Vol. 20 (Supplementry Issue 1A), March 2026, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2026.1s.15

Performance Evaluation of *Paspalum dilatatum* sp. leaf extracts from various solvents against the Selected Pathogenic Strains

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Abstract

The perennial species of grass *Paspalum dilatatum* is widely distributed and thrives in warm temperate to subtropical climates. Indigenous people have long utilized it for therapeutic purposes, demonstrating its significance in traditional medical practices. Its extensive root system improves erosion control, nutrient cycling, and soil aeration on slopes and disturbed areas. This shared knowledge has led to the increased application of *Paspalum dilatatum* in the treatment of various diseases and health issues, highlighting its importance in modern research settings. Empirical research and cross-cultural exchanges have helped to expand the knowledge of the bioactive compounds found in plant leaves and roots over time. The present work aims to separate and identify the bioactive ingredients found in various extraction with chloroform, acetone, ethanol, and petroleum ether of *Paspalum dilatatum* leaves. The resultant extracts were examined using Gas Chromatography Mass Spectrometry (GC-MS). The antibacterial activity revealed that all distinct concentration of extracts has strong inhibitory effect on bacterial growth. Further, insights from this research insists to perform therapeutic action and good candidate for breakthrough in medical field.

Keywords: *Paspalum dilatatum*, various extract, GC-MS, antibacterial activity, Therapeutical

Introduction

Paspalum dilatatum, commonly known as dallisgrass or paspalum, is a versatile perennial grass species in the Poaceae family. Native to South America, it has become naturalized across North America, Europe, Africa, and Oceania. Thriving in warm temperate to subtropical climates, dallisgrass prefers moist, fertile soils and is frequently found in pastures, lawns, roadsides, and other disturbed areas [1]. It forms dense clumps of broad, elongated blades thanks to its vigorous growth habit [2], which make it both an excellent forage—highly palatable and nutritious for cattle, horses, and other livestock and an effective means of soil stabilization. Its deep root system enhances soil aeration, nutrient cycling, and erosion control on slopes and disturbed sites [3, 4]. Dallisgrass also supports biodiversity by providing habitat and food for insects, birds, and other wildlife [5]. *Paspalum dilatatum* is nevertheless a flexible plant with a wide range of uses, such as feed production, decorative landscaping, environmental preservation, and soil stabilization, in spite of these disadvantages. Beyond agricultural uses, dallisgrass is cultivated ornamentally for its attractive foliage and low maintenance requirements[6], though it can become invasive, outcompeting native plants in managed landscapes. Its adaptability underpins applications in environmental conservation and landscape design, even as weed management strategies are sometimes necessary [7, 8]. A systematic review of 190 publications from the ScienceDirect database identified this plant's novel phytochemical profile, marking the first comprehensive report of its bioactive constituents in the literature [9, 10]. This study aims to isolate and evaluate the

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bioactive phytochemicals in *Paspalum dilatatum* and assess their efficacy against various pathogenic species.

Materials and Methods

Plant material

The fresh leaves of *Paspalum dilatatum* were collected from the Saveetha School of Engineering (SSE), Chennai in the month of November 2023. In order to get rid of the dirt and waste that has been deposited on the surface, the newly picked plant leaves are rinsed under running water. The extraction procedure with various solvents such as acetone, petroleum ether, ethanol and chloroform were performed at SSE laboratory. The resulting extract underwent analysis using Gas Chromatography Mass Spectrometry (GC-MS) at Neo Science Labs Private Limited. This laboratory, accredited by NABL, specializes in food and pharmaceutical testing.

Preparation of various extract

The plant leaves are allowed to air out for a week beneath the shade. The dried leaves were then thoroughly crushed into a fine powder, placed in an airtight container, and preserved in the freezer for later use. The corresponding sample was subjected to a cold maceration technique in order to obtain the crude extract. Glassware and equipment used in laboratories were sterilized before use. The experiment was conducted without ethical approval, and data were gathered and examined in triplicate.

For the extraction process, 20 grams of powdered plant material was combined separately with 200 milliliters of MERCK research-grade petroleum ether, ethanol, acetone and chloroform solventsand left for seven days. A Buchner funnel with Whatman and regular filter paper was used to filter the resultant combinations. The crude samples were heated in a hot air oven for thirty minutes in order to eliminate any remaining solvent. Then, the unrefined extracts were put in foil-sealed jars and left to rest at room temperature for further analysis.

