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Phytocompounds, antioxidant potential, and inhibitory actions of ethanolic leaf fraction of *Sida linifolia* Linn. (Malvaceae) on enzymes linked to inflammation, diabetes, and neurological disorders

Emmanuel Chimeh Ezeako[1*], Florence Nkechi Nworah[1] and Dionysius Obinna Osuji[1]

Abstract

Background *Sida linifolia* L. is a weed ubiquitously found in Africa with several folkloric applications. Traditional healers in the Southeastern part of Nigeria employ the alcoholic concoction of *S. linifolia* leaves as antidepressants, anti-malaria, antihypertensive, anti-abortifacients, and for managing painful whitlow; however, these claims lack scientific validation. The present study was aimed to explore the phytochemical profile of the plant, *S. linifolia* with special emphasis to its antioxidant and inhibitory actions on enzymes linked to inflammation, diabetes, and neurological disorders. Phytochemical profiling and in vitro antioxidant and enzyme inhibition assays were employed to assess the pharmacological profile of *S. linifolia* ethanolic leaf fraction (SLELF).

Results Preliminary phytochemical screening of SLELF revealed appreciable amounts of total phenolics (91.64 ± 7.61 mg GAE/g), total tannins (62.44 ± 3.86 mg TAE/g), and total flavonoids (27.35 ± 1.48 mg QE/g) present in SLELF. Results of HPLC analysis of SLELF revealed rich composition in bioactive compounds such as ellagic acid, quercetin, ferulic acid, 3,4-dimethoxy benzoic acid, gallic acid, 4-methoxy cinnamic acid, sinapic acid, vanillic acid, and chlorogenic acid. Enzymatic antioxidants (catalase and superoxide dismutase), non-enzymatic antioxidants (reduced glutathione (GSH), Vit A, C, and E), elemental minerals (Cu, Mn, Zn, Cr, Fe, and Ca), and γ-aminobutyric acid (GABA) were present in SLELF in appreciable levels. At various concentrations (0.2–1.0 mg/ml), SLELF exhibited potent and concentration-dependent hydrogen peroxide (H_2O_2) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS) radical scavenging activities and exerted moderate inhibitory actions on enzymes associated with inflammation (cyclooxogenase-2 (COX-2) and lipoxygenases (LOXs), diabetes (α-amylase, α-glucosidase), and neurological disorders (butyrylcholinesterase (BChE) and γ-aminobutyric acid transaminase (GABA-T), compared to respective standards (ascorbic acid, acarbose, indomethacin, galanthamine, and vigabatrin). Perhaps, the observed potent pharmacological activities of SLELF could be anchored to its phytoconstituents. Furthermore, the slightly higher ranges of IC_{50} values (0.57–0.87 mg/ml) of SLELF compared to standards (0.44–0.68 mg/ml) suggest moderation in enzyme inhibition that may preclude adverse side effects.

Conclusion This study lends credence to the folklore claims of *S. linifolia* leaves and revealed its potential as possible source of bioactive compounds for medicinal and pharmaceutical exploration.

*Correspondence:
Emmanuel Chimeh Ezeako
emmanuel.ezeako.188647@unn.edu.ng
Full list of author information is available at the end of the article

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Keywords *Sida linifolia*, Phytocompounds, Antioxidant, Anti-diabetic, Anti-inflammatory, Neuromodulatory

Background

The constant exposure of the human system to oxidants and harmful agents of endogenous or exogenous origin, capable of altering normal homeostasis and physiological status, results in disease conditions [1]. Oxidative stress ensues when the generated levels of reactive oxygen/nitrogen species overwhelm instituted antioxidant system meant to keep their oxidative activities in check and are characterized by oxidative destruction of cellular and subcellular entities [2]. The recent surge in research for antioxidants from natural sources could be due to the growing evidence that implicates oxidative stress in the pathophysiology of myriads of health aberrations, including inflammatory diseases, diabetes, and neurological disorders [3]. For decades, synthetic principles have been relied on as therapeutic agents for treating and managing chronic diseases. However, recent evidence linking synthetic drugs to several adverse side effects has prompted a surge in research for natural sources of drug candidates with improved efficacy and reduced toxicity, preferably derived from plants [4]. Coincidentally, despite the multiple oxidative assaults caused by biotic and abiotic stimuli, plants have adapted to thrive in their habitat by gaining genetic capabilities and key enzymatic pathways required to synthesize diverse classes of pharmacologically potent molecules to aid their survival [5]. In this regard, medicinal plants are a good source of phytoactive compounds with several pharmacological potentials [6]. Hence, recent research in pharmacology leverages efforts to identify and isolate bioactive secondary metabolites naturally distributed in plant leaves, stems, seeds, roots, or flowers [7]. Some phyto-principles such as polyphenolics, flavonoids, tannins, vitamins, elemental minerals, enzymes, peptides, and amino acids have been reported to exhibit excellent antioxidant, anti-inflammatory, neuromodulatory, and anti-diabetic potentials [7].

Inflammation is a series of events or reactions in the microenvironments of living tissues in the sight of injury or foreign agents, which manifests in fever, pain, redness, heat, and enlargement of affected tissues [8]. The mechanisms of inflammation entail a sequence of events in which arachidonic acid metabolism plays a crucial role [9]. Arachidonic acid is converted to important vasoactive molecules such as prostaglandins and thromboxane A2 via the cyclooxygenase (COX) pathway or to hydroperoxyeicosatetraenoic acids and leukotrienes via the lipoxygenases (LOXs) pathway involving 5-lipoxygenase (5-LOX), 12-LOX, and 15-LOX [10]. Prostaglandins are responsible for pain and are the complex inflammatory cascades associated with inflammation [10]. Modulation of the activity of key enzymes involved in the metabolism of arachidonic acid, including cyclooxygenases (COX) and lipoxygenases (LOXs), which are some of the several proposed mechanisms of anti-inflammatory agents [10]. Two isoforms of cyclooxygenase enzymes exist (COX-1 and COX-2). The isoform COX-1 is generally constitutive and plays housekeeping roles, such as cytoprotection of gastrointestinal layers and platelet aggregation. Conversely, COX-2, though constitutive in some tissues, is inducible in inflammatory cells, and its expression and activity are more susceptible to injurious external or internal stimuli, resulting in inflammatory events [11]. Hence, pharmacological agents that selectively repress the expression of COX-2 are preferred [11]. Similarly, the inhibition of LOXs is considered effective in treating inflammatory diseases, such as asthma, atherosclerosis, and some cancers [12].

Diabetes mellitus is an endocrine and metabolic disorder with a high mortality rate [13]. The mechanism of action of some pharmacologic agents used in the management of diabetes includes the inhibition of the human pancreatic α-amylase and α-glucosidase, which metabolize carbohydrates and elevate blood sugar levels and postprandial hyperglycemia symptoms [13].

Butyrylcholinesterase (BChE), a nonspecific cholinesterase produced in the brain and peripheral organs, has been linked to the etiology of diabetes and neurological disorders, including Alzheimer's disease (AD) [14]. Gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter released in the brain, is produced from glutamic acid by the action of glutamic acid decarboxylase. GABA binds to its receptors to induce relaxation and diminish anxiety [15]. GABA-transaminase (GABA-T) degrades GABA into succinate semialdehyde, a citric acid cycle substrate. The correlation between reduced GABA levels in physiological fluids with neurological disorders has been established [16]. Therefore, these enzymes represent potential drug targets in managing neurological disorders [14, 16]. Moreover, several anxiolytic herbs contain appreciable amounts of GABA [17].

The African community is blessed with medicinal herbs, which they rely on for food and disease panacea for humans and livestock. Moreover, the consumption of plant materials as food or medicine constitutes an integral part of the culture and beliefs of most African communities [18]. *Sida linifolia* L. commonly known

as flaxleaf fanpetals is a weed that belongs to the genus *Sida* and family Malvaceae that ubiquitously grows in dry forest areas in West Africa (specifically in Nigeria and Sierra Leone) and North America. It is referred to as Yaya in Yoruba (Nigeria), Kpakpa-niŋgivali (Sierra Leone), and Guanxuma (Brazil) [19]. Several species of the *Sida* genus, including *S. acuta*, *S. cordifolia*, *S. corymbosa*, *S. rhombifolia*, and *S. tiagii*, have been reported to show rich phytochemistry and exhibit numerous health benefits [20]; however, studies on the bioactivities of *S. linifolia* are still limited. From the little we have gathered, traditional healers employ the alcoholic or aqueous concoction of the leaves as antidepressants, aphrodisiacs, anti-malaria, antihypertensive, anti-abortifacients, and in managing painful whitlow [18]; however, these claims lack validation. Nevertheless, recent preliminary studies from our laboratory have demonstrated the antioxidant, anti-nociceptive, and anti-inflammatory potentials of the polar leaf extracts and a fraction of *S. linifolia* in vitro and in vivo [21, 22]. Furthermore, due to ethical concerns associated with the initial deployment of laboratory animals, investigators prefer to use in vitro experiments to mimic cellular responses during the early stages of drug discovery [23, 24]. Consequently, in furtherance to previous studies, the present study investigated the bioactive phytocomposition, in vitro radical scavenging activities, and inhibitory potential of *S. linifolia* leaf fraction on enzymes implicated in a number of disease pathologies.

Methods

Chemicals and reagents

All reagents used in this study were of analytical grade and purchased from reputable local dealers.

Collection and identification and plant materials

The weed, *S. linifolia,* was harvested in the month of July 2022 from a field in Nsukka metropolis, Nsukka, Enugu, Nigeria (located at latitude 6.8429°N and longitude 7.3733°E). The harvested plant leaves were left to dry in a shed out of sun reach. Authentication and identification of the plant were performed by Mr. Alfred Ozioko, a taxonomist resident at the Bio-resources Development and Conservation Program (BDCP) research center, Nsukka, Enugu, Nigeria (voucher no: BDCP20220704). The leaf sample was deposited in the herbarium to enable future retrieval.

Extraction and fractionation procedures

The pulverized plant material was extracted and fractionated according to the methods described by Parvin et al. [25] and Hwang et al. [26], respectively. Twenty-four-hour maceration of a known amount (3500 g) of the powdered plant leaves in 4 L of absolute ethanol (98%), followed by filtration using Whatman Number 1 filter paper, was performed, after which a rotary evaporator operated at 45 °C and low pressure was employed to concentrate the crude ethanolic extract. Subsequently, a fractionating column loaded with silica gel was employed to fractionate the resultant ethanolic extract using various solvents (n-hexane, ethyl acetate, and ethanol, respectively) with increasing polarity. The fractions thus formed were concentrated by evaporation (at a similar condition) and stored in appropriately labeled and sterile screw-capped vessels maintained at 3–5 °C until required. The ethanolic leaf fraction of *S. linifolia* (SLELF) was used for further determination. Our choice of fraction followed that of Nwankwo et al. [21].

