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Phytochemical and Pharmacological Evaluation of Cynodon Dactylon

Prashant Nayak[1*], Manohar Naik K[2], Arun Kumar R[3], Ramesh S Bhat[4], Janardhana Nayak[4], Anto Emmanuel DP[5]

[1*]Nitte (Deemed to be University), NGSM Institute of Pharmaceutical Sciences, Department of Pharmaceutics, Deralakatte, Mangaluru-575018, India.
[2]Department of Pharmacology, P A College of Pharmacy, Nadapadavu, Mangaluru-574199, Karnataka, India
[3]Holy Grace Academy of Pharmacy, Mala. Thrissur, India
[4]Department of Chemistry, NMAM Institute of Technology (NMAMIT), NITTE (Deemed to be University), Karnataka, 574110, India
[5]Department of Studies in Chemistry, St. Philomena College (Autonomous) Mysuru-570015, Karnataka, India

Corresponding author: [1*]Prashanth Nayak
[1*]Nitte (Deemed to be University), NGSM Institute of Pharmaceutical Sciences, Department of Pharmaceutics, Deralakatte, Mangaluru-575018, India.
ORCID ID: 0000-0002-2659-4893
Email:prashantn2001@nitte.edu.in

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ABSTRACT:

Cynodon dactylon, commonly known as Bermuda grass, has been extensively studied for its phytochemical and pharmacological properties. This study investigates the presence of various phytochemicals and evaluates the antioxidant and antibacterial activities of C. dactylon extracts. Phytochemical assays revealed high concentrations of proteins and reducing sugars, moderate concentrations of alkaloids and glycosides, and low concentrations of triterpenoids, steroids, tannins, and saponins. The antioxidant activity was assessed using DPPH radical scavenging, nitric oxide radical inhibition, and hydrogen peroxide scavenging assays, with IC50 values of 308 ± 1.05 µg/ml, 180 ± 1.34 µg/ml, and 600 ± 0.875 µg/ml, respectively. Although less potent than ascorbic acid, the extract demonstrated significant antioxidant potential. The antibacterial activity against Escherichia coli showed a zone of inhibition of 17 mm, comparable to the standard antibiotic neomycin's 19 mm. These findings suggest that C. dactylon possesses notable antioxidant and antibacterial properties, supporting its traditional medicinal use and highlighting its potential for developing natural therapeutic agents. Further research is needed to isolate specific bioactive compounds and elucidate their mechanisms of action.

Keywords: Antioxidant activity, Cynodon dactylon, Phytochemical study, and antimicrobial activity

1. Introduction

Cynodon dactylon, commonly known as Bermuda grass or durva grass, is a perennial grass species belonging to the Poaceae family. Widely distributed across tropical and subtropical regions, it is renowned for its medicinal properties deeply rooted in traditional medicine systems worldwide. With a rich history of therapeutic use, cynodon dactylon has been extensively studied for its diverse pharmacological activities, including its phytochemical, antioxidant, and antimicrobial properties [1].

Phytochemical properties:
Cynodon dactylon is a rich source of bioactive compounds, including alkaloids, flavonoids, saponins, tannins, steroids, and glycosides, among others. These phytochemicals contribute to the plant's medicinal properties and biological activities. Alkaloids, for instance, are known for

their potential therapeutic effects, including analgesic and anti-inflammatory properties. Flavonoids exhibit antioxidant activity and play a crucial role in scavenging free radicals, thus protecting cells from oxidative damage. Saponins possess antimicrobial and anti-inflammatory properties, while tannins are recognized for their astringent and antioxidant effects. Steroids and glycosides also contribute to the pharmacological profile of cynodon dactylon, enhancing its potential as a medicinal plant [2].

Antioxidant property:
Oxidative stress, resulting from an imbalance between free radicals and antioxidants in the body, is implicated in various chronic diseases, including cardiovascular disorders, cancer, and neurodegenerative conditions. Cynodon dactylon has garnered attention for its potent antioxidant properties, attributed to its rich phytochemical composition. Studies have shown that extracts of cynodon dactylon exhibit significant scavenging activity against free radicals, including DPPH radicals, nitric oxide radicals, and hydrogen peroxide, thus conferring protection against oxidative damage. These antioxidant effects highlight the potential of cynodon dactylon as a natural source of antioxidants for promoting health and preventing diseases associated with oxidative stress [3].