GC-MS Analysis

The Shimadzu GC QP 2010 Gas Chromatography Mass Spectrometer was used, with an HP5MS column length. The carrier gas was selected to be noble gas helium, with a flow rate of 1 mL per minute and an oven temperature gradient of 5 °C per minute at 250 °C. For identifying the corresponding molecules in each crude extract, the GC-MS spectra were juxtaposed to the NIST library.

Antibacterial Activity Assay

The antibacterial qualities were assessed using the disc diffusion method. Wells were cut under aseptic conditions on a previously sterilized nutrient agar plate after that carried set. The Saveetha University Laboratory provided all of the clinically isolated bacterial species, including gram-positive *Klebsiella pneumonia* and gram-negative *Salmonella typhi*. A reference was offered via commercial antibiotic discs containing 10 µg of ampicillin. After loading sterile filter-paper discs with crude extract at 25, 50, 75, and 100 µg per disc, the discs were put onto the inoculated agar and incubated for 24 hours at 37 °C. A summary of the measured inhibitory zones is provided.

Results

GC-MS Evaluation

Figure 1 (a-d) presents the GC-MS spectra of various compounds detected in the ethanol leaf extract of *Paspalum dilatatum*, and Tables 1-4 list the identified phytochemicals based on these spectra. Using reverse search indexing (RSI) against the NIST library, compounds with a similarity score of 900 or above were considered highly reliable matches, while those scoring between 800 and 900 were accepted as reasonably accurate identifications. In total, 24 peaks for chloroform and ethanolic extract, 29 peaks in acetone and petroleum ether were analyzed by associating their mass spectral shattering patterns with known reference compounds in the NIST database, which enabled the identification of several

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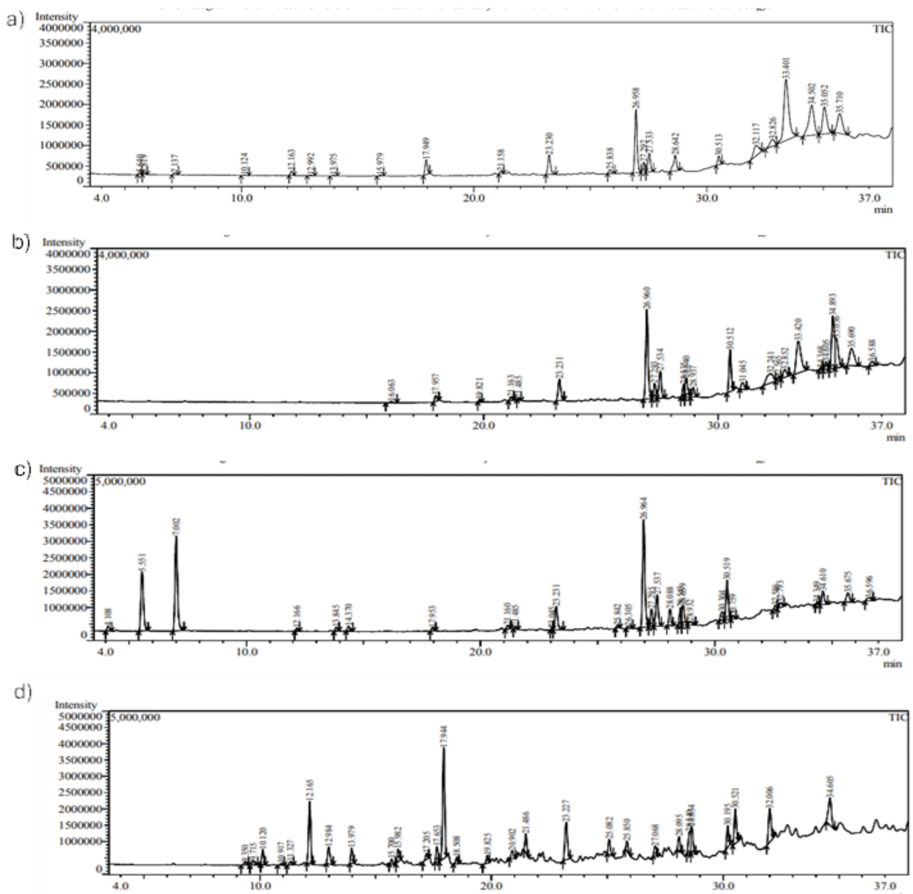

Fig 1: GC-MS spectra indicates a) Chloroform b) Ethanol c) Acetone d) Petroleum ether

bioactive constituents in the extract. This GC-MS analysis confirmed the presence of multiple phytochemicals, likely including alkaloids, flavonoids, phenolics, and terpenoids, in the *Paspalum dilatatum* leaf ethanol extract, highlighting its potential as a source of biologically active compounds. The presence of 9, Octadecenoic acid, hexadecenoic acid-methylester, hexa decanoic acid and 2-Pentanone, 4-hydroxy-4-methyl- are responsible for germination potential of ethanolic extract paspalum species reported in previously studied article [11], which are high bioactive compound for diet fibre. The phenolic compounds are involved in multiple health benefit.

