

Phytochemical Compositions and *In vitro* Assessments of Antioxidant and Antidiabetic Potentials of Fractions from *Ehretia cymosa* Thonn.

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ABSTRACT

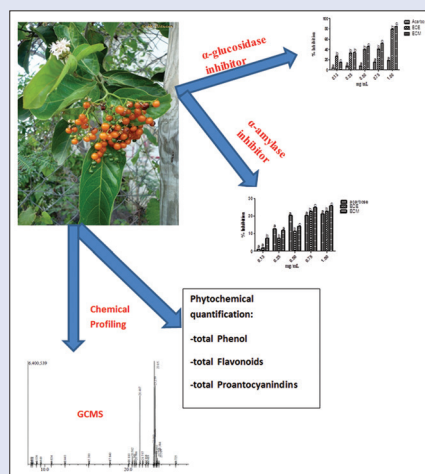
Background: *Ehretia cymosa* Thonn. is a popular medicinal plant used in different parts of West Africa for the treatment of various ailments including diabetes mellitus. **Objective:** The current study investigates bioactive constituents and *in vitro* antioxidant and antidiabetic potentials of fractions from extract of *E. cymosa*. **Materials and Methods:** Phytochemical investigation and antioxidant assays were carried out using standard procedures. Antidiabetic potential was assessed by evaluating the inhibitory effects of the fractions on the activities of α -amylase and α -glucosidase, while bioactive constituent's identification was carried out using gas chromatography-mass spectrometric (GC-MS) analysis. **Results:** The phytochemistry tests of the fractions revealed the presence of tannins, phenols, flavonoids, steroids, terpene, alkaloid, and cardiac glycosides. Methanol fraction shows higher phenolic (27.44 mg gallic acid/g) and flavonoid (235.31 mg quercetin/g) contents, while ethyl acetate fraction revealed higher proanthocyanidins (28.31 mg catechin/g). Methanol fraction displayed higher ($P < 0.05$) 1,1-diphenyl-2-picryl-hydrazyl (0.47 mg/mL), 2,2-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid (0.49 mg/mL), and hydroxyl radical (0.55 mg/mL) scavenging activities, while ethyl acetate exhibited strong metal chelating (0.61 mg/mL) and superoxide anion (1.68 mg/mL) scavenging activity. Methanol and ethyl acetate fractions displayed higher inhibition ($P < 0.05$) against α -glucosidase (0.60 mg/mL) and α -amylase (2.11 mg/mL), respectively. Methanol fraction also inhibited α -amylase and α -glucosidase in competitive and noncompetitive modes, respectively. The GC-MS chromatogram of the methanol fraction revealed 24 compounds, which include phytol (1.78%), stearic acid (1.02%), and 2-hexadecyloxirane (34.18%), which are known antidiabetic and antioxidant agents. **Conclusion:** The results indicate *E. cymosa* leaves as source of active phytochemicals with therapeutic potentials in the management of diabetes.

Key words: α -amylase, α -glucosidase, antioxidant, bioactive, diabetes, *Ehretia cymosa*

SUMMARY

- E. cymosa* fractions possess antioxidant and antidiabetic activities. Hence, it is a source of active phytochemicals with therapeutic potentials in the management of diabetes
- The high flavonoid, phenolic, and proanthocyanidin contents of fractions from *E. cymosa* also contribute to its antioxidant and antidiabetic properties

- Methanol fraction of *E. cymosa* displayed better antidiabetic activities compared to acarbose as revealed by their half maximal inhibitory concentration values
- Methanol fraction of *E. cymosa* extract contains phytol, hexadecyl oxirane, and stearic acid, which are reported to possess antidiabetic and antioxidant potentials.



Abbreviations used: ABTS: 2,2-Azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid, DPPH: 1,1-diphenyl-2-picryl-hydrazyl, PMS: Phenazine methosulfate, NBT: Nitroblue tetrazolium, NADH: Nicotinamide adenine dinucleotide, TCA: Trichloroacetic acid, TBA: Thiobarbituric acid, DNS: Dinitrosalicylic acid.

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INTRODUCTION

Diabetes mellitus is one of the common metabolic disorders with micro- and macro-vascular complications that result in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world.^[1,2] The disease results from dynamic interaction between defects in insulin secretion and insulin action. Such deficiency leads to increased concentrations of blood glucose, which in turn damage many of the body's systems, particularly the blood vessels. These disorders included retinopathy, nephropathy, neuropathy, and angiopathy.^[3] The current worldwide diabetic population is about 150 million, and this will be doubled by 2025.^[4] Africa is reported to have

the highest mortality due to the disease, with 4.3% prevalence rate.^[5] The disease is rapidly spreading in Africa today as a result of rapid and

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uncontrolled urbanization and westernization, unhealthy lifestyle, and dietary habits.^[6] In diabetes, the hyperglycemia generates reactive oxygen species (ROS) which in turn these species damage the cell membrane and cause lipid peroxidation, which leads to the secondary complications, such as heart attack, kidney failure, retinopathy, and nerve damage.^[7,8] Antioxidants have been shown to prevent the destruction of β -cells by inhibiting the peroxidation chain reaction, and thus, they may provide protection against the development of diabetes. Plants containing natural antioxidants (tannins, flavonoids, Vitamins C and E) can preserve β -cells function and prevent diabetes-induced ROS formation.^[9,10] One unique approach for decreasing postprandial hyperglycemia is to reduce or slow down dietary carbohydrate digestion. Inhibiting the enzymes involved, such as α -amylase and α -glucosidase enzymes, is a strong therapeutic goal of controlling the postprandial glycemic reaction.^[11-13] The inhibitors currently in the clinical use include acarbose, miglitol, and voglibose, which are known to inhibit wide range of glycosidases such as α -glucosidase and α -amylase. However, these hypoglycemic agents have serious side effects which include bloating, abdominal discomfort, diarrhea, and flatulence.^[14,15] Therefore, there is a need to search for new potent hypoglycemic agents with no or less side effects from natural source. Plant extracts have long been used for ethnomedicinal treatment of diabetes in various systems of medicine and are currently accepted as an alternative for diabetic therapy.^[16]

Ehretia cymosa (*Boraginaceae*) is a deciduous shrub or small tree that grows up to 7 m tall; it is commonly found in the Savanna and secondary jungle of West Africa which include Cameroon, Ghana, Gabon, Congo, and Nigeria. The leaves are ovates, while the fruits are black, ovoid to globose drupe of 2–6 mm long. It possesses opious terminal panicles of white flowers.^[17,18] The plant is locally called “Jaoko” among the Yoruba ethnic group of the Southwest Nigeria, it is known as “Lauso” among Akye tribe of Ivory Coast, and the plant is also referred to as “Okosua” by the Akan-Twi tribe from Ghana.^[19] The leaves of the plant are being used among the people of Southwestern Nigeria for the treatment of measles.^[20] The leaves have also been reported to be used as febrifuge, laxative, and painkiller, for paralysis, epilepsy, convulsions, and spasm.^[19] *E. cymosa* is used in traditional folklore medicine for the treatment of diarrhea.^[21] The use of the plant for the control of venereal diseases,^[22] epilepsy, dry cough, malaria, tonsils, mental problems, asthma, typhoid, wounds, and aphrodisiac has also been reported.^[23,24]

Sarkodie *et al.*^[17] reported the antibacterial activity of the ethanol extract from *E. cymosa* plant on both Gram-negative and Gram-positive bacteria. Furthermore, there is anecdotal report on the usage of the plant in the management of diabetes among the Akans and Ewe ethnics group in Ghana.^[17] Sarkodie *et al.*^[17] also reported the antioxidant activities and *in vivo* assessment of Antihyperglycemic effect of *E. cymosa* on Sprague–Dawley male rats. The report of previous phytochemical screening of *E. cymosa* revealed the presence alkaloids, saponins, glycosides, terpenoids, anthraquinones, phenolics, and flavonoids.^[24] However, to the best of our knowledge, there is no information on the *in vitro* assessment of *E. cymosa* inhibitory potentials on carbohydrate metabolizing enzymes, which is a convenient therapeutic approach for decreasing postprandial hyperglycemia. Hence, the present study sought to unravel this by investigating its carbohydrate metabolizing enzyme kinetics through *in vitro* α -amylase and α -glucosidase inhibitory models. In addition, *in vitro* antioxidant activities and phytochemical constituents of the plant were also examined to confirm the factors and agents that might be responsible for the possible antidiabetic activity.

