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Original Research Article

Phytochemical Profiling, Antioxidant, and Antidiabetic Activities of Defatted Ethanol Aerial Extract of *Heliotropium indicum*: Insights from GC-MS and In Vitro Studies

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ABSRTACT

Heliotropium indicum, a plant traditionally used in folk medicine, has been underexplored for its combined antioxidant and antidiabetic potential. This study investigates the phytochemical composition, antioxidant, and antidiabetic activities of the ethanol extract of *Heliotropium indicum*, marking the first comprehensive evaluation of these combined properties using advanced analytical techniques. Phytochemical screening, Fourier Transform Infrared Spectroscopy (FTIR), Gas Chromatography-Mass Spectrometry (GC-MS), and in vitro assays were employed to identify bioactive compounds and assess therapeutic potential. All tested phytochemicals were detected except anthocyanins, with quantitative analysis revealing significant levels of saponins (5.58 ± 0.18%), alkaloids (9.40 ± 0.29%), flavonoids (23.92 ± 0.16 mg QE/g), phenols (74.06 ± 0.48 mg GAE/g), and tannins (15.85 ± 0.20 mg GAE/g). FTIR analysis identified key functional groups (O-H, C=O, C-H), indicating the presence of diverse bioactive compounds. GC-MS analysis revealed 27 phytocompounds, with key compounds such as phytol and n-hexadecanoic acid exhibiting antioxidant and antidiabetic properties. The extract demonstrated strong antioxidant activity, with DPPH and FRAP IC_{50} values of 19.86 µg/mL and 38.70 µg/mL, respectively, compared to ascorbic acid (13.07 µg/mL), indicating efficacy comparable to standard antioxidants. Antidiabetic assays showed a dose-dependent response with an IC_{50} of 13.83 µg/mL, close to that of acarbose (10.59 µg/mL), suggesting near-equivalent antidiabetic potency. These findings highlight the multifunctional therapeutic potential of *Heliotropium indicum*, validating its traditional use and supporting further in vivo studies and drug formulation research for potential clinical applications.

Keywords: *Heliotropium indicum*, Antioxidant, Antidiabetic, Gas Chromatography-Mass Spectrometry, Phytochemicals.

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Introduction

Medicinal plants have been integral to traditional healthcare systems for centuries, serving as a rich source of bioactive compounds with therapeutic potential. These plants exhibit diverse pharmacological properties, including anti-inflammatory, antimicrobial, and antioxidant activities.[1] Antioxidants are particularly valued for their ability to neutralize free radicals, mitigating oxidative stress-related diseases.[2] Among plant-derived antioxidants, bioactive phenols, especially bioflavonoids, are of significant interest due to their potent free radical-scavenging capabilities. Approximately 25% of drugs prescribed in the United States are derived from plant-based natural products, with nearly 70% of modern pharmaceuticals originating from natural sources.[3,4] *Heliotropium indicum Linn*., an annual herb of the Boraginaceae family, is commonly found as a weed in waste areas and near human dwellings. It has been traditionally used across various regions to treat conditions such as malaria, abdominal pain, and dermatitis in Africa, and fevers, ulcers, venereal diseases, and sore throats in Jamaica.[5] Pharmacological studies have documented its antibacterial, antitumor, uterine stimulant, antifertility, wound-healing, anti-inflammatory, antinociceptive, and diuretic activities.[6]

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Recent studies have highlighted the anti-inflammatory and antioxidant potential of *H. indicum* extracts;[7] moreover, the antidiabetic properties of related Boraginaceae species have been demonstrated, offering a promising lead for future research and potential therapeutic applications.[8] Despite this broad spectrum of biological activities, research on the antidiabetic potential of *H. indicum* remains limited, representing a significant knowledge gap. Diabetes mellitus, a chronic metabolic disorder characterized by impaired insulin function and persistent hyperglycemia,[9] is a growing global health concern. The limitations of conventional antidiabetic therapies, including adverse side effects and drug resistance, underscore the need for alternative natural therapeutic agents. Plant-derived bioactive compounds with antidiabetic properties, particularly those inhibiting enzymes such as α-amylase and α-glucosidase, could offer novel strategies for managing diabetes.[10] This study aims to investigate the antidiabetic and antioxidant potential of the defatted ethanol extract of *Heliotropium indicum*. The selection of this extract is based on its polarity, which facilitates the extraction of bioactive compounds relevant to these pharmacological activities.[11] Phytochemical profiling of medicinal plant extracts is critical for identifying bioactive compounds responsible for therapeutic effects.[12] Gas Chromatography-Mass Spectrometry (GC-MS) is employed as a robust analytical technique to characterize volatile and semi-volatile compounds in the extract.[13] In vitro assays provide a controlled environment to evaluate antioxidant activity and antidiabetic effects, specifically through inhibition of α-amylase and α-glucosidase enzymes.[14] The objectives of this study are to characterize the phytochemical composition of the defatted ethanol extract of *H. indicum* using GC-MS analysis, evaluate its antioxidant activity through in vitro assays, and assess its antidiabetic potential in vitro. We hypothesize that the extract contains bioactive compounds

with significant antioxidant properties and exhibits antidiabetic effects via enzyme inhibition.

Materials and Methods

Reagents and chemicals used

All reagents and chemicals used were of the highest purity and were grouped based on their application for clarity.

n-Hexane, Ethanol, Diethyl ether, Acetic acid, Ammonium hydroxide, Sodium chloride, Folin-Ciocalteu reagent, Sodium carbonate, Gallic acid, Quercetin, Sodium nitrite, Aluminum chloride and Sodium hydroxide. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Trolox, 2,4,6-Tripyridyl-s-triazine (TPTZ), Ferric chloride hexahydrate and Acetate buffer Acarbose, α-Amylase enzyme, Tris-HCl buffer, Calcium chloride and Diclofenac

All reagents listed above and any other chemicals used were obtained from Sigma-Aldrich (Germany).