Determination of total phenolic, total flavonoids, and total tannins content

Sample preparation

A known amount (40 mg) of the leaf fraction was transferred into 5 ml methanol and sonicated at 40 °C for 45 min and then centrifuged for 10 min at 1000×*g*. The resultant supernatant was kept in an amber bottle until needed for analysis.

Total phenolics content

The Folin and Ciocalteu (F–C) test outlined by Singleton and Rossi [27] was used in this study. The principle of the test is based on the ability of phenolic compounds to reduce F–C reagent to molybdenum–tungsten blue, which absorbs at 765 nm. The amount of phenolics in the mixtures is directly proportional to the absorption of the reaction vessel as proposed by Swain and Hillis [28]. Spectrophotometric readings of the test sample and standard at 765 nm were taken against the reagent blank using a UV–Vis Spectrophotometer (Shimadzu, Kyoto, Japan). Preparation of the reaction mixture entailed diluting a volume (0.1 ml) of an aqueous solution of the test sample (1 mg/ml) with a volume of distilled water (7.9 ml) and then introducing a volume of 20% sodium carbonate (1.5 ml) and a volume of Folin–Ciocalteu's reagent (0.5 ml). Thereafter, the resultant mixture was allowed to stay in the dark for 2 h, after which the spectrophotometric readings of the mixture at 765 nm were taken against the control (having the same composition except for distilled water which replaced the test sample) using a UV-2600 spectrophotometer (Shimadzu, Kyoto, Japan). All determinations were performed in triplicates. The total phenolics composition of the test material was determined as the gallic acid equivalent of dry plant material (GAE/g of the test sample) using a gallic

acid standard curve (0.2–1.0 mg/L, $Y = 0.772$, $x - 0.144$, $R^2 = 0.992$).

Total flavonoids content

The total flavonoids content of the test sample was determined according to the aluminum chloride calorimetric method outlined by Afify et al. [29]. A volume (0.5 ml) of the test material (1 mg/ml) was introduced into a reaction vessel containing 1 ml of aluminum chloride (10%), 1 ml of potassium acetate (1 M), and 2.5 ml of distilled water. A calibration curve was plotted using various concentrations of quercetin. The absorbance of the mixtures was taken at 415 nm using a UV-spectrophotometer. All analyses were done in triplicates. The total flavonoids in the test sample were determined as the quercetin equivalent of the dry plant material (mg QE/g of the test sample) using a quercetin standard curve of (0.2–1.0 mg/L, $R^2 = 0.987$, $Y = 0.963x - 0.100$).

Total tannins content

This study was performed according to the protocol outlined by Babu et al. [30]. The reaction mixture comprised a volume (1 ml) of the test sample mixed with 0.5 ml of Folin–Ciocalteu's reagent 1 ml of 20% sodium carbonate solution, and a volume of distilled water (8 ml). The resultant mixture was left to stand at ambient temperature for 30 min; thereafter, the mixture was subjected to centrifugation for 10 min at 6000×*g*. The absorbance of the supernatant thus obtained was read with a UV–Visible Spectrophotometer at 725 nm. Spectrophotometric readings of the tannic acid standard at various concentrations were used to plot the tannic acid standard curve (0.2–1.0 mg/L, $R^2 = 0.999$, $Y = 1.003$, $x - 0.017$) of which the total tannin content of the test material was determined. The total tannin content of the test sample was determined as the tannic acid equivalent of the dry test sample (mg TAE/g).

High-performance liquid chromatography determination of polyphenolic compounds and flavonoids

This study was done according to the procedure outlined by Mizzi et al. [31]. The study began with the preparation of standard solutions of 18 studied phenolic compounds and flavonoids, viz. phenylacetic acid, luteolin, apigenin, *p*-coumaric acid, quercetin, chrysin, syringic acid, benzoic acids, sinapic acid, chlorogenic acid, 4-methoxycinnamic acid, 4-methoxycinnamic acid, 3,4-dimethoxybenzoic acid, ellagic acid, ferulic acid, gallic acid, vanillic acid, and caffeic acid (Sigma-Aldrich, Merck, Darmstadt, Germany stock). Stock solutions (100 mg/l) of standards were prepared, and the most suitable analytical wavelength within the UV–Vis region (180 and 480 nm) was established for the HPLC–DAD determinations. Quantification of the phenolic compounds and flavonoids in the test sample was performed with a Waters-2695 Alliance HPLC system (Milford, Connecticut, U.S.A.) attached to a UV–Vis Diode array detector (UV–Vis DAD). A 4.6 mm width, 250 mm length, and 5 µm particle size Waters SunfireTM system, attached to a C18 reverse-phase chromatographic column, was used to carry out the separation process. An autoinjector was used to inject equal volumes of the test sample and the phenolic standard separately into the system. The gradient arrived at after a series of prior studies employed acetonitrile solution as the mobile phase. The HPLC grade [≥ 99.9%] acetonitrile (Honeywell Seelze, Germany) served as the mobile phase A while mobile phase B (phosphoric acid) was made by adding drops of 85% orthophosphoric acid (Sigma-Aldrich, Germany) to HPLC grade water (Carlo-Erba) until a solution of pH 2 was attained. The analysis adopted a 0.5 ml/min constant flow rate 5 °C temperature, and a 6 min total runtime. Characteristic wavelengths (210, 280, and 360 nm) were selected for the analysis after taking the UV–Vis spectra of the various phenolic standards, utilizing the HPLC–DAD.

Antioxidant enzyme content

Sample preparation

A weighted sample (10 g) of the test material was transferred into a solution containing 50 ml of 100 mM sodium phosphate buffer (pH 7.0), ascorbic acid (1 mM), and polyvinylpyrrolidone (0.5% (w/v) and left to incubate at 4 °C for 5 min. The mixture thus obtained was filtered using three-layer cheesecloth, after which the filtrate was subjected to centrifugation at 5000×*g* for 15 min. The obtained supernatant was adopted for the antioxidant determinations.

Determination of catalase content

The catalase composition of the test sample was evaluated by estimating the activity of the enzyme in the test sample following the method outlined by Aebi [32]. The reaction mixture comprised 100 mM sodium phosphate buffer (pH 7.0), H_2O_2 (30 mM), and the test sample (100 µl), making a volume of 3.0 ml in total. Catalase (CAT) activity in the test sample was determined at room temperature by recording the decrease in absorbance of the reaction mixture at 240 nm resulting from H_2O_2 breakdown. One unit (U) of catalase activity denotes the enzyme amount that caused a change in absorbance of 0.001 min^{-1} under the assay condition.

Determination of superoxide dismutase content

The superoxide dismutase composition of the test sample was evaluated by estimating the activity of the enzyme

in the test sample according to the method proposed by Kumar et al. [33]. The activity of superoxide dismutase (SOD) in the test sample was evaluated by measuring the degree of photoreduction action of SOD on nitroblue tetrazolium (NBT). Briefly, EDTA (0.1 mM), sodium phosphate buffer (50 mM, pH 7.6), L-methionine (12 mM), sodium carbonate (50 mM), riboflavin (10 µM), NBT (50 µM), and the test sample (100 µl) were measured into a test tube making a volume of 3.0 ml in total. The control test tube was void of the test sample. Exposure of the reaction vessel to white light at room temperature for 15 min was used to initiate the enzyme reaction. Thereafter, the mixture was left to stand for 15 min at ambient temperature, after which spectrophotometric readings at 560 nm were recorded. One unit (U) of SOD activity denotes the enzyme amount that caused 50% attenuation of NBT photoreduction.

Determination of glutathione (GSH) content

The spectrophotometric method was used to assay for the glutathione (GSH) content of the leaf fraction following the procedure outlined by Sahoo et al. [34]. By principle, this assay entails the reduction of 5,5′-dithiol-bis (2-nitrobenzoic acid) (DTNB), a sulfhydryl-containing reagent by GSH, and the resultant yellowish derivative (5′-thio-2-nitrobenzoic acid [TNB]) thus formed was read spectrophotometrically at 412 nm. A weighted amount (0.2 g) of the test sample was introduced into a test tube containing EDTA (1 mM) and 2 ml of 6% metaphosphoric acid and mixed thoroughly. The mixture thus formed was centrifuged at 11,500×*g* for 15 min thereafter; 0.4 ml of the supernatant was introduced into 1 ml potassium phosphate buffer (0.5 M pH 7.5). Subsequently, respective volumes of DTNB (100 µl, 10 mM), BSA (200 µl, 10 mM), and NADH (100 µl, 0.5 mM) were dispensed into the mixture. The resultant mixture was left to incubate at 37 °C for 15 min; afterward, the reaction mixture was allowed to cool, and spectrophotometrical readings were taken at 412 nm.

Radical scavenging assays

ABTS radical scavenging assay

The radical quenching properties of the test materials were also evaluated using the modified ABTS radical cation decolorization test proposed by Re et al. [35]. The preparation of $ABTS^{\cdot+}$ radical cations was done by mixing crystals of a known mass (80 mg) of manganese dioxide with Na/K buffer (20 ml, 75 mM, pH 7) and an aqueous solution of $ABTS^{\cdot+}$ (5 mM). In a well-labeled separate test, either the test sample or standard antioxidant (ascorbic acid) at various concentrations (0.2–0.1 mg/ml) was diluted in Na/K buffer (pH 7), and the resultant mixture was transferred into sets of test tubes that contained 2000 µL solution of $ABTS^{\cdot+}$ radical cation. The control test tube contains all the reagents but was void of the test material. The resultant mixture was left to stay for 6 min thereafter; a spectrophotometer was employed to take the mixture's spectrophotometric readings at 734 nm. The antioxidant standard used in this assay was ascorbic acid. The degree (%) of $ABTS^{+}$ radical quenching activity of the test sample was evaluated with the equation:

$$\text{ABTS radical quenching activity (\%)} = 1 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Hydrogen peroxide scavenging assay

The hydrogen peroxide (H_2O_2) attenuating potential of the test sample was also evaluated using method outlined by Ruch et al. [36]. Briefly, a volume (0.6 ml) of freshly prepared solution of hydrogen peroxide (40 mM) in phosphate buffer (pH 7.4) was transferred into test tubes containing either the test sample or reference antioxidant (ascorbic acid) dissolved in ethanol at various concentrations (0.2–0.1 mg/ml). Subsequently, after allowing the mixture to stay for 10 min, absorbance (Abs) of the mixtures at 230 nm was read against a blank solution that consisted of phosphate buffer void of hydrogen peroxide. The % H_2O_2 radical attenuating activity of the test sample was evaluated using the formulae:

$$\%\ H_2O_2 \text{ quenching activity} = 1 - \frac{\text{Abs of Test Sample}}{\text{Abs of Control}} \times 100$$

