https://doi.org/ 10.33472/AFJBS.6.Si2.2024.783-793

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

"Chemical properties and bioactive properties of Cynodon dactylon (L.), from the plant to its active ingredients and phytochemicals"

Ajay Kumar Verma[1*], Ajay Kumar Singh Rawat[1], Karuna Shankar Shukla[2], Akash Ved[3]

[1]Maharishi School of Pharmaceutical Sciences, Maharishi University of Information Technology, Lucknow.

[2]Goel Institute of Pharmaceutical Sciences, Faizabad Road, Lucknow.

[3]Faculty of Pharmacy, Dr. A.P.J. Abdul Kalam Technical University, Lucknow

****Address for Correspondence***

Ajay Kumar Verma

Research Scholar, Maharishi School of Pharmaceutical Sciences, Maharishi University of Information Technology, Lucknow. Uttar Pradesh-India

Email: ajaykverma66@gmail.com

Phone No., +91-6394216650

Orcid: 0009-0004-5191-9225

Article History

Volume 6,Issue Si2, 2024

Received:10 Mar 2024

Accepted : 11 Apr 2024

doi: 10.33472/AFJBS.6.Si2.2024.783-793

Abstract: *Cynodon dactylon* (L.), commonly known as Bermuda grass, is a well-known herb among medicinal herbs. This study investigated the anticancer potential of *Cynodon dactylon* extracts obtained using petroleum ether, 50% hydroalcoholic, and aqueous solutions. In this paper, we evaluated the total phenolic content, total flavonoid content, and antioxidant power of the extracts. After that, in vitro, anticancer testing was performed using the MTT method on the A549 cell line. A549 cells were purchased from the National Center for Cell Sciences (NCCS), Pune, India, and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic solution. Different concentrations of Bermudagrass extract were applied to cells and untreated cells. After 24 hours of incubation, the MTT solution was added to the culture and incubated for another 2 hours. Our results indicate that Cynodon dactylon (L.). has important anti-inflammatory functions. Cynodon dactylon (L.) extract inhibits A549 cells, as evidenced by dose inhibition of cell proliferation. Use Graph Pad Prism software to calculate IC50 values and display the concentration at which 50% inhibition of cell growth occurs. This study supports the growing body of evidence supporting the medicinal potential of bermudagrass in treating cancer. The presence of phenols may play an important role in anti-inflammatory activity. Overall, our findings highlight Cynodon dactylon (L.) as an effective anti-cancer agent and highlight the importance of integrating traditional therapies with modern research methods in drug discovery.

Keywords: Cynodon dactylon, anti-cancer activity, A549 cell line, MTT assay, flavonoids, traditional medicine

Introduction: The research has shifted from synthetic compounds to herbal bioactive compounds in recent years. Herbal bioactive compounds are often obtained from natural sources such as plants, this plant has been used in traditional medicine f or centuries. Compared to synthetic compounds, they have lower toxicity profiles and are safer for consumption. It contains a wide range of chemical classes, including alkaloids, flavonoids, terpenoids, and phenolics, each with unique biological properties. *Cynodon dactylon* (L.) exhibits high genetic variability, with chromosome numbers ranging from 2n = 18 to 2n = 36, and both diploid and polyploid populations (**Poojary et al., 2021**). *Cynodon dactylon* (L.) can grow as both an annual and perennial grass, with growth patterns influenced by temperature, radiation, humidity, and soil fertility. *Cynodon dactylon* (L.), commonly referred to as Bermuda grass, is a perennial, warm-season grass that is widely distributed across the globe with rich antioxidant phytoconstituents (**Edwina K; Leela P. (2020**). Originating from Europe, Africa, Australia, and much of Asia, it has also been introduced to the Americas. Known by various names such as Dhoob, dūrvā grass, and Bahama grass, this species features grey-green blades, short stems, and clustered seed heads at its apex. *Cynodon dactylon* (L.) propagates via seeds, stolon's, and rhizomes, exhibiting optimal growth in temperatures ranging from 24 to 37 °C. Its flourishing is typically observed in warm climates with annual rainfall between 625 and 1,750 mm. With numerous cultivars adapted to diverse environments, it is extensively cultivated worldwide (***Cynodon Dactylon - Bugwoodwiki*, 2024**). *Cynodon dactylon* (L.) is a rich reservoir of phytochemical components dispersed throughout its different parts. Among these constituents are tannins, saponins, flavonoids, alkaloids, sterols, and fatty acids, each contributing to the therapeutic potential of C. dactylon. Noteworthy studies have unveiled the presence of linolenic acid, docosanoic acid ethyl ester, palmitic acid ethyl ester, and other bioactive compounds in extracts derived from this grass. The multifaceted nature of these phytoconstituents underscores their significance in the medicinal realm, manifesting in diverse therapeutic effects and antioxidant properties. Such attributes position Bermuda grass as a valuable natural resource in healthcare practices, offering a favorable alternative to synthetic drugs with fewer associated side effects (**Mozafari, A. A., Vafaee, Y., & Shahyad, M. (2018).** Therefore, in the present study, a sample of *Cynodon dactylon* (L.) was analyzed for its chemical characterization, and different organic extracts were analyzed for antioxidant and anti-cancer properties.

