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Investigating In Vitro α-Amylase Inhibitory and Free Radical Scavenging Activity Containing Compounds of *Gynura procumbens*

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Abstract

Gynura (G.) procumbens is a medicinal herb widely employed in Bangladesh to improve vitality. The main objective of this research was to characterize the bioactive constituents in *G. procumbens* leaves associated with both α-amylase inhibition and antioxidant potential. The extraction efficiency of *G. procumbens* was investigated using methanol, n-hexane, and different ethanol-water concentrations. The optimal solvent combination was determined by analyzing the relationship between the extraction yield and the potency of the resulting α-amylase inhibition and radical scavenging activities. The compounds were achieved through GC-MS, FTIR, and phytochemical analysis, which might be accountable for inhibition activity. The α-amylase inhibitory activity of methanolic and n-hexane extract were 9.54 and 8804.5 µg/mL, respectively. The total antioxidant capacities were estimated, and standard value of ascorbic acid was 0.358, whereas methanolic extract of *G. procumbens* was 0.244 and n-hexane was 0.072. A 100 % ethanol exhibited the highest scavenging potential (56.68 ± 0.40) µg/mL, and aqueous extract exhibited lowest potential (565.19 ± 0.38) µg/mL. Phenolics, carbohydrates, triterpenes and organic acids were identified as putative inhibitors against inhibitory activity. In conclusion, *G. procumbens* could be a potential herb used as a natural antioxidant and antidiabetic remedy.

Keywords: *G. procumbens*; Extraction; Inhibition activity; Antidiabetic; Antioxidant.

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1. Introduction

G. procumbens belongs to Gynura family which are traditionally used against different diseases in south Asian countries. In Bangladesh, the fresh plants of *G. procumbens* (Fig. 1) are commonly eaten raw with rice and the leaves are also used for cooking [1]. This medicinal plant has concerned noteworthy systematic attention because of its broad range of pharmacological properties and its long-standing use in traditional medicine. Its therapeutic versatility stems from a rich profile of bioactive secondary metabolites—notably phenolic acids, flavonoids, and terpenes. These compounds underpin the plant's documented antidiabetic, antimicrobial, and anti-inflammatory properties, while also showing significant promise in antioxidant and anticancer applications [2–6].

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Fig. 1. Image for *G. procumbens* leaves.

Traditionally consumed in diverse formats—ranging from crude raw material to processed infusions and extracts—this herb is now being scrutinized for its safety profile. Raw medicines may have inherent toxicity, but the rising tide of antibiotic resistance and the adverse possessions of synthetic drugs have turned scientific attention to natural alternatives. Consequently, there is a concerted effort to isolate and characterize the precise bioactive compounds within these plants to meet the increasing consumer demand for functional, health-optimized foods. While the path from natural source to clinical application is well-defined—involving extraction, screening, and characterization [7], the process is frequently hampered by technical inefficiencies. Specifically, traditional extraction methods often suffer from poor selectivity, high time consumption, and low total yields. Furthermore, the potential for bioactive degradation and the use of hazardous solvents present ongoing challenges to the sustainability and safety of these protocols. These factors directly influence environmental sustainability, economic feasibility, and the ultimate purity of the isolated compounds [8]. As the food, cosmetic, and pharmaceutical industries pivot toward 'green' and residue-free products, the limitations of traditional extraction methods have become increasingly apparent. This shift necessitates the adoption of non-conventional, eco-friendly alternatives. While the chemical profile and biological activities of *G. procumbens* are well-documented, existing literature reveals a notable reliance on conventional extraction techniques, highlighting a clear opportunity for methodological optimization.

The objective of this research is to outline the chemical composition of *G. procumbens* while assessing its antioxidant and antidiabetic properties using novel analytical techniques. Extracting bioactive compounds from *G. procumbens* via non-conventional techniques facilitates the development of residue-free products for the food, cosmetic, and pharmaceutical industries.

2. Materials and Methods

2.1. *Chemicals*

FCR (Folin-Ciocalteu reagent), methoxyamine, HCl, MSTFA (N-Methyl-N-(trimethylsilyl) trifluoroacetamide), methanol, sodium hydroxide, n-hexane, sodium nitrite, DPPH (2, 2-diphenyl-1-picrylhydrazyl) and Na_2CO_3 (sodium carbonate) were collected from Sigma-Aldrich (Germany). Catechin, sodium phosphate and ammonium molybdate were acquired from Sigma-chemical company (USA). Gallic acid was bought from Wako pure chemicals Ltd. (Japan). Ethanol, sulphuric acid, trichloroacetic acid and ascorbic acid were bought from Merck (Germany). $FeCl_3$ (ferric chloride) and $K_3[Fe(CN)_6]$ (potassium ferricyanide) were purchased from Matheson Coleman and Bell (America). A Milli-Q purification system (Millipore, Milford, MA) provided the ultrapure water used throughout the study. All additional reagents were of high analytical purity.

2.2. *Instruments*

A Shimadzu GC-MS QP-2020 system, equipped with both an AOS-20i autoinjector and an AOC-20S autosampler, was employed for the GC-MS analysis. The stationary phase consisted of an SH Rxi-5MS Sil column with dimensions of 30 m × 0.25 µm. UV-Spectrophotometer (SHIMADZU, USA) was used for measurement of UV absorption. FTIR (Fourier-transform infrared) spectra of the materials were perform a PerkinElmer FTIR spectrophotometer, model number FTIR-100 (made in Japan). Sonicator (Power sonic 505) and Vortex shaker (Digisystem, Model: VM-1000, Made in Taiwan) were used for extraction of *G. procumbens*.

2.3. *Plant material acquisition*

Fresh specimens of *G. procumbens* were harvested during the winter months (January–March) from the environs of the Rajshahi University campus in Bangladesh. Botanical validation was conducted at the Department of Botany, University of Rajshahi, where the material was assigned voucher specimen number 305.

2.4. *Preparation of plant materials*

Fresh leaves were sliced and cleaned under running water to confirm the elimination of exogenous contaminants. Then the plants were dried out in the air. Following the drying process, the sample was ground to a fine powdered by a crushing machine and preserved in airtight vials under refrigeration to prevent degradation of bioactive constituents. The initial weight of the plant material was 580 g, which yielded 554 g of fine powder after grinding, representing a 95.5 % recovery.

2.5. *Extraction method*

G. procumbens were extracted from leaves by sonication techniques using methanol and n-hexane. Adhering to the procedure established by Javedi *et al.* [9], 6 g of the sample was

dispersed in 200 mL of solvent within a 500 mL Erlenmeyer flask. This mixture underwent sonication for 30 min in a water bath, with the temperature strictly maintained below 40 °C. Following sonication, the solution was passed through Whatman No. 1 filter paper to isolate the extract from the solid residue. The resulting filtrate was concentrated to a dry state using a rotary evaporator under vacuum. The recovered crude extract was allowed to air-dry at ambient temperature and subsequently refrigerated for further experimental use.

The extractions were again carried out by utilizing two solvents such as, EtOH and DD water at different ratios (100 % EtOH, 80 % EtOH, 60 % EtOH, 40 % EtOH, 20 % EtOH, 0 % EtOH) through four replications. The sample was taken 6 g in conical flask and added 200 mL of each mixture ratio of ethanol and water. Each flask was enclosed with aluminium foil. Twenty-four samples underwent ultrasonic extraction at a constant temperature of 25 °C. To obtain the crude extracts, the solvents were eliminated via rotary evaporation under vacuum at a controlled temperature of 40 °C. These extracts were then air-dried at room temperature to ensure the removal of any remaining solvent traces before being stored for subsequent assays.

