbial, anti-inflammatory, and effects against obesity. Anthocyanins are also used for the prevention of cardiovascular diseases[48, 49]. As a flavylium ion, anthocyanins are present in grapes and wines[50].

Flavonoids are the largest group of naturally occurring phenolic compounds, which occur in different plant parts both in Free State and as glycosides. They are found to have many biological activities including anti-microbial, mitochondrial adhesion inhibition, antiulcer, antiarthritic, antiangiogenic, anticancer, protein kinase inhibition, etc.[51]. Flavonoids are particularly beneficial, acting as anti-oxidants and giving protection against cardiovascular disease, certain forms of cancer, and age-related degeneration of cell components. Their polyphenolic nature enables them to scavenge injurious free radicals such as superoxide and hydroxyl radicals[52]. A variety of dietary plant flavonoids inhibits tumor development in experimental animal models[53]. The present work shows that total phenolic contents (13.4703 ± 0.1494 mg/g), catalase (CAT) contents (40.2844 ± 0.1515 units/mg), total anthocyanin[2] contents (5.0166 ± 0.2966 g^{-1} FW) and total flavonoids content (TFC) (4.7863 ± 0.0442 µg/g) was found higher in the leaves of the *Cynodon dactylon* as compared to other parts of the plant organs as shown in Table 1 and Figure 2 (A–D). Our results were in accordance with that of previous work reported by[54–58]. Also few researchers have coded different total phenolic contents and total flavonoids contents for the aqueous extract of *Cynodon dactylon* (51.91 ± 1.20 mg GAE/g) and (23.45 ± 0.07 mg RE/g)[59] (Fig. 3A-C).

Peroxidases are the main anti-oxidant enzymes generally present in nature and catalyzed the oxidation of many electron-donor substrates associated with the disintegration of hydrogen peroxide (H_2O_2). In the paper and textile industry, they are effectively used for bio-pulping and bio-bleaching. Peroxidases are used in

