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Phytochemical profile of *Cynodon dactylon* L., evaluated using high performance liquid chromatography: potential as antimicrobial, preservative and additive agent

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Abstract

The challenges of antimicrobial resistance in agriculture and the health sector are a concern. Consumers' preference for organic preservatives and additives vis-a-vis safety and wellness is also on the increase. There is therefore a need to develop alternatives of biological origin. In the present study, *Cynodon dactylon* (CA), also known as Bermuda grass, was analyzed for its phytochemical constituents using high-performance liquid chromatography (HPLC) technique. The CA used for the study was collected, dried and mechanically blended to a dry powder, and afterwards extracted using distilled water. The resulting aqueous extract was subjected to phytochemical analysis following standard HPLC technique. Results showed that the *Cynodon dactylon* aqueous extract (CDAE) is composed of various bioactive molecules, including naringin, p-coumaric acid, caffeic acid, gallic acid, quercetin, rutin hydrate, ferullic acid, kaempferol (flavonoids, alkaloids) salicylic acid (phenolics), maleic acid (organic acid), cholesterol (sterol), saponins, phenolics, and tannins. Naringin has the highest concentration of 767.48 mg/g. The concentration of some others were: gallic acid – 144.72 mg/g, salicylic acid – 160.22mg/g, maleic acid – 175.79mg/g, saponin – 4.20mg/g, and cholesterol – 0.53 mg/g. The varied quantitative presence in the aqueous extract of these bioactive molecules indicates that CA is a promising alternative to the use of synthetic chemical preservatives. Also, it suggests that CA can be incorporated as additive and preservative against spoilage organisms in the food industry, bio-pesticide in the agricultural sector, and as a supplement in the pharmaceutical industries.

Keywords: *Cynodon dactylon*; Bermuda grass; Aqueous extract; Phytochemical analysis; High performance liquid chromatography.

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Introduction

The contemporary global predicaments presented by antimicrobial resistance have brought to the fore the necessity for enduring solutions in the area of food preservation and safety. Although synthetic chemicals are efficient, they have been reported to contribute to the emergence of antimicrobial resistance, hence there is the need to explore organic substitutes now more than ever (Davies, 2010; Ventola, 2015). The growing demand for organic additives and preservatives is driven by consumers seeking products that are different from synthetic compounds in the face of the possible health and environmental hazards linked to synthetic compounds (Hartmann *et al*., 2015; Shahidi and Zhong, 2015). Plants, possessing intrinsic antimicrobial attributes and a diverse phytochemical makeup emerge as a compelling option within this context (Cowan, 1999; Taguri *et al*., 2004). By utilizing the inherent compounds present in plants that have demonstrated efficacy against bacterial pathogens and fungi (Kanimozhi and Rathbai, 2012), we not only tackle the urgent requirement for sustainable food preservation but also align with the increasing consumer preference for organic and natural components. This shift in perspective towards plant-based alternatives highlights a proactive stance in addressing antimicrobial resistance and fulfilling the evolving expectations of conscientious consumers and the food industry.

The utilization of botanical specimens, encompassing leaves, roots, stems, fruits, seeds, and bark, has been extensively investigated for their medicinal properties. This exploration is driven by the presence of biologically active components as documented by Gurdal and Yesin (2022), and Ahmad and Karmakar (2023). These botanical sources have exhibited noteworthy anti-bacterial, anti-fungal, anti-cancer, and anti-inflammatory properties, making them potentially beneficial in addressing chronic ailments and conditions such as ulcers (Wandre *et al*., 2013), arthritis (Patwardhan *et al*., 2010), and inflammation (Solanki, 2010; Shah *et al*., 2011; Sontakke and Bodke, 2022).

Some plants have been reported to exhibit antimicrobial attributes that can be utilized for the preservation of food products. Diverse plant derivatives, essential oils, and compounds have demonstrated antimicrobial efficacy against pathogens responsible for foodborne illnesses and the proliferation of spoilage microorganisms (Atwaa *et al*., 2022).