Antimicrobial property:
In addition to its antioxidant activity, cynodon dactylon possesses notable antimicrobial properties, making it a promising candidate for the treatment of infectious diseases. Several studies have demonstrated the inhibitory effects of cynodon dactylon extracts against a wide range of pathogenic microorganisms, including bacteria, fungi, and viruses. The plant's antimicrobial activity is attributed to various bioactive compounds present in its aerial parts, which inhibit the growth and proliferation of microbial pathogens. The antimicrobial potential of cynodon dactylon underscores its traditional use in folk medicine for treating infections and highlights its therapeutic value in modern medicine [4].
In conclusion, cynodon dactylon is a valuable medicinal plant with diverse phytochemical, antioxidant, and antimicrobial properties. Its rich pharmacological profile makes it a promising candidate for the development of novel therapeutic agents for various health conditions. Further research into the mechanisms of action and clinical efficacy of cynodon dactylon is warranted to fully explore its therapeutic potential and promote its utilization in healthcare practices [5].

2. Materials and Methods

The Cynodon dactylon grasses were collected from Deralakatte Mangalore and these plant were collected and stored in the refrigerator.

Preparation of plant extract:
Plant materials collected were dried in the sun after drying the plant materials were made free from mud then these plant was used to remove the active constituents by decoction method. Water was used a beaker with plant material and kept in a heating mantle the resultant mixture was evaporated and decoction was concentrated. Later the mixture was filtered in a muslin cloth and filtrate obtained was taken in a china dish and the water was completely evaporated to get a concentrated extract of the plant
This concentrated extract was used for further analysis.

Preliminary Qualitative Phytochemical Investigations:
Cynodon dactylon extract was exposed to phytochemical assessment for portraying the constituents present in it. The tests were compared with the standard.

1.Test For Alkaloids [6]

Evaporate the concentrate independently to the build up and add dil HCl shake well and channel with filtrate do the accompanying test

A) Dragendroff's Test

2ml of filter and add potassium bismuth iodide where orange brown ppt is formed

B) Hagers Test

2ml of filtrate and add saturated solution of picric acid is formed where yellow ppt is formed

C) Wagner's Test

3-4ml of sample is added with drops of conc nitric acid is added then evaporated to dryness cool and add 2 drops of ammonium oxide where purple color is formed techniques.

D) Mayer's Test

2ml of filtrate with potassium mercuric iodide solution then cream ppt is formed

2.Test for Carbohydrates [7]

A] Test for Reducing Sugar

A] Molish's Test

Aqueous extract + drops of alpha naphthol in alcohol+ conc sulphuric acid {on the wall of the test tube} violet ring will form at the junction which shows carbohydrates present

B] Benedict's Test

Mix equal volumes of Benedict's reagents [Complex reagents of sod. Carbonates citrate copper sulphate pentaldheyde] and test solution in test tube+test arrangement in test tube Heat in water shower for 5 months depending on the reducing sugar the green yellow and red color formed.

C] Fehling Test

Mix 1ml feelings A [Copper sulfate -blue color aqueous solution] +Fehlings B [potassium sodium tartrate a base like NAOH] + add an equivalent volume of test + heat in bubbling water for 5-10 min where yellow to block red will be formed.

C] Tollen's Test

Take two clean, dry test tubes and add 1 ml of the test sample in one test tube and 1 ml of distilled water in another as blank. Add 2 ml of Tollen's reagent to both test tubes. Keep both the test tubes in a water bath for 1 min. Silver mirror is formed.

B] Test for Non-Reducing Sugar

I] Test solution does not give response to fehling and benedict test then

II] Hydrolyse Test Solution

Add hydrochloric acid+_boil and neutralise + Benedicts reagent which will form red precipitate

III]Test for Non Reducing Polysaccharides

Add 3ml sample+Iodine where blue colour appears and disappear on boiling and reappear on looking.