Antibacterial activity

The disc diffusion method evaluation of antibacterial efficacy, *Klebsiella pneumoniae* exhibited the biggest zone of inhibition, measuring 19 mm for almost all crude extract at 100 mg/mL (Fig. 2), while *Salmonella typhi* showed zones of inhibition of 18 and 17 mm for chloroform and petroleum ether respectively, at 100 mg/mL. All the other concentration revealed minimal zone of inhibition.

The ethanol and acetone extract against *Salmonella typhi* showed a minimal activity in all the concentrations, whereas chloroform and acetone extract had highest zone of inhibition at 100 mg/mL (Fig. 3). This

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Table 1: List of compounds present in the chloroform based crude extract was determined using GC-MS analysis

Retention Time (min)	Identified Compound	Peak Area (%)	Molecular Formula	Mol. Wt (g/mol)	Compound Class
5.64	3-Penten-2-one, 4-methyl-	0.24	$C_6H_{10}O$	98	Ketone
5.82	2-Hexanol, (R)-	0.24	$C_6H_{14}O$	102	Alcohol
7.14	2-Pentanone, 4-hydroxy-4-methyl-	0.48	$C_6H_{12}O_2$	116	Hydroxyketone
10.12	Bicyclo[2.2.1] heptane, 2,2-dimethyl-3-methylene-, (1S)	0.10	$C_{10}H_{16}$	136	Bicyclic hydrocarbon
12.16	Benzene, 1-methyl-3-(1-methylethyl)-	0.76	$C_{10}H_{14}$	134	Aromatic hydrocarbon
12.99	γ-Terpinene	0.18	$C_{10}H_{16}$	136	Monoterpene
13.98	Linalool	0.14	$C_{10}H_{18}O$	154	Monoterpene alcohol
15.98	L-α-Terpineol	0.16	$C_{10}H_{18}O$	154	Monoterpene alcohol
17.95	Thymol	2.90	$C_{10}H_{14}O$	150	Phenolic terpene
21.16	1-Dodecanol	0.54	$C_{12}H_{26}O$	186	Long-chain alcohol
23.23	Diethyl phthalate	4.36	$C_{12}H_{14}O_4$	222	Phthalate ester
25.84	Tetradecanoic acid (Myristic acid)	0.84	$C_{12}H_{28}O_2$	228	Fatty acid
26.96	Neophytadiene	12.29	$C_{20}H_{38}$	278	Diterpene
28.64	Dibutyl phthalate	4.11	$C_{16}H_{22}O_4$	278	Phthalate ester
30.51	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1.67	$C_{20}H_{40}O$	296	Diterpene alcohol
32.12	Campesterol, methyl ether	4.58	$C_{29}H_{50}O$	414	Steroidal derivative
32.83	Tetracosanol	2.73	$C_{24}H_{48}O$	352	Long-chain alcohol
33.40	β-Sitosterol acetate	25.69	$C_{31}H_{50}O_2$	454	Sterol derivative
34.50	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	13.82	$C_{42}H_{63}O_3P$	646	Antioxidant/phosphite
35.05	2-Methylhexacosane	9.77	$C_{27}H_{56}$	380	Alkane

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Table 2: List of compounds present in the ethanol based crude extract was determined using GC-MS analysis