Enzyme inhibition assays

Cyclooxygenase-2 inhibition assay

This test was performed following the methods described by Yoshimoto et al. [37] and Flower et al. [38]. Crude enzyme preparation from bovine seminal vesicle was made following the method of Nugteren et al. [39], while substrate preparation for the enzyme was done according to the protocol outlined by Harold et al. [40]. Spectrophotometric readings were taken at 278 nm and in triplicates determinations. Thereafter, degree (%) of COX-2 inhibition was evaluated using the expression as follows:

$$\%\ \text{Inhibition of COX} - 2 = 1 - \frac{\text{Abs of Test Sample}}{\text{Abs of Control Sample}} \times 100$$

Anti-lipoxygenases assay

The ability of the leaf fraction to inhibit LOXs was measured using the procedure outlined by Axelrod et al. [41] and Singsai et al. [42]. Crude enzyme preparation was obtained from pulverized soybean. Lipoxygenases inhibition potential of the test sample was determined using linoleic acid as substrate. Spectrophotometric readings were taken at 234 nm and in triplicates determinations. Thereafter, the % inhibition of the enzyme was determined using the equation:

$$\% \text{ Inhibition of LOXs} = 1 - \frac{\text{Abs of Test Sample}}{\text{Abs of Control Sample}} \times 100$$

Alpha-amylase inhibition assay

This study was done according to the procedure described by Kwon et al. [43]. A volume (500 µl) of either the test sample or acarbose (standard drug) at various concentrations (0.2–0.1 mg/ml) was prepared and mixed with an equal volume (500 µl) of 0.02 M sodium phosphate buffer solution (pH 6.9 prepared in 0.006 M NaCl) and α-amylase solution (0.5 mg/ml); thereafter, the resultant mixture was left to incubate for 10 min at 37 °C. Subsequently, 500 µl of 1% (w/v) soluble potato starch solution prepared with 0.02 M sodium phosphate buffer was introduced. Afterward, a volume (1.0 ml) of DNSA color reagent (1% 3, 5-dinitro salicylic acid) was added the resultant mixture and left to incubate for 15 min at 37 °C, after which the reaction was halted by introducing 12% sodium potassium tartrate in NaOH (0.4 M). Thereafter, the resultant mixture was made to incubate in a water bath at 100 °C for 5 min and then left to cool to ambient temperature, after which 10 ml of distilled water was introduced to dilute the solution. All determinations were done in triplicates. The absorbance readings of the resultant mixture were taken at 540 nm. The mean absorbance value was used to estimate the percentage α-amylase inhibition of the test sample as follows;

$$\% \text{ Alpha} - \text{amylase inhibition} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100$$

Alpha-glucosidase inhibition assay

This assay was performed according to the procedure described by Matsui et al. [44]. Various concentrations (0.2–1.0 mg/ml) of either the test sample or acarbose (reference drug) were allowed to incubate in 1 ml phosphate buffered solution (0.1 M, pH 7.2) containing a mass (0.5 mg) of the protein equivalent of crude α-glucosidase preparation, after which 45 mM sucrose (substrate) was introduced into the reaction mixture to initiate the reaction. Thereafter, incubation of reaction mixture at 37 °C for 30 min proceeded with the addition of a volume (1000 µl) of Tris base to halt the reaction. The propensity of the test sample to inhibit alpha-glucosidase was estimated in relation to the amount of reducing sugar resulting from the hydrolysis of sucrose by alpha-glucosidase, using glucose oxidase method. Spectrophotometric readings at 450 nm were used to measure the amount of glucose released. The % enzyme inhibition of α-glucosidase was determined using the expression;

$$\% \text{ Alpha} - \text{glucosidase inhibition} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100$$

Butyrylcholinesterase inhibition assay

The test adopted the Ellman et al. [45] modified spectrophotometrical method. The crude enzyme solution was derived from a volunteering human serum (BChE), while butyrylthiocholine chloride (Sigma, St. Louis, MO, USA) served as the substrate. Briefly, a volume (20 µl) of the freshly prepared crude enzyme was measured into sodium phosphate buffer solution (140 µl, 0.1 mM, pH 8.0), and 20 µl solution of either the test sample or standard drug (galanthamine) dissolved in ethanol at various concentrations (0.2–0.1 mg/ml) and allow to incubate for 30 min at room temperature. Thereafter, 10 µl of DTNB was introduced into the reaction mixture, after which 10 µL of butyrylthiocholine was introduced to initiate the enzymatic reaction. After 5 min of reaction, spectrophotometrical readings at 412 nm were taken to monitor the generation of a yellowish product (5-thio-2-nitrobenzoate anion) evolving from the reaction of thiocholines with DTNB, which corresponds to the rate of hydrolysis of butyrylthiocholine by the enzyme. Ethanol replaced the test sample in the negative control. The standard drug galanthamine dissolved in ethanol served as standard control. The percentage of BChE inhibition was evaluated using the formulae:

$$\text{Inhibition } (\%) = \frac{\text{Abs412(control)} \times \text{Abs412(fraction)} \times 100}{\text{Abs412(control)}}$$

GABA-T inhibition assay

The propensity of the test sample to inhibit GABA-T (EC 2.6.1.19) was determined following the method outlined by Awad et al. [46]. Rat brain homogenates mixed with various concentrations (0.2–0.1 mg/ml) of either the test material or vigabatrin (reference drug) were dispensed into test tubes containing 100 mM potassium pyrophosphate buffer (4 mM NAD, 5 mM α-ketoglutarate, 10 µM pyridoxal-5′-phosphates, 3.5 mM 2-mercaptoethanol, pH 8.6) at 37 °C for 15 min. Thereafter, 10 mM GABA was added, and then, the Vmax of the reaction was spectrophotometrically determined at ambient temperature by monitoring the production of NADH at 340 nm every 10 s for 5 min within the linear range. The expression below was used to estimate the % inhibition of GABA-T activity:

$$\% \text{ Inhibition of GABA} - \text{T} = \frac{(\text{Ts} - \text{Tb}) - (\text{C} - \text{Cb})}{\text{solvent control}} \times 100$$

where *Ts* is the test sample/drug (comprising of the enzyme, GABA, and test sample/reference drug), *Tb* is the test blank (comprising of buffer, GABA, and test sample/reference drug), *C* is the control (enzyme, water, and test sample/reference drug), and *Cb* is control-blank (water, buffer, and test sample/reference drug).

Determination of GABA content

This study adopted the procedure outlined by Zhou et al. [47]. The endogenous GABA content of the leaf fraction was determined according to the manufacturer's instructions that come with the plant γ-aminobutyric acid (GABA) assay kit (Suzhou-Comin Biotechnology Company Limited, Suzhou, China).

Determination of antioxidant vitamins

The antioxidant vitamin content of the test sample was evaluated using a spectrophotometric technique following the protocols outlined by the Association of Vitamin Chemists (AOVC) [48].

Determination of mineral content

Determination of some pharmacologically relevant elements (iron, manganese, calcium, copper, and zinc) in the test sample was done using atomic absorption spectrophotometer (AA-7000 model, Shimadzu, Japan) following the outlined procedure of AOAC [49]. Screening of individual elements was performed at their characteristic wavelength; thereafter, their concentration (presented in ppm) was obtained from the standard graph.

Statistical analysis

Numerical data from the study were statistically analyzed with one-way and two-way ANOVA on version 23.0 SPSS (Statistical Product and Service Solutions) (SPSS Inc., Chicago, Illinois, U.S.A.), and results obtained from the descriptive tables were presented as mean ± standard deviation. Duncan post hoc table was used to show significant differences in the result across the various groups. The significance level of the study was established at $p < 0.05$. Result presentation was done using GraphPad Prism version 6.5 (GraphPad Software, Inc., California, USA).

Results

Percentage Yield

The extraction process yielded 159.3 g of ethanolic extract, accounting for 4.55% of the mass (3500 g) of the pulverized leaves *S. linifolia*. Further fractionation gave a yield of 29.01 g (18.21%) n-Hexane fraction, 32.37 g (20.32%) ethyl acetate fraction 91.34 g (57.34%) ethanolic fraction, and residual mass of 6.58 g (4.13%). The ethanolic fraction had the highest yield.

Phytochemical composition of SLELF

The result (as presented in Table 1) showed the total phenolics content (91.64 ± 7.61 mg GAE/g), total tannins content (62.44 ± 3.86 mg TAE/g), and total flavonoids content (27.350 ± 1.48 mg QE/g) of SLELF. The composition of some antioxidant enzymes such as catalase (363.41 ± 11.23 U/g) and superoxide dismutase (192.59 ± 8.23 U/g) in the leaf fraction was also recorded in terms of their activity. In addition, the amount of reduced glutathione (GSH) (17.80 ± 0.23 µg/g) and gamma-aminobutyric acid (GABA) (153.06 ± 2.21 pg/ml) present in the leaf fraction were also recorded.

Table 1 Phytochemical composition of SLELF

Phytoconstituents	Content/activity
Total phenolics (mg GAE/g)	91.64 ± 7.61
Total tannins (mg TAE/g)	62.44 ± 3.86
Total flavonoids (mg QE/g)	27.35 ± 1.48
GSH (µg/g)	17.80 ± 0.23
GABA (pg/g)	153.06 ± 2.21
SOD (U/g)	192.59 ± 8.23
Catalase (U/g)	363.41 ± 11.23

High-performance liquid chromatography (HPLC) profile of composition of SLELF

High-performance liquid chromatography (HPLC) profile of polyphenolic compounds and flavonoids composition of SLELF

Presented in Table 2 are the polyphenolic compounds and flavonoids identified in SLELF using HPLC techniques. From the result, polyphenolics such as ellagic acid (5.46 ppm), quercetin (3.37 ppm), and ferulic acid (1.17 ppm), were detected in the leaf fraction at varying concentrations. In addition, different concentrations of flavonoids such as ellagic acid (1.23879 ppm), quercetin (0.616178 ppm), chlorogenic acid (0.056642 ppm), vanillic acid (0.053052 ppm), 3,4-dimethoxybenzoic acid (0.020722 ppm), gallic acid (0.012173 ppm), sinapic acid (0.001245 ppm), 4-methoxy cinnamic acid (0.000302 ppm), and one unidentified compound (0.000144 ppm) were detected in the leaf fraction. HPLC chromatograms showing the various peaks of the identified compounds are presented in Figs. 1 and 2.