Materials and methods

Plant material

Following the collection of the aerial portion of *Cynodon dactylon* (L.) from Lucknow in northern Uttar Pradesh in July 2022, all dirt and dry sections were eliminated. About 250 g of plant material is present. Reference specimen of a leaf from Cynodon dactylon: Ref. No., Boaz. 2022/1 stored in the herbaria of the Benares Hindu University in Varanasi. Verified by Dubey, Botany Department, Faculty of Science, BHU Varanasi, India, the botanical description provided by Professor N.K. Pulverize the material until it reaches a fine powder (around 20 mesh), blend to create a smooth sample, and keep it out of direct sunlight.

Preparation of organic and aqueous Extracts

Several aqueous techniques (water) and organic solvents (petroleum ether and 50% hydroalcoholic) were used in the extraction of the phytoconstituents from Cynodon dactylon (L.). Initially, 250 milliliters of petroleum ether were used to extract 10 grams of the material.

After being constantly swirled at 150 rpm for 48 hours at room temperature, the mixture was first applied to Whatman No. 541. It goes via a sintered glass funnel after passing through filter paper. Another 250 mL of petroleum ether was used to remove the leftover residue at the same time. At 40°C, the resultant extract was lowered in pressure and allowed to solidify and evaporate completely. Then, following the previously described protocol, sequential extractions were carried out with 50% hydroalcoholic and aqueous (water). The goal of this extensive extraction procedure was to provide a broad range (**Savadi et al., 2020**)

Chemical characterization

Method of Preliminary Phytochemical Screening of Cynodon dactylon (L.) leaves: A preliminary phytochemical screening of Cynodon dactylon (L.) leaves was conducted according to standard procedures. Petroleum ether, 50% alcohol, and aqueous extracts were prepared. Various chemical tests were performed to assess the presence of phytochemical constituents, including carbohydrates, alkaloids, saponin glycoside, anthraquinone glycosides, flavonoids, cardiac glycosides, steroids, phytosterols, proteins, amino acids, and tannins. The plant extracts were assayed for alkaloids, anthraquinone, glycosides, flavonoids, tannins, phenolic compounds, carbohydrates, saponins, phytosterols, and triterpenes. (**Mohammed et al., 2017**)

Bioactive compounds in the extracts

To measure phenolics, aliquots of various extracts (1 mL, concentration range 10 to 100 µg/mL) were combined with sodium carbonate solution (75 g/L, 4 mL) and Folinâ Ciocalteau reagent (5 mL, previously diluted 1:10 with water). v/v). To develop color, the mixture was vortexed for 15 seconds and then allowed to stand at 40°C for 30 minutes. Next, each sample's absorbance was calculated at 760 nm. A standard curve with a concentration range of 10 to 100 µg/mL was created using gallic acid, and the findings were reported in milligrams of gallic acid equivalent (GAE) per gram of extract. Singh & Associates, 2014). Aliquots of each extract (0.5 mL, concentration range: 10 to 100 µg/mL) were combined individually with distilled water (2 mL) to calculate the total flavonoids. (**Al-Sayyed et al., 2022**)

Evaluation of bioactive properties of the extracts

Determination of antioxidants using organic and aqueous extracts. The free radical scavenging activity of the organic and aqueous extract obtained from the Cynodon dactylon (L.) aerial part was evaluated using the stable radical compound 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH). This assay assesses the ability of different compounds within the extract to donate hydrogen atoms or electrons, resulting in a reduction in absorbance of the purple DPPH solution in methanol.

The free radical scavenging activity of organic and aqueous **extracts** from *C. dactylon* **leaves** was evaluated by(DPPH). This **test measures** the assessment of different compounds **in an** e xtract to donate hydrogen atoms or electrons, **which reduce the absorptance** of DPPH in me thanol.