3.Test for Flavanoids [8]

A) Shinoda Test

Drug powder was added in ethanol (5 ml) and to this conc HCL was added. A little piece of Mg alumina was added and afterward it was warmed. The presence of flavonoids was affirmed by pink tone

B) 3ml of sample with lead acetate solution is added where yellow precipitate is added

C)addition of invasing amount of NAOH to the residue where yellow colour which decolorizes upon addition of acid

4.Test for Saponins

A] Emulsion Test

In a test tube containing 5ml of Cynodon dactylon extract, a drop of sodium bi carbonate solution was added, shaken well and left for 3 min. Formation of honeycomb like froth confirmed the presence of saponins.

5.Test for Tannins and Phenolic Compounds

2-3ml of fluid/alcoholic concentrate to this add following reagents

A)5% ferric chloride solution forming of blue/black colour shows condensed tannins and green shows hydrolysable tannins

B) lead acetate solution shows white precipitate

C)Gelatine solution shows white ppt

D)Bromine water shows decolourization

E) Acetic acid solution shows red colour solution

F) Dill iodide solution shows transient red colour

G) Dil nitric acid shows reddish yellow colour

H) Dil KMNO4 shows decolourization

5.Test for Steriods [9]

a) Libermann Burchard Test

Around 3ml of test was dissolved in acidic anhydride. The arrangement was warmed and cooled, then, at that point, add at the edge of the test tube concentrated sulphuric acid is added. appearance of green tone affirms steroids are available.

b) Salkowski Test

In a test tube take test with chloroform to this sample put concentrated sulphuric acid at the edges of the test tube. In chloroform layer a red tone is seen which confirms steroids in the arrangement

c) Libermann

3ml of sample with 3ml acetic anhydride heat and cool with sulphuric acid where blue colour is formed

6. Test For Protiens [10]

A) Biuret's Test

3ml of test was taken, few drops of copper sulphate 1% with 4%NAOH was added. A violet red tone shaped distinguishes proteins in the example.

B) Millon's Test

For 3ml pf sample the 5ml Millon's reagent was added. A white colour precipitate which becomes red on heating shows proteins in the sample

C)Precipitation Test

3ml of sample with absolute alcohol with 5% copper sulphate solution where white colloidal precipitate is formed

Test for Amino Acid [11]

A) Ninhydrin Test

Heat 3ml of test and add 3 drops of ninhydrin disintegrate 0.2g of ninhydrin in 99.5ml of ethanol with 0.5ml of acidic corrosive arrangement in water shower for 10min where purple somewhat blue colour is formed

B) Test for Tyrosin

Heat 3ml of test with 3 drops of millons reagent where dull red tone is framed

C) Test for Cysteine

5ml of test with not many drops of 40% NAOH and add 10% of lead acetic acid derivation and dark ppt of lead sulphate is formed

Ii) Test for Fats/Oils

A) To ethanoic solution a few drops of copper sulphate with NAOH solution is added then the clear blue solution is formed.

B) sample is added with 2-3 drops of tincture alkane with red color formation

C)fine drops of sample is placed in filter paper where permanently stains filter paper.

Iiii) Test for Glycoside
I)Cardiac Glycoside
A) Keller-Killiani Test
2ml of test add glacial acidic corrosive with one drop of 5%ferric chloride and add conc sulphuric corrosive this prompts rosy earthy coloured tone shows up at the intersection of 2 fluid layer and copper layer seem somewhat blue green
B) Legal's Test (Cardenolides)
To fluid alcoholic concentrate and add 1ml of pyridine and add 1ml of sodium nitroprusside and pink red tone is framed
Ii)Anthraquinone Glycoside [12]
A) Borntrager's Test
3ml of test add dil sulphuric acid and channel to the cooled channel and add equivalent volume of benzene or chloroform shake well separate the natural dissolvable and add smelling salts where alkali layer becomes pink/red will frame

B) Modified Borntragers Test
5ml of test add 5ml of 5% ferric chloride and add 5ml of dil HCl then hotness for 5min in water shower cool and benzene or chloroform shake well separate natural layer then, at that point, add equivalent volume of weaken nitric corrosive is added where ammonical layer shows pinkish red tone is shaped.
Iii) Saponin Glycoside
A) Foam Test
3ml of sample is vigorously shaken with water where foam is formed
Iv)Cyanogenic Glycoside
A) Sample is added with 3% aqueous mercurous nitrate is added where metallic mercury is formed
B) Grignard Reaction
Splash a channel paper strip in 10% picric acid add 10% sodium carbonate with drug in cone like carafe place dampened powdered medication stopper it place it above channel paper strip in cut in plug where channel paper turns block red/maroon
C) Coumarin Glycoside
Alcoholic concentrate when made basic where blue/green fluorescence is formed.