Retention Time (min)	Identified Compound	Peak Area (%)	Molecular Formula	Mol. Wt (g/mol)	Compound Class
16.06	Dodecane	0.32	$C_{12}H_{26}$	170	Alkane
17.96	Thymol	1.10	$C_{10}H_{14}O$	150	Phenolic terpene
19.82	Tetradecane	0.30	$C_{14}H_{3}0$	198	Alkane
21.16	Decyl trifluoroacetate	0.67	$C_{12}H_{21}F_3O_2$	254	Ester
21.49	Eicosyl isopropyl ether	0.31	$C_{23}H_{48}O$	340	Ether
23.23	Diethyl phthalate	4.54	$C_{12}H_{14}O_4$	222	Phthalate ester
26.96	Neophytadiene	17.35	$C_{20}H_{38}$	278	Diterpene
27.29	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-	2.79	$C_{20}H_{40}O$	296	Diterpene alcohol
27.53	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	5.56	$C_{22}H_{42}O_2$	338	Diterpene alcohol
28.54	Pentadecanoic acid	2.08	$C_{15}H_{30}O_2$	242	Fatty acid
28.64	Dibutyl phthalate	4.25	$C_{16}H_{22}O_4$	278	Phthalate ester
28.94	Hexadecanoic acid, ethyl ester	2.04	$C_{18}H_{36}O_2$	284	Fatty acid ester
30.51	Neophytadiene	7.58	$C_{20}H_{38}$	278	Diterpene
31.05	9-Octadecenoic acid, 1,2,3-propanetriyl ester-	1.69	$C_{57}H_{104}O_6$	884	Triglyceride
32.24	(Steroidal derivative, methoxy-substituted) *	5.25	$C_{29}H_{50}O$	414	Steroidal compound
32.57	Hexacosanol	0.67	$C_{26}H_{52}O$	380	Long-chain alcohol
32.85	Triacontanol	2.38	$C_{30}H_{60}O$	436	Long-chain alcohol
33.42	Stigmasta-5,22-dien-3-ol acetate (β-Sitosterol acetate)	12.10	$C_{31}H_{50}O_2$	454	Sterol derivative
34.37	Eicosanal	0.30	$C_{20}H_{40}O$	296	Aldehyde
34.61	1,2-Dihydropyridine, 1-(1-oxobutyl)-	1.54	$C_9H_{13}NO$	151	Heterocyclic compound
34.89	Prasterone (Dehydroepiandrosterone, DHEA)	12.17	$C_{19}H_{28}O_2$	288	Steroid hormone
35.04	Tetratriacontane	6.99	$C_{34}H_{70}$	478	Alkane
35.69	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	6.86	$C_{33}H_{54}O_3$	498	Steroidal ester
36.59	Cholesterol 3β-O-[2-chloroethyl] ether	1.16	$C_{29}H_{49}C_{IO}$	448	Sterol derivative

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Table 3: List of compounds present in the acetone based crude extract was determined using GC-MS analysis

Retention Time (min)	Identified Compound	Peak Area (%)	Molecular Formula	Mol. Wt (g/mol)	Compound Class
4.11	4-Penten-2-one, 4-methyl-	0.88	$C_6H_{10}O$	98	Ketone
5.55	3-Penten-2-one, 4-methyl-	11.90	$C_6H_{10}O$	98	Ketone
7.00	2-Pentanone, 4-hydroxy-4-methyl-	18.72	$C_6H_{12}O_2$	116	Hydroxyketone
12.17	Benzene, 1-methyl-3-(1-methylethyl)-	0.48	$C_{10}H_{14}$	134	Aromatic hydrocarbon
13.85	3-Methyl-1,4,6,7-tetrahydro-pyrazolo [3,4-c] pyridin-5-one	0.66	$C_7H_9N_3O$	151	Heterocyclic compound
14.37	4-Piperidone, 2,2,6,6-tetramethyl-	0.89	$C_9H_{17}NO$	155	Lactam
17.95	Thymol	0.60	$C_{10}H_{14}O$	150	Phenolic terpene
21.16	Decyl trifluoroacetate	0.72	$C_{12}H_{21}F_3O_2$	254	Ester
21.49	Eicosyl isopropyl ether	0.43	$C_{23}H_{48}O$	340	Ether
23.05	3-Eicosene, (E)-	0.23	$C_{20}H_{40}$	280	Alkene
23.23	Diethyl phthalate	5.04	$C_{12}H_{14}O_4$	222	Phthalate ester
25.84	Tetradecanoic acid (Myristic acid)	0.40	$C_{14}H_{28}O_2$	228	Fatty acid
26.31	n-Nonadecanol-1	0.68	$C_{19}H_{40}O$	284	Long-chain alcohol
26.96	Neophytadiene	19.84	$C_{20}H_{38}$	278	Diterpene
28.09	Hexadecanoic acid, methyl ester	2.89	$C_{17}H_{34}O_2$	270	Fatty acid ester
28.54	Pentadecanoic acid	2.82	$C_{17}H_{34}O_2$	270	Fatty acid
28.64	Dibutyl phthalate	4.13	$C_{16}H_{22}O_4$	278	Phthalate ester
28.93	Tetradecyl trifluoroacetate	1.12	$C1_6H_{29}F_3O_2$	310	Ester
30.30	Linoleic acid methyl ester	1.89	$C_{19}H_{34}O_2$	294	Fatty acid ester
30.52	Neophytadiene	7.40	$C_{20}H_{38}$	278	Diterpene
30.76	Heptacos-1-ene	0.75	$C_{27}H_{54}$	378	Alkene
32.59	14-Methyl-14-(3-oxobutylroxy)-hexadec-15-enoic acid, methyl ester	0.72	$C_{22}H_{38}O_5$	382	Fatty acid ester
32.79	Hexacontane	1.24	$C_{60}H_{122}$	842	Alkane