Plant collection and identification

The aerial parts of *Heliotropium indicum* were collected from farmland in the Aroje area, Ogbomoso, Oyo State, Nigeria, located at approximately 8.0313°N latitude and 3.5394°E longitude. Identification and authentication were performed by Prof. A.T.J. Ogunkunle from the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso. A voucher specimen (No. LHO 392,627) was deposited in the university herbarium.

Extraction of plant material

The plant material was washed with distilled water, shade-dried, pulverized, and stored in an airtight container. A total of 700 g of the powdered material was subjected to sequential extraction to isolate a broad spectrum of bioactive compounds. The extraction process began with defatting using n-hexane for 72 hours, followed by extraction with ethyl acetate for 72 hours. Finally, ethanol was used for 72 hours to extract polar phytochemicals. Each extraction step was conducted at room temperature with intermittent shaking. After each step, the solvent was filtered using Whatman No. 1 filter paper and concentrated under reduced pressure using an IKA RV 10 digital rotary evaporator, model number Ident. No. 0010005195, manufactured by IKA-Werke GmbH & Co. KG in Germany, set at 40°C and 175 mbar vacuum pressure. The yields of the extracts were quantitatively recorded as follows: 14.16 g for *n*-hexane, 12.48 g for ethyl acetate, and 15.24 g for ethanol. All extracts were stored in airtight containers for future use. The ethanolic extract was selected for subsequent analyses, while the *n*-hexane and ethyl acetate extracts were reserved for potential further investigation.

Preliminary phytochemical screening

Standard qualitative chemical tests were employed to identify phytochemicals in the extract. Tests for terpenoids, saponins, phenols, coumarins, steroids, cardiac glycosides, alkaloids, tannins, flavonoids, quinones, glycosides, anthocyanins, and anthraquinones were conducted following previously described methods.[15]

Quantitative Phytochemical Screening

Determination of Total Alkaloid Content

The alkaloid content was determined following an established protocol. Briefly, 5 g of the sample was mixed with 200 mL of 10% acetic acid in ethanol in a 250 mL beaker. The mixture was incubated for 4 hours and then filtered. The filtrate was concentrated in a water bath (Bio Techno Lab Serological Water Bath, Model BTL-10, India) to approximately 25% of its original volume. Concentrated ammonium hydroxide was gradually added to induce precipitation. The resulting precipitate was treated with dilute ammonium hydroxide, filtered, dried, and weighed using an analytical balance (Mettler Toledo XPR205, Switzerland) to determine the alkaloid content.[16]

Determination of Total Saponin Content

The saponin content was determined using a previously established method. A 20 g sample was macerated with 100 cm^3 of 20% aqueous ethanol in a conical flask and heated at 55°C for 4 hours in a water bath (Bio Techno Lab Serological Water Bath, Model BTL-10, India). The mixture was filtered, and the residue was re-extracted with 200 mL of 20% aqueous ethanol. The combined extracts were concentrated to 40 mL in a water bath at 90°C. Thereafter, 20 mL of diethyl ether was added, and the aqueous layer was separated and washed twice with 10 mL of 5% aqueous sodium chloride. The final solution was heated to a constant weight and weighed using an analytical balance (Mettler Toledo XPR205, Switzerland) to determine the saponin content.[17]

Determination of Total Phenolic Content

The total phenolic content was determined using a previously established method. A reaction mixture was prepared in a 25 mL volumetric flask by combining 1 mL of plant extract, 1 mL of Folin-Ciocalteu reagent, and 9 mL of distilled water. After a 5-minute reaction time, 10 mL of a 7% sodium carbonate solution was added. Gallic acid standards (20–100 µg/mL) were prepared using the same procedure. After a 90-minute incubation at room temperature, the absorbance of the test and standard solutions was measured at 550 nm using a UV/Visible spectrophotometer (LAMBDA 1050+ (PerkinElmer, USA). The total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per gram of extract.[18]

Determination of Total Tannin Content

The tannin content was determined using the Folin-Ciocalteu method. In a 10 mL volumetric flask, 0.1 mL of the sample extract was mixed with 7.5 mL of distilled water, 0.5 mL of Folin-Ciocalteu reagent, and 1 mL of 35% sodium carbonate (Na_2CO_3). The mixture was shaken and incubated for 30 minutes. Gallic acid standard solutions (20–100 µg/mL) were prepared and treated similarly. Absorbance was measured at 725 nm using a UV/Visible spectrophotometer (LAMBDA 1050+, PerkinElmer, USA). Tannin content was expressed as milligrams of gallic acid equivalent (GAE) per gram of extract.[19]

Determination of Total Flavonoid Content

The total flavonoid content was determined using the aluminum chloride colorimetric method. A solution was prepared in a 10 mL volumetric flask by combining 1 mL of extract with 4 mL of distilled water. After a 5-minute reaction time, 0.3 mL of 5% sodium nitrite and 0.3 mL of 10% aluminum chloride were added. Following an additional 5-minute reaction time, 2 mL of 1 M sodium hydroxide was added, and the solution was diluted to 10 mL with distilled water. The same procedure was applied to standard quercetin solutions (20–100 µg/mL). The absorbance of the test and standard solutions was measured at 510 nm using a UV/Visible spectrophotometer (LAMBDA 1050+ (PerkinElmer, USA), with the reagent blank as a reference. The flavonoid content was expressed as milligrams of quercetin equivalent (QE) per gram of extract.[20]

FTIR Spectrum Analysis

The ethanolic extract was mixed with potassium bromide (KBr) salt using a mortar and pestle and compressed into a thin pellet. Infrared spectra were recorded on a Shimadzu FTIR Spectrometer 8000 series, over a range of 4000–400 cm^{-1}.