Pharmacological Effects
The grass has different pharmacological exercises. The dried concentrates of aeronautical pieces of this wasanalysed for CNS exercises in mice. Antidiabetic, antiulcer, pain relieving and hostile to pyretic, diuretic and antimicrobial action are a portion of its different fundamental functions. This is extremely compelling in snakebite treatment and the anti-snake toxin from the plant extricate is exceptionally powerful to treat patients who are chomped by a snake. The grass is utilized as a customary society medication in India and numerous different spots for the treatment for different illnesses and issues. Other unmistakable movement incorporates calming and cell reinforcement action.

Dpph Radical Scavenging Activity [13]
The examples were assessed for the DPPH revolutionary rummaging conduct as per the strategy proposed by Barros et al. In 5 mL of a 100 μM arrangement of DPPH (in methanol), 50 μL of the different plant separates focuses in ethanol fused. The ascorbic corrosive was utilized as a norm and ready as referenced above without the concentrates. For 30 minutes, the examples were brooded at room temperature, and later absorbance of the relative multitude of

tests, including clear, was estimated at 517 nm utilizing Shimadzu UV spectrophotometer 1300 series. Determined the level of restraint action from the accompanying condition:
% inhibition= [(Ao-A1)/Ao] x100
Where Ao is the control absorbance,
A1 is the concentrate/standard absorbance Here ascorbic corrosive was utilized as a norm. The analyses were acted in three-fold.

Nitric Oxide Radical Inhibition Assay [13]
The utilization of the Griess Illosvoy response is feasible to assess NO° hindrance. The test was completed by an adjusted GriessIl losvoy reagent utilizing 0.1 % N-(1-Naphthyl) ethylene diamine dihydrochloride and ascorbic corrosive as standard. The examples were estimated at 540 nm using Shimadzu uv-spectrophotometer 1300 series frequency and were contrasted with the clear utilizing the accompanying recipe:
Rummaging action of Nitric oxide extremist = (Aoblank - A1sample) × 100/Ao
Blank Ao is the clear absorbance, and A1 is the example absorbance Ascorbic corrosive was utilized as standard.

Antibacterial Activity [13]
A few concentrates of plants were performed on antibacterial movement, essentially to Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923.The technique for circle dissemination had the option to evaluate the antibacterial action of B. flabellifer. We arranged the way of life plates by pouring 20mL (supplement media containing Müller Hinton Agar, MHA) liquid media into sterile Petri plates, with ciprofloxacin (5 mg) standard or control. The trial included concentrates of B. flabellifer in the openings of immunized plates at 37o C for 24 hours, posteriorly restraint zones around the area of openings were estimated in millimetres.

3. Results and Discussion

TABLE 1 PHYTOCHEMICAL ASSAYS OF CYNODON DACTYLON EXTRACT

Sl. no	Tests	Observation	Cynodon dactylon extract
1	Alkaloids a) Dragendroff'stest b) Hager's test c) Wagner's test d) Mayer's test	a) Brick red precipitate b) Yellow precipitate c) Reddish brown precipitate d) Milky precipitate	++ ++ ++ ++
2	Reducing sugars,a)Moloch's test b) Benedict's test c)Fehling's test d)Tollen's test	a) A brown coloration observed the interface b) Yellow to bright precipitate c) Presence of a brick red precipitate d) Silver precipitate	+++ +++ +++ +++
3	Flavonoids a) Shinoda test	No reddish color	+++
4	Saponins Emulsion test	Emulsion formed	+
5	Tannins lead sub acetate test	Presence of cream gelatinous precipitate	+