2.6. *Antidiabetic test*

Iinhibitory activity of the α-amylase was assessed via adapting the methodology of Jabir *et al.* [10] with several modifications. Extract samples (500 µL; 50–500 µL concentrations) were incubated with 500 µL of 0.5 mg/mL α-amylase in Na_3PO_4 buffer (0.02 M, 6.0 mM NaCl, pH 6.9) for 10 min at 37 °C. Subsequently, 500 µL aliquot of 1 % (w/v) soluble starch was introduced into each reaction mixture, which was then subjected to an additional 10 min incubation period. The reaction was halted through the addition of 1.0 mL of 3,5-dinitrosalicylic acid (DNS) solution. The enzymatic reaction was quenched by the addition of 1 M HCl (20 µL), followed by color development with 100 µL of iodine reagent (5 mM I_2 and 5 mM KI). Once the chromatic shift was evident, spectrophotometric readings were taken at 540 nm. The resulting data were expressed as percent inhibition (I_{ac}) and calculated using the formula provided in Eq. 1.

$$\% \text{ of } I_{ac} = [(A_c - A_s)/A_c] \times 100 \quad \text{.....(1)}$$

where,

A_s = the measured absorbance value of the sample

A_c = the measured absorbance value of the control

2.7. *Antioxidant activity*

2.7.1. *Total antioxidant capacity (TAC)*

Overall antioxidant potential was evaluated via a modified phosphomolybdenum assay based on the protocol by Prieto *et al*. [11]. Extract aliquots (0.5 mL) were combined with (3 mL) of reagent (0.8 M H_2SO_4, 14 mM Na_3PO_4, and 0.4 % ammonium molybdate) and incubated at 95 °C for 10 min. Once the samples reached ambient temperature, the optical

density was measured at 695 nm. The results were compared to an ascorbic acid standard curve. Higher absorbance readings indicated superior antioxidant performance. The experiment was performed three times to ensure data consistency.

2.7.2. *Total phenolic content (TPC)*

TPC was quantified spectrophotometrically, following the methodologies of Slinkard *et al*. [12] and McDonald *et al.* [13], respectively, with several adjustments. For the evaluation of total phenolic content, a stock solution was prepared, from which a 300 µL aliquot was transported to a test tube for analysis. Subsequently, 2.25 mL of FCR, previously diluted in 20 mL of distilled water, was introduced and allowed to react for 5 min at room temperature. Following the addition of 2.25 mL of 6 % (Na_2CO_3), the reaction was maintained at room temperature for 90 min. Following the incubation period, the absorbance was read at 760 nm. The TPC of the two extracts was then quantified as specified in Eq. 2.

$$C = (c \times V)/M \ldots\ldots\ldots (2)$$

Where, C represents the total phenolic content (mg GAE/g dry weight), c is the gallic acid concentration, V denotes the extract volume (mL), and M signifies the mass of the pure extract (g). The phenolic concentration was quantified using a gallic acid-derived linear regression model ($y = 0.0148x + 0.0022$, $R^2 = 0.9896$). Results are presented as milligrams of gallic acid equivalents (GAE) per gram of dry extract, based on the mean of three independent measurements.

2.7.3. *Total flavonoids content (TFC)*

Estimation of the TFC was performed using an aluminium chloride-based colorimetric assay, adapted from the methodology described by Olajire *et al*. [14].

A 0.5 mL sample aliquot was placed in a test tube and diluted with 2.25 mL of distilled water. To this, 0.15 mL of 5 % of $NaNO_2$ was added, allowing the mixture to equilibrate at room temperature for 6 min. Subsequently, 0.3 mL (300 µL) of 10 % $AlCl_3.6H_2O$ was introduced and left for 5 min, followed by the addition of 1.0 mL of 1 M NaOH (0.406 g/mL). The solution was thoroughly homogenized using a vortex mixer, and the absorbance was immediately measured at 510 nm via UV-visible spectrophotometry. Flavonoid levels were quantified as catechin equivalents per gram of dry weight (mg CA/g) using Eq. 2 and a catechin-based regression model ($y = 0.0022x + 0.0119$, $R^2 = 0.9985$). All assays were performed in triplicate to ensure precision.

2.7.4. *Ferric reducing antioxidant power (FRAP)*

The reductive potential was assessed following a modified version of the protocol established by Oyaizu *et al*. [15]. Briefly, 250 µL of the extract at varying concentrations (10-100 mL) was combined with 0.625 mL of potassium phosphate buffer (pH 6.7) and

0.625 mL of 1 % potassium ferricyanide [$K_3Fe(CN)_6$]. After a 20 min incubation at 50 ºC, 1.0 mL of 10 % trichloroacetic acid was added to the solution. A 1.0 mL portion of this mixture was then diluted with an equal volume of distilled water and treated with 0.2 mL of 0.1 % ferric chloride ($FeCl_3$). The absorbance value of the reaction mixture is indicative of its reducing capacity. Ascorbic acid standards at different concentrations were prepared in a similar manner for comparative analysis. Spectrophotometric measurements were carried out at a wavelength of 700 nm.

2.7.5. *DPPH free radical scavenging activity*

The free radical scavenging potential of the extracts was evaluated using the DPPH assay, following a modified protocol by Shahwar *et al*. [16]. Briefly, 3.0 mL aliquots of the extracts at various concentrations (10, 20, 30, 50, and 100 µg/mL) were combined with 3.0 mL of a 0.1 mM methanolic DPPH solution. The resulting mixtures were incubated in the dark at room temperature for 30 minutes. Subsequently, the absorbance was measured at 517 nm, and the percentage of radical inhibition was calculated as specified in Eq. 1.

Scavenging activity (%) of DPPH radical was graphed as a function of concentration, and IC_{50} was evaluated from the plot. Ascorbic acid was utilized as a positive control for comparative purposes.

2.8. *Identification*

2.8.1. *Phytochemical analysis*

The presence of major phytochemical constituents, such as alkaloids, flavonoids, phenols, saponins, tannins, steroids, and glycosides, in *G. procumbens* was evaluated using established qualitative methods [17-19] with slight modifications. Major secondary metabolites were identified by the formation of characteristic colors and precipitates after treatment with selective reagents.

2.8.1.1. *Test of saponins*

Frothing test: Saponins were identified by treating 5.0 mL of the sample with a drop of $NaHCO_3$ solution. After being shaken well and left to stand for 3 min, the mixture was observed for the formation of honeycomb-like froth, signaling a positive result.

2.8.1.2. *Test of tannins*

Following the addition of 5 % (w/v) $FeCl_3$ drops to 1.0-2.0 mL of extract, the mixture was observed for chromatic shifts. Gallo-tannins were identified by a green reaction product, while pseudo-tannins were characterized by a brown coloration.