The organic extract was dissolved in methanol at a final concentration of 100 µg/mL, and the aqueous extract was dissolved in water at the same concentration. These solutions were further diluted to various concentrations for different antioxidant activity assays. The DPPH radical-scavenging activity was determined using an ELX800 microplate reader (Bio-Tek Instruments, Inc.; Winooski, VT, USA). The percentage of DPPH discoloration was calculated using the formula: $[(A_{BLANKk} - A_{SAMPLE})/A_{BLANK}] \times 100$, where $A_{BLANK} - A_{SAMPLE}$

Represents the absorbance of the sample and **blank** at 515 nm, respectively. To conduct the assay, 2 milliliters of each concentration of the extract were added to 2 mL of 0.004% DPPH solution in methanol. The mixture was then left at room temperature in the dark for 90 minutes. The absorbance of the sample at 517 nm was then measured against the control. For comparison, the synthetic antioxidant butylated hydroxyanisole (BHA) was used at a concentration of 100 ppm. (**Dashipour et al., 2014**)

Cytotoxicity in human tumor cell line

Cytotoxicity of extract against A549 (purchased from NCCS Pune) cell line was determined by MTT assay. (Levitz & Diamond, 1 985).

Cells (10,000 cells/well) were plated in 96well plates in DMEM medium (Dulbecco's Modifi ed Eagle MediumAT1491L) supplemented with 10% FBS (Fel Bovine SerumHIMEDIARM1 0432) for 24 hours) and 1% antibiotics, 5% CO2 at 37 °C. The next day, cells were treated wi th different concentrations (0, 1, 10, 50, 100, 250, 500, 1000 µg/mL). Untreated cells were co nsidered as controls. (Liu & Dalgleish, 2009) After 24 hours of incubation, MTT solution (0, 1,10,50,100,250,500,1000 µg/m) was added to the cell culture and incubated for another 2 ho urs (Fotakis & Timbrill, 2006). At the end of the experiment, the culture supernatant was rem oved and the cell layer matrix was dissolved in 100 µl of dimethyl sulfoxide (DMSOSRLcat. no.67685) and placed in an Elisa microplate reader (iMark, Biorad, USA).) Read 540 nm and 660 nm. IC50 was calculated with Graph Pad Prism6 software. Images were captured using a camera (AmScope Digital Camera 10 MP Aptima CMOS) on an inverted microscope (Olym pus ek2) using a Camera (AmScope digital camera 10 MP Aptima CMOS). (Pop et al., 2022)

Statistical analysis

Analysis of variance (ANOVA) was performed for total phenolic content and total flavonoid content using the overall treatment design (random blocks). Data were analyzed in GenStat 7 (version 7.2.0.220). Experimental results are presented graphically. Student's t test, linear regression analysis (performed in SPSS version 16.0.2007) and Excel plotting were used to investigate relationships between variables.

Results and discussion

Chemical characterization of *Cynodon dactylon* (L.)

Table. 01 Screening of Phytochemical of different extracts of *Cynodon dactylon* aerial parts

Phytochemical	**Results**		
	Petroleum ether extract	**50% Hydroalcoholic Extract**	**Aqueous Extract**
Carbohydrates: Molish test	-	+	+
Benedict’s test	+	+	-
Fehling’s test	-	+	+

Alkaloids:Mayer's test	-	+	+
Wagner's test	-	+	+
Dragendroff's test	-	+	+
Hager's test	-	+	+
Saponin	+	+	+
Glycosides	-	-	+
Foam test			
Haemolysis test			
Anthraquinone	+	+	+
Glycosides	-	+	+
Borntrager's			
Modified Borntrager's			
Flavonoids: Shinoda test	+	+	+
Ferric chloride	-	+	+
Cardiac Glycosides	-	+	+
Legal test	-	+	+
Baljet test	-	+	+
Keller kiliani test			
Phytosterols	+	-	-
Salkowski's			
Xanthoproteic			
Proteins and	+	+	+
Amino Acids	+	+	+
Ninhydrin	-	+	+
Millon's			
Tannins	-	+	+
Ferric chloride			

The result of the phytochemical characterization of *Cynodon dactylon* namely alkaloids, Saponin, Glycosides, Anthraquinones Glycosides, Flavonoids, Cardiac glycosides, Phytosterols, and macronutrients such as Protein, Amino Acids, Carbohydrates are presented in table. Preliminary phytochemical screening of *Cynodon dactylon* (L.) leaves revealed the presence of various phytochemicals. Carbohydrates were detected in 50% alcohol and aqueous extracts, while alkaloids were found in the 50% alcohol and aqueous extracts. Saponin glycosides were present in all extracts, confirmed by positive foam tests. Flavonoids were detected in all extracts, as indicated by positive Shinoda tests and ferric chloride tests in the 50% alcohol and aqueous extracts. Cardiac glycosides were present in the 50% alcohol and aqueous extracts, confirmed by positive legal, Baljet, and Keller Kiliani tests. Steroids were detected only in the petroleum ether extract. Phytosterols were found in the petroleum ether extract but were absent in the other extracts. Proteins and amino acids were present in all