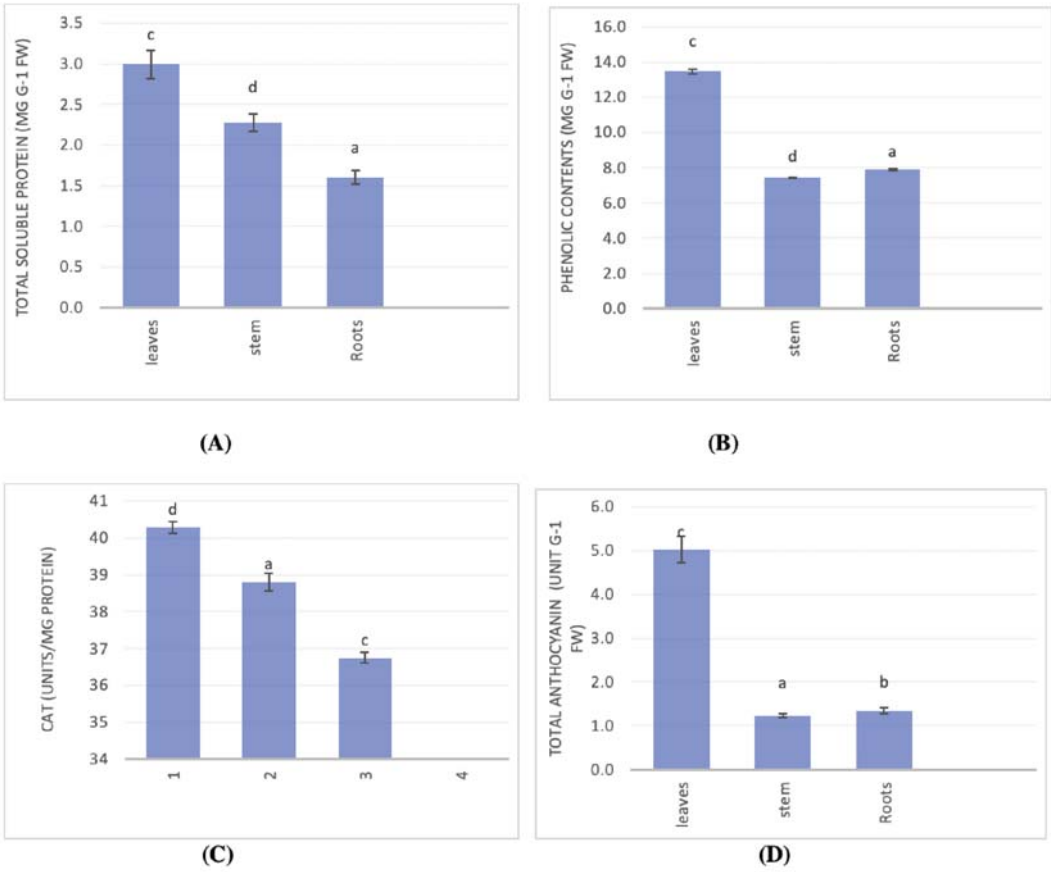

Figure 2. (A) Comparative analysis of total soluble proteins in various organs of *Cynodon dactylon*. (B) Comparative analysis of Total Phenolic Contents in various organs of *Cynodon dactylon*. (C) Comparative analysis of Catalase in various organs of *Cynodon dactylon*. Values are mean ±SE of three replicates. (D) Comparative analysis of Total anthocyanin in various organs of *Cynodon dactylon*

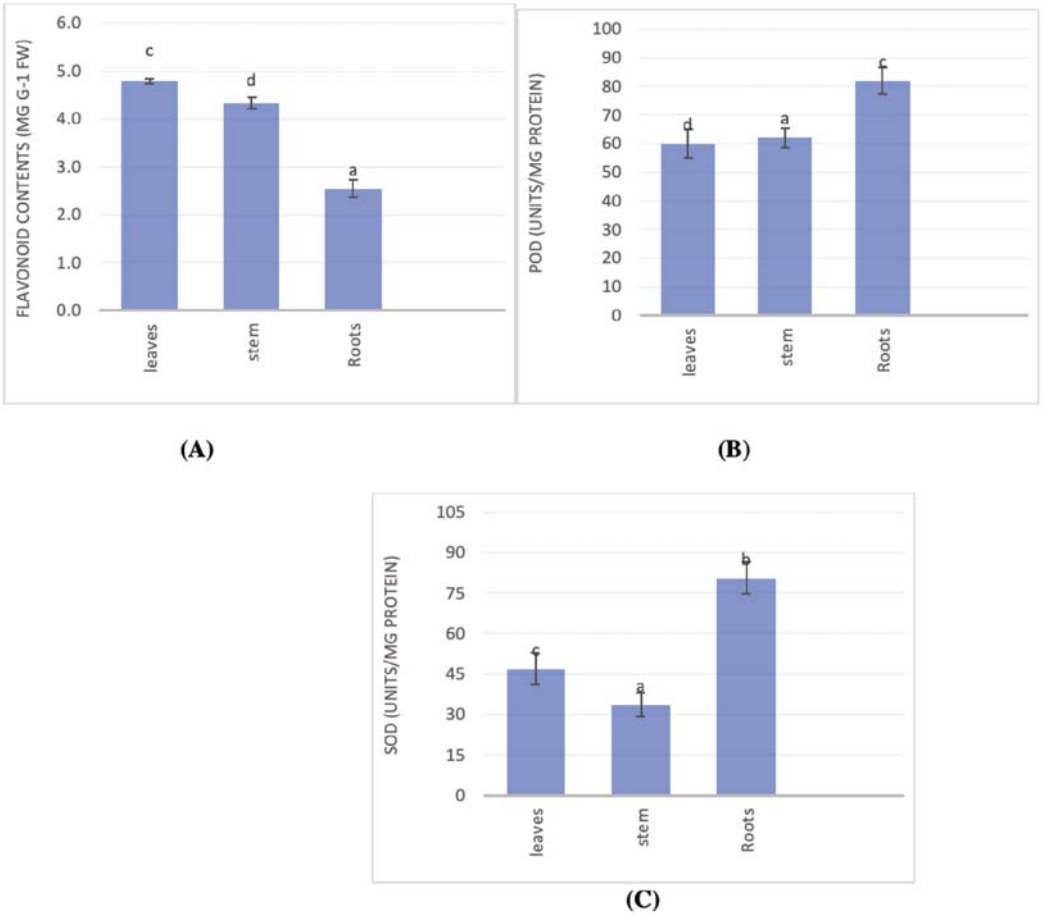

Figure 3. (A) Comparative analysis of Total Flavonoids in various organs of *Cynodon dactylon*. (B) Comparative analysis of peroxidase in various organs of *Cynodon dactylon* (C) Comparative analysis of Super oxidase in various organs of *Cynodon dactylon*