6	Steroids a) Liebermann-Burchard test b) Salkowski test	Green blue color Bluish red to purple color	+ +
7	Proteins a)Biuret test b)Millon's test	a) White precipitate Protein present b) White precipitate Protein present	+++ +++
8	Tri terpenoids a)Liebermann Burchard	Light green to dark green color	+
9	Glycosides I)Cardiac Glycoside a) Keller-Killiani Test b) Legal's Test (Cardenolides) II)Anthraquinone Glycoside a) Borntrager's Test b) Modified Borntragers Test III) Saponin Glycoside a) Foam Test IV) Cyanogenic Glycoside a) Grignard Reaction b) Coumarin Glycoside	a) Reddish brown colour appears at the junction of two liquid layer and copper layer appear bluish green b) Pink red color is formed a) Ammonia layer turns pink/red will Form b) Ammonical layer shows pinkish red color is formed. a) Foam is formed a) Metallic mercury is formed b)Filter paper turns brick red/maroon	++ ++ ++ ++ ++ ++ ++

Key–Absent
+ Present in low concentration ++ Present in moderate concentration
+++ Present in high-concentration
As sown in table 1: Cynodon dactylon showed macronutrients showed the presence of following proteins and reducing sugar in high concentration based on the intensity of color observed in the solution and also the precipitation upon screening
The secondary metabolites like glycoside and alkaloids were present in the moderate concentration. Some other phytochemical concentrations like triterpenoids, steroids, tannins, and saponins were present in low concentrations.

Results of Antioxidant analysis of Cynodon dactylon
The antioxidant activity of Cynodon dactylon extract was assessed through various assays, including DPPH radical scavenging, nitric oxide radical inhibition assay, and hydrogen peroxide scavenging. The IC50 values for assay are presented below in Table 2 and figure 1
DPPH Radical Scavenging:
Cynodon dactylon extract: 308 ± 1.05 µg/ml
Ascorbic acid (standard): 42 ± µg/ml
Nitric Oxide Radical Inhibition Assay:
Cynodon dactylon extract: 180 ± 1.34 µg/ml
Ascorbic acid (standard): 65 ± 0.984 µg/ml

Scavenging of Hydrogen Peroxide:
Cynodon dactylon extract: 600 ± 0.875 µg/ml
Ascorbic acid (standard): 150 ± 0.729 µg/ml

Discussion:

The antioxidant activity of Cynodon dactylon extract was evident in all three assays, albeit with higher IC50 values compared to the standard antioxidant, ascorbic acid. The DPPH radical scavenging assay revealed that the extract possesses moderate antioxidant potential, with an IC50 value of 308 ± 1.05 µg/ml, indicating its ability to neutralize free radicals.

Similarly, in the nitric oxide radical inhibition assay, the extract exhibited significant antioxidant activity with an IC50 value of 180 ± 1.34 µg/ml, indicating its ability to inhibit nitric oxide production, a marker of oxidative stress.

In the scavenging of hydrogen peroxide assay, the Cynodon dactylon extract demonstrated moderate antioxidant activity, as indicated by its IC50 value of 600 ± 0.875 µg/ml. Hydrogen peroxide is a reactive oxygen species, and the ability of the extract to scavenge it reflects its potential to mitigate oxidative damage.

Although the IC50 values of the Cynodon dactylon extract were higher than those of the standard ascorbic acid, it is important to consider that natural extracts often contain a complex mixture of phytochemicals, each contributing to overall antioxidant activity. Thus, the observed antioxidant potential of Cynodon dactylon extract suggests its utility as a natural source of antioxidants, which may contribute to its medicinal properties and potential health benefits.