Table 2 High-performance liquid chromatography (HPLC) profile of polyphenolics and flavonoids composition of SLELF

Peak ID	Retention time (min)	Peak height	Peak area	Conc. (ppm)*
Polyphenolics				
Ellagic acid	1.607	37,242.449	2,001,188.750	5.46
Quercetin	3.098	15,813.879	1,232,989.500	3.37
Ferulic acid	2.732	15,614.772	428,381.125	1.17
Flavonoids				
Ellagic acid	2.657	94,729.250	1,909,336.125	1.23879
Quercetin	2.898	79,751.523	2,346,381.250	0.616178
Chlorogenic acid	2.773	62,934.246	215,689.000	0.056642
Vanillic acid	2.707	58,791.965	202,020.719	0.053052
3,4-Dimethoxybenzoic acid	1.273	28,171.299	78,909.594	0.020722
Gallic acid	1.332	14,966.476	46,353.305	0.012173
Sinapic acid	2.098	1979.141	4741.750	0.001245
4-Methoxycinnamic acid	2.040	981.600	1148.800	0.000302
Unidentified	0.615	192.667	550.200	0.000144

* 1 ppm = 10^{-1} mg/100 g = 1 μg/g = 1 μg/ml = 1 mg/L

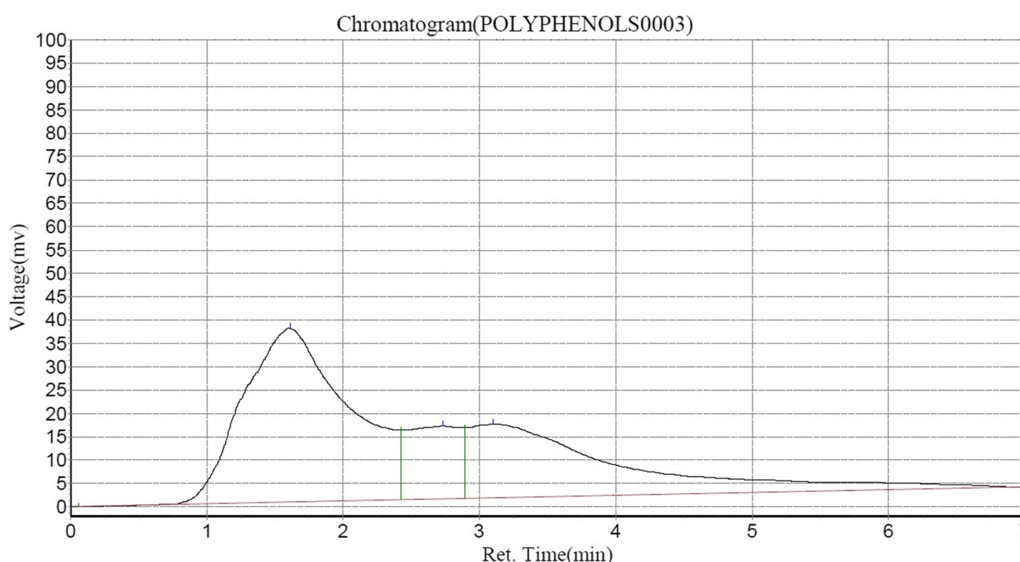

Fig. 1 High-performance liquid chromatography (HPLC) chromatogram of polyphenolic compounds in SLELF

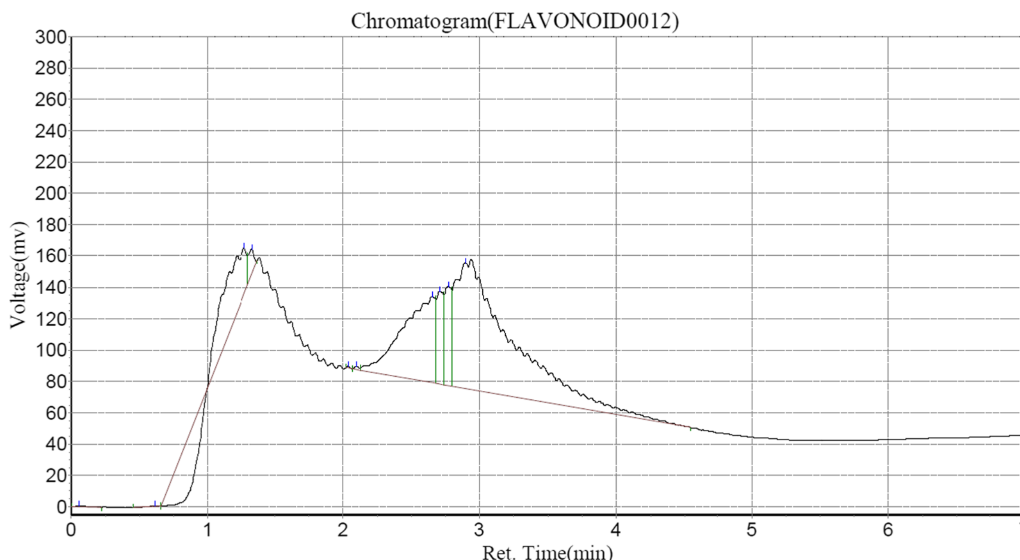

Fig. 2 High-performance liquid chromatography (HPLC) chromatogram of flavonoids in SLELF

Antioxidant vitamins and mineral composition of SLELF

Presented in Fig. 3 are the antioxidant vitamins and minerals recorded in SLELF. From the result, vitamin C (1.42 ± 0.17 ppm), vitamin E (0.038 ± 0.006 ppm), and vitamin A (0.023 ± 0.009 ppm) were present in the leaf fraction at various concentrations. The result also revealed the presence of some pharmacologically relevant minerals such as Ca (1.73 ± 0.08 ppm), Cu (1.32 ± 0.05 ppm), Mn (1.28 ± 0.03 ppm), Zn (0.75 ± 0.06 ppm), Cr (0.18 ± 0.007 ppm), and Fe (0.07 ± 0.009 ppm) in the leaf fraction at various concentrations.

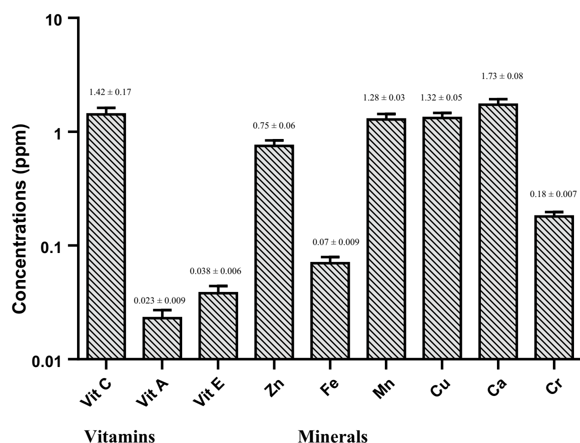

Fig. 3 Antioxidant vitamins and mineral composition of SLELF (n = 3)

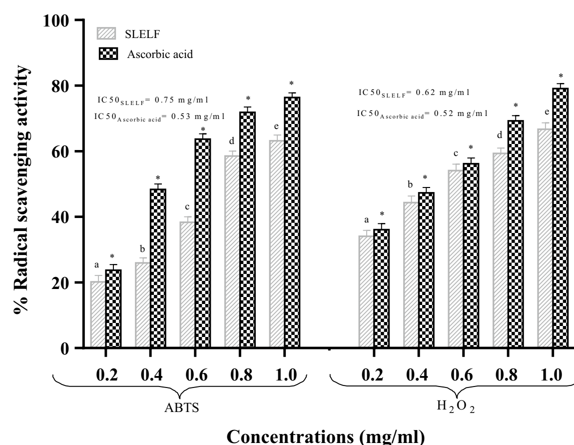

Fig. 4 Percentage (%) 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{+}$) and hydrogen peroxide (H_2O_2) radical scavenging activities of SLELF. Values are presented as mean ± standard deviation of triplicate determinations. Columns with different lowercase alphabets differed from each other significantly ($p < 0.05$), while subsets of a paired column with asterisks (*) differed significantly ($p < 0.05$) from each other

Radical scavenging activity of SLELF

The free radical scavenging potential of SLELF is presented in Fig. 4. From the result, SLELF scavenged $ABTS^{\cdot+}$ radical in a concentration-dependent fashion and was comparable to ascorbic acid. At lower concentrations (0.2, 0.4, and 0.6 mg/ml), SLELF exhibited moderate (< 50%) $ABTS^{\cdot+}$ radical scavenging activities (20.15 ± 1.98, 25.94 ± 1.64 and 38.35 ± 1.64%),

respectively, and was less potent ($p < 0.05$) compared to the scavenging activities (23.71 ± 1.73, 48.33 ± 1.71, and 63.67 ± 1.64%) produced with ascorbic acid at similar concentrations. Similarly, ascorbic acid exhibited significantly ($p < 0.05$) higher scavenging activities (71.83 ± 1.64 and 76.36 ± 1.42%) compared to SLELF (58.51 ± 1.53 and 63.18 ± 1.75%) at higher concentrations (0.8 and 1.0 mg/ml). Nevertheless, at higher concentrations (0.8 and 1.0 mg/ml), the $ABTS^{\cdot +}$ radical quenching activities of SLELF improved (> 50%) significantly ($p < 0.05$). The peak $ABTS^{\cdot +}$ radical attenuating activity (63.18 ± 1.75%) of SLELF occurred at the highest concentration (1.0 mg/ml); however, this was significantly ($p < 0.05$) lower compared to the $ABTS^{\cdot +}$ scavenging activity (76.35 ± 1.42%) produced with ascorbic acid at the same concentration (1.0 mg/ml). In addition, SLELF had a higher IC_{50} value (0.75 mg/ml) compared to ascorbic acid (which has an IC_{50} value of 0.53 mg/ml).

Furthermore, SLELF was potent in mopping up H_2O_2 pro-oxidant in a concentration-dependent manner and was on par with ascorbic acid (as shown in Fig. 4). At lower concentrations (0.2 and 0.4 mg/ml), the leaf fraction exhibited moderate (< 50%) H_2O_2-attenuating activities (34.08 ± 1.80 and 44.33 ± 1.00%), respectively, but was lower ($p < 0.05$) compared to the scavenging activities (36.06 ± 1.87 and 47.31 ± 1.62%) produced with ascorbic acid at similar concentrations. However, at higher concentrations (0.6, 0.8, and 1.0 mg/ml), SLELF exhibited more potent (> 50%) H_2O_2 neutralizing activities (54.09 ± 1.00, 59.31 ± 0.66, and 66.67 ± 0.38%), yet at similar concentrations, ascorbic acid showed greater ($p < 0.05$) scavenging activities (56.12 ± 0.76, 69.23 ± 0.50, and 79.07 ± 0.52%). Additionally, ascorbic acid exhibited significantly ($p < 0.05$) higher H_2O_2 radical scavenging activity across all concentrations when compared to SLELF. The maximum H_2O_2 radical scavenging activity of SLELF (66.66 ± 1.38%) occurred at the highest concentration (1.0 mg/ml). In addition, the IC_{50} value (0.62 mg/ml) of SLELF showed that the leaf fraction possesses potent H_2O_2 radical scavenging ability; however, ascorbic acid exhibited higher scavenging activity (with an IC_{50} value of 0.52 mg/ml) compared to SLELF.