several analytical applications in diagnostic kits (ELISA). In bioremediation and organic synthesis, peroxidases have also been used extensively. Biosensors based on the peroxidase have found application in analytical systems for the accurate determination of hydrogen peroxide (H_2O_2), alcohol, glucose, glutamate, and choline etc.[60].

Superoxidase dismutase (SODs) is a significant defensive anti-oxidant against oxidative stress in the organism's body[61]. The physiological and therapeutic potential of superoxidase dismutase (SOD) has been revealed by several studies[62]. SOD can be served as an anti-inflammatory agent and it can also stop pre-cancerous cell

Table 1. Comparison of different parameters of *Cynodon dactylon*

Sr No	Parameters	ROOT (Mean ± SE)	STEM (Mean ± SE)	LEAVES (Mean ± SE)
1	Chlorophyll a (mg g^{-1} FW)	3.7708 ± 0.1528	3.3153 ± 0.0605	0.5946 ± 0.0737
2	Chlorophyl b (mg g^{-1} FW)	2.3768 ± 0.1365	2.4881 ± 0.1326	0.5515 ± 0.0841
3	Total Chlorophyll (mg g^{-1} FW)	6.1460 ± 0.2915	5.8018 ± 0.3469	1.2194 ± 0.1195
4	Carotenoids (mg g^{-1} FW)	0.3884 ± 0.0172	0.3724 ± 0.0185	0.0909 ± 0.0145
5	Total soluble proteins (g^{-1} FW)	2.9916 ± 0.1734	2.2795 ± 0.1075	1.6053 ± 0.0844
6	Total phenolic contents (mg g^{-1} FW)	13.4703 ± 0.1494	7.4402 ± 0.0120	7.9009 ± 0.0630
7	Catalase (µg g^{-1} FW)	40.2844 ± 0.1515	38.8145 ± 0.2368	36.7531 ± 0.1442
8	Total Anthocyanin Contents (µg g^{-1} FW)	5.0166 ± 0.2966	1.2333 ± 0.0536	1.3444 ± 0.0619
9	Flavonoids (units/mg protein)	4.7863 ± 0.0442	4.3340 ± 0.1107	2.5482 ± 0.1774
10	POD (units/mg protein)	59.8962 ± 4.9553	61.9938 ± 3.4437	81.8763 ± 4.6609
11	SOD (units/mg protein)	46.9419 ± 5.8214	33.54532 ± 4.3642	80.4346 ± 5.9367

Table 2. Anti-bacterial Potential of *Cynodon dactylon* against *Staphylococcus aureus* and *Escherichia coli*

Sr. No	Sample Fraction	Microbial Strain/ Culture	Reference Standard	Zone of Inhibition[7]		
				Reference standard value	Test sample 1 (TS1)	Test Sample 2 (TS2)
1.	Aqueous	*Escherichia coli*	Amikacin	21.31	21.01	19.16
		Staphylococcus. Aureus	Gentamicin	27.41	25.37	21.26
2.	Methanol	*Escherichia coli*	Amikacin	27.55	26.87	24.91
		Staphylococcus. Aureus	Gentamicin	26.99	25.79	23.35
3.	n-hexane	*Escherichia coli*	Amikacin	20.01	19.09	17.05
		Staphylococcus. Aureus	Gentamicin	26.97	26.03	23.35
4.	Chloroform	*Escherichia coli*	Amikacin	18.35	18.98	15.80
		Staphylococcus. Aureus	Gentamicin	26.87	26.07	24.35
5.	Ethyl acetate	*Escherichia coli*	Amikacin	18.78	18.64	15.27
		Staphylococcus. Aureus	Gentamicin	27.35	26.45	24.3593