2.8.1.3. *Test of glycosides*

Molisch's test: Initially, 2 mg of the extract was homogenized in 10.0 mL of distilled water and filtered. The filtrate was treated with 2-3 drops of Molisch's reagent, and 2 mL of concentrated H_2SO_4 was carefully introduced along the tube's side. In a parallel procedure, 2 mL of each extract was combined with 2.0 mL of 0.1 mM DPPH in methanol; the mixture was then maintained at room temperature for a 30 min incubation period. Spectrophotometric measurements were performed at 517 nm at room temperature. Using Eq. 1, the scavenging efficiency was calculated; IC_{50} values were then interpolated from the plot of activity versus concentration, with catechin serving as the standard. Simultaneously, the presence of glycosides in the extracts was confirmed by the development of a characteristic reddish-violet ring during the chemical assay.

2.8.1.4. *Test of steroids*

Salkowski reaction: The presence of steroids was evaluated by mixing 2 mg of the dry extract with chloroform, followed by the slow addition of H_2SO_4 (conc.) via the side of the tube. A positive result was signaled for steroid by the development of a distinct red color within the chloroform phase.

Liebermann-Burchard's test: A 2.0 mg portion of the extract was boiled in acetic anhydride and allowed to cool. Subsequently, 1.0 mL of H_2SO_4 was layered carefully along the tube wall. Steroids were identified by the formation of a characteristic green color, indicating a positive reaction.

2.8.1.5. *Test of alkaloid*

Dragendorff's test: The presence of alkaloids was evaluated by dissolving 2 mg of the sample in 5 mL of distilled water, followed by treatment with 2 M HCl. Upon adding 1 mL of Dragendorff's reagent, the formation of a characteristic orange-red precipitate was recorded, confirming the presence of alkaloids.

Hager's test: A 2 mg portion of the extract was treated with several drops of Hager's reagent. The formation of a characteristic yellow precipitate served as a positive indicator, confirming the presence of alkaloidal constituents.

2.8.1.6. *Test of Phenol*

The Folin–Ciocalteu colorimetric assay was utilized to determine the total phenolic content (TPC) of the extracts, following established protocols [12,13]. About 0.30 mL of each trial prepared at 1000 µg/mL in methanol was pipetted into separate vials in triplicate. A calibration curve was generated using gallic acid standards at concentrations ranging from 5 to 100 µg/mL. The total phenolic content was determined by plotting concentration on the x-axis (abscissa) against absorbance on the y-axis (ordinate).

2.8.1.7. *Test of flavonoids*

Shinoda's test: The presence of flavonoids was evaluated by dissolving 2.0 mg of the extract in 5.0 mL of ethanol. After adding ten drops of dilute hydrochloric acid and a small piece of magnesium metal, the reaction mixture was observed for the development of a pink color, confirming a positive result.

2.8.2. *GC-MS analysis*

2.8.2.1. *Derivatization technique*

Samples were derivatized for GC–MS based on the method by Robinson *et al.* [20]. Briefly, 12.5 mg of sample was sonicated in pyridine (25 µL) for 10 min, followed by methoximation using 50.0 µL of methoxyamine hydrochloride (20 mg/mL) at 60 °C for 2 h. Silylation was then achieved by adding 150 µL of MSTFA ((N-methyl-N-(trimethylsilyl) trifluoroacetamide)) and incubating at 60 °C for a further 30 min. After syringe filtration and an overnight standing period at room temperature, the clarified samples were subjected to GC–MS analysis.

2.8.2.2. *GC-MS analysis*

GC–MS analysis utilized helium (1.72 mL/min) as the mobile phase. The column temperature was programmed from 80 °C (2 min hold) to 150 °C (5 min hold) at 5 °C/min, and finally to 280 °C (8 min hold). The injector and ion source were set to 230 °C and 280 °C. Samples (6 µL) were introduced via a 20:1 split injection. Mass spectra were recorded at 70 eV within a scan range of 45-350 m/z. The method employed a 3.2 min solvent delay and a total analysis duration of 55 min. Identification of constituents was facilitated by comparison with NIST08s, NIST08, and NIST14 reference libraries.

2.8.3. *FT-IR analysis*

A small quantity of the sample powder, equivalent to 0.1–2 % of the KBr weight or just covering the spatula tip was taken. The sample was then mixed with KBr powder. After mixing subsequently, the mixture was grinded for 3-5 min with a mortar-pestle. Then it was performed for IR spectroscopy at wavelength 4000-400 cm^{-1}.

2.9. *Statistical analysis*

One-way ANOVA and Tukey’s comparison tests were employed to determine statistical significance using Minitab 16 software. All analyses were performed at a 95 % confidence interval, where a p-value of less than 0.05 was defined as the threshold for significance.

3. Results and Discussion

3.1. *Screening of various solvent extract of G. procumbens according to extraction yield and biological activities*

3.1.1. *Percentage of yield and Antidiabetic activity*

Table 1 details the percentage yields of *G. procumbens* leaf extracts across the various solvents employed in this study. Sonication-assisted extraction yields were (26.55 ± 0.68)% for the methanolic extract and (2.89 ± 0.06)% for the n-hexane extract, demonstrating a significant difference in solvent efficiency. The % yields of methanolic extraction of *G. procumbens* is higher than that of n-hexane. Therefore, methanol is considered a suitable solvent to achieve a higher extraction yield. Consistent with previous studies [21,22],

Table 1. Comparison of extraction yield, α-amylase inhibition and antioxidant activity of *G. procumbens* extracts prepared with methanol and n-hexane.

Name of the solvent	Extraction yield (%) ± SD	IC_{50} (µg/mL) ± SD(DPPH)	(%) of inhibition (IC_{50} value ± SD) (α-amylase)
Methanol	26.55 ± 0.68	56.86 ± 0.38	9.54 ± 0.42
n-hexane	2.89 ± 0.06	113.95 ± 0.28	8840.75 ± 0.10

Values represent the means ± standard deviation (SD), n = 3

aqueous methanol provided the highest extraction yields for *G. procumbens*. The methanolic extract also showed the strongest radical scavenging activity, indicating that the plant's antioxidant potential is dictated by solvent polarity. This suggests that the bioactive compounds involved in neutralizing oxidative species are more soluble in polar media. This observation supports the findings of Tunna *et al*. [23], where methanol significantly surpassed nonpolar solvents in antioxidant performance. The extraction yields (%) and corresponding IC_{50} values for the methanol and n-hexane extracts are summarized in Table 1.

Percentage of extraction yield = (extract weight /sample weight) × 100 ……(3)

Extraction yields (%) for aqueous and ethanolic extracts were determined using Eq. (3), which are largely dependent on solvent characteristics.

Previous research has established that methanolic extracts possess significantly greater radical scavenging activity compared to those obtained with n-hexane [21].

The radical scavenging mechanism involves the conversion of DPPH into a non-radical form upon the acquisition of a hydrogen radical [24]. By donating hydrogen atoms to species such as lipid peroxides, antioxidants effectively terminate the propagation phase of lipid peroxidation [25]. This reductive capacity was monitored spectrophotometrically by the attenuation of absorbance at 517 nm. Experimental data demonstrated that the antioxidant effects of *G. procumbens* and the standards were concentration-dependent. The scavenging profiles were benchmarked against a positive control, where the IC_{50} served as an inverse indicator of antioxidant strength.