MATERIALS AND METHODS

Fresh leaves of *E. cymosa* were collected from a location in Amuloko area, Ibadan, Oyo state, Nigeria, in May 2015. The plant was authenticated

at the Herbarium of the Forestry Research Institute of Nigeria, Ibadan, Nigeria, where a voucher specimen FHI 110188 was deposited for future reference. The leaves were air-dried and pulverized. The plant material was then hermetically sealed in a plastic bag and stored at room temperature until the time of the extraction.

Preparation of plant extract and fractions

The pulverized plant material (500 g) was subjected to cold extraction by percolation for 1 week using 2.0 L of 99% ethanol. The extract was filtered and concentrated to dryness using a rotary evaporator at 35°C to yield 126.34 g ethanol crude extract (25.268% w/w of dry plant material). The ethanol crude extract (126.34 g) was then macerated using a Coor porcelain mortar and pestle (Aldrich and Sigma, Germany) with ethyl acetate (3 mL \times 100 mL) and methanol (3 mL \times 100 mL) successively, to ensure the fractionation of the relatively polar and polar constituents, respectively. Each of the resulting fractions was separately concentrated to dryness using rotary evaporator at 35°C to give ethyl acetate (35.46 g) and methanol (53.95 g) fractions. The respective fractions were weighed and kept inside labeled sample bottle and stored in the refrigerator.

Chemicals and reagents

Silymarin, gallic acid, rutin, quercetin, catechin, 2,2-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric chloride, potassium ferricyanide, porcine pancreatic α -amylase, rat intestinal α -glucosidase, and p-nitrophenyl- α -D-glucopyranoside (pNPG) were procured from Sigma Chemical Co., St. Louis, Missouri, USA. Starch, dinitrosalicylic acid (DNS), and acarbose were purchased from Bayer Medical Co. (Germany). Distilled water was obtained from the Phytomedicine and Phytopharmacology Research Group Laboratory, Plant Sciences Department, QwaQwa Campus, South Africa. All other chemicals and reagents used were of analytical grade.

Phytochemical analysis

Phytochemical screening for different compounds

Chemical tests for the screening and identification of secondary metabolites present in the fractions of *E. cymosa* were carried for flavonoids, alkaloids, cardiac glycosides, steroids phenols terpenoids, saponins, tannins, and anthraquinones, using standard procedures.^[25-27]

Assessment of total phenolic content

The quantification of phenolic content of *E. cymosa* fractions was carried out using the procedure previously reported.^[28] An aliquot of the fractions (1 mL) was mixed with 5 mL Folin–Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 mL (75 g/L) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 30 min at 40°C for color development. Absorbance was read at 765 nm using a spectrophotometer (Beckman, DU 7400, USA). Fractions were evaluated at a final concentration of 1 mg/mL. Gallic acid was used as standard. Total phenolic content was expressed as mg/g gallic acid equivalent using the equation obtained from a calibration curve of gallic acid.

Determination of total flavonoids

Total flavonoids were determined using the method already adopted.^[29] Briefly, to 0.5 mL of the sample and standard (1 mg/mL), 2 mL distilled water was added followed by 0.15 mL of 5% NaNO₂ and allowed to stand at 25°C for 5–6 min. 0.15 mL of 10% AlCl₃ was added and allowed to stand for another 6 min. After which 1 mL of 4% NaOH was added to the mixture and make up to 5 mL with distilled water, the reaction mixture was vortexed for 15 min and color change was observed. The absorbance was measured at 420 nm using the spectrophotometer.

Total flavonoid contents were calculated as quercetin (mg/g) equivalent using the equation obtained from a calibration curve of quercetin.

Determination of total proanthocyanidins

Total proanthocyanidins of the fractions from *E. cymosa* were estimated using a reported method.^[30] A volume of 0.5 mL of 1 mg/mL fractions solution was mixed with 3 mL of 4% vanillin-methanol solution and 1.5 mL hydrochloric acid (HCl). The mixture was allowed to stand for 15 min before the absorbance was measured at 500 nm. Total proanthocyanidin contents were expressed as catechin equivalents (mg/g) using the equation obtained from a calibration curve of proanthocyanidin.

In vitro antioxidant assay

2,2-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid radical determination

The ability of the fractions to scavenge ABTS cation chromophore obtained from the oxidation of ABTS solution and potassium persulfate was determined according to the already adopted method.^[31] Briefly, 50 mL each of 7 mmol/L aqueous ABTS and 2.45 mM potassium persulfate were prepared and allowed to react in the dark for 16 h. The mixture was then adjusted with ethanol to 0.700 at 734 nm using a microplate reader (Bio-Rad, Model 680, Japan). 20 μ L aliquot was then mixed with 200 μ L ABTS solution in a 96-well microtiter plate, and absorbance was read at 734 nm using the microplate reader (BIO-RAD, Model 680, Japan) after 15 min of incubation. The percentage inhibition activity was obtained using $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard.

The half maximal inhibitory concentration (IC_{50}) value was calculated and obtained from the linear regression equation using $y = mx + c$, where y is the percentage activity and equals 50, m is the slope, c is the intercept, and x is the IC_{50} value.

Metal chelating assay

The chelating of ferrous ions by the fractions from *E. cymosa* was estimated as previously described.^[32] Briefly, 40 μ L of the different concentrations of the fractions and standards (0.13–1.00 mg/mL) was taken and emptied into a 96-well microtiter plate; 200 μ L of 2 mM $FeCl_2$ solution was afterward added to the mixture. The reaction was initiated by the addition of 80 μ L 5 mmol/L ferrozine and the mixture was shaken vigorously and left standing at room temperature for 10 min. The absorbance of the solution was then read using the Bio-Rad (Model 680, Japan) microplate reader at 562 nm. The percentage of inhibition of ferrozine- Fe^{2+} complex formation was calculated by $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard. The IC_{50} value was calculated and obtained from the linear regression equation using $y = mx + c$, where y is the percentage activity and equals 50, m is the slope, c is the intercept, and x is the IC_{50} value.