extracts, evidenced by positive Xanthoproteic and Ninhydrin tests. Tannins were detected in the 50% alcohol and aqueous extracts, confirmed by positive ferric chloride and gelatin tests. These findings suggest a diverse chemical composition in Cynodon dactylon (L.) leaves, which may contribute to its potential medicinal properties. Further detailed analysis is necessary to identify specific compounds and understand their biological activities.

Bioactive compounds in different *Cynodon dactylon* (L.) extract

Phenolic compounds play an important role in the free radical oxidation process and protect tissues from various diseases such as inflammatory diseases, cancer, diabetes, myocardial infarction, and Alzheimer's disease. The relationship between the antioxidant effects of phenolic compounds and the level of phenolic compounds in aqueous and alcohol-based extracts should be identified and measured. Jenny et al. Major and minor phenolics such as glycerol (38.49%) and phytol (4.89%) were identified in the cyandon dactylon water extract (Savadi et al., 2020). In another study by Kumar and Chandel, glycerol (38.5%), thymol (1.15%), ethyl glucopyranoside (8.42%), and phytol (4.5%) were identified. (Nallathambi et al., 2019)The content of total phenols and total flavonoids in different *Cynodon dactylon* (L.) aerial parts are listed in the table. The 50% hydroalcoholic extract had the highest content of total phenols (7.78 ± 0.063 mg GAE per gram of extract) and total flavonoids (3.155 ± 0.12 mg quercetin per gram of extract). Aqueous extract (2.208 ± 0.26 mg quercetin per gram of extract). The leaves contained only 4.23 ± 0.042 mg GAE per gram of extract. 50% is reported to be a good solvent for the extraction of phenolics and flavonoids.The total phenolic content of 50% hydroalcoholic extract was 7.78±0.063mg/G, and the total phenolic content in the water extract was 4.23±0.042mg/G. As seen in Figure 2, it increases as the absorbance level increases. The extract concentration was increased from 10 µg/mL to 250 µg/mL. There is a 95% probability that the total amount of extract was increased compared to the control group of 0.18 mg gallic acid/g extract ($p < 0.05$). It is sufficient to show that as the concentration of the extract increases, the phenolic compounds improve, and their antioxidant capacity increases. According to the literature, its high phenolic content has antioxidant activity. Many studies have shown a relationship between phenolic compound levels and the antioxidant properties of plants. According to (unver et al., 2009), unnver et al. The antioxidant activity of plant extracts was found to be related to the total phenolic content.

