

Phytochemical Characterization of *Digitaria horizontalis* Willd leaf and stem extracts using GC-MS Analysis

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Abstract

Jamaican crabgrass or *Digitaria horizontalis* Willd (Poaceae), is a slender monocotyledonous plant that can grow either annually or perennially. The phytochemical composition of the methanol extract of the stem and leaves of *Digitaria horizontalis* was investigated in this study using gas chromatography-mass spectrometry (GC-MS). *Digitaria horizontalis* Willd. (Poaceae), possesses anti-inflammatory, antioxidant, antibacterial, anti-tumor, and anti-diabetic pharmacological qualities. Stems and leaves were collected and extracted using methanol in Kharawar, Rohtak (Haryana), India. To identify bioactive compounds, methanolic extracts of *Digitaria horizontalis* stem and leaves were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The leaf extract included 115 chemical components, while the stem extract contained 76. Several noteworthy phytochemicals were found in the stem extract, including n-hexadecanoic acid, diosgenin, 9,19-cyclolanost-24-en-ol (3 β)-, 24-noroleana-3,12-diene, stigmasterol, cyclopropa-octanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl], gamma-sitostenone, and (+)-longicamphenylone. These phytochemicals demonstrated a range of pharmacological uses, including cytotoxic, anti-inflammatory, and antibacterial qualities. n-hexadecanoic acid, cyclohexanemethanol, 4-ethenyl-alpha, phytol, 7-epi-a-Eudesmol, neophytadiene, gamma-sitosterol, 2-((2R,8R,8As)-8,8a-Dimethyl-1,2,3,4,6-, Z-5,17-octadecadien-1-ol acetate, glutinol, and 14-hydroxycaryophyllene provided additional antimicrobial, antioxidant, and anticancer properties. According to the phytochemicals discovered, *Digitaria horizontalis* may be used medicinally to treat a variety of ailments. These findings could pave the way for the creation of novel drugs.

Key words : GC-MS, phytochemicals, *Digitaria horizontalis*, pharmacological applications.

Digitaria horizontalis Willd (Poaceae), commonly referred to as Jamaican crabgrass is a slim monocotyledonous plant that can grow as an annual or perennial. Although it is frequently thought of as a lawn weed, it is frequently found in pastures, lawns, and as fodder¹. *Digitaria horizontalis* is indigenous to tropical parts of Africa and South America. It spreads by roots at nodes that come into contact with the soil and by propagating through seeds². *Digitaria horizontalis* is an annual or perennial plant that grows well in humid tropical habitats. It has several uses, including as food, traditional medicine, and animal feed. The tropical areas of West and West Central Africa, Cape Verde, and Tropical and Subtropical America are the native habitats of this species. The leaf blade has a corrugated edge, a tapering base, and a sharp tip. It has triangular racemes with rough (scabrous) edges and small wings. The top glume tapers to a point and turns brown when mature, while the spikelets have a fusiform shape. Five to seven noticeable ciliated ribbed veins define the lemma³.

It is easy to identify *Digitaria horizontalis* because of its long, finger-like inflorescences. A plant decoction is used in traditional African medicine to cure a number of ailments, including problems of the neurological system, gonorrhoea, cataracts, general weakness, and inducing vomiting. Studies on ethnomedicine have demonstrated the plant's extensive application in the treatment of neurological disorders and pain. Studies on the neuropharmacological characteristics of *Digitaria horizontalis* in mice have shown anxiolytic, antinociceptive, hypnotic, and antidepressant effects, indicating possible use in the treatment

of conditions like Alzheimer's disease⁴.

Investigating the phytochemical composition of medicinal plants is essential to identifying their bioactive compounds and learning more about their potential therapeutic uses. Compared to other analytical techniques used for phytochemical profiling, gas chromatography-mass spectrometry (GC-MS) is a powerful tool for detecting and measuring a wide range of chemical components in plant extracts⁵. The objective of this research is to fill in current knowledge gaps by employing GC-MS to analyze the phytochemical components of *Digitaria horizontalis* leaf and stem extracts. Our objective is to determine the chemical foundation of the plant's medicinal qualities by identifying and measuring bioactive components. In addition to supporting its potential uses in the pharmaceutical and nutraceutical industries, this study may deepen our understanding of *Digitaria horizontalis*'s medical potential.

Plant material collection and identification:

Digitaria horizontalis stems were gathered from Kharawar village in the District of Rohtak, Haryana, India. At the National Bureau of Plant Genetic Resources' Pusa Campus in New Delhi, the plant sample was subsequently identified. Healthy and mature leaves and stems were the sole ones carefully chosen to ensure minimal contamination and harm.