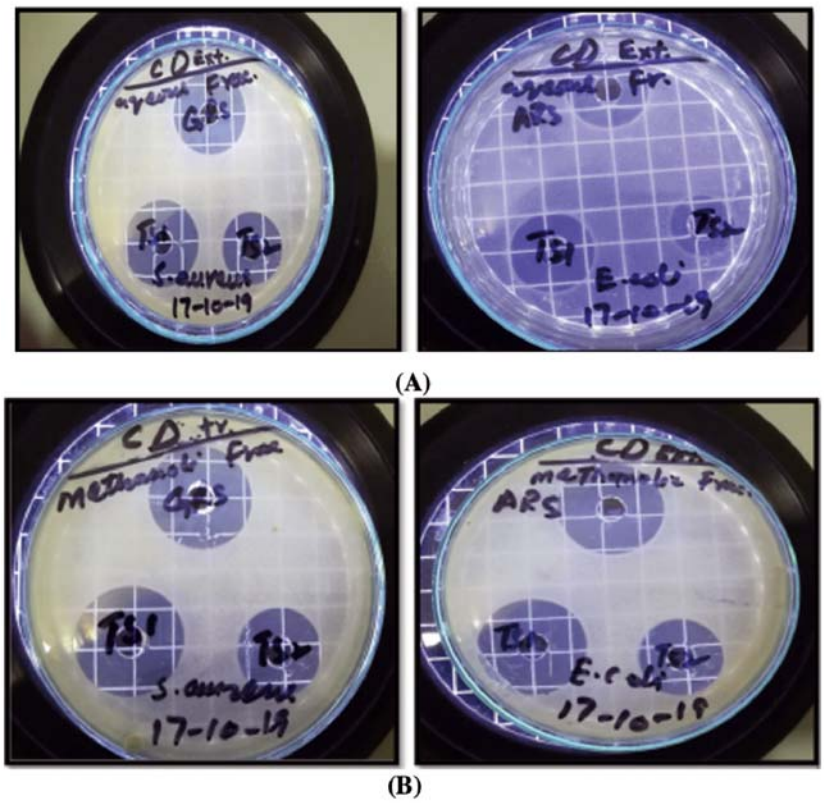

Figure 4. (A) Antibacterial potential of *Cynodon dactylon* aqueous extract against *Escherichia coli* and *Staphylococcus aureus* (B) Antibacterial potential of *Cynodon dactylon* methanolic extract against *Escherichia coli* and *Staphylococcus aureus*

variations[63]. As an anti-aging component superoxidase dismutase (SOD) is used in cosmetics and also in personal care products. Due to its anti-oxidant nature, it also helps in the healing of wounds, softening of scars tissues, reducing age spots, and signs of aging, and providing protection against UV rays[64].

Peroxidase (POD) contents (81.8763 ± 4.6609 units/mg) and superoxidase dismutase (SOD) activity (80.4346 ± 5.9367 units/mg) were investigated higher in the roots of *Cynodon dactylon*. Our results were in accordance with the remarks of[54, 55].

Anti-bacterial Activity

Anti-bacterial activity of *Cynodon dactylon* was performed against the Gram-Negative bacteria *Escherichia coli* ATCC# 8739 and Gram-Positive bacteria *Staphylococcus aureus* ATCC# 6538 by well diffusion method. Gram--Negative bacteria *Escherichia coli* and Gram-Positive bacteria *Staphylococcus aureus* was susceptible to five different extracts resulting into significant inhibition zones as shown in Table 2. Among all the plant extracts, the methanolic extracts showed maximum inhibition zone (26.87 mm) against anti-bacterial strain *Escherichia coli* whereas ethyl acetate extract showed maximum inhibition