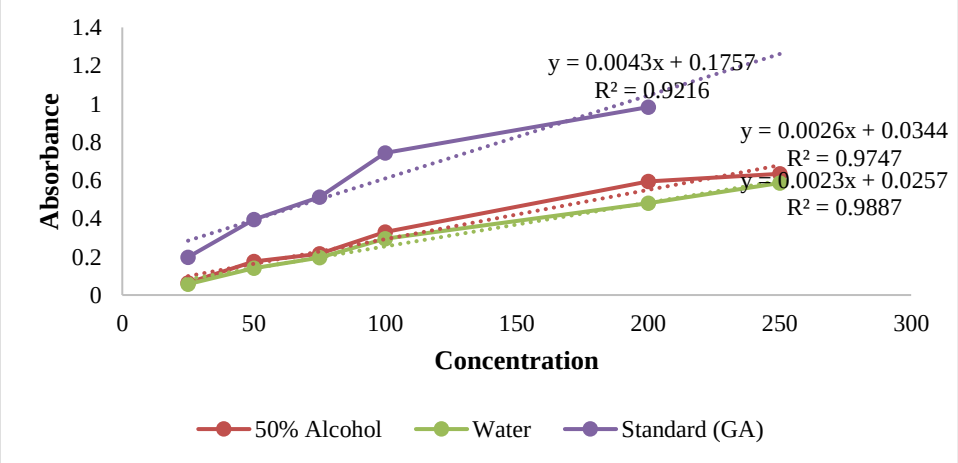


Fig.01 Absorbance at (760 nm) of various concentrations (µg/mL) of

standard (gallic Acid) and Cynodon dactylon (L.) in total phenolic content

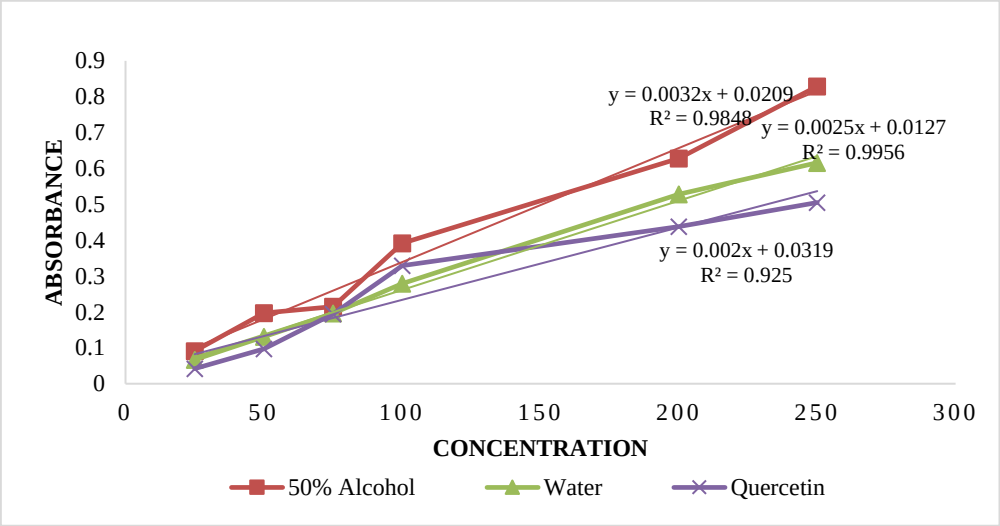


Fig.02 Absorbance at (760 nm) of various concentrations (µg/mL) concerning Quercetin Equivalent

Table.02 Total Phenolic and total Flavonoid content

Extracts	Organic Extracts	Aqueous Extracts
	50 % Hydroalcoholic	**Water**
Total quantity of Polyphenols (mg GAE per g extract)	7.78±0.063	4.23±0.042
Total quantity of Flavonoids (mg quercetin per g extract)	3.155±0.12	2.208±0.26

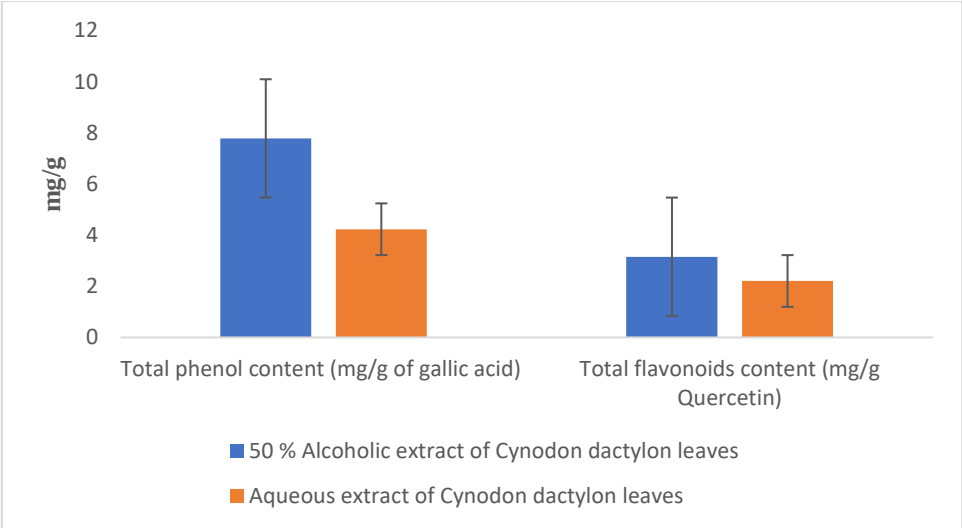


Fig.03 Total Phenolic and total Flavonoid content

Bioactive properties of different *Cynodon dactylon* extracts

The in vitro antioxidant and cytotoxic properties of different extracts of Cynodon dactylon were evaluated, and the results are presented in Table 3. The antioxidant activity was determined by free radical (DPPH) scavenging activity. The cytotoxicity was tested against human tumor cell lines (lung carcinomas) using a porcine liver primary cell culture.