Inhibitory effect of SLELF on alpha-amylase and alpha-glucosidase activity

The inhibitory action of SLELF on alpha-amylase and alpha-glucosidase activity is presented in Fig. 5. From the result, SLELF showed potent inhibitory action on α-amylase activity following a concentration-dependent trend akin to a standard drug (acarbose). At lower concentrations (0.2 and 0.4 mg/ml), SLELF exhibited moderate (< 50%) inhibitory action (35.05 ± 1.46 and 45.66 ± 1.33%), respectively, on α-amylase activity and was on par with acarbose (37.61 ± 0.43 and 45.03 ± 0.68%) at a similar concentration. Conversely, at higher concentrations (0.6, 0.8, and 1.0 mg/ml), SLELF showed improvement (> 50%) in α-amylase inhibition (51.66 ± 1.26, 60.79 ± 0.91, 64.50 ± 0.71%), respectively; however, at similar concentrations, acarbose exhibited significantly ($p < 0.05$) higher α-amylase inhibition (53.42 ± 0.34, 63.24 ± 0.60, and 68.09 ± 0.43%). The maximal inhibitory effect of SLELF on α-amylase activity (64.50 ± 0.71%) occurred at the highest concentration (1.0 mg/ml); however, at this concentration, acarbose exhibited significantly ($p < 0.05$) higher % inhibition of α-amylase (68.09 ± 0.43%). In addition, the IC_{50} value (0.56 mg/ml) of SLELF in inhibiting α-amylase was higher when compared to that of acarbose (0.44 mg/ml).

Furthermore, the result (as shown in Fig. 5) also revealed that SLELF exerted remarkable inhibitory actions on α-glucosidase activity akin to acarbose (a standard inhibitor). The inhibitory potential exhibited by the leaf fraction on α-glucosidase activity was concentration-dependent and followed a similar trend as that of acarbose. At a low concentration (0.2 mg/ml), SLELF showed moderate (< 50%) inhibitory action (37.86 ± 1.05%) on α-glucosidase activity, which did not differ significantly ($p > 0.05$) when compared with the inhibition (37.86 ± 0.88%) produced with acarbose at the same concentration. However, the inhibitory potential of SLELF on α-glucosidase increased with increase in concentration. The % inhibitory action of SLELF on α-glucosidase activity increased to 51.93 ± 1.05 at a

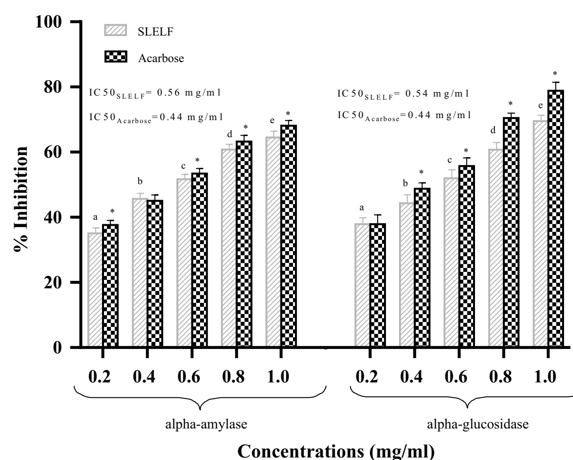

Fig. 5 Percentage (%) inhibitory action of SLELF on alpha-amylase and alpha-glucosidase activity. Values are presented as mean ± standard deviation of triplicate determinations. Columns with different lowercase alphabets differed from each other significantly ($p < 0.05$), while subsets of a paired column with asterisks (*) differed significantly ($p < 0.05$) from each other

concentration of 0.4 mg/ml; however, at this concentration, acarbose exhibited significantly ($p < 0.05$) higher inhibition (55.70 ± 0.52%). At higher concentrations (0.8 and 1.0 mg/ml), SLELF showed potent (> 50%) inhibitory actions (60.72 ± 2.20 and 69.51 ± 0.38%) on α-glucosidase activity, respectively; however, acarbose produced significantly ($p < 0.05$) higher α-amylase inhibition (70.44 ± 0.52 and 78.81 ± 0.63). The maximal α-glucosidase inhibition exhibited by SLELF (69.51 ± 0.38%) occurred at the highest concentration (1.0 mg/ml); however, at a similar concentration, acarbose exerted significantly ($p < 0.05$) higher α-glucosidase inhibition (78.81 ± 0.63). In addition, SLELF had a higher IC_{50} value (0.54 mg/ml) when compared to acarbose (with an IC_{50} value of 0.44 mg/ml).

Inhibitory action of SLELF on cyclooxygenase-2 and lipoxygenases activity

The inhibitory action of SLELF on cyclooxygenase-2 and lipoxygenases activity is presented in Fig. 6. The result showed that SLELF suppressed COX-2 activity in a dose-dependent manner, similar to that produced with indomethacin. At lower concentrations (0.2, 0.4, and 0.6 mg/ml), SLELF showed moderate (< 50%) inhibitory effects (29.62 ± 1.21, 35.74 ± 1.20, 44.84 ± 1.76%) on COX-2 activity, respectively, but exhibited more potent (> 50%) inhibition (54.15 ± 0.63 and 66.76 ± 1.54%) at higher concentrations (0.8 and 1.0 mg/ml), respectively. The % maximum inhibitory action of SLELF on COX-2 activity occurred at the highest concentration. Similarly, at lower concentrations (0.2 and 0.4 mg/ml), indomethacin exhibited moderate (< 50%) inhibitory effects (26.93 ± 0.76 and 38.24 ± 2.00%) on COX-2 activity, respectively, and exerted more potent inhibitory actions on COX-2 activity (60.37 ± 0.63, 65.37 ± 1.54, and 74.57 ± 0.76%) at higher concentrations (0.8–1.0 mg/ml), respectively. From the result, SLELF displayed significantly ($p < 0.05$) higher COX-2 inhibition at the lowest concentration (0.2 mg/ml); however, at higher concentrations (0.4–0.8 mg/ml), indomethacin exhibited significantly ($p < 0.05$) higher inhibitory actions on COX-2 activity compared to SLELF. In addition, SLELF showed a higher IC_{50} value (0.68 mg/ml) compared to indomethacin (which had an IC_{50} value of 0.58 mg/ml).

Furthermore, SLELF effectively suppressed LOXs activity in a concentration-dependent manner akin to indomethacin (as shown in Fig. 6). At lower concentrations (0.2 and 0.4 mg/ml), SLELF exhibited moderate (< 50%) inhibitory actions (26.51 ± 1.13 and 32.45 ± 0.81%) on LOXs activity, respectively; however, the inhibitory effects of SLELF on LOXs activity (39.33 ± 0.53, 47.62 ± 0.64, and 51.38 ± 0.57%) improved significantly ($p < 0.05$), at higher concentrations (0.6, 0.8, and 1.0 mg/ml), respectively. Similarly, at lower concentrations (0.2,

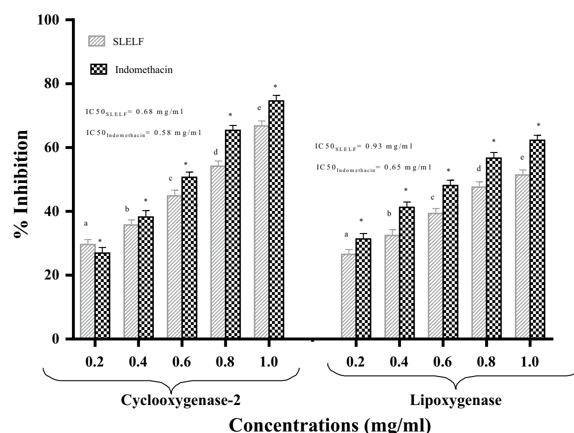

Fig. 6 Percentage (%) inhibitory action of SLELF on cyclooxygenase and lipoxygenase activity. Values are presented as mean ± standard deviation of triplicate determinations. Columns with different lowercase alphabets differed from each other significantly ($p < 0.05$), while subsets of a paired column with asterisks (*) differed significantly ($p < 0.05$) from each other

0.4, and 0.6 mg/ml), indomethacin exhibited moderate (< 50%) inhibitory actions (31.33 ± 0.71, 41.27 ± 0.64, and 48.09 ± 0.67%) on LOXs activity, respectively, and exerted more potent inhibitory effects on LOXs activity (56.67 ± 0.27 and 62.26 ± 0.47%) at higher concentrations (0.8 and 1.0 mg/ml), respectively. From the result, indomethacin exhibited significantly ($p < 0.05$) higher inhibitory actions on LOXs activity when compared to SLELF at all concentrations. However, SLELF exhibited its peak % LOXs inhibition at the highest concentration (1.0 mg/ml). In addition, SLELF showed a higher IC_{50} value (0.93 mg/ml) compared to indomethacin (which had an IC_{50} value of 0.65 mg/ml).

Inhibitory effect of SLELF on BChE and GABA-T activity

The inhibitory action of SLELF on BChE and GABA-T activity is presented in Fig. 7. The result showed that SLELF effectively suppressed the activity of BChE in a concentration-dependent manner and was on par with a reference drug (galanthamine). At lower concentrations (0.2 and 0.4 mg/ml), SLELF exhibited moderate (< 50%) inhibitory actions (43.60 ± 0.48 and 47.92 ± 0.61%) on BChE activity, respectively; however, galanthamine exhibited significantly ($p < 0.05$) more potent inhibitory actions on BChE activity (46.06 ± 0.46 and 57.18 ± 0.83%) at similar concentrations. Furthermore, SLELF showed improvement in inhibitory actions on BChE activity (55.01 ± 0.48, 62.04 ± 0.83, and 66.20 ± 0.61%) at higher concentrations (0.6, 0.8, and 1.0 mg/ml), respectively; yet, galanthamine exhibited significantly ($p < 0.05$) higher inhibitory actions on BChE activity (66.51 ± 0.58,

73.84 ± 0.69, and 80.71 ± 0.48%) at similar concentrations. The peak % BChE inhibition (66.20 ± 0.61%) exhibited by SLELF occurred at the highest concentration (1.0 mg/ml); still, galanthamine at the same concentration (1.0 mg/ml) produced significantly ($p < 0.05$) higher BChE inhibition (80.71 ± 0.48%). In addition, SLELF showed a higher IC_{50} value (0.43 mg/ml) compared to galanthamine (which had an IC_{50} value of 0.22 mg/ml).

Furthermore, SLELF exhibited potent GABA-T inhibition in a concentration-dependent manner akin to the reference drug (vigabatrin) (as presented in Fig. 7). The leaf fraction exerted moderate (< 50%) inhibitory effects on GABA-T activity (35.16 ± 0.36, 40.99 ± 0.21, and 47.56 ± 0.57%) at lower concentrations (0.2, 0.4, and 0.6 mg/ml), respectively, which was lower ($p < 0.05$) when compared to that of vigabatrin (37.80 ± 0.21, 42.27 ± 0.27, and 50.11 ± 0.44), at similar concentrations. However, at higher concentrations (0.8 and 1.0 mg/ml), SLELF showed relatively higher inhibitory effects on GABA-T activity (51.57 ± 0.41 and 54.95 ± 0.44%), respectively; nevertheless, vigabatrin displayed significantly ($p < 0.05$) more potent GABA-T % inhibition (57.00 ± 0.48 and 62.15 ± 0.42%) at similar concentrations. The leaf fraction exhibited its maximal GABA-T % inhibition at the highest concentration (1.0 mg/ml), although, at this concentration, vigabatrin was more potent ($p < 0.05$). In addition, SLELF showed a higher IC_{50} value (0.76 mg/ml) compared to vigabatrin (which had an IC_{50} value of 0.60 mg/ml).