Superoxide anion scavenging assay

Measurement of superoxide anion scavenging activity of the various extracts was based on the method described by Liu *et al.*^[33] Superoxide radicals were generated in 50 mL of Tris-HCl buffer (16 mM, pH 8.0) containing 50 mL of nitroblue tetrazolium (50 mM) solution, 50 mL nicotinamide adenine dinucleotide (78 mM) solution, and different concentrations (0.13–1 mg/mL) of *E. cymosa* fractions. The reaction started by adding 1 mL of phenazine methosulfate (PMS) solution (10 mM) to the mixture. The reaction mixture was incubated at 25°C for 5 min, and the absorbance measured at 560 nm. Superoxide anion radical scavenging ability of a standard antioxidant was also tested by replacing the fractions with gallic acid (0.13–1 mg/mL). IC_{50} was then evaluated from calibration curve following estimation of percentage superoxide

anion scavenging capacity of the tested fractions from *E. cymosa* using the expression:

$$\text{Percentage scavenging (S\%)} = [(A_{\text{control}} - A_{\text{extract}})/A_{\text{control}}] \times 100,$$

where A_{control} is the absorbance of the control and A_{extract} is the absorbance of the standard. The IC_{50} value was obtained from the linear regression equation using $y = mx + c$, where y is the percentage activity and equals 50, m is the slope, c is the intercept, and x is the IC_{50} value.

Hydroxyl radical scavenging ability

The ability of the plant fractions to prevent Fe^{2+}/H_2O_2 -induced decomposition of deoxyribose was carried out using the modified method of Sindhu and Emilia-Abraham.^[34] Briefly, 100 μ L aliquots, 120 μ L of 20 mM deoxyribose, 400 μ L of 0.1 M phosphate buffer, 40 μ L of 20 mM hydrogen peroxide, and 40 μ L of 500 μ M ferrous sulfate each were taken and mixed in 2 mL Eppendorf tubes. Subsequently, 100 μ L of distilled water was added and incubated for 30 min at 37°C; 0.5 mL 2.8% trichloroacetic acid and 400 μ L 0.6% thiobarbituric acid solutions were added to stop the reaction. From the mixture, 300 μ L was transferred to a 96-well microtiter plate and absorbance was taken at 532 nm using a microplate reader (Bio-Rad, Model 680, Japan) after incubating in boiling water for 20 min. The percentage inhibition activity was obtained using $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the fraction/standard. The IC_{50} value was calculated and obtained from the linear regression equation using $y = mx + c$, where y is the percentage activity and equals 50, m is the slope, c is the intercept, and x is the IC_{50} value.

1,1-diphenyl-2-picrylhydrazyl radical scavenging activity

The free radical scavenging activity of the fractions from *E. cymosa*, based on the scavenging activity of the stable DPPH free radical, was determined by the method described by Braca *et al.*^[35] Briefly, 150 μ L of the varying concentration of plant fractions (0.13–1.00 mg/mL) was added separately to 150 μ L of 0.004% methanolic solution of DPPH in a 96-well microtiter plate. The absorbance at 517 nm was determined after 30 min using a 96-well microplate reader (Bio-Rad, Model 680, Japan), and the percentage inhibition activity was calculated using $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the fraction/standard. The IC_{50} value was calculated and obtained from the linear regression equation using $y = mx + c$, where y is the percentage activity and equals 50, m is the slope, c is the intercept, and x is the IC_{50} value.

In vitro antidiabetic assay

α -amylase inhibitory assay

This assay was carried out using a modified procedure.^[36] A total of 250 mL of each fractions (3.13–100 mg/mL) was placed in a test tube and 250 mL of 0.02 M sodium phosphate buffer (pH 6.9) containing α -amylase solution was added. This solution was preincubated at 25°C for 10 min; after that, 250 mL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals and then incubated at 25°C for 10 min. The reaction was terminated by adding 500 mL of DNS reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 5 mL distilled water and the absorbance was measured at 540 nm using the microplate reader (Bio-Rad, Model 680, Japan). The control was prepared using the same procedure replacing the fraction with distilled water while activity of the standard was tested by replacing the fractions with acarbose (3.13–1.00 mg/mL). The α -amylase inhibitory activity was calculated as percentage inhibition, thus;

$$\% \text{ Inhibition} = (\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}) / \text{Abs}_{\text{control}} \times 100$$

Concentrations of fractions resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically.

α-Glucosidase inhibitory assay

The effect of the plant fractions on *α*-glucosidase activity was determined according to the method described by Elsnoussi *et al.*^[37] In brief, different concentrations (0.13–1 mg/mL) of *E. cymosa* fractions were prepared in distilled water. Then, 50 mL from the stock solution was mixed with 100 mL of 0.1 M phosphate buffer (pH 6.9) containing 1.0 M of *α*-glucosidase solution. The mixtures were then incubated in 96-well plates at 25°C for 10 min. Following this, 50 mL of 5 mM p-nitrophenyl-*α*-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25°C for 5 min. The enzyme activities were determined by measuring the absorbance of the reaction mixtures at 405 nm. The control was prepared using the same procedure replacing the fraction with distilled water, while activity of the standard was tested by replacing the fractions with acarbose (0.13–1 mg/mL). The experiments were conducted in triplicate and the *α*-glucosidase inhibitory activity was expressed as % inhibition using the expression:

$$\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{extract}}) / A_{\text{control}}] \times 100,$$

where A_{control} and A_{extract} are the absorbances of the control and fractions, respectively. Concentrations of fractions resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically.

Mode of *α*-amylase inhibition

The methanol fraction of *E. cymosa* was selected to determine its mode of enzymatic inhibition because it exhibited good antidiabetic activities as compared to ethyl acetate fraction, due to its potent and mild inhibition of *α*-glucosidase and *α*-amylase, respectively.^[38] The experiment was conducted using a modified method.^[39,40] Briefly, 250 mL of the (100 mg/mL) methanol fraction of *E. cymosa* was preincubated with 250 mL of *α*-amylase solution for 10 min at 25°C in one set of tubes, while *α*-amylase was preincubated with 250 mL of phosphate buffer (pH 6.9) in another set of tubes. The reaction of the two sets of the mixtures was initiated by adding 250 mL of starch solution at increasing concentrations (0.31–5.00 mg/mL). The mixture was then incubated for 10 min at 25°C, followed by addition of DNS (500 mL). The reaction was terminated after boiling for 5 min. The amount of reducing sugars released was determined spectrophotometrically using maltose standard curve and converted to reaction velocities. A double-reciprocal (Lineweaver–Burk) plot (1/v versus 1/[S]), where v is reaction velocity and [S] is substrate concentration, was plotted to determine the mode of inhibition.

Mode of *α*-glucosidase inhibition

For the enzyme kinetics on inhibition of *α*-glucosidase activity by methanol fraction of *E. cymosa*, a modified method was adopted.^[41,42] Briefly, 50 μ L of 5 mg/mL methanol fraction was preincubated with 100 μ L of *α*-glucosidase solution for 10 min at 25°C in one set of tubes. In another set of tubes, *α*-glucosidase was preincubated with 50 μ L of phosphate buffer (pH 6.9). 50 mL of pNPG at concentrations (0.31 – 5.00 mg/mL) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 10 min at 25°C, and 500 mL of Na_2CO_3 was added to stop the reaction. The amount of reducing sugars released was determined colorimetrically using a p-nitrophenol standard curve. Reaction rates (v) were thereafter calculated and double reciprocal plots of enzyme kinetics were constructed according to Lineweaver and Burk method to study the nature of inhibition.^[43]

Gas chromatography-mass spectrometric analysis of *Ehretia cymosa* methanol fraction

The gas chromatography-mass spectrometric (GC-MS) analysis of the methanol fraction from *E. cymosa* was carried out using an Agilent