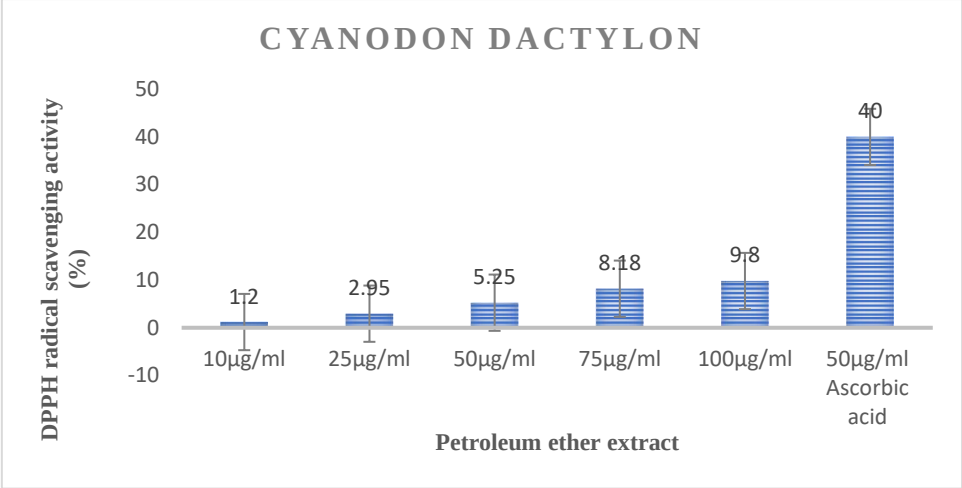
The in vitro antioxidant and cytotoxic properties of different extracts of C. dactylon were evaluated and the results are listed in Table 3. Cytotoxicity against human tumor cell lin es (cancer cells) was tested using porcine liver primary cell lines.

Antioxidant determination was made by DPPH assay. DPPH measures the activity of organic and aqueous extracts of Bluetooth roots and leaves at different concentrations. In our data ana lysis, we determined that scratching activity increased as the concentration of hydroalcoholic and aqueous extracts increased. Scavenging activity at 10 ug/mL was 10.58 % and level up to 64.52 as the extract concentration increased to 100 10 ug/mL in 50 % hydroalcoholic extract and at 25,50,75,100 ug/mL was 17.85, 29.26,39 % respectively.

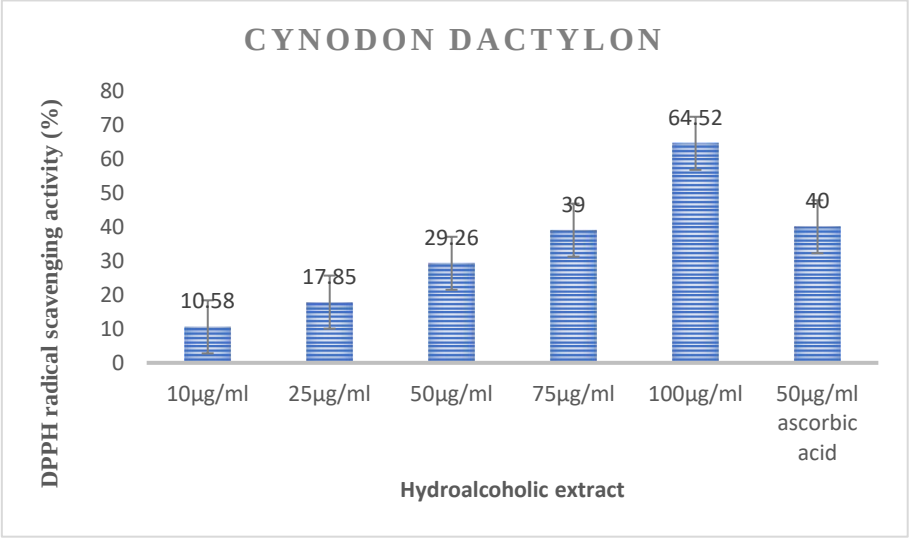
On the other side, an aqueous extract shows scavenging activity at 10 ug/mL 12.16 % compared to 50 % hydroalcoholic at 10 % which is a higher value but does not consistently increase like 50 % hydroalcoholic extract from 50 to 100 ug/mL. Compare the scavenging activity of different concentrations of 50 % hydroalcoholic and aqueous extract with 50 ug/mL Ascorbic acid and found that 50 ug/mL has lower anti-oxidant than that of 100 ug/mL of 50 % hydroalcoholic and aqueous extract. This signifies that high scavenging activity is observed at high concentrations. This data indicates may be a phenolic compound responsible for anti-oxidant activity. We may say that as if phenolic concentration increases then anti-oxidant increases.