Acarbose, an α-amylase inhibitor, was employed as a positive control. The data in Table 1 demonstrated that both methanolic and n-hexane extract possessed α-amylase inhibitory activity. In the starch–iodine color evaluates, the methanolic extract exhibited a higher percentage of α-amylase inhibition than the n-hexane extract, although lower than the standard acarbose. The α-amylase inhibitory activity order of *G. procumbens* were following as:

Methanol > Acarbose > n-hexane

Above the consequences, it is clear that, methanolic extract of *G. procumbens* shows superior activity in all cases, as per many antioxidants, enzyme-modulating and anti-diabetic phytochemicals (flavonoids, phenols, terpenoids, sterols) are soluble in methanol better than pure water and n-hexane.

The current research investigated the antidiabetic activity of *G. procumbens*, a plant traditionally used for the treatment of various diseases. To the best of our knowledge, this herb has not previously been evaluated for its in vitro antidiabetic activity in this way. The findings of this study may have potential implications for human health.

3.1.2. *Antioxidant*

3.1.2.1. *Total phenolic (TPC) and total flavonoid content (TFC)*

Table 2 displays the TPC and TFC of the methanol and n-hexane extracts. These concentrations were calculated as gallic acid equivalents (GAE/g) and catechin equivalents (CE/g) based on the weight of the dried sample. The TPC of the methanolic extract was found (70.07 ± 4.28) GAE/g, whereas the n-hexane was (9.51 ± 1.24) GAE/g. Thus, the TPC was highest in methanolic extract.

The methanolic extract of *G. procumbens* was contained TFC of (219.73 ± 9.97) CE/g of dried-up materials and the n-hexane extract demarcated the TFC (101.25 ± 1.46) CE/g. These results demonstrated that methanolic extract of *G. procumbens* contained higher number of flavonoids. An excellent linear correlation was obtained for total flavonoid content (R^2 = 0.9985). It is noteworthy that total flavonoid content displayed a slightly stronger correlation than total phenolic content [26-30]. The results indicate that antioxidant components in *G. procumbens* leaf extract are accountable for the observed effect. The extract of *G. procumbens* is a more potential medicinal source for the treatment of diseases because it contains high phenolic as well as high flavonoids content. However, this finding contrasts with traditional medicinal practices, where the leaves are widely used for treating various diseases.

Data presented in Table 2 reveals that methanolic extracts exhibit significantly higher TPC and TFC levels compared to n-hexane extracts.

Table 2. Total phenolic and total flavonoids content of methanol and n-hexane extracts.

Name of the solvent	TPC (GAE/g)	TFC (CE/g)
Methanol	70.07±4.28	219.73±9.97

n-hexane	9.51±1.24	101.25±1.46

Each value in the table is represented as mean ± SD

3.1.2.2. *Total antioxidant capacity (TAC)*

Evaluation of the TAC (Fig. 2) revealed a concentration-dependent increase for both extracts. However, the methanolic extract of *G. procumbens* showed the highest capacity (0.244 ± 0.002) at 100 µg/mL, whereas the n-hexane extract yielded a much lower value of (0.072 ± 0.002). On the other hand, (0.358 ± 0.005) was for standard ascorbic acid.

Variations in antioxidant activity among plant parts are influenced by the mechanism of action and function of phenolic constituents. The standard ascorbic acid was used in this study. For the determination of the TAC in n-hexane extract of *G. procumbens* is not so convenient solvent. In contrast, methanol is a good solvent due to its high extraction efficiency and its ability to dissolve a broad range of bioactive compounds because of its polarity.

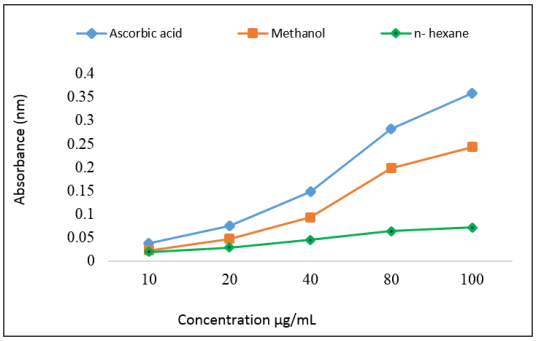

Fig. 2. Total antioxidant capacity of leaves extract of *G. procumbens*.

3.1.2.3. *Ferric reducing antioxidant power (FRAP)*

Among the various methods available, the FRAP assay is a widely adopted approach for assessing the protective antioxidant properties found in fruits, vegetables, and herbal medicines. The FRAP of *G. procumbens* leaf extract is shown in Table 3. The standard ascorbic acid whose value was 0.616 µg/mL. The value of FRAP for methanolic extracts of *G. procumbens* was 0.529 and n-hexane was 0.043 µg/mL (Fig. 3). Methanolic extracts of *G. procumbens* is shown the highest FRAP value than n-hexane. The results indicated significant variation in FRAP values depending on the solvent used for extracting *G. procumbens*.

The decreasing order of the standard and extracts:

Ascorbic acid > Metahnol > n-hexane

According to the current results, methanol demonstrated greater extraction efficiency compared with n-hexane.

The electron-donating capacity of antioxidants is frequently evaluated using the reducing power assay [31]. This reducing capacity serves as a critical marker for the

potential antioxidant activity of plant-derived compounds [32]. Consistent with this, prior research has established a strong linear correlation between the reducing potential and the overall antioxidant efficacy of various botanical extracts [33].

The root extract of *G. procumbens* showed superior ferric reducing ability relative to the other plant extracts. A similar pattern of ferric reducing capacity was observed in both plants, with values in order of root, stem, and leaf. A comparable pattern was observed for *G. procumbens* callus, where the reducing capacity followed an ascending sequence from leaf to stem, and finally to root callus. Overall, among all extracts that the leaf, stems, plants, and root consistently showed the highest ferric reducing antioxidant capacity.

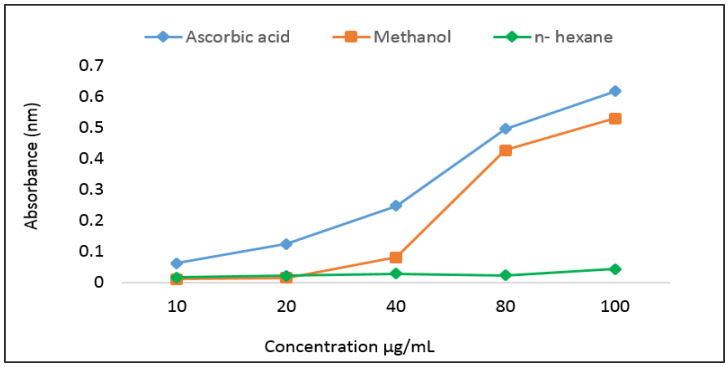

Fig. 3. Ferric reducing power capacity of leaves extract of *G. procumbens.*

Table 3. Determination of the ferric reducing capacity of the *G. procumbens* leaves extract of *G. procumbens*.

Name of the sample	Conc.(µg/mL)	Absorbance (nm) a	b	c	Absorbance (nm) Mean ± STD
Ascorbic acid (standard)	10	0.060	0.061	0.064	0.062 ± 0.002
	20	0.121	0.130	0.122	0.124± 0.005
	40	0.242	0.251	0.244	0.246 ± 0.005
	80	0.483	0.515	0.487	0.495 ± 0.017
	100	0.614	0.619	0.614	0.616 ± 0.003
Methanolic extract	10	0.010	0.011	0.012	0.011 ± 0.001
	20	0.013	0.014	0.015	0.014 ± 0.001
	40	0.114	0.115	0.013	0.080 ± 0.058
	80	0.428	0.427	0.425	0.426 ± 0.001
	100	0.507	0.551	0.530	0.529 ± 0.022
n-hexane	10	0.018	0.016	0.015	0.016 ± 0.001
	20	0.020	0.021	0.022	0.021 ± 0.001
	40	0.023	0.032	0.030	0.028± 0.004
	80	0.025	0.0025	0.020	0.023 ± 0.002
	100	0.045	0.040	0.044	0.043 ± 0.002

3.1.2.4. *DPPH free radical scavenging activity*

Following the initial assessment of yield and biological potency for the methanolic and n-hexane extracts, the DPPH free radical scavenging activities of various water–ethanol solvent ratios were evaluated, with results summarized in Table 4.