Gas chromatography-mass spectrometry Analysis

Gas chromatography-mass spectrometry analysis of the ethanolic extract was performed using an Agilent Technologies 7890A system equipped with a DB-35MS capillary column (30 m × 0.25 mm ID × 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injector was operated at 250°C, and 1 µL of the sample was injected in split mode to ensure optimal resolution and peak detection. The oven temperature was programmed to increase at a rate of 3°C/min until it reached 280°C. Identification of the components was achieved by comparing the mass spectra with those in the NIST and Wiley spectral libraries, as well as using retention indices obtained from literature values.

Antioxidant Screening

DPPH Radical Scavenging Activity

The free radical scavenging and antioxidant activities of the ethanolic extract were evaluated using a methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) at concentrations of 10, 20, 30, and 40 µg/mL,

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following a previously reported method. Absorbance was measured at 517 nm using a spectrophotometer (LAMBDA 1050+ PerkinElmer, USA) after 30 minutes of incubation at room temperature in the dark, with ascorbic acid as the reference standard. [21] The percentage inhibition for each concentration of the extract and standard was calculated using the following equation:

$$\% \text{ Inhibition} = \left(\frac{Ab-As}{Ab}\right) \times 100 \quad \text{(equation 1)}$$

where A_s is the absorbance of various concentrations of the ethanolic extracts and A_b is the absorbance of the blank solution. The extract and the standard's IC_{50} values were computed after the dose-response curve was plotted.

Determination of Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing antioxidant power (FRAP) assay was performed according to a previously described method with minor modifications. Stock solutions included a 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 mL of acetic acid, pH 3.6) and a 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution. The working FRAP solution was prepared by combining 25 mL of acetate buffer, 2.5 mL of TPTZ solution, and 2.5 mL of $FeCl_3 \cdot 6H_2O$ solution. Plant extract samples (10, 20, 30, and 40 µg/mL) were mixed with the FRAP solution and incubated for 5 minutes in the dark. The absorbance of the resulting ferrous tripyridyltriazine complex was measured at 593 nm using using a spectrophotometer (LAMBDA 1050+ PerkinElmer, USA). Results were expressed as milligrams of ascorbic acid equivalents per gram of extract (mg AAE/g).[15]

Antidiabetic Screening

Mode of α-Amylase Inhibition

The α-amylase inhibition assay was carried out based on a previously established method, with minor modifications. The α-amylase enzyme (from swine pancreas, ≥99% purity, Sigma-Aldrich) was used. A substrate solution was prepared by suspending 2 mg of starch azure in 0.2 mL of 0.5 M Tris-HCl buffer (pH 6.9) containing 0.01 M $CaCl_2$. The substrate solution was preincubated at 37°C for 5 minutes. A 10 mg extract sample was dissolved in DMSO to prepare various concentrations. Then, 0.2 mL of the extract solution was added to the substrate solution, followed by 0.1 mL of swine pancreatic amylase in Tris-HCl buffer. The reaction was incubated at 37°C for 10 minutes and stopped by adding 0.5 mL of 50% acetic acid. The mixture was centrifuged at 3000 rpm for 5 minutes at 4°C, and the absorbance of the supernatant was measured at 595 nm using a spectrophotometer (LAMBDA 1050+ PerkinElmer, USA). Acarbose, a known α-amylase inhibitor, was used as the standard. The experiments were performed in triplicate. The α-amylase inhibitory activity was calculated using the following formula:[22]

$$\text{The } \alpha - \text{amylase inhibitoryactivity} = \frac{[(Ac+)-(Ac-)]-[(As-Ab)]}{[(Ac+)-(Ac-)]} \times 100 \quad \text{(equation 2)}$$

where Ac+ = Absorbance of the positive control, Ac- = Absorbance of the reagent blank, As = Absorbance of the test extract and Ab = Absorbance of the extract blank.

Statistical Analysis

All experiments were conducted in triplicate (n = 3), and results are expressed as mean ± standard deviation (SD). Statistical comparisons were evaluated using one-way ANOVA followed by Student's t-test for pairwise analysis, where applicable. A p-value less than 0.05 ($p < 0.05$) was considered statistically significant.

Results and Discussion

The preliminary phytochemical screening of the defatted ethanolic extract of the aerial parts of *Heliotropium indicum* revealed the presence of various bioactive compounds, including saponins, alkaloids, flavonoids, tannins, coumarins, steroids, terpenoids, cardiac glycosides, glycosides, quinones, and phenols, while anthraquinones were absent, as shown in Table 1. These phytochemicals are known for their significant pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, and antidiabetic properties. These findings are consistent with previous studies on *Heliotropium indicum*, reinforcing its medicinal potential.[5,6]

Table 1: Preliminary phytochemical composition of defatted ethanol extract *of Heliotropium indicum*

Phytochemicals	Test	Result
Saponins	Frothing Test	+
Alkaloids	Mayer's Test	+
Flavonoids	Alkaline reagent Test	+
Tannins	Braymer's Test	+
Coumarins	Sodium hydroxide Test	+
Steroids	Salkowski Test	+
Terpenoids	Liebermann Test	+
Cardiac glycosides	Keller-killiani Test	+
Glycosides	Sulphuric acid Test	+
Quinones	Sulphuric acid Test	+
Anthocyanins	Hydrochloric acid Test	+
Anthraquinones	Borntrager's Test	-
Phenol		+