Table 03 Comparison of free radical scavenging activity of different extracts of Cynodon dactylon aerial parts at different concentrations and ascorbic acid

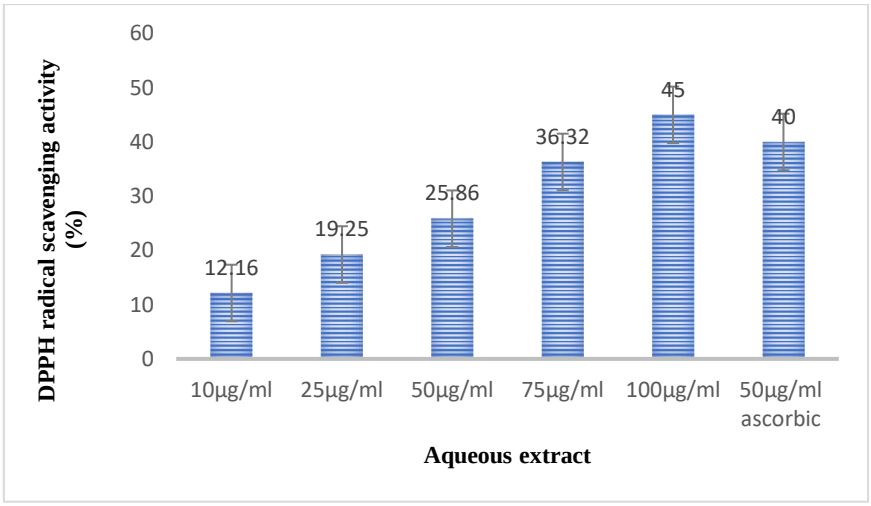
Concentration	Petroleum ether	50 % Hydroalcoholic	Aqueous
10µg/ml	1.2	10.58	12.16
25µg/ml	2.95	17.85	19.25
50µg/ml	5.25	29.26	25.86
75µg/ml	8.18	39	36.32
100µg/ml	9.8	64.52	45
50µg/ml ascorbic	40	40	40



Fig,04 Comparison of free radical scavenging activity of petroleum extracts of Cynodon dactylon leaves at different concentrations and ascorbic acid



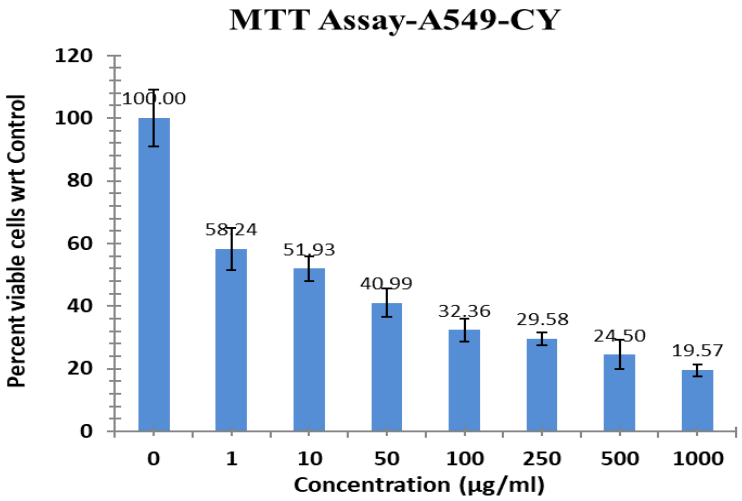
Fig,05 Comparison of free radical scavenging activity of hydroalcoholic extracts of *Cynodon dactylon* aerial parts at different concentrations and ascorbic acid



Fig,06 Comparison of free radical scavenging activity of Aqueous extracts of Cynodon dactylon leaves at different concentrations with ascorbic acid

The extract was then concentrated and **tested for** antioxidant activity on A549 cancer **cells vi a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium** bromide **(MTT).** According to the MTT assay results, the cytotoxic activity of the *Cynodon dactylon* sample was estimated (IC50 = 31.02 ± 0.26 µg/ml) and A549 cells were found to exhibit different concentrations of 50% hydroalcoholic Cyanide root extract. The CY sample was found to be less cytotoxic. IC50, the concentration of inhibitor/sample/preparation of cells used is reduced by half.

Sample code	IC_{50} value (µg/ml)
CY	31.02 ± 0.26



Conclusion

Based on the above experiment result and data, we can clearly say that *Cynodon dactylon* aerial part is rich in carbohydrates, alkaloids, flavonoids, and phenolic compounds. In general, the organic and aqueous extract showed anti-oxidant and tested for cytotoxicity against human tumor cell lines. The 50 % hydroalcoholic extract showed the highest anti-oxidant potential in the DPPH assay. The 50 % hydroalcoholic showed also good in vitro anti-cancer activity. the obtained results support the traditional uses of Cynodon dactylon.