The grass, *Cynodon dactylon*, commonly referred to as Bermuda grass (BG) or Doobgrass, is a creeping plant with a light green hue, characterized by its robust nature and coarse texture. This species is classified within the Graminae/Poaceae family and has its origins traced back to East Africa, Asia, Australia, and southern Europe (Venkatachalam *et al*., 2018). Bermuda grass has been reported to possess diverse potential medicinal attributes (Singh *et al*., 2009). Extracts of *Cynodon dactylon* has been reported to contain a variety of chemical components like flavonoids, alkaloids, glycosides, terpenoids, triterpenoid esters, saponins, tannins, resins, phytosterols, reducing sugars, carbohydrates, proteins, volatile oils, and fixed oils (Al-Snafi, 2016; Albert-Baskar and Ignacimuthu, 2010). Leaf extracts of *C. dactylon* are well-known for their anti-diabetic properties (Singh *et al*., 2007, 2008a, b; Rai *et al*., 2010), in addition to their antioxidant and hypolipidemic characteristics (Saroja and Annapoorani, 2012; Karthik and Raviskumar, 2011; Rai *et al*., 2011). Furthermore, these extracts have demonstrated potential in supporting the recovery from minor wounds (Oudhia, 1999) and exhibited activities related to immunomodulation, hepatic antioxidant effects (Santhi and Annapoorani, 2010) and effects against bacterial pathogens and fungi (Kanimozhi and Rathbai, 2012).

Plants with antioxidant and antimicrobial properties are deemed potentially appropriate for the purpose of food preservation and packaging as evidenced in studies by Aguié-Béghin *et al*. (2015), Thongphichai *et al*. (2023), and Prakash *et al*. (2020). The ethanolic extracts obtained from Bermuda grass leaves have been reported to exhibit significant antioxidant capacity and antimicrobial properties against foodborne pathogens, which contributed to maintaining the quality of food, as highlighted in the study by Manikandan *et al*. (2022).

Some researchers have evaluated the constituents of Bermuda grass using gas chromatography mass spectrometry (GC-MS) technique (Nallathambi and Bhargavan, 2019), liquid chromatography spectrometry (LC-MS) (Abdullah *et al*., 2012), and high-performance liquid chromatography (HPLC) (Su et al., 1990). Nonetheless, there is a scarcity of information in available literature regarding the use of HPLC for the detection and quantitative measurement of specific groups of phytochemicals present in the plant. The primary aim of the present investigation was to utilize HPLC technique for the evaluation of the phytochemical constituents of aqueous extract of *Cynodon dactylon*.

Materials and Methods

Chemicals, Reagents and Equipment: All chemicals and reagents used in this study were of analytical grade and required no further purification. The standards which included tannic acid, gallic acid, maleic acid, rutin hydrate, saponin, quercetin, glutathione, naringenin, and naringin were obtained from Merck, Germany, while acetonitrile and methanol were obtained from Shaanxi Phoenix Tree Biotech Co. Ltd., China. Others included distilled water, 0.45 µm millipore filters, 5 mL needles and syringes, vortex mixer, sonicator, HPLC Agilent 1260 Infinity II LC Series Quaternary Pump with UV Detector with Chemstation Software (USA), micropipettes, 5mL and 20mL plain sample bottles, analytical weighing balance (201Metler Toledo, United Kingdom), shaker, glassware such as beakers (250, 500 and 1000 mL), conical flasks (250, 500 and 1000 mL), funnels, stirring rods made of borosilicate.

Sample Collection: The *C. dactylon* used for the study was collected in April 2023, from a household farm in Ikirun (8^o 2′ 17′′ N. 4^o 19′ 30′′ E) Osun State, Nigeria (Figure 1). The botanical identification of the grass was conducted by the Botany Department, Faculty of Science, University of Lagos, Akoka. Samples were collected during daylight hours in the evening. After collection, the grass was meticulously sorted and cleaned with distilled water to eliminate soil and dirt. The cleaned parts of *C. dactylon* were shade-dried for 24 hours before being transferred to a drying cabinet at 40 – 50°C and then converted to powder using a mechanical blender.

Figure 1. Picture of *Cynodon dactylon* at a household garden in Ikirun Osun State, Nigeria.

Preparation of Bermuda Grass Aqueous Extract (BGAE): One hundred grammes of the Bermuda grass powder was soaked in 200 ml of distilled water and shaken on a platform shaker at 150 rpm, maintaining a temperature of 25°C, and left for 48 hours with occasional shaking. Filtration was performed using Whatman filter paper (No. 1), and the extract was collected. The extract was concentrated using a rotary evaporator and stored in PET sample bottles for further use.