Technologies 6890 Series gas chromatograph coupled with an Agilent 5973 Mass Selective Detector, driven by Agilent Chemstation software. An eHP-5MS capillary column (Agilent Technologies, Stevens Creek Blvd, Santa Clara, USA) was used (30 m \times 0.25 mm internal diameter, 0.25 μ m film thickness). The carrier gas was ultrapure helium at a flow rate of 0.57 mL/min and a linear velocity of 27.5 cm/s. The injector temperature was set at 250°C. The initial oven temperature was at 50.0°C, which was programmed to increase to 250°C at the rate of 15°C/min with a hold time of 4 min at each increment. Injections of 1 μ L were made in the splitless mode with a split ratio of 20:1. The mass spectrometer was operated in the electron ionization mode at 70 eV and electron multiplier voltage at 1859 V. Other MS operating parameters were as follows: ion source temperature 230°C, quadrupole temperature 150°C, solvent delay 4 min, and scan range 50–700 amu. Identification of the constituents was achieved by comparing the retention times, mass spectral data, and fragmentation pattern of the unknown constituents of the sample analyzed with those from the National Institute of Standards and Technology and Wiley Libraries having more than 75,000 compounds. The name, molecular weight, structure, and relative percentage of each component were thereafter confirmed.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 Statistical Package (GraphPad Software, USA). The data were analyzed by one-way analysis of variance (ANOVA), followed by Bonferroni test. *In vitro* IC_{50} results were expressed as mean \pm standard error of mean for triplicate determinations. Free radical scavenging activities were expressed in percentage; phytochemicals quantification was expressed as mean \pm standard deviation. Statistical significance was considered at $P < 0.05$.

RESULTS

Qualitative and quantitative phytochemical analysis

The results of the phytochemical screening of the tested fractions from *E. cymosa* indicated that tannins, phenols, flavonoid, saponin, and steroids in both fractions alkaloids and cardiac glycosides were present only in methanol fraction while terpene was present in ethyl acetate fraction. However, anthraquinone and saponin were absent in two fractions. The detailed results of the phytochemical screening are presented in Table 1, while phytochemicals quantifications of phenols, flavonoids, and proanthocyanidins from *E. cymosa* fractions are displayed in Table 2. Methanol fraction displayed highest quantity of phenol (27.44 mg gallic acid/g) and flavonoid (235.31 mg quercetin/g), while the proanthocyanidins contents of ethyl acetate fraction (28.01 mg catechin/g) were higher as compared to that of methanol fraction.

Table 1: Phytochemicals of fractions from *Ehretia cymosa* leaves

Phytochemicals	ECE	ECM
Tannins	+	+
Phenols	+	+
Saponin	-	-
Flavonoids	+	+
Alkaloids	-	+
Terpenes	+	-
Anthraquinone	-	-
Steroids	+	+
Cardiac glycosides	-	+

+: Present; -: Absent; ECE: *Ehretia cymosa* ethyl acetate fraction; ECM: *Ehretia cymosa* methanol fraction

In vitro assay

Antioxidant assay

Figures 1-5 displayed the *in vitro* antioxidant potentials of ethyl acetate and methanol fractions from *E. cymosa* leaves. The percentage inhibitions of methanol fraction against ABTS [Figure 1] scavenging activities were significantly higher ($P < 0.05$) than the standards (silymarin and gallic acid) at all concentrations. It

Table 2: Total phenolic, flavonoid and proanthocyanidins contents of *Ehretia cymosa* leaves fraction

Phytochemicals	ECE	ECM
Total phenol (mg gallic acid/g)	25.27±0.002	27.44±0.001
Total flavonoid (mg quercetin/g)	221.44±0.003	235.31±0.003
Total proanthocyanidins (mg catechin/g)	28.01±0.002	18.08±0.003

Value were expressed per g of plant fraction and are means of triplicate determinations±SD, ECE: *Ehretia cymosa* ethyl acetate fraction; ECM: *Ehretia cymosa* methanol fraction; SD: Standard deviation

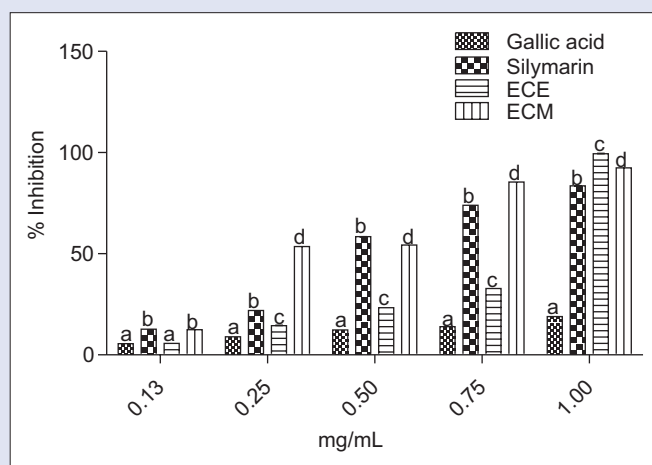


Figure 1: 2,2-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid radical scavenging effect of *Ehretia cymosa* leaves fractions. The values are expressed as means ± standard error of the mean of triplicate determinations. Bars not sharing a common letter at the same concentration are significantly different ($P < 0.05$). ECE: *Ehretia cymosa* ethyl acetate fraction, ECM: *Ehretia cymosa* methanol fraction

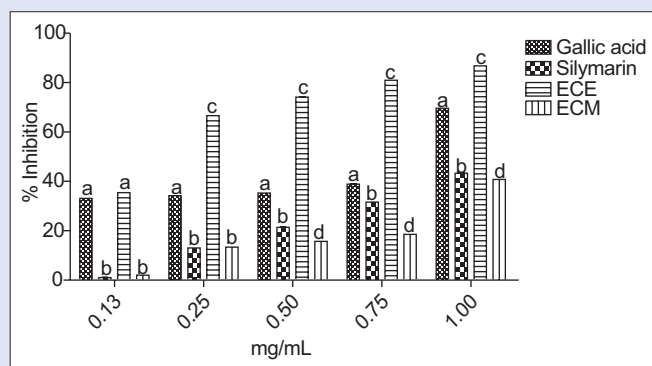


Figure 3: Metal chelating potential of *Ehretia cymosa* leaves fraction. The values are expressed as means ± standard error of the mean of triplicate determinations. Bars not sharing a common letter at the same concentration are significantly different ($P < 0.05$). ECE: *Ehretia cymosa* ethyl acetate fraction, ECM: *Ehretia cymosa* methanol fraction

was also significantly higher ($P < 0.05$) than ethyl acetate fraction at 0.25–0.75 mg/mL.

The percentage inhibition of methanol fraction was significantly higher ($P < 0.05$) than ethyl acetate and standards at lower concentration (0.13–0.50 mg/mL) for hydroxyl radical scavenging potential [Figure 2], while that of ethyl acetate was significantly higher ($P < 0.05$) than methanol and standards at higher concentration (1.0 mg/mL). There was no significant difference between the percentage inhibition of the two standards at 0.13 and 0.50–0.75 mg/mL [Figure 2].

The result of metal chelating potential of fractions from *E. cymosa* [Figure 3] revealed a significant higher ($P < 0.05$) percentage inhibition by ethyl acetate fraction compared to methanol and standards at all concentrations, except gallic acid, that show no significant difference at 0.13 mg/mL. Gallic acid was significantly higher ($P < 0.05$) than methanol fraction at all concentrations, while silymarin was significantly higher ($P < 0.05$) than methanol at higher concentration (0.5–1.00 mg/mL).