Table 4. Comparison of extraction yields and IC_{50} value of *G. procumbens* in different ratios of water and ethanol solvent.

Name of the solvent	Extraction yield (%) ± SD	IC_{50} (µg/mL) ± SD (DPPH)
100% ethanol	22.45 ± 1.02	56.68 ± 0.40
80% ethanol	14.80 ± 0.58	275.00 ± 0.50
60% ethanol	11.60 ± 0.43	355.00 ± 0.36
40% ethanol	15.55 ± 0.68	256.58 ± 0.17
20% ethanol	9.75 ± 0.66	398.98 ± 0.10
0% ethanol	8.45 ± 1.17	565.19 ± 0.38

Data are expressed as the mean ± standard deviation (n = 3). Within each column, means followed by distinct superscript letters indicate statistically significant differences ($p < 0.05$).

The ethanol extract of *G. procumbens* showed the highest extraction yield (22.45 ± 1.02) %, whereas the water extract had the lowest yield (8.45 ± 1.17)%. The extraction yields followed the order: 100 % ethanol > 80 % ethanol > 60 % ethanol > 40 % ethanol > 20 % ethanol > 0 % ethanol, reliable with prior reports by Javadi *et al*. [9] and Sultana *et al*. [21].

In this study, IC_{50} value of ethanol-water ratio was determined at several concentrations (50, 100, 200, 400 and 800 µg/mL). Hence, 100 % ethanol-water ratio has exhibited highest IC_{50} value, and 0% ethanol-water ratio showed the lowest IC_{50} value. On the other hand, 40% ethanol water ratio showed better result than 80 % ethanol-water ratio. Several recent studies have reported lower DPPH free radical scavenging activity in aqueous extracts at various ratios, consistent with the findings of this study on medicinal plants [9,21,22].

Inhibitory capacity was quantified via IC_{50} values for six solvent ratios (0–100% ethanol). The *G. procumbens* extracts reached IC_{50} values as low as (56.68 ± 0.40) µg/mL, significantly outperforming the Acarbose standard (617.5 ± 0.61) µg/mL). The efficacy of the extracts was ranked as follows: 100 % ethanol > 40 % ethanol > 80 % ethanol > 60 % ethanol > 20 % ethanol > water. Consistent with literature [9,24,25], the water-based extract showed the weakest performance, confirming that higher ethanol concentrations generally enhance the recovery of bioactive inhibitors.

This observation aligns with earlier reports, where water–organic solvent mixtures (e.g., water and ethanol) were found to optimize extraction by dissolving both polar and less polar compounds, thereby facilitating the isolation of antioxidant constituents [25–30].

This study demonstrated that the binary solvent system (100 %–0 %) exhibited greater radical scavenging activity than single solvents, consistent with the findings of Lim *et al*. [33] and showed correlation between antioxidant and antidiabetic properties that was reported previously [23,34-37].

3.2. *Identification of compounds*

3.2.1. *Phytochemical analysis*

The qualitative phytochemical profiles of *G. procumbens* methanolic and n-hexane extracts are summarized in Table 5. While both extracts tested positive for tannins, steroids, and glycosides, alkaloids were notably absent from both. Significant differences were observed in the distribution of other secondary metabolites; specifically, saponins, phenols, and flavonoids were identified exclusively in the methanolic extract and remained undetected in the n-hexane fraction. This enriched phytochemical profile likely accounts for the superior antioxidant activity of the methanolic extract, a conclusion supported by the work of Kulkula *et al*. [38]. These results align with previous literature highlighting the role of phenolics, flavonoids, and glycosides in the biological efficacy of *G. procumbens* [39].

Glycosides were detected in the crude extract and all fractions, whereas alkaloids were not identified in any of the tested samples. The crude extract and some of its partitions were found to contain saponins, tannins, steroids, and flavonoids, in agreement with earlier studies on this plant [40].

Table 5. Phytochemical analysis of the methanolic and n-hexene extracts of *G. procumbens.*

Phytochemical constituents	Name of test	Extract of methanol	Extract of n-hexene
Saponins	Frothing test	+++	-
Tannins	Lead acetate test	+++	+++
Glycosides	General test	+++	++
	Molisch's test	+++	++
Steroids	Libermann-Burchard's test	+++	++
	Salkowski test	+++	+++
Alkaloids	Color test	-	-
	Hager's test	-	-
Phenols	General test	++	-
Flavonoids	Shinoda test	++	-

3.2.2. *Identification of compounds by GC-MS*

Bioactive antioxidant compounds in *G. procumbens* were detected by GC-MS investigation. The analysis was carried out on *G. procumbens* leaves extracts such as methanol, n-hexane and ethanol-water (0 %-100 %) ratio. GC-MS chromatogram of *G. procumbens* for the spectral pattern of recognised metabolites is shown in Fig. 4. Methanolic extract *G. procumbens* contained six significant compounds and these are listed with name of the tentative compounds in Table 6.

Phenolic compounds, hexadecanoic acid, 3,7,11,15-tetramethyl-2-hexadecen-1-ol**,** 9-octadecadienoic acid, squalene, and 9-borabicyclo[3.3.1] nonane were identified as significant compounds with the retention times (RT) are 3.817, 29.865, 33.985,45.482, 45.971, and 48.745, respectively.

Table 6. The compounds contained in methanol extract by using GC-MS of *G. procumbens*.

No.	Tentative compounds	RT (min)	m/z	Conc. (%)
1	Phenolic compounds	3.817	94.00	6.316
2	Hexadecanoic acid, methyl ester	29.865	74.00	6.299
3	3,7,11,15-tetramethyl-2-hexadecen-1-ol	33.985	71.00	4.685
4	9-octadecadienoic acid	45.482	59.00	20.851
5	Squalene	45.971	69.00	61.117
6	9-borabicyclo[3.3.1] nonane, 9-boromo-	48.745	70.00	0.253

GC-MS chromatogram of *G. procumbens* from leaves extracts in n-hexene for the spectral pattern of recognised metabolites is shown in Fig. 5. A total of twenty significant compounds were detected from n-hexane extract of *G. procumbens* by GC-MS and these components are listed in Table 7. The RT of the significant compounds of trans-beta-Ionone (flavonoids), hexadecanoic acid-methyl ester, methyl 18-methylnonadecanoate, 1, 6, 10, 14, 18, 22-tetracosahexaen-3-ol, 2,6,10 is 15.568 min, 29.865 min, 38.20 min, and 47.508 min, respectively, which were reported to having antioxidants, antibacterial and antifungal activity [39]. Stigmasterol had a retention time of 53.935 min and is used for thyroid inhibitory, antioxidant activity, anti-diabetic, and anticancer activity [41].