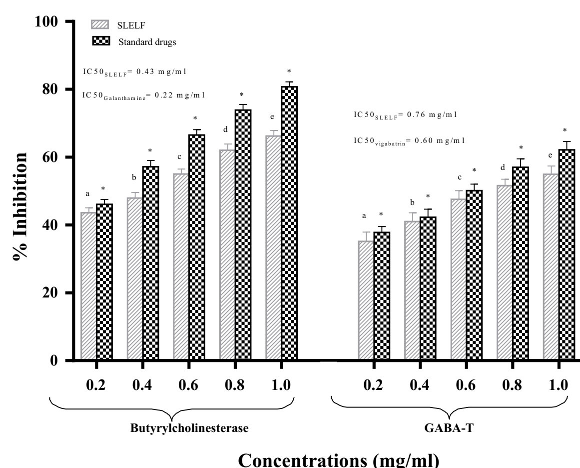

Fig. 7 Percentage (%) inhibitory action of SLELF on butyrylcholinesterase (BChE) and γ-aminobutyric acid transaminase (GABA-T) activity. Values are presented as mean ± standard deviation of triplicate determinations. Columns with different lowercase alphabets differed from each other significantly ($p < 0.05$), while subsets of a paired column with asterisks (*) differed significantly ($p < 0.05$) from each other

Discussion

The rich composition of bioactive secondary metabolites constituent in medicinal plants makes them valuable sources of pharmacologically relevant phytocompounds and templates for redesigning novel pharmacologic agents and modern-day pharmaceutics [7]. These justify the recent surge in research for plant-based drug candidates to substitute the use of synthetic remedies, which are almost not void of adverse effects when employed in managing diseases. *Sida linifolia* L. is an underexplored medicinal weed with several folkloric applications in African traditional medicine, yet not many scientific reports on the bioactivities are available. Herein, we assessed the bioactive compounds, in vitro free radical scavenging actions, and inhibitory potential of *S. linifolia* ethanolic leaf fraction on enzymes associated with diabetes, inflammation, and neurological disorder.

The present study was designed to replicate the alcoholic decoction of the *Sida linifolia* plant, as used by traditional healers in African folklore medicine. The ethanolic leaf fraction used in the study gave an appreciable yield of 57.34%. The % yield obtained in this study was higher than that of Nwankwo et al. [36], which reported a 40.91% yield of the plant leaves, using similar extraction and fractionation paradigm. Studies have proposed that the percentage yield after extraction depends on many determinants, including the drying temperature, the season of harvest, post-harvest condition, the fineness of the pulverized test material, the extraction time, and the choice of solvent [7]. In addition, polar fractions are usually rich in phenolics because of their polar chemistry. The enrichment of polyphenolics in polar fractions also influenced our choice of plant fraction. Moreover, the choice of leaf fraction aligns with Debalke et al. [50] and Nwankwo et al. [21], which demonstrated excellent pharmacological activities using the aerial parts of *Sida rhombifolia* and ethanolic leaf fraction *S. linifolia* in their studies, respectively.

Results of the phytochemical analysis of SLELF revealed appreciable amounts of total phenolics (91.64 ± 7.61 mg GAE/g), total tannins (62.44 ± 3.86 mg TAE/g), and total flavonoids (27.350 ± 1.48 mg QE/g). This result corresponds to de Oliveira et al. [51], which reported relatively higher levels of total phenolics (88.311 ± 2.660 mg·GAE/g) in the ethyl acetate leaf fraction of *Sida rhombifolia*. Nwankwo et al. [22] also reported appreciable amounts of tannins, flavonoids, phenols, alkaloids, terpenoids, cyanogenic compounds, steroids, glycosides, and saponins in the ethanolic leaf fraction of *S. linifolia*. Another study by Subramanya et al. [52] recorded considerable amounts of total phenolic, total tannins, and total flavonoids in very close relatives of

the study plant, such as *S. rhombifolia*, *Sida cordifolia*, *S. acuta*, *S. indica*, *S. spinosa*, *S. cordata*, *S. cordifolia*, *S. retusa*, and *S. mysorensis*. The methanolic leaf extract of *Sida cordifolia* showed relatively higher phenolics, tannins, and flavonoids content [52]. Polyphenolics such as flavonoids, tannins, and other phenols are well known for their pharmacological potentials, including antioxidant, anti-inflammatory, anti-diabetic, and neuromodulatory activities [53]. In connection with this, Zahoor et al. [54] reported a positive correlation between free radical scavenging potential (DPPH and ABTS), anti-cholinesterase (AChE and BChE), and anti-diabetic (α-glucosidase and α-amylase) actions versus total phenolics and total flavonoid content of *Grewia optiva* extracts. Therefore, the rich composition of SLELF in phenolics, flavonoids, and tannins implies that the plant could possess an excellent pharmacological profile.

We have previously reported appreciable amounts of bioactive compounds such as phenols (proanthocyanin, resveratrol), flavonoids (catechin, epicatechin, rutin, kaempferol, flavanones, naringenin, flavone, and flavo-3-ol), tannins, steroids, alkaloids, saponins (sapogenin), glycosides (lunamarin), oxalate, and phytate in the ethanolic leaf fraction of *S. linifolia* using GC-FID techniques (Additional file 1) [21]. Reports have linked some of these compounds with a number of pharmacological properties, including anti-inflammatory, antioxidant, immunomodulatory, neuromodulatory, anticancer, hepatoprotective, anti-diabetic, and cardio-protective potentials [54, 55]. In line with this, a correlation between the phytocomposition of plant extracts and their pharmacological properties has been previously reported [7]. The presence of these phytoactive compounds in SLELF sheds light on its potent pharmacological profile of the leaf fraction.

Furthermore, the results of HPLC analysis revealed appreciable amounts of polyphenolics such as ellagic acid, quercetin, and ferulic acid in the leaf fraction. In addition, several phytoactive flavonoids such as 3,4-dimethoxybenzoic acid, gallic acid, 4-methoxycinnamic acid, sinapic acid, vanillic acid, chlorogenic acid, and one unidentified flavonoid were also detected in the leaf fraction in appreciable amounts by HPLC technique. These further suggest that the leaf fraction may possess promising bioactivities. For instance, ferulic acid is reported to possess many pharmacological potentials, including anti-diabetic, anti-inflammatory, antioxidant, antimicrobial, and anticancer properties. Ferulic acid has been extensively employed in the pharmaceutical, food, and cosmetics industries [56]. Moreover, ellagic acid displayed pharmacological effects such as antiallergic, antiatherosclerotic, cardio-protective, hepatoprotective, nephroprotective, and neuroprotective in various in vitro and in vivo models [57]. Sinapic acid detected in SLELF has also been reported to possess antioxidant, anti-inflammatory, anti-glycemic, neuroprotective, antibacterial, and anticancer potentials [58]. Similarly, vanillic acid is well known to exhibit antioxidant, neuroprotective, and anti-inflammatory effects [59]. Interestingly, a wide range of potential health benefits linked to chlorogenic acids found in SLELF, such as anti-carcinogenic, anti-diabetic, anti-inflammatory, and anti-obesity effects, suggests its potential to provide a non-pharmacological and noninvasive approach for managing chronic diseases [60]. In addition, quercetin has been shown to demonstrate pharmacological activities such as its anti-carcinogenic, anti-inflammatory, and antiviral activities, as well as the ability to halt lipid peroxidation, capillary permeability, and platelet aggregation [61]. Several health benefits associated with gallic acid and 3,4-dimethoxy benzoic acid include anti-inflammatory, antioxidant, and antineoplastic actions. These compounds are well known to exhibit therapeutic activities in gastrointestinal, neuropsychological, metabolic, and cardiovascular disorders [62].

Furthermore, the human body possesses a potent and effective enzymatic antioxidant system, including catalase, superoxide dismutase, peroxidase, and polyphenol oxidase, which synergistically function in scavenging free radicals. This antioxidant system helps to scavenge ROS and protect against oxidative damage to cellular components [2]. Coincidentally, plants also possess similar enzymatic antioxidants system, which help to attenuate oxidative damage and maintain their cellular Redox status [63, 64]. Fortunately, studies have shown that these antioxidant enzymes could be exogenously supplemented in humans through dietary consumption of food and medicinal formulations rich in these antioxidant enzymes [64, 65]. Our data showed appreciably high catalase (363.41 ± 11.23 U/g) and superoxide dismutase (192.59 ± 8.23 U/g) activity in SLELF. These findings align with the work of Arshad et al. [67], which also reported appreciable catalase (CAT) (106.00 ± 15.00 U/g) and superoxide dismutase (SOD) (64.00 ± 1.50 U/g) activity in *S. cordifolia* leaves. Another recent study by Johri and Khan [68] reported excellent superoxide radical dismutation activity of superoxide dismutase in n-butanol and aqueous extracts of *Triticum aestivum* grass. In addition, catalase activity in a crude extract of Verdolaga's leaves (60.25 ± 0.25 U/g), root strips (23 ± 0.22 U/g), and stem strips (15.13 ± 0.64 U/g) has also been reported in a recent study [69].

Furthermore, our result revealed a considerable amount (17.80 ± 0.23 μg/g) of reduced glutathione in SLELF, indicative of a potent antioxidant capacity of SLELF. Our findings agree with Malar et al. [70], which

reported a moderate level (9.0 ± 0.2 μg/ml) of reduced glutathione in the ethanolic leaf extract of *Lepidium sativum,* as well as excellent antioxidant properties of the extract in vitro. Reduced glutathione (GSH), an essential thiol peptide, is well known to accumulate in the subcellular compartments of plants, animals, and microorganisms and is involved in maintaining the cellular redox status and protecting the living cell from oxidative damage by directly playing antioxidant roles against oxidative stress or indirectly quenching ROS as a co-enzyme to antioxidant enzymes [1]. As an essential component of the ascorbate–glutathione cycle, GSH also aids the attenuation of hydrogen peroxide by recycling oxidized ascorbate to reduced form in a reaction catalyzed by dehydroascorbate reductase [71]. Reduced glutathione can also scavenge hydroxyl or superoxide radicals and attenuate the oxidation of other cellular components [71]. Moreover, the consumption of sulfur-rich vegetables is highly encouraged due to its potential to supplement serum reduce glutathione levels to avert oxidative stress [70]. Moreover, glutathione, and other antioxidant enzymes (catalase, superoxide dismutase), enhances plant tolerance to different abiotic stresses, including drought, salinity, temperature variations, and toxic metals [72]. Perhaps, the considerably high level of GSH and activity of CAT and SOD in the leaf fraction of the study plant could be anchored as one of several mechanisms of stress response employed by the plant in the face of environmental stress [72].