The ethyl acetate fraction displayed significantly higher ($P < 0.05$) percentage inhibition than methanol and the standards at all

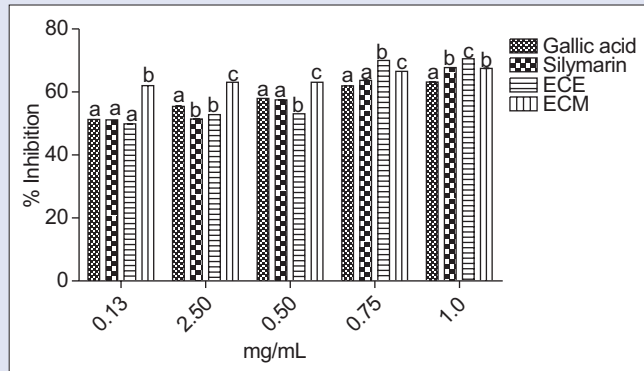


Figure 2: Hydroxyl (OH) radical scavenging effect of *Ehretia cymosa* leaves fraction. The values are expressed as means ± standard error of the mean of triplicate determinations. Bars not sharing a common letter at the same concentration are significantly different ($P < 0.05$). ECE: *Ehretia cymosa* ethyl acetate fraction, ECM: *Ehretia cymosa* methanol fraction

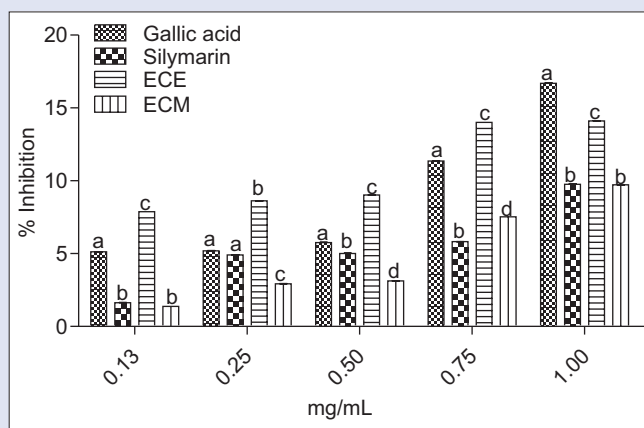


Figure 4: Superoxide anion radical scavenging effect of *Ehretia cymosa* leaves fractions. The values are expressed as means ± standard error of the mean of triplicate determinations. Bars not sharing a common letter at the same concentration are significantly different ($P < 0.05$). MBE: *Ehretia cymosa* ethyl acetate fraction, ECM: *Ehretia cymosa* methanol fraction

concentrations for superoxide anion scavenging activity [Figure 4], except gallic acid which was significantly higher ($P < 0.05$) than ethyl acetate at 1.0 mg/mL. Gallic acid exhibited higher percentage inhibition ($P < 0.05$) than methanol fraction and silymarin at all tested concentrations.

The DPPH radical scavenging activity [Figure 5] revealed that methanol fraction has significantly higher ($P < 0.05$) percentage inhibition than ethyl acetate fraction and the standards; ethyl acetate fraction also displayed significantly higher ($P < 0.05$) percentage inhibition than gallic acid and silymarin, while silymarin was significantly higher ($P < 0.05$) than gallic acid.

Table 3 displays the IC_{50} values of free radical scavenging and metal chelating potentials of *E. cymosa* fractions. Methanol fraction (0.61 mg/mL), for metal chelating potential, exhibited significantly lower ($P < 0.05$) IC_{50} than standards and ethyl acetate fraction. However, IC_{50} of silymarin (0.75 mg/mL) was significantly lower ($P < 0.05$) than that of gallic acid, while that of gallic acid (2.21 mg/mL) was significantly lower ($P < 0.05$) than ethyl acetate fraction for metal chelating activity. The result of DPPH radical scavenging potential revealed the IC_{50} of the gallic acid (0.47 mg/mL) to be significantly lower ($P < 0.05$) than the tested fractions and silymarin. Furthermore, the IC_{50} of ethyl acetate (0.56 mg/mL) and methanol (0.60 mg/mL) fractions for DPPH scavenging activity was significantly higher ($P < 0.05$) than silymarin. There was no significant difference between the IC_{50} of ethyl acetate and methanol fractions. Silymarin (0.09 mg/mL) displayed a significantly higher IC_{50} ($P < 0.05$) for hydroxyl radical scavenging activity than the tested fractions and gallic acid. The IC_{50} of gallic acid (1.06 mg/mL) for DPPH scavenging potential was also significantly higher ($P < 0.05$) than ethyl acetate and methanol fractions, while methanol fraction (0.55 mg/mL) was significantly lower ($P < 0.05$) than ethyl acetate fraction. Furthermore, ethyl acetate fraction (0.49 mg/mL) exhibited significantly lower ($P < 0.05$) IC_{50} value for superoxide anion than methanol fraction and the standards for ABTS scavenging activity; it also exhibited significantly lower ($P < 0.05$) IC_{50} than methanol and silymarin in the case of superoxide anion scavenging potentials with the IC_{50} value of 0.49 mg/mL.

In vitro enzyme kinetic inhibitory activity

The inhibitory activity of the ethyl acetate and methanol fraction from *E. cymosa* against α -amylase and α -glucosidase is shown in Figures 6 and 7, respectively. The results of enzymatic inhibitory

assay revealed that the inhibition activities of the *E. cymosa* fractions against α -amylase and α -glucosidase to be dose-dependent, strong inhibition were observed at the highest dose investigated, while minimum inhibitions were observed at the lowest dose of 0.13 mg/mL. Methanol and ethyl acetate fractions exhibited significantly higher ($P < 0.05$) percentage inhibition against α -amylase than acarbose (standard antidiabetic drug) at higher concentrations (0.75–1.00 mg/mL) [Figure 6]. The percentage inhibition of methanol fraction for α -amylase inhibition was significantly higher ($P < 0.05$) than ethyl acetate at all tested concentrations. However, acarbose was significantly higher ($P < 0.05$) than methanol and ethyl acetate fraction at lower concentrations (0.25–0.50 mg/mL).

The percentage inhibition of ethyl acetate and methanol fractions against α -glucosidase was significantly higher ($P < 0.05$) than acarbose at every tested dosage [Figure 7]. Methanol fraction was also significantly higher ($P < 0.05$) than ethyl acetate fraction for α -glucosidase inhibition at all concentrations, except at 0.13 mg/mL.

The IC_{50} of α -amylase and α -glucosidase inhibition by ethyl acetate and methanol fractions from *E. cymosa* is shown in Table 4. Methanol (0.60 mg/mL) fraction displayed significantly lower ($P < 0.05$) IC_{50} for α -glucosidase inhibition compared to ethyl acetate and acarbose. Furthermore, ethyl acetate (2.11 mg/mL) fraction exhibited significantly lower ($P < 0.05$) IC_{50} than methanol fraction and acarbose for α -amylase inhibition.