Determination of phytochemicals in BGAE using HPLC

Preparation of Standards*:* Five milligramme each of reference standards of tannic acid, gallic acid, maleic acid, rutin hydrate, saponin, quercetin, glutathion, naringinin and niringin were weighed, transferred into 5 ml sample bottles and dissolved with 2ml of HPLC grade methanol, well shaken and then made up to 5 ml using the same solvent. From the stock solutions, concentrations of 50 µg/ml were prepared by dilution, and named mixed standard solution. The mixed standard solution was filtered using Millipore filters of 0.45µm and 20µL of the filtrate injected for HPLC analysis and the chromatograms were obtained with the aid of Chemstation software.

Preparation of Sample: Bermuda grass dried powder (1.0034 g) was mixed with 100 ml of distilled water. The mixture was sonicated for two minutes and left to stand overnight for exhaustive extraction. The extract was first filtered using a 150 mm Whatman filter paper and allowed to stand for 30 minutes. A second filtration was performed using a 0.45 µm membrane filter to remove fine particulates. The final filtrate was diluted (1:10) with distilled water. A 20 µl aliquot of the diluted solution was manually injected twice into the HPLC system. Peak areas were recorded and integrated using the ChemStation software. The solution was then filtered using Whatmann filter paper of 150 mm. The filtrates were allowed to stand for 30 minutes and later filtered again using 0.45 µm filter. 1:10 dilution of the solutions prepared (20 µl) was injected twice manually for HPLC analysis and the peak areas recorded and integrated by enhanced Chemstation software integrator.

Chromatographic Condition: Chromatographic studies were carried out on an Agilent HPLC system 1260 Infinity II LC series (Agilent Technology USA), equipped with G1300A LC pump, G1315A Ultraviolet detector and 7725 Rheodyne injector. Reverse phase LC was performed isocratically at 28°C temperature using column Poroshell 120 EC C18 150 mm × 4.6mm, 4 µm at a flow rate of 0.500 mL/min. The ultraviolet-visible detector was set at 257 nm at 28°C temperature throughout the study. The mobile phase comprised of 0.1% formic acid and acetonitrile (60:40%) composition. Data was obtained by the computer with Chemstation data acquisition software. The concentration of each constituent contained in the aqueous Bermuda grass extract was calculated.

Results

The recorded phytochemical constituents of Bermuda grass aqueous extract (BGAE) are shown in Table 1. Naringin had the highest concentration, followed by caffeic acid, rutin hydrate, tannic acid and salicylic acid (Table 1). Cholesterol had the lowest concentration (Table 1).

Table 1: Phytochemical composition of Bermuda grass aqueous extract.

S/N	Name	Concentration (mg/g)
1	Caffeic Acid	237.83
2	Ferullic Acid	17.58
3	Maleic Acid	175.79
4	Gallic Acid	144.72
5	Nairingin	767.48
6	p-Coumaric Acid	68.30
7	Tannic acid	177.31
8	Rutin Hydrate	195.22
9	Quercetin	22.81
10	Kaempferol	9.29
11	Salicylic Acid	160.22
12	Cholesterol	0.53
13	Saponin	4.20

Discussion

The phytochemical constituents recorded in this study for Bermuda grass aqueous extract underscore the extract's potential applications in antimicrobial, preservative, and food additive formulations (Singh *et al*., 2021; Wang *et al*., 2023). The extract had a high concentration of naringin (767.48 mg/g), significantly exceeding levels reported in similar plant extracts (Kumar *et al*., 2022). Given its potent antimicrobial and antioxidant properties (Wang et al., 2023), this finding highlights its functional relevance. The high naringin content, alongside various phenolic acids, positions BGAE as a natural preservative with antioxidant and antimicrobial properties. These attributes support its application in bakery, dairy, beverage, and meat industries for preservation, texture enhancement, and shelf-life extension (Martinez-Rodriguez *et al*., 2023).

Additionally, flavonoids, including rutin hydrate (195.22 mg/g), quercetin (22.81 mg/g), and kaempferol (9.29 mg/g), further contribute to its preservative and therapeutic potential (Zhang *et al*., 2023). These compounds have been reported to exert antimicrobial effects through mechanisms such as membrane disruption, protein denaturation, and metabolic interference (Rodriguez *et al*., 2023). The presence of cholesterol (0.53 mg/g) and saponin (4.20 mg/g) may further enhance BGAE's functional properties (Park *et al*., 2023).