Table 7. The compounds contained in n-hexane extract by using GC-MS of *G. procumbens*.

No.	Tentative Compounds	RT (min)	m/z	Conc. (%)
1	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4-trimethyl-, (R)-	14.772	111.00	0.499
2	trans-beta-Ionone	15.568	177.00	0.310
3	2-Methoxy-4-methyl-bicyclo[3.2.1]oct-2-ene	16.836	111.00	1.773
4	2H-Tetrazole, 2-(1,3-dioxolan-4 methyl)	29.684	87.00	0.634
5	Hexadecanoic acid, methyl ester	29.865	74.00	6.299
6	9,12-Octadecadienoic acid, methyl ester	33.637	67.00	4.575
7	9,12,15-Octadecatrienoic acid, methyl ester, Z, Z)-	33.758	79.00	7.216
8	Phytol	34.009	71.00	9.635
9	Octadecanoic acid, methyl ester, methyl- (E,E)-	35.453	69.00	1.67
10	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	36.896	57.00	0.408
11	Icosapent	38.142	91.00	0.398
12	Methyl 18-methylnonadecanoate	38.202	74.00	0.329
13	1,2-Benzenedicarboxylic acid, diisooctyl ester	41.641	149.00	0.433
14	Squalene	45.992	69.00	54.333
15	2-Methylhexacosane	47.236	57.00	0.581
16	1,6,10,14,18,22 Tetracosahexaen-3-ol, 2,6,10,15 methyl-(E,E)-	47.508	69.00	2.829
17	(2E,6E,10E)-3,7,11,15-Tetramethylhexadecanoic,10,14-tetraen-1-yl formate	47.657	69.00	1.141
18	1,6,10,14,18,22Tetracosahexaen-3ol,2,6,10,19,23hexamethyl	48.563	69.00	0.471
19	alpha-Tocopherolquinone	51.344	165.00	0.989
20	Stigmasterol	53.935	55.00	0.765

GC-MS chromatogram of *G. procumbens* from leaves extracts in 100 % ethanol for the spectral pattern of recognised metabolites is shown in Fig. 6. In Table 8, GC-MS chromatogram of 100 % ethanolic extract of *G. procumbens* is revealed total 9 compounds. Hexadecanoic acid having a retention time of 29.85 min may be responsible for antioxidant, antibacterial, and antifungal activity. Methyl 8, 11, 14-heptadecatrienoate having the retention time of 33.74 min is used for antioxidant activity. Squalene having the retention time of 45.97 min reported for inhibition activity, antioxidant activity. In this study, the compounds stigmasterol have the retention time of 53.935 min and is responsible for anti-diabetic activity, thyroid inhibitory, antioxidant activity, free radical scavenging activity and anticancer activity by Zawistowski *et al.* [42] which has been already reported.

Table 8: The compounds contain 100 % ethanol extract by using GC-MS of *G. procumbens*.

No.	Tentative Compounds	RT (min)	m/z	Conc. (%)
1	Trisiloxane, 1,1,1,5,5,5-hexamethyl 3[trimethyl-silyl	6.844	-	2.77
2	Hexadecanoic acid, methyl ester	29.858	-	16.157
3	9,12-Octadecadienoic acid, methyl ester	33.629	-	7.328
4	9,12,15-Octadecatrienoic acid, methyl ester,Z,Z)-	33.758	-	7.216
5	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	33.991	-	4.201
6	Tetradecanoic acid, 12-methyl-, methyl ester	34.362	-	1.202
7	Di-n-octyl phthalate	41.635	-	2.734
8	13-decosenamide, (Z)-	45.498	-	21.779
9	Squalene	45.992	-	29.841

GC-MS chromatogram of *G. procumbens* from leaves extracts in 0% ethanol for the spectral pattern of recognised metabolites is shown in Fig. 7. In Table 9, GC-MS chromatogram of 0 % ethanolic extract of *G. procumbens* presents total 10 significant compounds. 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- having the retention time of 7.111, hexadecanoic acid having the retention time of 29.871 min may be accountable for antioxidant activity. Methyl 1,8,11,14-heptadecatetraene, (Z, Z, Z)-having the retention time of 33.755 min was reported for antioxidant activity [43].

Table 9. The compounds contain 0 % ethanol extract by using GC-MS of *G. procumbens*.

No.	Tentative Compounds	RT (min)	m/z	Conc. (%)
1	3-methoxy-2,2-dimethyloxiran	3.816	59.00	1.351
2	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	7.111	144.00	9.708
3	Hexadecanoic acid, methyl ester	29.871	74.00	6.329
4	Dibutyl phthalate	30.495	149.00	6.329
5	1,8,11,14-heptadecatetraene, (Z,Z,Z)-	33.755	79.00	1.835
6	9-octadecanoic acid (Z)-, methyl ester	33.799	55.00	1.082
7	Oleanitrile	40.998	55.00	1.967
8	1.2-benzenedicarboxilic acid, Diisooctyl ester	41.649	149.00	5.855
9	13-decosenamide, (Z)-	45.506	59.00	42.707
10	Squalene	45.985	69.00	26.737

From GC-MS, it is shown that hexadecanoic acid, 9,12-octadecadienoic acid, 9,12,15-octadecatrienoic acid, (Z, Z)-Phytol and stigmasterol respectively as a major part. The presence of phytol may be accountable for antioxidants and antidiabetic activity.

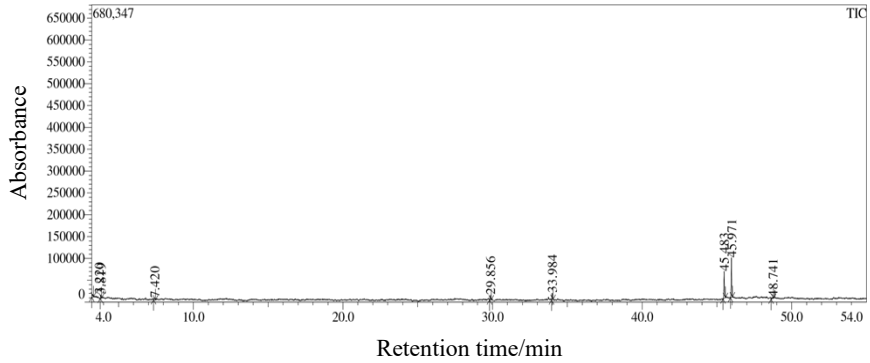

Fig. 4. GC-MS chromatogram of *G. procumbens* from leaves extracts in methanol.

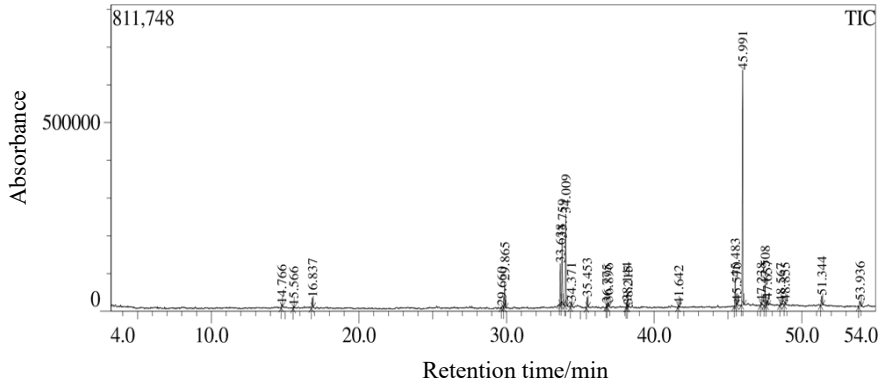

Fig. 5. GC-MS chromatogram of *G. procumbens* from leaves extracts in n-hexene.