Table 3: Radical scavenging and metal chelating activities of *Ehretia cymosa* leaves fractions for *in vitro* antioxidant assays

Assay	Fractions (IC_{50} mg/mL)			
	Ethyl acetate	Methanol	Gallic acid	Silymarin
DPPH	0.56±0.05 ^a	0.47±0.02 ^a	2.09±0.01 ^b	1.60±0.03 ^c
Metal chelating	0.61±0.02 ^a	2.93±0.01 ^b	0.75±0.03 ^c	2.21±0.03 ^d
Hydroxyl radical	0.71±0.04 ^a	0.55±0.01 ^b	0.86±0.01 ^c	0.91±0.04 ^d
Superoxide anion	1.68±0.05 ^a	4.59±0.04 ^b	3.40±0.04 ^c	3.61±0.01 ^d
ABTS	0.65±0.02 ^a	0.49±0.05 ^b	3.23±0.05 ^c	0.60±0.02 ^d

Each value of IC_{50} obtained by linear regression equation is presented as mean±SEM (where $n=3$); Values with different superscripts in the same row for each parameter are significant ($P < 0.05$) to each other. DPPH: 1,1-Diphenyl-2-picrylhydrazyl; ABTS: 2,2-Azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid; SEM: Standard error of mean, IC_{50} : Half maximal inhibitory concentration

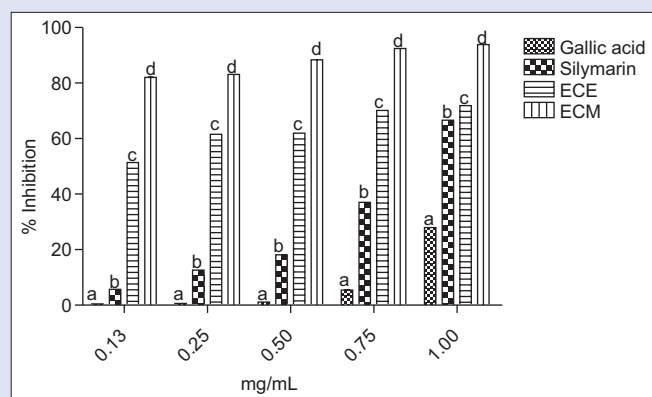


Figure 5: 1,1-diphenyl-2-picrylhydrazyl radical scavenging effect of *Ehretia cymosa* leaves fractions. The values are expressed as means ± standard error of the mean of triplicate determinations. Bars not sharing a common letter at the same concentration are significantly different ($P < 0.05$), ECE: *Ehretia cymosa* ethyl acetate fraction, ECM: *Ehretia cymosa* methanol fraction

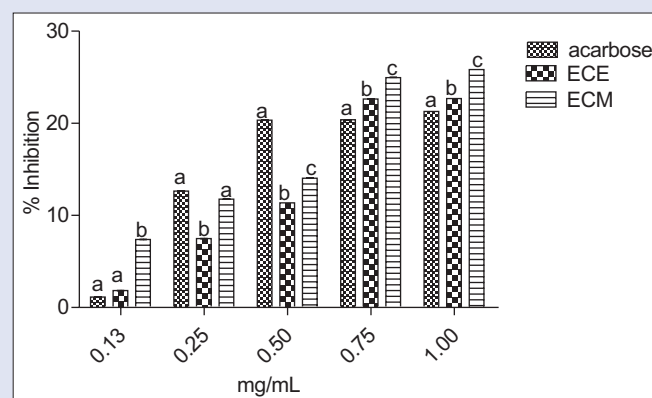


Figure 6: Inhibitory potency of *Ehretia cymosa* leaves fractions against α -amylase activity. The values are expressed as means ± standard error of the mean of triplicate determinations. Bars not sharing a common letter at the same concentration are significantly different ($P < 0.05$). ECE: *Ehretia cymosa* ethyl acetate fraction, ECM: *Ehretia cymosa* methanol fraction

Modes of α -glucosidase and α -amylase inhibition

Figures 8 and 9 display the Lineweaver–Burk double reciprocal which explains the mechanism of inhibition of α -glucosidase and α -amylase, respectively, by methanol fraction of *E. cymosa*. Methanol fraction

Table 4: IC₅₀ values of α -amylase and α -glucosidase inhibition by *Ehretia cymosa* leaves fractions

Extracts/control	Extracts (IC ₅₀ mg/mL)	
	α -amylase	α -glucosidase
Acarbose	2.26±0.05 ^a	3.02±0.02 ^a
Ethyl acetate	2.11±0.01 ^b	0.66±0.04 ^b
Methanol	2.75±0.03 ^c	0.60±0.01 ^b

Each value of IC₅₀ obtained by linear regression equation is presented as mean±SEM (where $n=3$); Values with different superscripts in the same row for each parameter are significant ($P<0.05$) to each other. SEM: Standard error of mean, IC₅₀: Half maximal inhibitory concentration

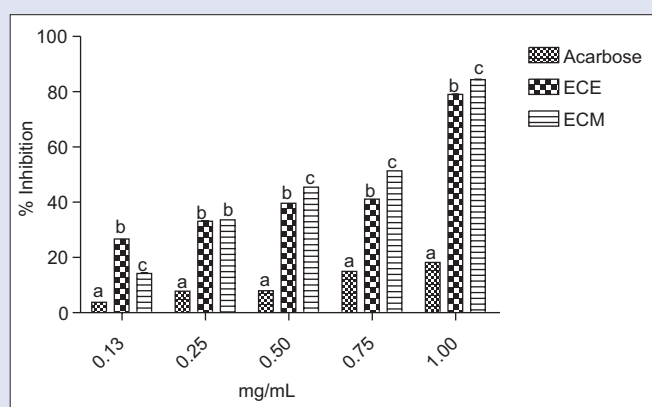


Figure 7: Inhibitory potency of *Ehretia cymosa* leaves fractions against α -glucosidase activity. The values are expressed as means \pm standard error of the mean of triplicate determinations. Bars not sharing a common letter at the same concentration are significantly different ($P < 0.05$), ECE: *Ehretia cymosa* ethyl acetate fraction, ECM: *Ehretia cymosa* methanol fraction

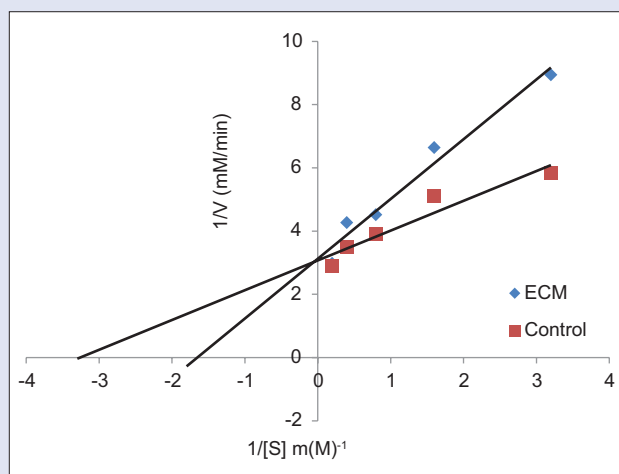


Figure 9: Lineweaver–Burk plot of *Ehretia cymosa* methanol fraction eliciting competitive inhibition on α -amylase activity. Results represent mean \pm standard error of the mean ($n = 3$); ($P < 0.05$). ECM: *Ehretia cymosa* methanol fraction

displayed competitive and noncompetitive modes of inhibition against α -amylase and α -glucosidase, respectively.

Result of gas chromatography-mass spectrometric analysis of methanol fraction from *E. cymosa*

Table 5 and Figure 10 show the identities of 24 compounds identified in the methanol fraction of *E. cymosa*, accounting for 99.9% of the total methanol fraction contents. The major constituents were 2-hexadecyl oxirane (34.18%), methyl linolenate (28.93%), methyl hexadecanoate (17.45%), and methyl linolelaidate (4.97%). Compounds present in a significant quantity include phytol (1.78%), stearic acid (1.02%), and 1-oxacyclopropyl-3,4-epoxycyclohexane (2.18%). Other compounds were present in a traceable amount ($<1\%$).