Key: Present = + and Absent = -

Quantitative phytochemical analysis of the defatted ethanolic extract of the aerial parts of *Heliotropium indicum* was conducted in triplicate, revealing high concentrations of phenolic compounds (74.06 ± 0.48 mg GAE/g), along with appreciable amounts of flavonoids (23.92 ± 0.16 mg QE/g), saponins (5.58 ± 0.18%), tannins (15.85 ± 0.20 mg GAE/g), and alkaloids (9.40 ± 0.29%), as shown in Figure 1. Phenolic compounds and their derivatives are widely recognized for their antioxidant and free radical-scavenging properties, which contribute to their protective effects against oxidative stress-related diseases.[23] The antioxidant activity of medicinal plants is strongly linked to their phytochemical composition, particularly the presence of polyphenols, flavonoids, and other bioactive compounds.[24] The elevated phenolic and flavonoid content in *Heliotropium indicum* likely underpins its traditional use in disease management by mitigating lipid peroxidation, which is implicated in atherogenesis and carcinogenesis. [2,23]

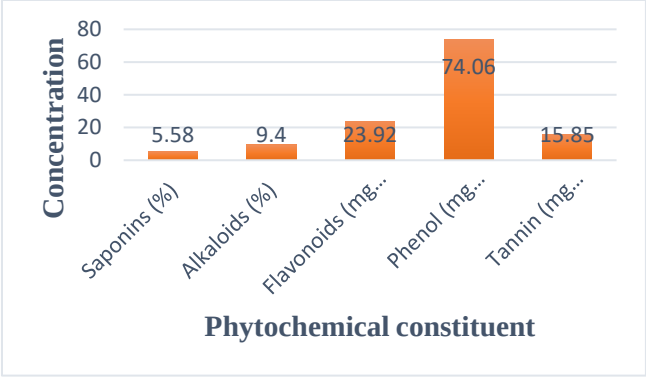

Figure 1: Quantitative phytochemical composition of aerial ethanol extract of *Heliotropium indicum*

Tannins, as polyphenolic compounds, exhibit antidiarrheal and antidiuretic properties, further supporting their pharmacological significance.[25] Alkaloids are known for their anti-inflammatory, analgesic, and antihypertensive activities, making them valuable therapeutic agents[26]. Saponins have been reported to possess antifungal, antibacterial, and antioxidant properties, which could contribute to the

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medicinal value of *Heliotropium indicum*.[27] The synergistic interaction of these polyphenolic compounds likely enhances the overall antioxidant potential of the plant extract.

These findings are consistent with previous research identifying *Heliotropium indicum* as a rich source of bioactive compounds with notable therapeutic potential.[5] The presence of these phytoconstituents further validates the traditional use of this plant in managing various health conditions.

The antioxidant potential of the defatted ethanolic extract of *Heliotropium indicum* was assessed using two widely recognized assays: the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and the FRAP (ferric reducing antioxidant power) assay. These methods provide a comprehensive evaluation by measuring both free radical scavenging capacity and reducing power.[28] Given the presence of known antioxidant phytochemicals identified through qualitative and quantitative analyses, this study aimed to establish a direct correlation between the extract's phytochemical composition and its antioxidant activity.

In the DPPH assay, which evaluates the extract's ability to donate electrons or hydrogen atoms to neutralize free radicals,[21] the extract showed a concentration-dependent increase in scavenging activity, with inhibition ranging from 55.28 ± 1.21% at 10 µg/mL to 68.56 ± 0.53% at 40 µg/mL (Figure 2). The IC_{50} value, indicating the concentration required to inhibit 50% of radicals, was determined to be 19.86 µg/mL. Although the extract demonstrated significant radical scavenging potential, its activity was slightly lower than that of the standard antioxidant, ascorbic acid (IC_{50} = 13.07 µg/mL) (Figure 4). This activity is likely attributable to flavonoids, phenolic compounds, and key bioactive constituents identified in the GC-MS analysis, including phytol, 9,12,15-octadecatrienoic acid, methyl ester, 9,12-octadecadienoic acid, 9,12,15-octadecatrien-1-ol, n-hexadecanoic acid, and 4-methylpentyl 2-methylbutanoate, which are known for their antioxidant properties.

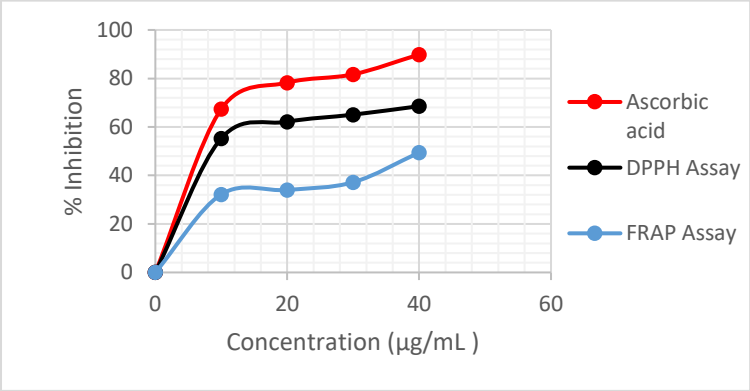

Figure 2: DPPH and FRAP antioxidant assays showing dose-dependent scavenging activity of the extract compared to standard (ascorbic acid)

The FRAP assay measures the extract's ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), reflecting its electron-donating capacity.[29] The extract exhibited a concentration-dependent increase in ferric ion reduction, with absorbance values ranging from 32.20 ± 1.78% at 10 µg/mL to 49.44 ± 1.34% at 40 µg/mL (Figure 2). The IC_{50} value for the FRAP assay was 38.70 µg/mL, indicating moderate reducing potential compared to ascorbic acid (IC_{50} = 13.07 µg/mL) (Figure 4). The disparity between the DPPH and FRAP results may be attributed to the different mechanisms assessed: DPPH measures direct free radical scavenging, whereas FRAP evaluates reducing power. Since these assays measure distinct aspects of antioxidant activity, their results may not always correlate linearly.