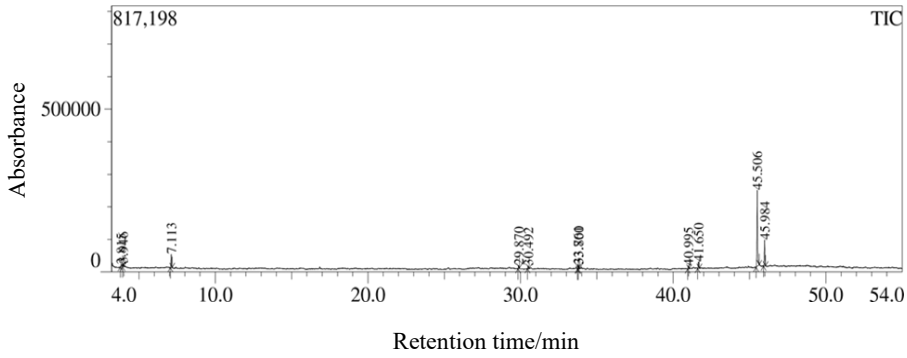

Fig. 6. GC-MS chromatogram of *G. procumbens* from leaves extracts in 100 % ethanol.

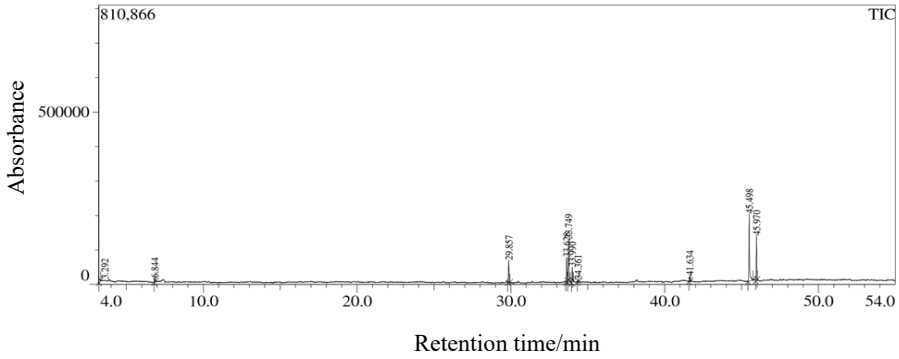

Fig. 7. GC-MS chromatogram of *G. procumbens* from leaves extracts in 0 % ethanol.

3.2.3. *FTIR analysis*

The FTIR analysis of methanolic, n-hexane and ethanolic extracts were illustrated in Fig. 8-11, and data listed out in Tables 10 to 12.

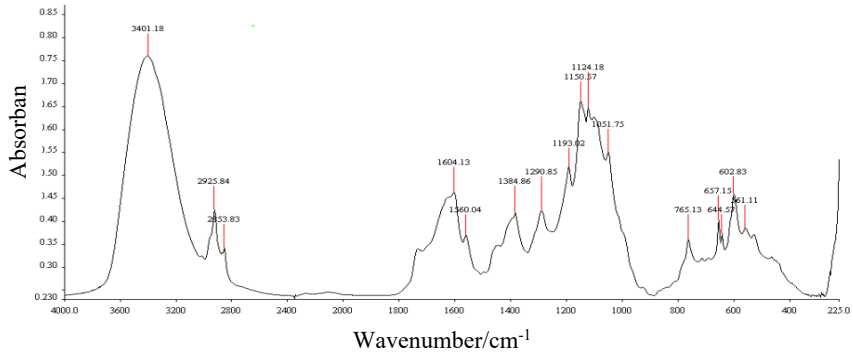

Fig. 8. FTIR spectra of methanolic extract of *G. procumbens*.

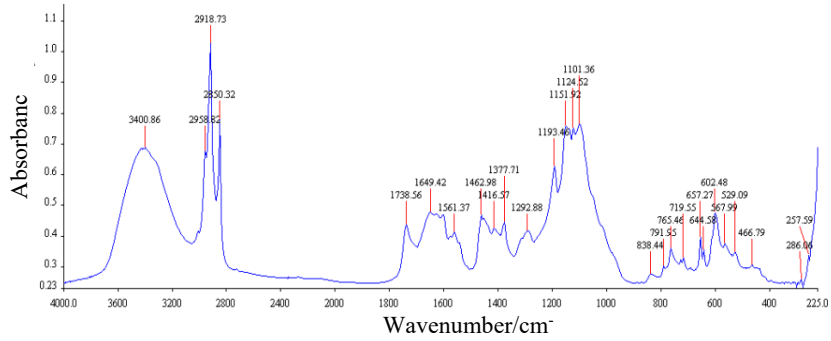

Fig. 9. FTIR spectra of n-hexane extract of *G. procumbens*.

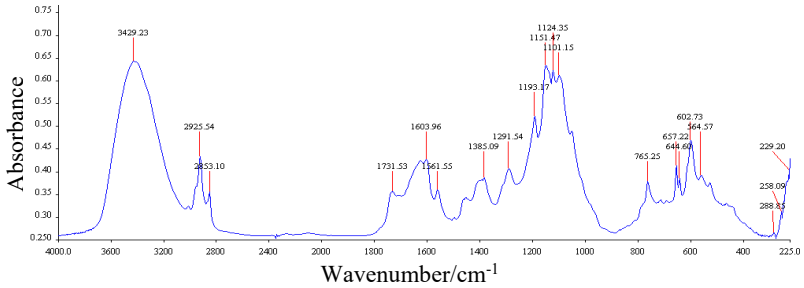

Fig. 10. FTIR spectra of 100 % ethanolic extract of *G. procumbens*.

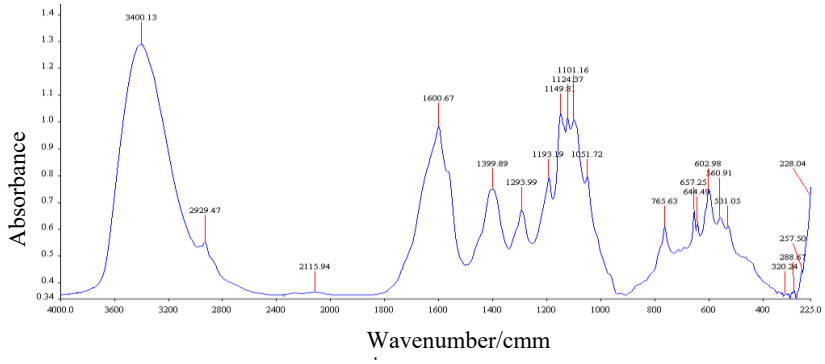

Fig. 11. FTIR spectra of 0 % ethanolic extract (water extract) of *G. procumbens*.

Table 10. Functional groups and modes of vibration in methanol extracts of *G. procumbens*.