DISCUSSION

The leading cause of mortality in diabetics has been linked to oxidative stress,^[44] and antioxidants have been considered as treatments.^[45-48] Plants often contain substantial amounts of antioxidants, including

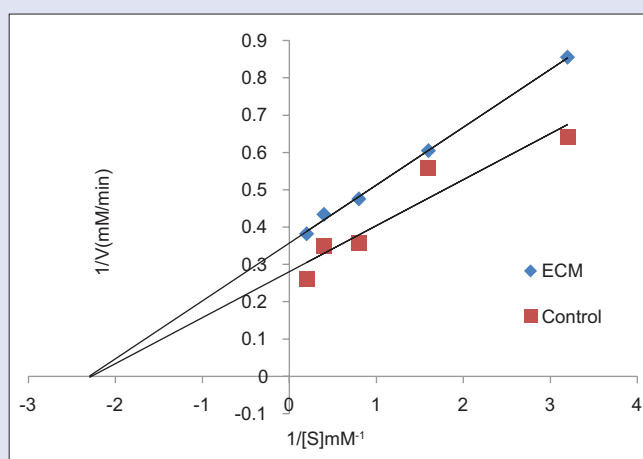


Figure 8: Lineweaver–Burk plot of *Ehretia cymosa* methanol fraction eliciting noncompetitive inhibition on α -glucosidase activity. Results represent mean \pm standard error of the mean ($n = 3$); ($P < 0.05$). ECM: *Ehretia cymosa* methanol fraction

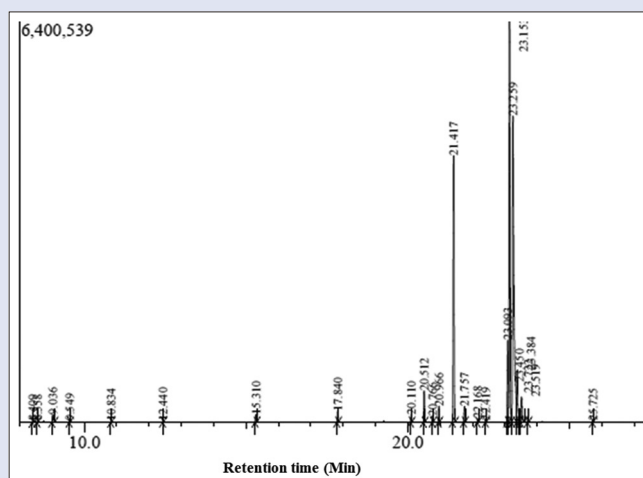


Figure 10: Gas chromatography-mass spectrometric chromatogram of *Ehretia cymosa* methanol fraction

Table 5: Compounds identified from the methanol fraction of *Ehretia cymosa* leaves

Constituents	Retention time (s)	Area (%)	Molecular formula	Molecular weight (g/mol)
2,2-Bis (4-nitrobenzyl)-1-phenylbutane-1,3-dione	8.41	0.06	C ₂₄ H ₂₀ N ₂ O ₆	432
Benzamide	8.56	0.06	C ₃₃ H ₂₈ N ₆ O ₈	442
Phenethyl alcohol	9.04	0.70	C ₁₂ H ₁₈ O	178
S-Phenyl (2S,1'S)-2-(2-Nitro-1-phenylethyl)-1-(1-phenylethyl) aziridine-2-carbothioate	9.55	0.06	C ₂₅ H ₂₄ N ₂ O ₃ S	432
Diglycidyl ether	10.83	0.04	C ₆ H ₁₀ O ₃	130
2,2,6,6-Tetramethyl-4-(4-nitrobenzyl) heptane-3,5-dione	12.44	0.04	C ₁₈ H ₂₅ NO ₄	319
n-Pentadecane	15.31	0.46	C ₁₅ H ₃₂	212
n-Undecane	17.84	0.67	C ₁₁ H ₂₄	156
n-Docosane	20.11	0.43	C ₂₂ H ₄₆	310
Phytol*	20.51	1.78	C ₃₀ H ₆₀ O	296
Citronellyl propionate	20.77	0.31	C ₁₃ H ₂₄ O ₂	212
Lavandulyl acetate	20.97	0.81	C ₁₂ H ₂₀ O ₂	196
Methyl hexadecanoate	21.42	17.45	C ₁₇ H ₃₄ O ₂	270
Stearic acid*	21.76	1.02	C ₁₈ H ₃₆ O ₂	284
n-Hexadecane	22.17	0.17	C ₁₆ H ₃₄	226
Methyl heptanoate	22.42	0.08	C ₈ H ₁₆ O ₂	144
Methyl linolelaidate	23.09	4.97	C ₁₉ H ₃₄ O ₂	294
Methyl linolenate	23.15	28.93	C ₁₉ H ₃₂ O ₂	292
2-Hexadecyloxirane*	23.26	34.18	C ₁₈ H ₃₆ O	268
Methyl octadecanoate	23.38	4.51	C ₁₉ H ₃₈ O ₂	298
2-Dodecenyl acetate	23.45	0.94	C ₁₄ H ₂₆ O ₂	226
1-Oxacyclopropyl-3,4-epoxycyclohexane	23.52	2.18	C ₈ H ₁₂ O ₂	140
6-hepten-3-one	23.72	0.10	C ₇ H ₁₂ O	112
4-Methylvaleric acid	25.73	0.04	C ₈ H ₁₂ O ₂	116
Total			99.99	

*Compounds reported to have antioxidative, antidiabetic, and other biological activities

tocopherols (Vitamin E), carotenoids, ascorbic acid, flavonoids, and tannins, which implies that antioxidant action may be an important property of plant medicines associated with diabetes.^[49] We assessed five complementary antioxidant assays on the ethyl acetate and methanol fractions from *E. cymosa*, namely, DPPH, ABTS, hydroxyl radical, superoxide anion scavenging abilities, and metal chelating potential, to have a full evaluation of the antioxidative capabilities of the extract fractions due to their different mechanisms of actions.^[50] Ethyl acetate fraction displayed significantly higher ($P < 0.05$) percentage inhibition for superoxide anion scavenging activity [Figure 4] and metal chelating potential [Figure 3] than methanol fractions as well as gallic acid and silymarin used as standards, while methanol fractions has significantly higher ($P < 0.05$) percentage inhibition for DPPH [Figure 5] and ABTS [Figure 1] scavenging activities compared to ethyl acetate fraction and the standards. The percentage inhibition for the two fractions against hydroxyl radical scavenging activity [Figure 2] compared favorably that the methanol fraction was significantly higher at lower (0.13–0.50 mg/mL) concentration while ethyl acetate was significantly higher at high concentration (0.75–1.00 mg/mL). 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical with an unpaired electron that is delocalized over the entire molecule.^[51] DPPH possesses a purple color, with a maximum absorption at 519 nm in ethanol; hence, scavenging the DPPH radical by antioxidants will result in decolorization measured by decrease in absorption readings over time; the extent of decrease in DPPH absorption is proportional to the concentration of radicals that are being scavenged. The ABTS assay utilizes a free radical, monocation of 2,2'-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid (ABTS⁺), which has a blue/green color with maximum absorption spectra at 734 nm in water. The more hydrophilic free radical (ABTS^{•+}) is decolorized when reduced in the presence of the test sample. This implies that the methanol fraction decolorized the purple color of DPPH and blue/green color of mono-radical cation of ABTS most and so possessed the best ability to scavenge the DPPH radicals and ABTS radicals