Table 2: IC 50 values of the compound and standard

Name of the compound	**IC_{50} values**		
	DPPH (µg/ml)	**Nitric oxide radical inhibition assay (µg/ml)**	**Scavenging of hydrogen peroxide**
Cynodon dactylon	308 ± 1.05	180 ± 1.34	600 ± 0.875
Ascorbic acid	42 ± 0.63	65 ± 0.984	150 ± 0.729

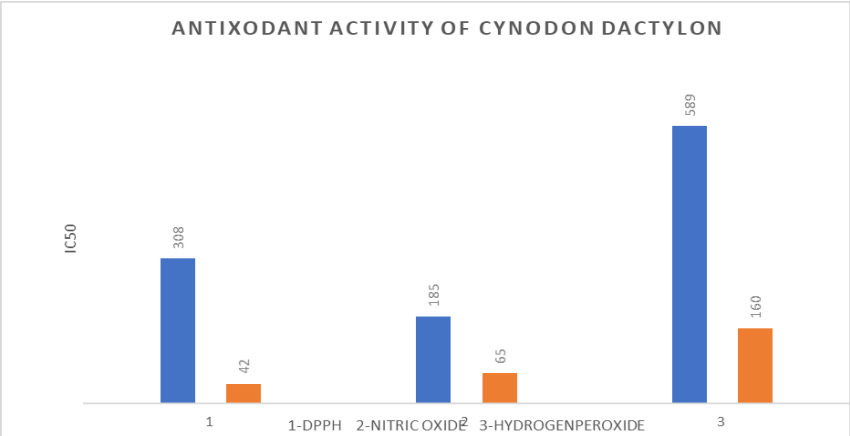

FIG.1Antioxidant activity of Cynodont Dactylon

As shown in figure 2 The antibacterial activity of the extract of Cynodon dactylon against Escherichia coli (E. coli), a gram-negative bacterium, was evaluated using the cup plate method. The diameter of the zone of inhibition was measured as an indicator of bacterial growth

suppression. The extract exhibited a growth inhibition property with a zone of inhibition measuring 17 mm.

The observed antibacterial activity of the Cynodon dactylon extract against E. coli is noteworthy, particularly considering its gram-negative nature, which often poses challenges for antimicrobial interventions. The zone of inhibition of 17 mm indicates a significant suppression of bacterial growth.

Comparison with the standard antibiotic neomycin, which exhibited a zone of inhibition of 19 mm, suggests that the extract's antibacterial efficacy is comparable to that of the standard drug. This finding is promising as it implies that Cynodon dactylon extract may serve as a potential alternative or adjunct to conventional antibiotics for combating bacterial infections.

The comparable results between the extract and neomycin highlight the extract's potential as a natural antimicrobial agent. Furthermore, the fact that the extract demonstrated significant antibacterial activity against E. coli underscores its broad spectrum of action, which may extend to other pathogenic bacteria.Overall, these results support the traditional use of Cynodon dactylon in ethno medicine for the treatment of bacterial infections. Further studies are warranted to elucidate the specific bioactive compounds responsible for its antibacterial activity and to explore its mechanism of action, safety profile, and potential applications in clinical settings.

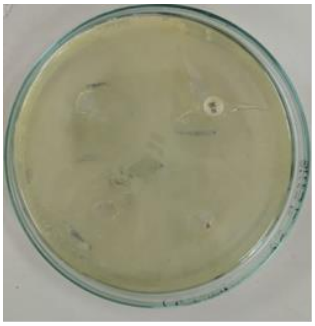

Fig: 2 antimicrobial activity of Cynodon dactylon extract

4. Conclusion

In conclusion, the comprehensive phytochemical screening of Cynodon dactylon extract revealed the presence of various bioactive compounds, including proteins and reducing sugars in high concentrations, while glycosides and alkaloids were present moderately. Additionally, triterpenoids, steroids, tannins, and saponins were detected in low concentrations. These findings suggest the potential health benefits of Cynodon dactylon due to its rich phytochemical profile.

Moreover, the antioxidant analysis demonstrated moderate antioxidant activity, as evidenced by its IC50 values in DPPH, nitric oxide radical inhibition assay, and scavenging of hydrogen peroxide. Although not as potent as ascorbic acid, Cynodon dactylon still exhibited significant antioxidant properties.

The observed inhibition of bacterial growth suggests the potential of Cynodon dactylon extract as a natural antimicrobial agent.

In essence, the findings underscore the medicinal potential of Cynodon dactylon, supporting its traditional use in various therapeutic applications

Conflict of Interest

The authors have no conflicts of interest regarding this investigation.

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