The strong antioxidant activity observed can be attributed to the extract's high levels of phenolic (74.06 ± 0.48 mg GAE/g) and flavonoid (23.92 ± 0.16 mg QE/g) compounds, which are widely recognized for their roles in mitigating oxidative stress. Phenolics exert antioxidant effects primarily by donating hydrogen atoms to neutralize free radicals, while flavonoids contribute through metal-chelating activity and inhibition of oxidative stress-related pathways.[30,31] Additionally, tannins (15.85 ± 0.20 mg GAE/g) may enhance antioxidant activity by interacting with and neutralizing free radicals.[32]

The GC-MS analysis further supports these findings, identifying bioactive compounds such as phytol, 9,12,15-octadecatrienoic acid, methyl ester, 9,12-octadecadienoic acid, 9,12,15-octadecatrien-1-ol, n-hexadecanoic acid, and 4-methylpentyl 2-methylbutanoate. Phytol, a diterpene alcohol, has documented free radical-scavenging properties [33], while 9,12,15-octadecatrienoic acid, methyl ester [30], 9,12-octadecadienoic acid, 9,12,15-octadecatrien-1-ol [34], n-hexadecanoic acid, and 4-methylpentyl 2-methylbutanoate have been shown to inhibit lipid peroxidation, a key process in oxidative stress-related disorders.[35,36]

Ascorbic acid, a well-established antioxidant, was used as the reference standard (Figure 4). Although *Heliotropium indicum* exhibited slightly lower activity, its rich phytochemical profile suggests a potential synergistic effect that could enhance its antioxidant efficacy in biological systems. These findings confirm the antioxidant potential of *Heliotropium indicum*, reinforcing its traditional medicinal use and highlighting its potential in managing oxidative stress-related conditions.

The increasing prevalence of diabetes and the limitations of existing treatments necessitate the search for alternative antidiabetic agents, particularly from natural sources. Given the presence of bioactive compounds such as flavonoids and saponins in *Heliotropium indicum*, which have been linked to antidiabetic properties, an α-amylase inhibition assay was conducted to evaluate its potential in modulating carbohydrate metabolism.[37] The defatted ethanolic extract exhibited a concentration-dependent increase in α-amylase inhibition, with inhibition percentages ranging from 69.06 ± 1.09% at 10 µg/mL to 77.06 ± 0.53% at 40 µg/mL (Figure 3). The IC_{50} value, representing the concentration required to inhibit 50% of the enzyme's activity, was 13.83 µg/mL, compared to 10.59 µg/mL for acarbose, the standard α-amylase inhibitor (Figure 5). While the extract showed slightly lower inhibitory activity than acarbose, its efficacy suggests a promising role as a natural enzyme inhibitor, especially when compared to other plant-based extracts, such as Adenanthera pavonina (IC50 = 16.16 µg/mL). These results indicate that *Heliotropium indicum* exhibits enzyme inhibition activity within an effective range.

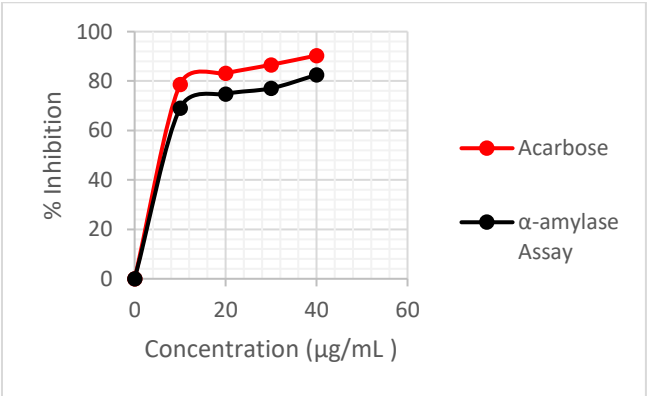

Figure 3: Antidiabetic activity of aerial ethanol extract of *Heliotropium indicum*

The observed α-amylase inhibition can be attributed to key phytochemicals, notably flavonoids, phenolic compounds, and saponins. Flavonoids interact with the active site of α-amylase, reducing its catalytic activity and delaying carbohydrate digestion.[38] Phenolic compounds stabilize the enzyme structure, further inhibiting its function, [39] while saponins regulate glucose metabolism by inhibiting digestive enzymes.[40] GC-MS analysis identified compounds such as 9,12-octadecadienoic acid, n-hexadecanoic acid, 4-methylpentyl 2-

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methylbutanoate, and phytol, which are linked to glucose metabolism regulation.[41-44] Notably, n-hexadecanoic acid enhances insulin sensitivity and inhibits carbohydrate-metabolizing enzymes,[45] while both n-hexadecanoic acid and phytol exhibit anti-inflammatory properties that may indirectly support glucose homeostasis. [46,47]
Acarbose was used as the standard for comparison due to its established role as an α-amylase inhibitor in diabetes management. The defatted ethanolic extract exhibited slightly lower inhibition than acarbose but demonstrated significant enzyme inhibitory potential (Figure 5). These findings support the traditional use of *Heliotropium indicum* in diabetes management and warrant further research to isolate and characterize the specific compounds responsible for its antidiabetic activity.