Our data also showed appreciable amounts of vitamin C (1.42 ± 0.17 ppm), vitamin E (0.038 ± 0.003 ppm), and vitamin A (0.023 ± 0.009 ppm) in SLELF. A similar study by Nwankpa et al. [74] reported a relatively higher amount of Vit C (2.427 ppm) and a lower level of Vit E (0.185 ppm) in the ethanolic leaf extract of *S. acuta.* Another study by Shittu and Alagbe [75] also reported a relatively higher level of Vit C (3.017 ppm) in the aqueous leaf extract of *Sida acuta*. The variations in the composition of antioxidant vitamins could be due to several environmental and phylogenetic factors. However, the amounts of these antioxidant vitamins in SLELF were in considerable amounts akin to the levels reported in the literature for plant extracts with good antioxidant properties [76].

Furthermore, varied amounts of pharmacologically relevant minerals such as Ca (1.73 ± 0.05 ppm), Cu (1.32 ± 0.07 ppm), Mn (1.28 ± 0.06 ppm), Zn (0.75 ± 0.06 ppm), Cr (0.18 ± 0.03 ppm), and Fe (0.07 ± 0.03 ppm) were also detected in SLELF. Our result was lower than that previously reported in *S. acuta*, a closely related species. In fact, Nwankpa et al. [74] reported relatively higher levels of Ca (14.428 ± 0.02 ppm), Mg (12.211 ± 0.01 ppm), and Zn (32.512 ± 0.02) in the ethanolic leaf extract of *S. acuta.* Another study by Enin et al. [77] reported 4.432 ppm of Ca, 0.101 ppm of Fe, 0.107 ppm of Zn, and 1.440 ppm of Mg in *S. acuta* leaf extract. Similarly, in a separate study, Shittu and Alagbe [75] reported 12.76 ppm of Ca, 10.21 ppm of Mg, 0.214 ppm of Fe, 0.060 ppm of Mn, and 0.004 ppm of Cu in aqueous leaf extract of *Sida acuta*. This study is the first of its kind. To the best of our knowledge and as of the time of the study, no report was available online on the mineral composition of *S. linifolia*. Several factors could be responsible for the slight discrepancy in mineral composition observed in the results. These could be due to phylogenic variations, cultivation site differences, and the screening methodology and instrumentation [78]. However, the moderate levels of these minerals in SLELF suggest its safety profile in terms of toxicity, as higher amounts of these heavy metals could be deleterious to health.

Oxidative stress is implicated in the pathophysiology of several diseases, either as a causative factor or as a consequence. Oxidative stress results when the production of free radicals in the living system upsets instituted antioxidant mechanisms that neutralize them. Excessive pro-oxidant build-up could lead to indiscriminate denaturation and distortion of the native structure and redox of macromolecules, such as proteins, nucleic acids, lipids, and carbohydrates, resulting in an overall progressive decline in physiological functions [2]. Hence, the need for exogenous supplementation of antioxidants to help attenuate oxidative chain reactions and disease progression in the human system. The antioxidant properties of SLELF were tested and compared with standard antioxidant. The $ABTS^{\cdot+}$ test is an effective screening method for electron or hydrogen-donating antioxidants (aqueous phase radical scavengers) and chain-breaking antioxidants (scavenging lipid peroxyl radicals) [79]. Our data revealed that SLELF exhibited appreciable and concentration-dependent $ABTS^{\cdot+}$ radical quenching properties and was on par with ascorbic acid. The potent $ABTS^{\bullet+}$ radical quenching activity recorded for SLELF could be attributable to its rich polyphenolics (flavonoids, tannins, and phenolics) composition. Perhaps, the rich composition and variety of polyphenolic compounds in SLELF could justify its radical quenching activities. Extensive research on the antiradical and antioxidant actions of low-molecular weight phenolics such as flavonoids and phenolic acids has demonstrated their radical attenuating properties [51, 69]. Besides, Riedl and Hagerman [80] proposed that phenolics with higher molecular weight, e.g., tannins, are better free radicals quenchers and that the radical scavenging ability of phenolics is dependent on the number of aromatic rings, molecular

weight, and natural hydroxyl group substitution rather than the specific functional groups. The presence of antioxidant vitamins (Vit A, C, and E) in SLELF could also account for the observed potent free radical scavenging activities exhibited by the leaf fraction. These synergistic radical quenching activities of vitamins C and E have been documented in the literature [81]. The presence of hydrophobic chain of polyene units capable of neutralizing thiyl radicals, quenching singlet oxygen, and combining with and stabilizing peroxyl radicals confers vitamin A its potent antioxidant potential [29]. These vitamins are effective scavengers against reactive oxygen and nitrogen species, such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\cdot}$), singlet oxygen ($^1O_2$), nitroxyl anion (NO^-), nitric oxide radical ($NO^\bullet$), and nitrosonium cation (NO^+), and defend cellular and subcellular components from free radical-induced damage [29]. The $ABTS^+$ radical cation assay is efficient for screening most antioxidants, including high- and low-molecular weight phenolics [82]. However, its pitfalls, such as the non-existence of $ABTS^{\cdot+}$ radical in the biological system and the high steric hindrance around the nitrogen-centered atom of ABTS compound (as in DPPH molecule), make it a poor model for highly reactive radicals, namely hydroxyl radical, nitric oxide radical, superoxide radical, or peroxy radical ($LOO^\bullet$), which are commonly present in the biological system [83]. Additionally, the higher IC_{50} value of SLELF (0.75 mg/ml) in scavenging $ABTS^{\cdot+}$ radicals when compared with ascorbic acid (with an IC_{50} value of 0.53 mg/ml) suggests that the leaf fraction may possess lesser toxicity.

Our data also showed that SLELF effectively neutralized hydrogen peroxide pro-oxidant in a concentration-dependent manner akin to ascorbic acid. This suggests the ability of the leaf fraction to maintain cellular redox and protect against free radical-induced DNA damage. Hydrogen peroxide is a non-free radical precursor that reacts in the presence of free ferrous iron (Fe^{2+}) to produce hydroxyl radicals, which can permeate the nuclear membrane and attack the DNA. The H_2O_2 assay complements the ABTS test as it demonstrates the propensity of antioxidants to attenuate H_2O_2 molecules, a precursor molecule for the production of hydroxyl and superoxide radicals, which cannot be tested effectively with ABTS due to high steric hindrance [82]. The appreciable amounts of non-enzymatic antioxidants, such as polyphenols, glutathione, vitamins A, C, and E, and the considerable activity of antioxidant enzymes (catalase, superoxide dismutase) recorded in SLELF, could explain the excellent free radical scavenging properties it displayed. Perhaps, it could be that the various antioxidant phytocompounds in the leaf fraction acted synergistically or additively in exerting the observed potent antioxidant properties. This aligns with the work of Yao et al. [84], which opined on the synergistic activities of antioxidant phytoconstituents. Our result also aligns with the submissions of Subramanya et al. [52], Nwankwo et al. [21], and Nwankwo et al. [22], which have demonstrated potent antioxidant activities of some members of the *Sida* genus, including *S. linifolia*. In the same vein, Panduranga et al. [85] also reported the ability of extract from *Sida glutinosa* to up-regulate the levels of GSH in vivo. Furthermore, the higher IC_{50} value of SLELF (0.62 mg/ml) in neutralizing H_2O_2 radicals compared to that of ascorbic acid (0.52 mg/ml) further implies a moderate radical scavenging action of the leaf fraction, which may preclude adverse side effect.

Screening plant materials for their enzyme inhibition potential is a valuable pharmacological approach that provides an early indication of their efficacy against some targeted diseases [86]. Diabetes mellitus, characterized by insulin-signaling dysfunction, is a life-threatening metabolic disorder with an alarmingly high mortality rate in recent times [13]. Postprandial hyperglycemia, which is responsible for the modality of diabetes, is mainly caused by two carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase. Alpha-amylase hydrolyzes the 1,4-glycosidic bonds of polysaccharides (starch, glycogen) to disaccharides (such as dextrins and maltose), while α-glucosidase converts disaccharides to monosaccharides, which culminates into elevated blood glucose level [13]. In that effect, α-amylase and α-glucosidase inhibitors have been shown to be effective against hyperglycemia symptoms as they delay carbohydrate digestion and lower postprandial plasma glucose levels [13]. In addition, inhibition of α-amylase is proposed to be a better suppressor of postprandial hyperglycemia when compared to α-glucosidase because of its ability to prevent excessive build-up of maltose [13]. From our findings, SLELF was effective against α-amylase and α-glucosidase. The inhibitory action of SLELF was directly proportional to concentration and was on par with the standard drug, acarbose. In addition, our data showed that SLELF exerted a relatively mild inhibitory effect on α-amylase and α-glucosidase as evident in the higher IC_{50} values (0.56 and 0.54 mg/ml, respectively) compared to that of the acarbose (0.44 mg/ml), which implies less toxicity compared to the reference drug. Similarly, Shankar et al. [87] demonstrated a dose-dependent inhibitory activity of *S. acuta* (a closely related plant) on α-amylase and α-glucosidase. Likewise, Dinlakanont et al. [88] reported good inhibition action of three Malvaceous weeds, *S. acuta, A. indicum,* and *M. coromandelianum* on the activity

of α-amylase and α-glucosidase. In line with these, a study by Bati et al. [89] reported that the ethanolic leaf extract of *S. rhombifolia* showed potent inhibitory effects on α-amylase and α-glucosidase. Also, a previous submission by Ahmad et al. [90] showed that *S. cordifolia* alcoholic extract produced a dose-dependent anti-diabetic effect in streptozotocin-induced diabetic rats. At the time of this study, no anti-diabetic study has been reported for *S linifolia* plant. Our study is the first of its kind. The observed bioactivity of SLELF could be due to its rich composition in flavonoids, tannins, and phenolics, which are well known to inhibit these carbohydrate-metabolizing enzymes and reduce blood glucose levels [13, 92]. In line with our findings, Mahnashi et al. [91] previously reported a binding interaction that was favorable between human pancreatic amylase and some flavonoids, such as epicatechin, rutin, naringenin, and kaempferol, which have been previously reported in *S. linifolia* leaves [21]. More so, GC-FID result, reported by Nwankwo et al. [21], identified several bioactive compounds notable for excellent anti-diabetic activity, as evident in the literature. Among these compounds, kaempferol, anthocyanin, epicatechin, naringenin, catechin, and rutin are potent radical quenchers and hypoglycemic agents [92]. Naringenin inhibits hyperglycemia by decreasing glucose reabsorption in the kidney, decreasing intestinal glucose absorption via the brush border, and increasing glucose uptake and usage in the adipocytes and muscle cells [93]. Furthermore, rutin has been reported to modulate hyperglycemia by promoting glucose uptake in the peripheral tissues, inhibiting gluconeogenesis in hepatocytes, and stimulating β-cells exocytosis of insulin granules [94]. The anti-DM properties of rutin could also be due to its potent antioxidant and anti-inflammatory potential [95]. In the same vein, catechin has been shown to exert an anti-diabetic effect via its ability to boost the antioxidant profile and could improve diabetes-related nephropathy [96]. Additionally, kaempferol has been shown to suppress gluconeogenesis and enhance glucose metabolism [13]. Our present HPLC data also revealed the presence of quercetin in SLELF. Quercetin and flavan-3-ols (catechin and epicatechin) have been shown to inhibit some mammalian α-amylases and α-glucosidase similarly as standard synthetic and natural anti-diabetic agents such as acarbose [97, 98]. Thus, the pharmacological potential of *S. linifolia* ethanolic leaf fraction could be anchored on these phytoconstituents.