Conflicts of Interest

The authors declare no conflicts of interest

References

1. Poojary, R., Kumar, N. A., Kumarchandra, R., & Sanjeev, G.. (2021). *Cynodon dactylon alleviates radiation-induced behavioral and biochemical changes in the cerebral cortex of mice*. *14*(5). https://doi.org/10.52711/0974-360X.2021.00452
2. Edwina K; Leela P. (2020). *Phytotoxic Effect Of Cyanodon Dactylon (L.) Pers. and Cyperus Rotundus L. On Growth And Biochemical Changes of Vigna Radiata (L.) R. Wilczek*. *2*, 90–96. https://doi.org/10.47392/IRJASH.2020.166
3. *Cynodon dactylon - Bugwoodwiki*. (2024, March 26). *Cynodon dactylon - Bugwoodwiki*. https://wiki.bugwood.org/Cynodon_dactylon
4. Mozafari, A. A., Vafaee, Y., & Shahyad, M. (2018). Phytochemical composition and in vitro antioxidant potential of *Cynodon dactylon* leaf and rhizome extracts as affected

by drying methods and temperatures. *Journal of food science and technology*, *55*(6), 2220–2229. https://doi.org/10.1007/s13197-018-3139-5

5. Savadi, S., Vazifedoost, M., Didar, Z., Nematshahi, M. M., & Jahed, E.. (2020). *Phytochemical Analysis and Antimicrobial/Antioxidant Activity of Cynodon dactylon (L.) Pers. Rhizome Methanolic Extract*. *2020*. https://doi.org/10.1155/2020/5946541
6. Mohammed, S., Suleiman, H., Abubakar, M., Sagir, Sule, S., Balarabe, B., & Mohammed, K.. (2017). *Preliminary phytochemical screening of Parkinsonia aculeata Leaf extracts*. *5*(2).
7. Singh, H., Dixit, A., Sharma, R., & Sharma, A.. (2014). *Comparative evaluation of Total Phenolic Content, Total Flavonoid Content and DPPH free radical scavenging activity of Different Plant Parts of Vitex negundo L. COMPARATIVE EVALUATION OF TOTAL PHENOLIC CONTENT, TOTAL FLAVONOID CONTENT AND DPPH FREE RADICAL*. *7*(2).
8. Al-Sayyed, H. F., Al-Kurd, R. A., Mahmoud, I. F., AbdelQader, S. M., Sweidan, D., Rizeq, L. T., Arafat, T., & Mwalla, M. M.. (2022). *Developing a database for total phenolic content, total flavonoid content, and antioxidant activity of Jordanian crops*. *25*(1). https://doi.org/10.1080/10942912.2022.2077369
9. Dashipour, A., Khaksar, R., Hosseini, H., Shojaee-Aliabadi, S., & Ghanati, K.. (2014). *Physical, Antioxidant and Antimicrobial Characteristics of Carboxymethyl Cellulose Edible Film Cooperated with Clove Essential Oil*. *16*(8).
10. Levitz, S. M., & Diamond, R. D.. (1985). *A Rapid Colorimetric Assay of Fungal Viability with the Tetrazolium Salt MTT*. *152*(5). https://doi.org/10.1093/INFDIS/152.5.938
11. Liu, W. M., & Dalgleish, A. G.. (2009). *MTT assays can underestimate cell numbers*. *64*(4). https://doi.org/10.1007/S00280-009-1047-0
12. Fotakis, G., & Timbrell, J. A.. (2006). *In vitro cytotoxicity assays: comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride.*. *160*(2). https://doi.org/10.1016/J.TOXLET.2005.07.001
13. Pop, O. L., Kerezsi, A. D., & Ciont, C.. (2022). *A Comprehensive Review of Moringa oleifera Bioactive Compounds—Cytotoxicity Evaluation and Their Encapsulation*. *11*(23). https://doi.org/10.3390/foods11233787
14. Savadi, S., Vazifedoost, M., Didar, Z., Nematshahi, M. M., & Jahed, E. (2020). Phytochemical Analysis and Antimicrobial/Antioxidant Activity of Cynodon dactylon (L.) Pers. Rhizome Methanolic Extract. *Journal of Food Quality*, *2020*, 5946541. https://doi.org/10.1155/2020/5946541
15. Nallathambi, A., Nallathambi, A., Bhargavan, R., & Bhargavan, R.. (2019). *GC/MS Analysis of Bioactive Compounds in Aqueous Extract of Cynodon Dactylon* (Vol. 10). https://doi.org/10.37506/v10/i12/2019/ijphrd/192194
16. Ünver, A., Arslan, D., Özcan, M. M., & Akbulut, M.. (2009). *Phenolic Content and Antioxidant Activity of Some Spices*. *6*(3).