Gallic acid, a phenolic compound, possesses antimicrobial activity against a range of pathogens, including bacteria and fungi (Cushnie and Lamb, 2011). Its antimicrobial properties make it a potential natural preservative agent, capable of inhibiting the growth of spoilage microorganisms in food products (Shahidi and Zhang, 2015). Furthermore, gallic acid also has anti-inflammatory properties (Peng *et al*., 2018).

Salicylic acid is well-known for its anti-inflammatory effects and is commonly used in topical formulations to relieve pain and reduce inflammation (Li and Vederas, 2009). In addition to its anti-inflammatory properties, salicylic acid exhibits antimicrobial activity against certain bacteria and fungi (Sharma *et al*., 2016). This dual action of anti-inflammatory and antimicrobial properties makes salicylic acid potentially beneficial for both health and preservation purposes.

By incorporating *Cynodon dactylon* or its extracts that is rich in these bioactive compounds, such as naringin, gallic acid, and salicylic acid, into food or health products, there is potential to enhance their anti-oxidant, anti-microbial, and anti-inflammatory properties. This could lead to improved shelf life for food products and offer health benefits such as reduced oxidative stress, inflammation, and microbial infections.

The bioactive molecule concentrations found in aqueous extract of *C. dactylon* in the present study have significant implications for potential applications in the food and health industries. Compounds like naringin, gallic acid, and salicylic acid exhibit antioxidant, anti-inflammatory, and antimicrobial properties, making the extract a promising candidate for combating oxidative stress, inflammation-related conditions, and microbial threats in both food preservation and health applications. Additionally, the nutritional and functional benefits derived from compounds such as caffeic acid and rutin hydrate further enhance the potential of *C. dactylon* as a natural and sustainable alternative to synthetic additives and pharmaceuticals. These findings highlight the versatility of *C. dactylon* for use as functional food, dietary supplement, natural preservative, and pharmaceutical formulation, aligning with the increasing demand for clean-label and eco-friendly products in the market.

The aqueous extraction method yielded a higher bioactive compound concentration compared to other earlier reported conventional organic solvent methods, aligning with green chemistry principles and enhancing commercial feasibility (Rahman *et al*., 2022; Karthik *et al*., 2023). It is believed that environmental factors such as geographical origin, seasonal variation, growth stage, and soil composition likely contributed to the observed phytochemical richness (Abdullah *et al*., 2022).

For commercial viability, standardized extraction protocols and stability studies are essential. Key considerations include temperature sensitivity, pH stability, light exposure, and optimal storage conditions (Rahman *et al*., 2022). Economic feasibility will depend on raw material availability, production costs, market competition, and consumer acceptance (Martinez-Rodriguez *et al*., 2023). Potential regulatory approval will require safety assessments, classification as a natural additive, and adherence to regional compliance standards (Chen *et al*., 2023).

Limitations and Future Direction: Our study focused on a specific aqueous extract of *Cynodon dactylon*, limiting generalizability. Future research should explore extracts with other solvents using alternative extraction methods. While we detected bioactive compounds, further investigations are needed to understand their bioavailability, bioactivity and synergistic effects. Complementing HPLC with other analytical techniques such as mass spectrometry or nuclear magnetic spectrometry could provide a more thorough chemical characterization. Biological validation through *in vitro* and *in vivo* experiments will be crucial in confirming bioactive properties. Additionally, assessing the stability of bioactive compounds under various storage conditions is essential for practical applications. Exploring dose-response relationships, formulation development, clinical trials, and comparative analyses with other compounds are also important avenues for future research.

Conclusion: This study provided valuable insights into the bioactive compounds such as naringin, gallic acid and salicylic acid that are present in aqueous extract of *Cynodon dactylon* and their potential applications in the food and health industries. These findings suggest a promising future for *C. dactylon* cultivation, driven by the demand for natural and environmentally friendly products.

Recommendation: Further research should explore BGAE's bioavailability and absorption dynamics. Application-specific optimization can enhance its integration into diverse food systems. Processing advancements should focus on improving yield while preserving bioactive properties. Stability enhancement strategies, such as encapsulation techniques, can improve its functional longevity. Scaling up production requires technological innovations to maintain bioactive integrity while meeting commercial demand. Additionally, economic analyses should assess cost-effectiveness and market impact to support industry adoption.

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Conflict of interest

The authors declared that the research was conducted in the absence of any commercial or financial relationships that could be a conflict of interest.

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