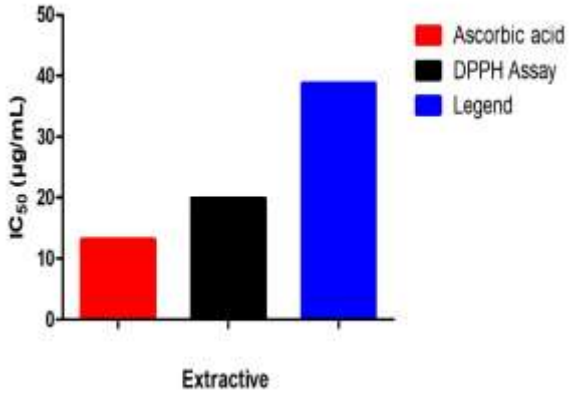

Figure 4: IC_{50} value of the antioxidant activity

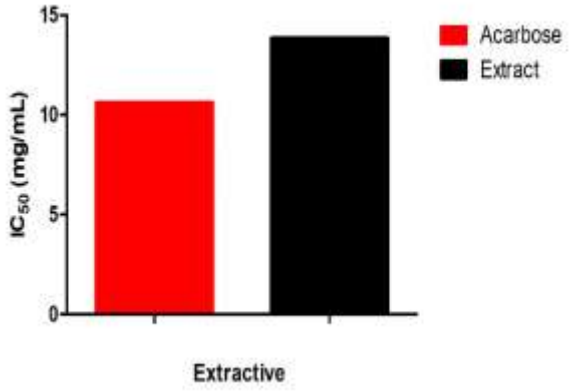

Figure 5: IC_{50} value of the antidiabetic activity

Fourier Transform Infrared (FTIR) spectroscopy was used to identify functional groups in the defatted ethanolic extract of *Heliotropium indicum*, providing molecular insights into the bioactive compounds responsible for its pharmacological effects.[48] The FTIR spectra revealed key functional groups, complementing the phytochemical screening and GC-MS results and substantiating the extract's antioxidant and antidiabetic potential (Figure 6, Table 2). A broad, well-defined peak at 3350.99 cm^{-1} corresponded to O-H stretching vibrations, characteristic of hydroxyl groups in phenols and alcohols, indicating the presence of flavonoids, phenolic acids, and other polyphenolic compounds. These compounds, present in substantial amounts, are recognized for their antioxidant properties, primarily through hydrogen atom donation to neutralize free radicals, as supported by previous research.[49] This aligns with the DPPH and FRAP assay results, confirming their contribution to free radical scavenging activity.

Table 2: FTIR Peak values of ethanolic aerial extract of *Heliotropium indicum*.

Peak No.	Frequency cm^{-1}	Functional group Assignment
1	3350.99	O-H stretch, H- bond
2	2934.70	C-H stretch
3	1631.50	C=O stretch
4	1348.47	C-H rock
5	1030.69	C-O stretch
6	590.11	C-Cl
7	482.22	C–Cl

A peak at 2934.70 cm^{-1} corresponded to C-H stretching vibrations, commonly associated with alkanes in fatty acids, terpenoids, and sterols, which contribute to anti-inflammatory and antimicrobial activities. The GC-MS analysis identified n-hexadecanoic acid and 9,12-octadecadienoic acid, both containing C-H functional groups, supporting the extract's therapeutic potential. A distinct peak at 1631.50 cm^{-1} indicated C=O stretching vibrations, characteristic of carbonyl-containing compounds such as flavonoids, aldehydes, ketones, and phenolic acids, which contribute to antioxidant and antidiabetic properties. Similar C=O stretching peaks have been associated with flavonoids involved in glucose metabolism, which supports the observed α-amylase inhibition activity.[50]
An absorption band at 1348.47 cm^{-1} represented C-H rocking vibrations, typically found in alkanes and attributed to terpenoids and steroids, which have analgesic, anti-inflammatory, and antimicrobial effects. The identification of phytol via GC-MS supports this, as phytol is a bioactive diterpene alcohol with antioxidant and anti-inflammatory properties. A peak at 1030.69 cm^{-1} indicated C-O stretching vibrations, suggesting the presence of esters, such as glycosides (including flavonoid glycosides and cardiac glycosides), known for antioxidant and cardiovascular benefits. Flavonoid glycosides have been linked to α-amylase inhibition, further supporting the antidiabetic activity. A weaker peak at 590.11 cm^{-1} corresponded to C-Cl stretching vibrations, suggesting organohalogen compounds, which may contribute to antimicrobial properties. A final peak at 482.22 cm^{-1} corresponded to C=C bending vibrations, characteristic of alkenes in unsaturated hydrocarbons, which may contribute to free radical stabilization, as reported in previous studies. [51]
The FTIR analysis confirms the presence of key functional groups correlating with bioactive compounds identified in the extract. The O-H and C=O groups support the abundance of flavonoids and phenolic acids, while C-H and C-O groups correspond to fatty acids, glycosides, and terpenoids identified via GC-MS, such as 9,12-octadecadienoic acid, n-hexadecanoic acid, and phytol, all known to modulate oxidative stress and glucose metabolism. These findings validate the traditional use of *Heliotropium indicum* and highlight its potential as a natural source of therapeutic agents for oxidative stress-related and metabolic disorders.
Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the defatted ethanolic extract of *Heliotropium indicum* identified 27 bioactive compounds, many contributing to its antioxidant and antidiabetic potential. Identification was based on retention time, peak area, molecular weight, and molecular formula (Figures 7 and 8; Table 3). Key compounds with significant pharmacological activities were identified, reinforcing the plant's traditional medicinal applications.
Phytol (1.12%), a diterpene alcohol, is recognized for its strong antioxidant properties, acting as a free radical scavenger to prevent lipid peroxidation and oxidative damage, as confirmed by previous studies.[52,53] The O-H functional group detected by FTIR at 3350.99 cm^{-1} aligns with phytol's presence, corroborating its antioxidant potential. n-Hexadecanoic acid (10.45%), a saturated fatty acid, has reported antioxidant and hypocholesterolemic effects, reducing lipid