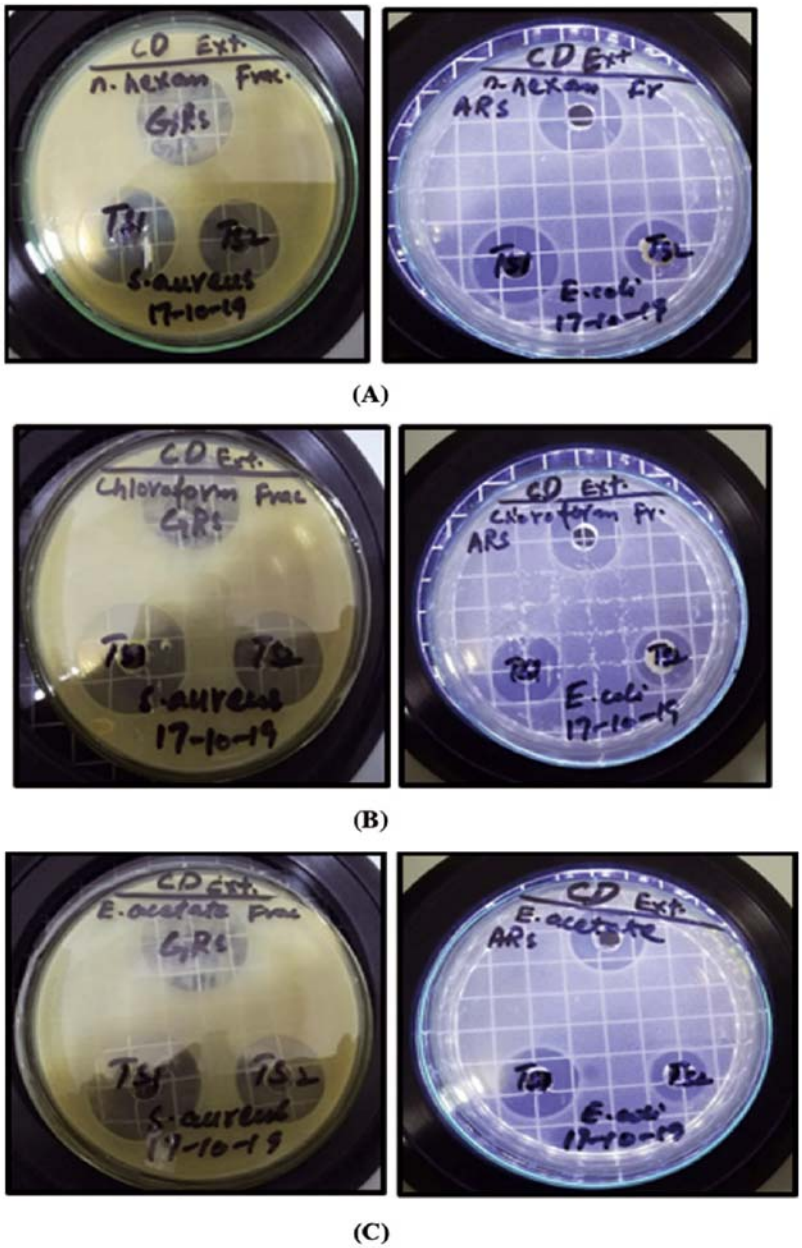

Figure 5. (A) Antibacterial potential of *Cynodon dactylon* n-hexane extract against *Escherichia coli* and *Staphylococcus aureus* (B) Antibacterial potential of *Cynodon dactylon* chloroform extract against *Escherichia coli* and *Staphylococcus aureus* (C) Anti--bacterial potential of *Cynodon dactylon* ethyl acetate against *Escherichia coli* and *Staphylococcu aureus*

Table 3. Anti-fungal potential of *Cynodon dactylon* against *Candida albicans*

Sr. No	Sample Fraction	Microbial Strain/ Culture	Reference Standard	Zone of Inhibition[7]		
				Reference standard value	Test sample 1 (TS1)	Test Sample 2 (TS2)
1.	Aqueous	*Candida albicans*	Nystatin	19.00	17.36	15.16
2.	Methanol	*Candida albicans*	Nystatin	18.01	17.35	15.25
3.	n-hexane	*Candida albicans*	Nystatin	18.32	17.88	16.27
4.	Chloroform	*Candida albicans*	Nystatin	17.03	16.73	15.08
5.	Ethyl acetate	*Candida albicans*	Nystatin	17.28	17.57	16.12