compared to ethyl acetate fraction, silymarin, and gallic acid. The result is in agreement with Gil *et al.*,^[52] which reported that results of ABTS assay should be comparable to results found in the DPPH assay and may be viewed as confirmation of the DPPH assay. The significantly higher percentage of inhibition exhibited by ethyl acetate fraction against superoxide anion scavenging activity and metal chelating compared to methanol fraction, and the standards indicate that the fraction effectively scavenged the superoxide radical anion (O₂⁻) which originates from the one-electron reduction of free molecular oxygen by nicotinamide adenine dinucleotide phosphate oxidase, which is the membrane-bound enzyme.^[53] It also serves as metal ion pro-oxidant chelator by deactivating transition metals such as Fe²⁺, thereby prevents such metals from participating in the initiation of lipid peroxidation and oxidative stress through metal-catalyzed reaction.^[50] The significantly higher ($P < 0.05$) percentage inhibition displayed by methanol and ethyl acetate fraction at lower (0.13–0.50 mg/mL) and higher concentration (0.75–1.00 mg/mL) for hydroxyl radical scavenging activities, respectively, signify their potentials in scavenging the hydroxyl radical, which is one of the most reactive free radicals in a biological system, thereby preventing it from causing enormous biological damage in the living cell.^[54] The significantly lower ($P < 0.05$) IC₅₀ displayed by methanol fractions for DPPH (0.47 mg/mL), hydroxyl radical (0.55 mg/mL), and ABTS (0.49 mg/mL) scavenging activities is an indication of its potency in scavenging the DPPH, ABTS, and hydroxyl radicals more than ethyl acetate and the standards [Table 3]. Similarly, ethyl acetate shows significantly lower IC₅₀ for metal chelating (0.61 mg/mL) and superoxide anion (1.68 mg/mL), which signify its stronger ability to serve as metal ion pro-oxidant chelators and to effectively scavenge the superoxide radical anion (O₂⁻), respectively, compared to methanol fraction and standards [Table 3]. The various antioxidants activities displayed by the methanol and ethyl acetate fraction of *E. cymosa* may be due to the presence of high contents of phenols, flavonoids, and proanthocyanidin present. Polyphenolic compounds have been reported as important plant components which defend the body from

diverse types of oxidative damage.^[55,56] Their scavenging action is mainly due to their hydroxyl groups. Tung *et al.*^[57] reported radical scavenging properties as one of the spectrum of biological and chemical activities of flavonoids. An effective approach to manage the diabetics disorder is to strongly inhibit intestinal activity of α -glucosidases and mildly inhibit pancreatic α -amylase activity, which in turn reduce postprandial hyperglycemia and consequently alleviate potential chronic vascular complications.^[58,59] Plant-derived antioxidants are potential α -amylase and α -glucosidase inhibitors, which indicate their great potentials in the management of diabetics.^[60] The results of α -glucosidase [Figure 10] and α -amylase inhibitory assays revealed a dose-dependent inhibition by ethyl acetate and methanol fractions of *E. cymosa*, with most potent inhibition observed at higher concentrations, while the lowest concentration of 0.13 mg/mL revealed the lowest inhibition. Methanol fraction revealed significantly higher inhibition than ethyl acetate and acarbose at all concentrations against α -glucosidase. It also exhibited significantly higher inhibition than ethyl acetate and acarbose at higher concentrations (0.75–1.00 mg/mL) and competed favorably at lower concentrations with ethyl acetate and acarbose for α -amylase inhibitory activity. The displayed activity by the methanol fractions compared to ethyl acetate may be due to its phenolic (27.44 mg gallic acid/g) and flavonoids (235.31 mg quercetin/g) content which are higher than that of ethyl acetate [Table 2]. Other phytochemicals, namely, alkaloids, tannins, steroids, and cardiac glycosides present in the methanol fraction [Table 1] might have also contributed to its enhanced inhibitory activity. The antidiabetic capability of this phytochemicals have been documented.^[58,59,61,62] Methanol fraction exhibited good antidiabetic property with potent inhibition against α -glucosidase and mild inhibition against α -amylase at the respective IC₅₀ of 0.60 and 2.75 mg/mL. The IC₅₀ of methanol fraction (2.75 mg/mL) against α -amylase inhibition is significantly higher ($P < 0.05$) than acarbose which is a synthetic standard antidiabetic drug. Furthermore, IC₅₀ of methanol against inhibition of α -glucosidase (0.60 mg/mL) was significantly lower ($P < 0.05$) than acarbose. The activity demonstrated by the methanol fraction against α -amylase and α -glucosidase makes it suitable as antidiabetic drug candidate with no or reduced negative side effects, which are normally observed in the usage of synthetic antidiabetic agents such as abdominal distention and hypoglycemia due to excessive inhibition of these enzymes.^[63] The current report on the α -glucosidase inhibition by methanol fraction of *E. cymosa* is in agreement with other findings,^[63,64] where moderate α -amylase inhibition with potent α -glucosidase inhibitory activity was proposed as better therapeutic approach to delay availability of dietary carbohydrate substrate for glucose production in the gut.

The Lineweaver–Burk plot of the mode of inhibition of α -amylase and α -glucosidase by the methanol fractions of *E. cymosa* displayed competitive [Figure 8] and noncompetitive [Figure 9] modes of inhibition, respectively. The competitive inhibition of α -amylase by the methanol fraction of *E. cymosa* suggests that the inhibitory component of the fraction binds reversibly to the active site of the enzyme and occupies it in a mutually exclusive manner with the substrate.^[65] The noncompetitive inhibition of α -glucosidase by the methanol fraction of *E. cymosa* indicated that the active components in the fraction also binds to a site other than the active site of the enzyme and combines with either free enzyme or the enzyme–substrate complex, possibly interfering with the action of both.^[66] However, the inhibitor had equal affinity for both the free enzyme and enzyme–substrate complex. The antidiabetic potentials displayed by the fractions of *E. cymosa* may be due to the presence of phytochemicals such as tannins, saponins, phenolics, sterols, and flavonoids. Studies have shown the antioxidant and antidiabetic properties of saponins from different medicinal plants.^[67,68] Tannins and phenolics have been found to induce phosphorylation of insulin

receptors and translocation of glucose transporter, thereby helping in the reduction of blood glucose level.^[69] Besides that, the phytoconstituents of the fraction as revealed from its GC-MS result have been implicated in diverse therapeutically significant biological activities.^[70-74] Its antioxidant and antidiabetic properties as significantly elicited in this study may be specifically attributable to its phytol. While 2-hexadecyloxirane and stearic acid have been demonstrated to having good antioxidant potentials,^[71,73] the antioxidant and hypoglycemic activities of phytol have also been reported.^[72,75] The presence of these identified bioactive constituents in *E. cymosa* might be accountable for the elicited potential in this study.

CONCLUSION

From the result of the present study, it can be concluded that the *E. cymosa* exhibited promising antidiabetic effect. Our findings suggest that *E. cymosa* has both hypoglycemic and *in vitro* antioxidant activity. This study also establishes a correlation between antidiabetic and antioxidant potential. The data obtained from this study suggest that the *E. cymosa* leaves contain active phytochemicals that could serve as antioxidative agents and inhibitors of α -glucosidase and α -amylase, indicating its therapeutic potential for the control of postprandial blood glucose levels, type 2 diabetics, and other chronic vascular complications that are associated with diabetes.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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