Preparation of plant extract :

To get rid of any dirt or debris, the collected *Digitaria horizontalis* leaves and stems were carefully cleaned with distilled

water. They were allowed to air dry at ambient temperature before being mechanically milled into a fine powder. Since methanol is a powerful solvent for removing a variety of phytochemicals, the powdered plant material was then extracted using a Soxhlet equipment. The procedure was carried out effectively to optimize the extraction of bioactive substances. A concentrated extract was obtained by filtering the resultant methanol extract and using a rotary evaporator to evaporate the solvent at lower pressure.

GC-MS Analysis of Phytochemical compounds :

In this study, the phytochemical components found in the methanol extract of *Digitaria horizontalis* leaves and stems were analyzed and characterized using GC-MS. To obtain the right concentration for the best detection, the extract was diluted in methanol before analysis. To guarantee effective separation and precise identification of specific compounds, the GC-MS system was set up with carefully chosen parameters, such as detector settings, temperature programming, and column type⁶. In order to get a clear resolution of components, chromatographic settings were carefully tuned. By comparing the observed retention times and mass spectra with those in the NIST Mass spectrum Library (2023), compounds were identified after mass spectrum data were obtained using the electron ionization (EI) mode⁷. This approach ensured accurate and reliable identification of phytochemical constituents within the *Digitaria horizontalis* leaves and stem methanol extract.

Digitaria horizontalis methanolic extract was evaluated by GC-MS analysis,

which verified the existence of various volatile compound chemical classes 76 chemicals (phytochemical ingredients) were found in the stem and 115 in the leaves of *Digitaria horizontalis*, according to GC-MS analysis. These substances may have contributed to the plant's characteristics. To identify the phytochemical substances, three parameters were examined: peak area%, retention duration (RT), and molecular formula (MW). Based on their high peak area%, retention time (RT), molecular weight, and molecular formula, we identified 10 unique compounds from a total of 79 bioactive compounds of the stem and 115 bioactive compounds of the leaves and stem.

Bioactive compounds analysis from stem methanolic extract of Digitaria horizontalis:

The existence of 76 chemical components was discovered in *Digitaria horizontalis* methanol stem extract. In Figure 1, the stem methanolic extract's GCMS chromatogram is displayed. According to Jisha *et al.*,⁸, n-hexadecanoic acid, one of the substances found in the stem extract (Table-1), has anti-inflammatory and antioxidant properties and is also utilized as a pesticide and nematocide. Subramonian *et al.*,⁹, 9,19-Cyclolanost-24-enol, (3 beta): it has anti-tumor, anti-microbial, and antioxidant qualities.

According to Semwal *et al.*,¹⁰, diosgenin (8.63%) and diosgenin (7.04%) have significant antioxidant, neuroprotective, anticancer, antiatherosclerosis, antiasthmatic, and hepatoprotective properties. Ullah *et al.*,¹¹, 24-Noroleana-3,12-diene has anti-cancerous properties. Among the pharmacological possibilities of stigmasterol that have been

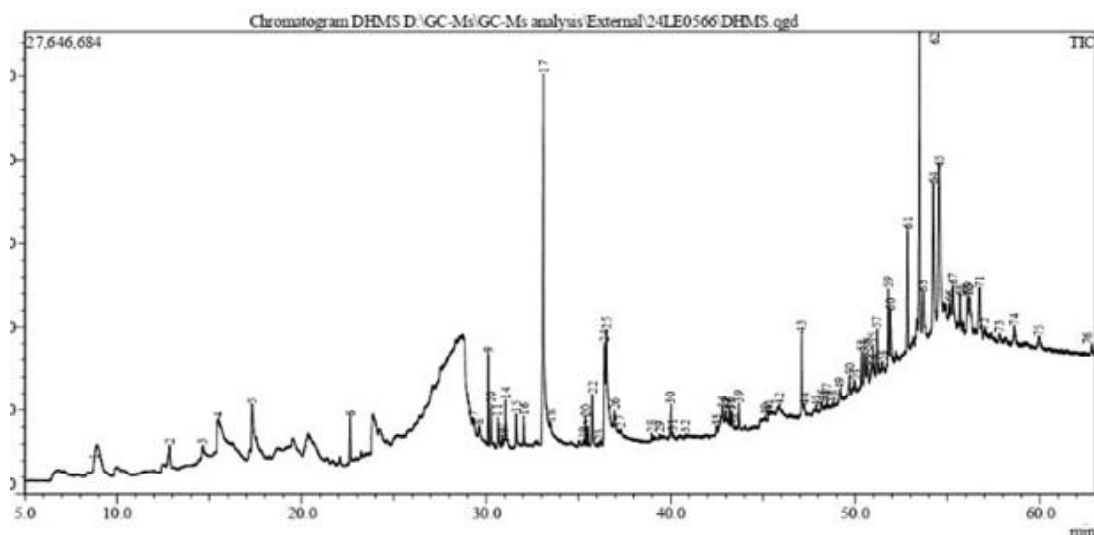


Figure 1. GC-MS chromatogram of *Digitaria horizontalis* methanolic stem extract.

investigated are antiosteoarthritic, antihypercholesterolemic, cytotoxic, antitumor, hypoglycaemic, antimutagenic, antioxidant, anti-inflammatory, and neurological actions¹². Cyclopropaocanoic acid, 2-[[2-[(2-ethylcyclopropyl) methyl] cyclopropyl] methyl] No biological activity has been discovered.