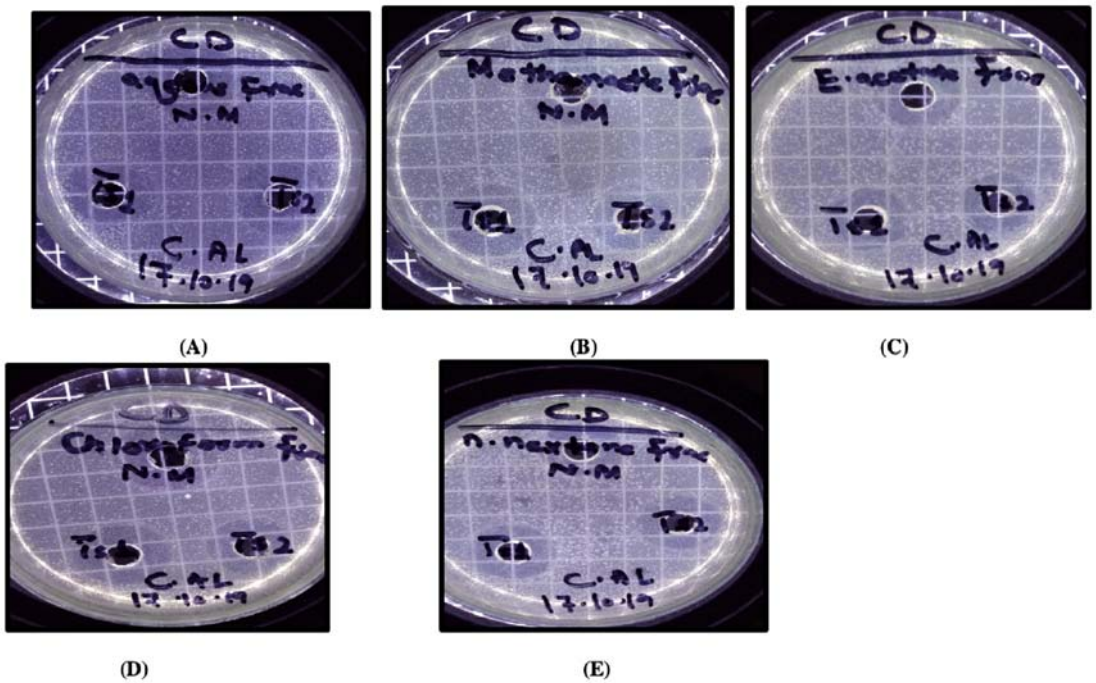

Figure 6. Anti-fungal potential of *Cynodon dactylon* aqueous extract against *Candida albicans* (B) Anti-fungal potential of *Cynodon dactylon* methanolic extract against *Candida albicans* (C) Anti-fungal potential of *Cynodon dactylon* ethyl acetate extract against *Candida albicans* (D) Anti-fungal potential of *Cynodon dactylon* chloroform extract against *Candida albicans*. (E) Anti-fungal potential of *Cynodon dactylon* n-hexane extract against *Candida albicans*

zone (26.45 mm) against *Staphylococcus aureus*. Our results were partially homologous with that of reported by[65–68] (Fig. 4 and 5).

Anti-fungal activity

Anti-fungal activity of *Cynodon dactylon* was determined against *Candida albicans* ATCC# 10231. Anti-fungal reference standard Discs Nystatin 100 units was used. The screening of anti-fungal activity of *Cynodon dactylon* (aqueous, methanolic, n-hexane, chloroform, ethyl acetate) showed that all extracts have anti-fungal potential against *Candida albicans*. The n-hexane extract demonstrated maximum efficacy (17.88) against anti-fungal strain *Candida albicans* (Fig. 6 and Table 3). Our results were matched with that of reported by various studies[75].

CONCLUSION

It could be concluded that Cynodon dactylon stem, leaves and roots showed a larger capacity for antioxidant potential used in pharmaceutical industry due to the presence of total soluble proteins, total phenolic and antioxidants activity. In some cases, these modifications favored increasing the medicinal value of extracts such as anti-fungal activity, anti-bacterial. The methanolic extracts exhibited maximum inhibition zone (26.87 mm) against anti-bacterial strain Escherichia coli whereas ethyl acetate extract showed maximum inhibition zone (26.45 mm) against Staphylococcus aureus. The n-hexane extract showed maximum inhibition zone (17.88 mm) against anti-fungal strain Candida albicans.

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