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peroxidation and enhancing cardiovascular health, as documented in earlier research. [54,55] The C=O functional group at 1710.45 cm^{-1} in the FTIR analysis supports its presence.

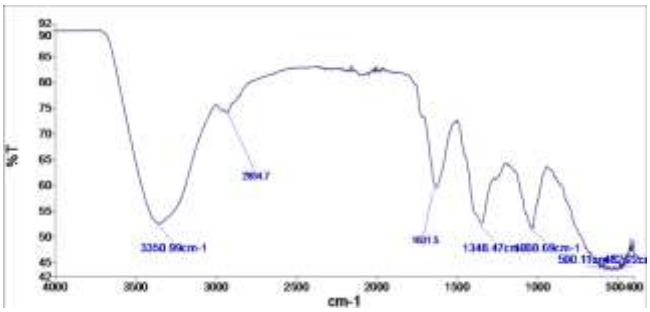

Figure 6: FTIR spectral of defatted ethanolic aerial extract of *Heliotropium indicum*.

9,12-Octadecadienoic acid (5.88%), a polyunsaturated omega-6 fatty acid, exhibits potent antioxidant activity, reducing oxidative damage and enhancing cellular defense mechanisms, as shown in prior study. [56] It likely contributes to the observed α-amylase inhibition, demonstrating antidiabetic activity. Additionally, 9,12,15-octadecatrien-1-ol (6.76%) and 9,12,15-octadecatrienoic acid, methyl ester (1.63%) were identified, both linked to antioxidant activities.[52] 4-Methylpentyl 2-methylbutanoate (33.88%), a branched-chain fatty acid ester, may contribute to bioactivity through membrane permeability, antimicrobial, or anti-inflammatory functions, as structurally similar esters exhibit mild antimicrobial or enzyme-modulating activity.[57]
While direct studies on this compound are limited, structurally similar esters such as isobutyl isobutyrate and isoamyl acetate have been reported to exhibit mild antimicrobial or enzyme-modulating activity, likely due to their hydrophobic chains facilitating interaction with lipid membranes or proteins.[58,59] Given its abundance, it is plausible that 4-Methylpentyl 2-methylbutanoate contributes to the bioactivity profile of the extract, possibly through membrane disruption in microbes **or** modulation of oxidative stress pathways**.** Future studies may investigate this hypothesis using isolated compound assays or structural analog screening.
The synergistic effects of these compounds enhance the overall bioactivity of *Heliotropium indicum*. The combined antioxidant and antidiabetic effects observed in the in vitro assays are likely due to the interaction of multiple phytochemicals.[6] The presence of key functional groups detected by FTIR, such as O-H and C=O, aligns with these bioactive compounds, substantiating their pharmacological significance. These findings reinforce *Heliotropium indicum* as a potential source of natural therapeutic agents for diabetes and oxidative stress-related conditions.[7,8]

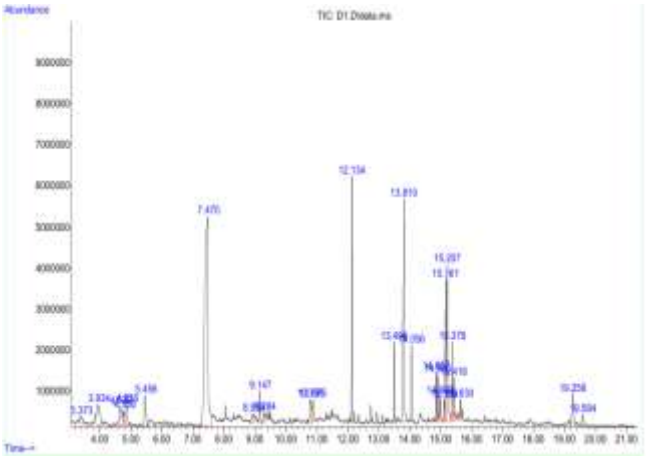

Figure 7: GC-MS spectral of defatted ethanolic aerial extract of *Heliotropium indicum*

Table 3: List of compounds present in defatted ethanolic aerial extract of *Heliotropium indicum*