Gamma the antibacterial properties of sitostenone¹³. In the food sector, cosmetics, and cosmetics, 4-vinylphenol demonstrated cytotoxicity, anti-elastase, and anti-tyrosinase properties¹⁴, whereas (+)-Longicamphenylone demonstrated termite antifeedant properties¹⁵.

Table-1. The GC-MS analysis of bioactive substances found in the methanolic extracts of *Digitaria horizontalis* stem

S. No.	Name of the compound	RT	Peak Area%	Mol. Wt.	Mol. Formula	Biological activity
1.	n-Hexadecanoic acid	33.105	9.83%	256	C ₁₆ H ₃₂ O	anti inflammatory, antioxidant
2.	9,19-Cyclolanost-24-en-ol,(3 beta)-	53.474	8.99%	426	C ₃₀ H ₅₀ O	antioxidant, antimicrobial and anti-tumour
3.	Diosgenin	54.544	8.63%	414	C ₂₇ H ₄₂ O ₃	antioxidant, neuroprotective, anticancer, antiatherosclerosis, antiasthmatic, hepatoprotective
4.	Diosgenin	54.231	7.04%	414	C ₂₇ H ₄₂ O ₃	-Same-

5.	24-Noroleana-3,12-diene	55.287	4.23%	394	C ₂₉ H ₄₆	anti-cancerous
6.	Stigmasterol	53.692	4.14%	412	C ₂₉ H ₄₈ O	antiosteoarthritic, hypoglycemic, cytotoxic, antitumor, anti-hypercholesterolemic, antimutagenic, antioxidant, and anti-inflammatory
7.	Cyclopropaeoctanoic acid,2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]	36.934	3.66%	334	C ₂₂ H ₃₈ O ₂	No activity
8.	gamma Sitostenone	56.740	3.44%	412	C ₂₉ H ₄₈ O	antibacterial
9.	4-vinylphenol	15.491	3.19%	120	C ₈ H ₈ O	cytotoxicity, anti-elastase and anti-tyrosinase, cosmetics
10.	(+)-Longicamp-henylone	52.835	3.02%	206	C ₁₄ H ₂₂ O	antifeedant activities for termites

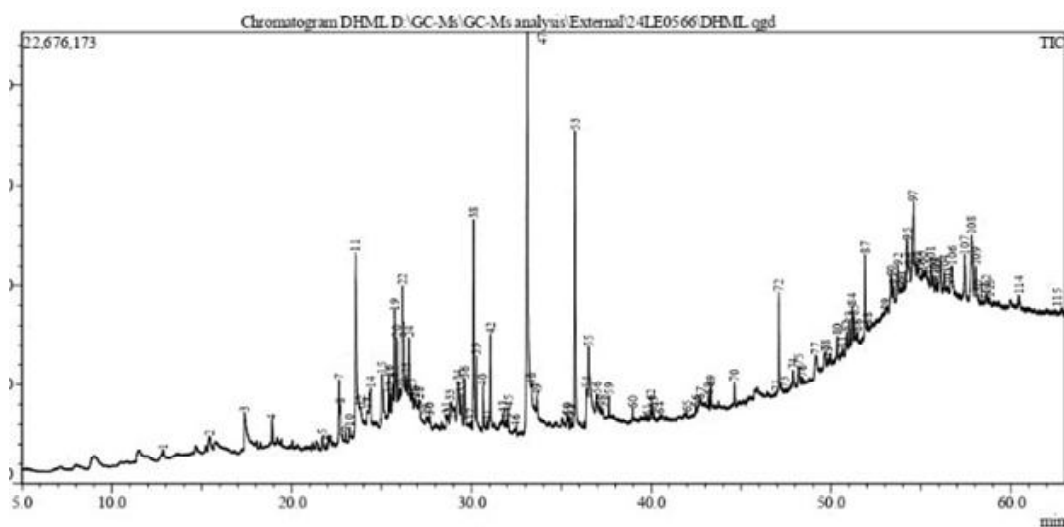


Figure 2. GC-MS chromatogram of *Digitaria horizontalis* methanolic leaves extract.