Wavenumber (cm^{-1})	Functional group	Vibration mode
3401	O-H (Alcohol)	Stretching
2925	C-H (Alkene)	Stretching
2853	-C-H (Aldehyde)	Stretching
1604	C=C (Alkene)	Stretching
1560	N-H (primary and secondary amines and amides)	Bending
1384	S=O (SO_2), C=X, N=O (NO_2)	Stretching
1290	C-O (Alcohols, ethers, esters, carboxylic acids, anhydrides)	Stretching
1193-1051	C=X, S=O (SO_2), C=N (NH_2), C=O (OH, COOH)	Stretching

According to Table 10 and Fig. 8, the methanolic extract of *G. procumbens* leaves exhibits a broad, intense absorption band at 3401 cm^{-1}, characteristic of O-H stretching in alcohols and phenols involved in hydrogen bonding. Peaks identified at 2925 cm^{-1} and 2853 cm^{-1} are attributed to sp^3 and sp^2 C-H stretching vibrations from methyl and aldehyde groups, respectively. Additionally, the absorption at 1604 cm^{-1} signifies C=C stretching, while the 1560 cm^{-1} peak corresponds to the bending vibrations of primary and secondary amides. Finally, the range of 1193-1051 cm^{-1} encompasses S=O vibrations (sulfoxides), C-O stretching (alcohols and carboxylic acids), and C-N vibrations (amines).

Table 11. Functional groups and modes of vibration in n-hexane extracts of *G. procumbens*.

Wavenumber (cm^{-1})	Functional group	Modes of vibration
3429	O-H (Alcohol)	Stretching
2925	-C-(CH_2)	Stretching
1731	C=O (CHO, $COOCH_3$), Carbonyl (C=O)	Stretching
1603	N=H (NH_2), C=C (alkene)	Stretching, Bending
1291	C-O (Aromatic ester)	Stretching
1385	O-H (Phenol)	Bending
(1193-1101)	C=X, S=O (SO_2), C=N (NH_2), C=O (OH, COOH)	Stretching
(765-564)	C=X (halogen) Vibration of halogen & out of plane vibration of di-substituted aromatics	Stretching

The absorption bands of FTIR spectra (Fig. 9) were examined in bio-reduction procedure detected in the region between 400-4000 cm^{-1} and the bands are 3429, 2925, 1731, 1604, 1385, 765 and 564 cm^{-1}, respectively (Table 11). Major absorption observed at 3400 cm^{-1} indicates –O–H stretching associated with alcoholic hydroxyl groups. Based on the analysis, the principal functional group identified in *G. procumbens* leaf extract is –O–H, characteristic of alcohols. The methanol extract also exhibited the presence of other functional groups. However, the methanolic extract of *G. procumbens* exhibited the highest biological activities, including antioxidant and antidiabetic effects.

Table 12. Functional groups and modes of vibration in 100 % and 0 % ethanol extract of *G. procumbens*.

Wavenumber (cm^{-1})	Functional group	Modes of vibration
3429	O-H (Alcohol)	Stretching
2925	-C-(CH_2)	Stretching
1731	C=O (CHO, COOCH3) Carbonyl (C=O)	Stretching
1603	N=H (NH_2), C=C (alkene)	Stretching, Bending
1291	C-O (Aromatic ester)	Stretching
1385	O-H (Phenol)	Bending
(1193-1101)	C=X, S=O (SO_2), C=N (NH_2), C=O (OH, COOH)	Stretching
(765-564)	C=X (halogen) Vibration of halogen & out of plane vibration of di-substituted aromatics.	Stretching

The FTIR spectra for the 100 % and 0 % (aqueous) ethanol extracts are illustrated in Figs. 10 and 11, with their respective functional groups detailed in Table 12. By analyzing characteristic absorption peaks, the bioactive constituents within the extracts were identified. This spectroscopic evaluation confirmed the presence of several key chemical functionalities, including alcohols, aldehydes, alkynes, and alkenes, as well as amines and esters.

The chemical constituents of the *G. procumbens* ethanolic extract were further elucidated through FTIR spectroscopy, which identified various moieties such as alcohols, phenols, and aromatics. The presence of alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, nitro compounds, and amines was also established, consistent with previous reports [44].

The identified compounds predominantly consisted of polyhydroxy compounds, triterpenes, and organic acids. The identified polyhydroxy constituents likely play a significant role in the observed biological effects. The inhibitory potential of polyhydroxy compounds has led to widespread use in foods and nutraceuticals [44,45]. These biological activities generally depend on the structure of the chemical compound, the number and position of hydroxyl groups, and the nature of the substituents [45]. Several studies have reported methyl methyl 1,8,11,14-heptadecatetraene, stigmasterol as potential inhibitors against the α-amylase enzyme and antioxidant activity [42,43]. However, the involvement of additional compounds in mediating α-amylase inhibition and antioxidant activity has not yet been explored. It is possible that these constituents operate through a unique pathway to potentiate the activity.

The enzyme inhibitory and antioxidant properties of the compound are probably due to the presence of hydroxyl groups in its chemical framework. There are currently no reports describing the in vitro enzyme inhibitory or antioxidant activities of compounds such as hexadecanoic acid, 9,12-octadecadienoic acid, 9,12,15-octadecatrienoic acid (Z,Z,Z), phytol, icosapent, squalene, and stigmasterol. Various studies have shown that natural medicines exert antidiabetic effects via diverse mechanisms. Currently available anti-diabetic drugs exert their activity through various mechanisms. One such mechanism involves the regulation of postprandial blood glucose levels. Plants have been reported to reduce glucose absorption by inhibiting key digestive enzymes, such as α-amylase and α-glucosidase, in the pancreas and intestine, thereby preventing sharp postprandial increases in blood glucose levels [46–50]. The inhibitory activity against these enzymes is often linked to antioxidant phytochemicals such as flavonoids and phenolic compounds [51]. Phenolic compounds and flavonoids, as natural antioxidants, play a crucial role in preventing oxidative stress and may help delay the development and progression of diabetes mellitus [48]. In addition to their role in reducing oxidative stress, natural antioxidants can exert inhibitory activity against digestive enzymes [48,51]. Based on these results, we investigated the antioxidant properties of *G. procumbens* through the determination of TFC, TPC, DPPH radical scavenging activity, and α-amylase inhibition. Additional studies are required to confirm the efficacy of these compounds as inhibitors and to explore their potential use as therapeutic agents in managing hyperglycemia and oxidative damage. Taken together, the present findings justify additional research into *G. procumbens* as a potential antidiabetic agent mediated by α-amylase inhibitory and antioxidant effects. Individual evaluation of the identified metabolites may provide stronger evidence regarding their biological activity. This study may contribute to elucidating the pharmacological mechanisms underlying the development of functional foods, nutraceuticals, and medicinal formulations for diabetes and its associated complications.

4. Conclusion

In this study, the phytochemical profile of *G. procumbens* extracts was obtained via sonication at various ethanol–water ratios (0-100%) was characterized using a synergistic,

GC-MS, and FTIR approach. Among the samples, the 100 % ethanolic extract demonstrated the most pronounced free radical scavenging activity. Key bioactive compounds, including hexadecanoic acid, 9,12-octadecadienoic acid, 9,12,15-octadecatrienoic acid (Z,Z,)-, phytol, icosapent, squalene, and stigmasterol, were identified as major contributors to both α-amylase inhibition and antioxidant effects. Ultimately, this research provides a scientific basis for the development of *G. procumbens* as a therapeutic agent. These findings support the advancement of functional foods and nutraceuticals aimed at managing diabetes and oxidative stress-related disorders.

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