S/N	Compound Name	MF	MW	RT	Area %	SI
1	Acetic acid, phenyl ester	$C_8H_8O_2$	136.15	3.373	1.01	49
2	Phthalic anhydride	$C_8H_4O_3$	148.11	3.934	3.02	50
3	Indole	C_8H_7N	117.15	4.632	2.21	94
4	Benzoic acid, methyl ester	$C_8H_8O_2$	136.15	4.758	0.77	76
5	Benzoic acid, methyl ester	$C_8H_8O_2$	136.15	4.838	2.27	41
6	Butanoic acid, 2-ethyl-, butyl ester	$C_{10}H_{20}O_2$	172.26	5.456	2.13	37
7	4-Methylpentyl 2-methylbutanoate	$C_{11}H_{22}O_2$	186.29	7.470	33.88	37
8	(R*,R*)-5-Hydroxy-4-methyl-3-heptanone	C8H16O2	144.21	8.935	0.84	27
9	Cyclohexene, 1,3,4,5,6-pentachloro-, .gamma.-	$C_6H_5Cl_5$	254.40	9.147	1.30	60
10	Cyclohexanone, 3,3,5-trimethyl-	$C_9H_{16}O$	140.22	9.283	0.69	50
11	Ethyl.alpha.-d-glucopyranoside	$C_9H_{18}O_8$	254.23	10.795	1.71	45
12	Ethyl.alpha.-d-glucopyranoside	$C_9H_{18}O_8$	254.23	10.875	1.66	68
13	Lindane	$C_6H_6Cl_6$	290.80	12.134	6.90	99
14	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	279.50	13.496	2.33	98
15	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	13.810	10.45	99
16	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.50	14.056	2.35	99
17	Methyl 10-trans,12-cis-octadecadienoate	$C_{19}H_{34}O_2$	294.50	14.857	1.62	99
18	9,12,15-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292.50	14.903	1.63	99
19	Phytol	$C_{20}H_{40}O$	296.50	14.983	1.12	90
20	Methyl stearate	$C_{19}H_{38}O_2$	298.50	15.109	0.82	97
21	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280.40	15.161	5.88	99
22	9,12,15-Octadecatrien-1-ol	$C_{18}H_{32}O$	264.40	15.207	6.76	93
23	Octadecanoic acid	$C_{18}H_{36}O_2$	284.50	15.378	2.90	99

24	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310.50	15.41	1.39	93
25	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312.50	15.63	0.94	97
26	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.50	19.25	2.46	87
27	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390.60	19.58	0.96	87

MF-Molecular Formula, MW-Molecular Weight, RT-Retension Time, SI-Soft Ionization Value

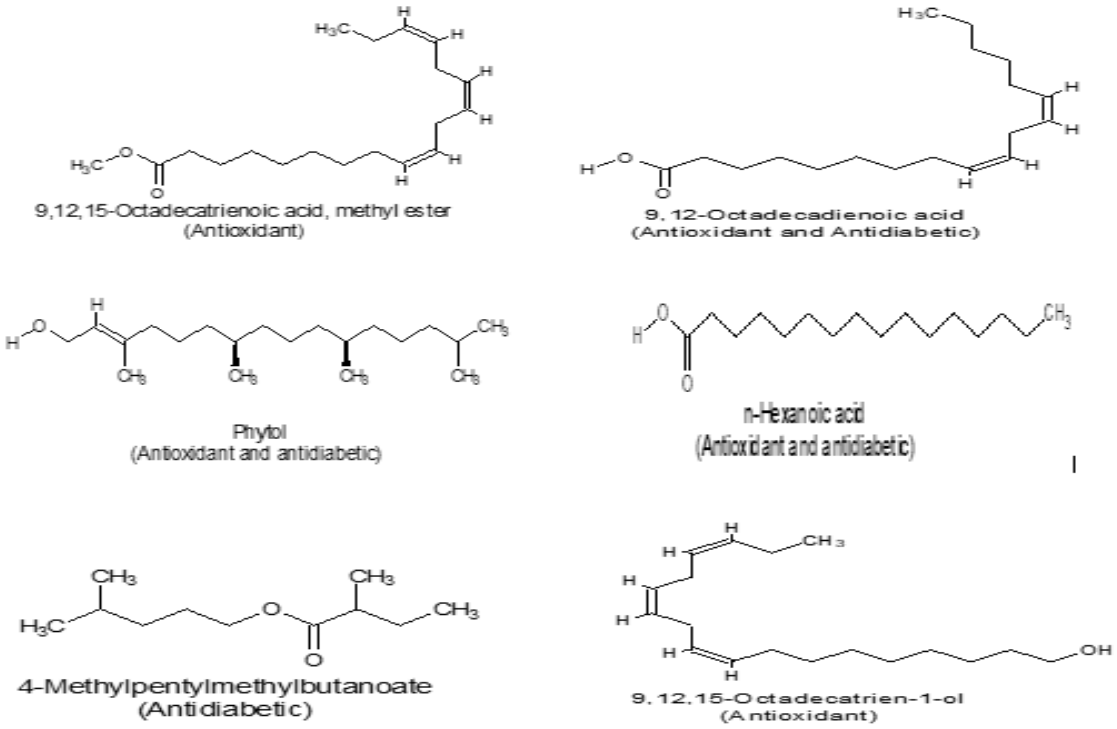

Figure 8: Structure of compounds identified by GC-MS responsible for antioxidant and anti-inflammatory properties

Conclusion

This study highlights the phytochemical richness and therapeutic potential of the defatted ethanolic extract of *Heliotropium indicum*. The extract exhibited potent antioxidant activity, as demonstrated by DPPH and FRAP assays, and notable α-amylase inhibitory activity, comparable to acarbose. Phytochemical screening confirmed the presence of bioactive compounds, including flavonoids, phenols, alkaloids, saponins, and tannins, all contributing to antioxidant and antidiabetic effects. GC-MS analysis identified 27 compounds, including phytol, 9,12,15-octadecatrienoic acid, n-hexadecanoic acid, and 9,12-octadecadienoic acid, associated with antioxidant and antidiabetic activities. These findings validate the traditional medicinal use of *Heliotropium indicum* and suggest its potential as a natural source of therapeutic agents for managing oxidative stress and metabolic disorders. Future studies should focus on isolating these active constituents, elucidating their mechanisms of action, and conducting in vivo evaluations to confirm their potential for drug development.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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