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34.35	2-Bromopropionic acid, pentadecyl ester	0.23	$C_{18}H_{35}BrO_2$	362	Brominated ester
34.61	1,2-Dihydropyridine, 1-(1-oxobutyl)-	2.73	$C_9H_{13}NO$	151	Heterocyclic compound
35.68	Tetratetracontane	2.21	$C_{44}H_90$	618	Alkane
36.60	Cholesta-3,5-diene	1.03	$C_{27}H_{44}$	368	Sterol derivative

Table 4: List of compounds present in the petroleum ether based crude extract was determined using GC-MS analysis

Retention Time (min)	Identified Compound	Peak Area (%)	Molecular Formula	Mol. Wt (g/mol)	Compound Class
9.35	Camphene	0.47	$C_{10}H_{16}$	136	Monoterpene hydrocarbon
9.72	α-Pinene	0.80	$C_{10}H_{16}$	136	Monoterpene hydrocarbon
10.12	Sabinene	2.38	$C_{10}H_{16}$	136	Monoterpene hydrocarbon
10.92	Octane, 2,3,3-trimethyl-	0.32	$C_{11}H_{24}$	156	Alkane
11.33	β-Myrcene	0.57	$C_{10}H_{16}$	136	Monoterpene hydrocarbon
12.17	m-Cymene	10.58	$C_{10}H_{14}$	134	Aromatic hydrocarbon
12.98	Tetradecane, 1-chloro-	3.37	$C_{14}H_{29}Cl$	232	Chlorinated alkane
13.98	Linalool	2.44	$C_{10}H_{18}O$	154	Monoterpene alcohol
15.70	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	0.70	$C_{10}H_{18}O$	154	Monoterpene alcohol
15.98	L-α-Terpineol	2.03	$C_{10}H_{18}O$	154	Monoterpene alcohol
17.21	Benzene, 1,3-bis(1,1-dimethylethyl)-	0.93	$C_{14}H_{22}$	190	Aromatic hydrocarbon
17.65	Dodecane, 4,6-dimethyl-	2.30	$C_{14}H_{30}$	198	Alkane
17.94	Thymol	18.72	$C_{10}H_{14}O$	150	Phenolic terpene
18.51	Nonane, 5-(2-methylpropyl)-	1.09	$C_{13}H_{28}$	184	Alkane
19.83	Tetradecane	1.24	$C_{15}H_{32}$	212	Alkane
20.90	2,6,10-Trimethyltridecane	1.45	$C_{16}H_{34}$	226	Branched alkane
21.49	Eicosane	2.86	$C_{20}H_{42}$	282	Alkane
23.23	Diethyl phthalate	7.59	$C_{12}H_{14}O_4$	222	Phthalate ester
25.08	Eicosane	2.61	$C_{20}H_{42}$	282	Alkane
25.85	Tetradecanoic acid (Myristic acid)	1.45	$C_{14}H_{28}O_2$	228	Fatty acid

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27.07	2-Pentadecanone, 6,10,14-trimethyl-	1.72	$C_{18}H_{36}O$	268	Ketone
28.10	Eicosane	2.35	$C_{20}H_{42}$	282	Alkane
28.54	Pentadecanoic acid	4.25	$C_{15}H_{30}O_2$	242	Fatty acid
28.63	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	4.35	$C_{16}H_{22}O_4$	278	Phthalate ester
30.20	Hexadecanoic acid, propyl ester	3.49	$C_{19}H_{38}O_2$	298	Fatty acid ester
30.52	Neophytadiene	6.08	$C_{20}H_{38}$	278	Diterpene
32.01	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	7.05	$C_{19}H_{34}O_2$	294	Unsaturated diol
34.61	1,2-Dihydropyridine, 1-(1-oxobutyl)-	6.69	$C_9H_{13}NO$	151	Heterocyclic compound