Bioactive compounds analysis from leaves methanolic extract of Digitaria horizontalis:

Digitaria horizontalis methanolic leaves extract identified 115 chemical components. Figure 2 displays the GCMS chromatogram of the methanolic extract of

leaves. The chemicals found in the leaf extracts (Table-2) include n-hexadecanoic acid, which is used as a pesticide and nematode and is said to have anti-inflammatory and antioxidant properties⁸. 4-ethenyl-alpha and cyclohexane-methanol have not been shown to have any biological activity. Antianxiety, cytotoxicity,

antimicrobial, anti-inflammatory, immunomodulating, autophagy and apoptosis-inducing, metabolism-modulating, and antioxidant qualities are only a few of the many bioactivities that phytol possesses¹⁶.

7-epi-a-Eudesmol's function as a volatile oil component and plant metabolite¹⁷. Neophytadiene has been shown to have sedative, depressive, and anxiolytic-like effects¹⁸. According to Naikwadi *et al.*,¹⁹, gamma-sitosterol exhibits anti-inflammatory properties.

According to Yuyama *et al.*,²⁰, 2-((2R,8R,8As)-8,8a-Dimethyl-1,2,3,4,6-) function as eremophilanes and primarily have phytotoxic, antibacterial, anticancer, and immunomodulatory qualities. It has been discovered that Z-5,17-octadecadien-1-ol acetate exhibits antioxidant properties²¹. 14-Hydroxycaryophyllene revealed antibacterial and antifungal action²² glutinol demonstrated antifungal activity²³ and anticancer properties against human ovarian cancer²⁴.

Table 2. The GC-MS analysis of bioactive substances found in the methanolic extracts of *Digitaria horizontalis* leaves

S. No.	Name of the compound	RT	Peak Area%	Mol. Wt.	Mol. Formula	Biological activity
1.	n-Hexadecanoic acid	33.119	12.46%	256	C ₁₆ H ₃₂ O	anti inflammatory, antioxidant
2.	Cyclohexanemethanol, 4-ethenyl-alpha	23.553	6.57%	222	C ₁₅ H ₂₆ O	No activity
3.	Phytol	35.757	5.22%	296	C ₂₀ H ₄₀ O	anti-inflammatory, anti-anxiety, cytotoxic, immunomodulating, metabolism-modifying, antioxidant, autophagy- and apoptosis-inducing,
4.	7-epi-a-Eudesmol	26.169	4.81%	222	C ₁₅ H ₂₆ O	plant metabolite, volatile oil component
5.	Neophytadiene	30.124	3.44%	278	C ₂₀ H ₃₈	anxiolytic-like activity, sedative properties, and antidepressant-like action
6.	gamma-Sitosterol	54.580	3.26%	414	C ₂₉ H ₅₀ O	anti-inflammatory
7.	2-((2R,8R,8As)-8,8a-Dimethyl-1,2,3,4,6-	25.726	2.69%	222	C ₁₅ H ₂₆ O	antimicrobial, anticancer, immunomodulatory
8.	Z-5,17-octadecadien-1-ol acetate	36.524	2.62%	308	C ₂₀ H ₃₆ O ₂	Antioxidant
9.	Glutinol	57.835	2.40%	426	C ₃₀ H ₅₀ O	Antifungal
10.	14-Hydroxycaryophyllene	26.519	2.06%	220	C ₁₅ H ₂₄ O	Antibacterial, antifungal

The methanolic extracts of *Digitaria horizontalis* stem and leaves were subjected to GC-MS profiling, which identified a wide range of bioactive chemicals with diverse biological activity. Ten main components, including n-Hexadecanoic acid, Diosgenin, Stigmasterol, and 9,19-Cyclolanost-24-en-ol, were found in the stem extract. These compounds are known to have pharmacological activities such as anti-inflammatory, anti-cancer, antibacterial, and antioxidant effects. Interestingly, diosgenin was found in two peaks with significant peak areas, suggesting that it is quite abundant and may have therapeutic value. With the identification of 115 compounds, including n-hexadecanoic acid, phytol, gamma-sitosterol, neophytadiene, glutinol, and 14-hydroxycaryophyllene, the leaf extract demonstrated a more comprehensive chemical profile. These substances further supported the plant's therapeutic usefulness by displaying a variety of bioactivities, including cytotoxic, antifungal, antibacterial, neuroactive, anti-inflammatory, and antioxidant properties. Certain components, such as 2-((2R,8R,8As)-8,8a-Dimethyl-1,2,3,4,6-, also showed immunomodulatory and phytotoxic qualities, highlighting the plant's phytochemicals multipurpose character.

The findings support *Digitaria horizontalis* traditional medical use and demonstrate its potential as a source of bioactive chemicals for use in pharmaceutical and therapeutic settings. Bioactive chemicals that have been identified may be used in the pharmaceutical industry to create novel medications.

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