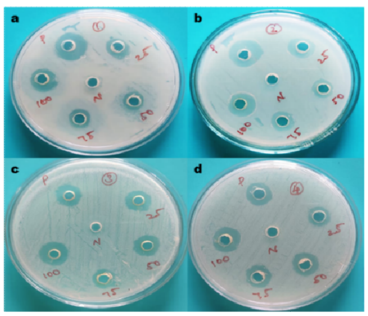

Fig 2: Antibacterial effect against *Klebsiella pneumonia* of *P. dilatatum* (a) Chloroform (b) ethanol, (c) Acetone and (d) petroleum ether extracts. P and N depicts the antibiotic and neutral wells.

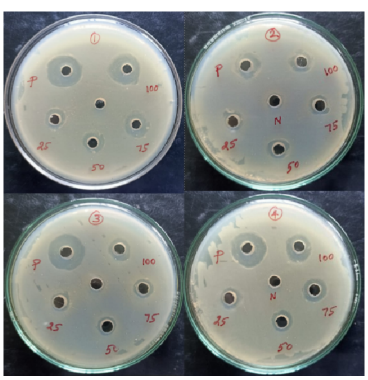

Fig 3: Antibacterial effect against *Salmonella typhi.* (a) Chloroform (b) ethanol, (c) Acetone and (d) petroleum ether extracts. P and N depicts the antibiotic and neutral wells.

effect shows the potential of p. dilatatum as antibacterial efficacy and future scope in pharmaceutical behaviour in various health related issues.

Discussion

Paspalum dilatatum, known as Dallis grass, is spread over the world's tropical, subtropical, and temperate climates. It is a strong, tenacious, and very tasty species that withstands intensive grazing. [12]. Studies have investigated the antifungal proteins (such as glucanases and chitinases) introduced into dallisgrass to confer resistance against *Claviceps paspali* (ergot fungus). These efforts focus on improving disease resistance through transgenic approaches rather than identifying natural antimicrobial compounds produced by dallisgrass itself [13]. While other grasses in the Paspalum genus (e.g., *Paspalum conjugatum*, or carabao grass) have demonstrated notable antimicrobial activity, especially against *Staphylococcus aureus*, current literature does not report similar antibacterial or broad-spectrum antimicrobial properties for *Paspalum dilatatum* [14]. The study explores the anti-inflammatory properties of *Paspalum thunbergii* extract using LC-MS, researchers identified 15 chemical constituents, including rosmarinic acid and isoquercitrin, exhibited strong antioxidant activity in DPPH and ABTS assays [15]. Similarly, Afrose et al., 2024 conducted experiment with *Paspalum conjugatum* leaves were sequentially extracted using various

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solvents, revealing alkaloids, tannins, flavonoids, and glycosides in n-hexane and ethanol extracts. Proximate analysis and antioxidant tests showed notable bioactivity, with the ethanol extract displaying the highest antioxidant potential due to its rich phenolic and flavonoid content, supporting its medicinal value[16]. The copper oxide nanoparticles (CuONPs) green synthesis using *Paspalum scrobiculatum* ethanolic extract showed antibacterial activity against *Pseudomonas aeruginosa* but not *Proteus vulgaris*, highlighting their potential as eco-friendly antibacterial agents [17]. Another study highlights the allelopathic impact of the exotic grass *Paspalum dilatatum* on native plant species in Egypt's urban garden represents the reduced native plant diversity, coverage, and biomass. The findings suggest allelopathy as a key factor in *P. dilatatum*'s negative effect on native urban flora [18]. The presence of variety of phenolic compounds, terpenoids and alkaloid metabolites in GC-MS analysis, the grass can be used in antioxidants defense action and bioremediation. The bahia grass Paspalum notatum resulted in antifungal approach and as well withstand the cadmium stress soil [19]. There is no detailed report till date on *P. dilatatum* against many microbial. The current study provided with evidence of antibacterial activity against gram positive and negative bacterium class. Though, there is need for more research in association with compounds responsible in this concern of pharmacology sector.

Conclusion

In conclusion, *Paspalum dilatatum* is a fascinating plant with a variety of noteworthy properties related to its therapeutic action and a promising future in the field of antimicrobial research. Its intrinsic antibacterial properties make it a promising candidate for future medical and therapeutic advances, which is why scientists continue to be interested in and explore it.

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