In addition, elemental minerals such as Ca, Mn, Zn, Fe, and Cr recorded in the leaf fraction are known to function as enzyme cofactors and stabilizing components of functional proteins [140]. From the standpoint of diabetes mellitus, trace minerals play vital roles in modulating insulin signaling. For instance, homeostatic regulation of calcium coordinates insulin secretion, cell recognition, and actions. Abnormal regulation of calcium levels reduces the β-cell function, which could distort glucose homeostasis [99]. Moreover, the critical role of Zn in the maximal maturation of insulin secretory granules and its role in facilitating insulin secretion and sensitivity have been reported [100]. Zinc has also been shown to inhibit cytokine-induced destruction of islet cells to a reasonable extent preventing the progression of diabetes and improving insulin production, storage, and release by the β-cells [100]. Besides, a disorder in zinc metabolism could result in the upregulation of microangiopathic complications in diabetic patients [99]. Also, ferritin, the form in which iron is stored, has emerged as a valuable and independent indicator for assessing glucose tolerance, since the degree of insulin sensitivity, vascular resistance, and oxidative damage depends on iron levels [101]. Chromium has also been shown to play roles in the regulation of insulin action and its effects on carbohydrate, protein, and lipid metabolism [102]. Furthermore, from the viewpoint of free radical attenuation and anti-inflammatory actions, these elemental minerals show excellent pharmacological potential. For example, zinc plays a crucial role in cell-mediated immunity, antioxidant, and anti-inflammatory actions [103]. Manganese (Mn) complexes have been shown to attenuate superoxide anion radicals and render support to superoxide dismutase against superoxide toxicity [104]. In addition, manganese is part of the antioxidant enzyme superoxide dismutase (SOD), which plays a vital role as a therapeutic agent against inflammatory disorders [104]. The antioxidant potential of dietary chromium has also been reported [105].

The COX enzymes convert arachidonic acid to prostaglandin H2. Prostaglandin plays an important role in inflammation. Currently available non-steroidal anti-inflammatory drugs (NSAIDs) act by inhibiting the activity of COX enzymes. However, their reported adverse effects have warranted the search for alternative anti-inflammatory drug candidates, preferably of plant origin. In the current study, SLELF demonstrated potent anti-inflammatory property by inhibiting COX-2 activity. The observed COX-2 inhibitory activity of the leaf fraction followed a concentration-dependent trend similar to that observed with the NSAID, indomethacin. Based on this report, the anti-inflammatory mechanism of SLELF could be anchored on its ability to inhibit COX-2 and consequently halt the synthesis of prostaglandins. Moreover, the observed higher IC_{50} value (0.68 mg/ml) of SLELF in inhibiting COX-2, compared to that of indomethacin

(0.58 mg/ml), depicts mild inhibitory action of SLELF on the enzyme, with less lethal side effects. In consonance with our study, Nwankwo et al. [21] reported that the ethanolic leaf fraction of *S. linifolia* inhibited phospholipase A2 in vitro as well as inhibited carrageenan and egg albumin-induced inflammation in rat models, suggesting its anti-inflammatory potential. The anti-inflammatory action exhibited by SLELF may be attributable to its phytoconstituents. Flavonoids are reported to inhibit COX enzymes and cytokine/chemokine production in human whole blood [106]. Another more recent study demonstrated the potential of flavonoids as dual inhibitors of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX), in vitro and in silico [107]. Flavones present in the leaf fraction could also be implicated in the observed anti-inflammatory activity. For instance, a study by Kim et al. [108] comparing the structural activity relationships between several flavone derivatives versus COXs and LOXs inhibition submitted that most flavones were potent COX inhibitors, while some flavonol derivatives, including quercetin, were selectively LOXs inhibitors. Kaempferol and quercetin were also reported to inhibit COXs isolated from rat peritoneal macrophages [108]. In addition, a more recent study has demonstrated the inhibitory activity of flavonoids on COX, mostly COX-1 [109]. In relation to this, Abubakar et al. [110] reported that kaempferol is a more potent inhibitor of COX-2 than COX-1. However, Tanumihadja et al. [111] recently reported that the active compounds of *Sida rhombifolia* exhibited an anti-inflammatory effect by significantly inhibiting COX-1 and COX-2 non-selectively.

The action of 5-LOX on arachidonic acid results in the formation of 5-hydroperoxyeicosatetraenoic acid (HPETE) and further converts into leukotrienes, a potent constrictor of the bronchioles, implicated in asthma and chronic bronchitis [12]. On the other hand, the 15-Hydroxyeicosatetraenoic acid produced by 15-LOX plays a significant role in inflammation, cell differentiation, atherogenesis, and carcinogenesis [12]. In the present study, SLELF suppressed LOXs activity in a concentration-dependent manner, akin to indomethacin. The inhibitory action of SLELF on LOXs could be linked to its rich phenolics and flavonoid composition. Phenolics have been previously shown to inhibit LOXs activity hence, suggesting a beneficial role in the dietary intake of polyphenols [112]. Our result aligns with the work of Preethidan et al. [113], which recorded potent lipoxygenase inhibitory activity of isolated compounds from six members of *Sida* species, namely *S. acuta*, *S. cordata*, *S. mysorensis*, *S. alnifolia*, *S. cordifolia,* and *S. rhomboidea*. Our data also showed a greater IC_{50} value (0.93 mg/ml) of SLELF in inhibiting LOXs when compared to that of indomethacin (0.66 mg/ml), which implies moderation in the inhibitory action of the leaf fraction on LOXs and lesser toxicity.

The etiology of neurological disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) has been linked to perturbation in cholinergic devices, which affects memory, cognitive, and attention functions [114, 115]. In addition, it has been opined that less pharmacological advantage would be obtainable if cholinesterase inhibitors (ChEI) selectively inhibit AChE; on the other hand, agents that selectively inhibit BChE may elicit better efficacy in managing AD patients. Potent cholinergic inhibitors, such as tacrine, huperzine A, and galanthamine, are effective in managing AD; however, they are not without adverse side effects [115]. Because of these, screening plant materials for their propensity to inhibit cholinergic enzymes like butyrylcholinesterase (BChE), relevant to Alzheimer's disease, is an important approach to finding novel drug candidates to combat the disease. This informed the choice of screening the *S. linifolia* plant for novel butyrylcholinesterase inhibitors. Interestingly, from the data obtained, SLELF moderately inhibited BChE activity following a concentration-dependent trend and was on par with the conventional antipsychotic drug galanthamine. In fact, the higher IC_{50} value (0.43 mg/ml) of SLELF suggests a mild anti-BChE activity compared to that of galanthamine (0.22 mg/ml). The promising neuromodulatory actions of the leaf fraction could be due to its rich phenolic content. The inhibition effects of the phenolic compounds against AChE and BChE activities have been reported in the literature [169,170]. A recent study by Kundo et al. [116] reported a strong correlation between phenolics, flavonoids, and proanthocyanidin content with cholinesterase inhibition activities. Naringenin found in the leaf fraction has been reported to exhibit effective anti-BChE activity [116]. In addition, gallic acid, quercetin, ferulic acid, and vanillic acid present in the leaf fraction are reported to exhibit ChEs inhibitory activities [117]. Moreover, our report agrees with the work of Panduranga et al. [85], which reported potent antioxidant and neuroprotective properties of *Sida glutinosa*, a closely related plant.

The inhibitory neurotransmitter γ-aminobutyric acid (GABA) is the major mediator of neuronal inhibition in the central nervous system (CNS). When the brain's GABA level decreases below a certain threshold, the excessive neuronal excitation elicited by other excitatory neurotransmitters could result in convulsion [17]. The imbalance in the proportion of these neurotransmitters toward excitation could be corrected by inhibiting γ-aminobutyric acid aminotransferase (GABA-T), the enzyme which converts GABA to the excitatory neurotransmitter L-glutamic acid or by exogenous supplementation of GABA present in anxiolytic agents

[17]. Hence, the present study evaluated the propensity of SLELF to modulate GABA-T activity in vitro. From the result, SLELF moderately inhibited GABA-T activity in a concentration-dependent fashion and was on par with a conventional GABA-T inhibitor, vigabatrin. The peak GABA-T inhibition (54.95%) exhibited by SLELF occurred at the highest concentration (1.0 mg/ml). In addition, the higher IC_{50} value (0.76 mg/ml) of SLELF indicated that the leaf fraction possesses GABA-T inhibition property but was less potent when compared to vigabatrin (with an IC_{50} value of 0.60 mg/ml). The anti-GABA-T effect of the leaf fraction could be attributable to its rich phenolic compounds and other phytoconstituents. In relation to our findings, phytochemicals such as flavonoids, alkaloids, phenolic acids, saponins, and terpenes have shown to demonstrate good anxiolytic effect in several animal models by exhibiting potent binding affinity with GABA-A receptors at both the benzodiazepine (BZD) and non-BZD binding sites [119]. Several phenolic acids, including chlorogenic acid, sinapic acid, cinnamic acid, vanillic acid, ferulic acid, and gallic acid, have been associated with anxiolytic and antidepressant activities [120]. Moreover, the bioactive compounds present in famous anxiolytic plants such as *Ilex paraguariensis*, *Humulus lupulus* (Hops), *Centella asiatica*, *Ginkgo biloba*, and *Matricaria recutita* were phenolic compounds, chlorogenic acid, theobromine, caffeic acid, caffeine, gallic acid, and rutin [121], some of which were detected in SLELF. In connection with our data, Ibironke et al. [122] reported that the ethanolic leaf extract of *S. acuta*, which is of the same genus as our study plant, showed antidepressant-like properties in a rat model, proving that the plant species (*S. acuta*) contains psychoactive substances. Similarly, Datusalia et al. [123] submitted that *S. tiagii* exhibited potent anxiolytic and anticonvulsant activities. Our data also reported appreciable amounts of GABA in SLELF. These further suggest that *S. linifolia* leaves may possess anxiolytic potentials, perhaps, via the modulation of GABA-T activity and the additive effect of its GABA content.

Conclusion

The result of the present study showed that *S. linifolia* ethanolic leaf fraction demonstrated appreciable antioxidant, anti-diabetic, anti-inflammatory, and neuromodulatory potentials, which could be anchored on its rich phytochemistry. Perhaps, the plant leaves could be a rich source of bioactive phytoconstituents which will be of valuable essence to the health and pharmaceutical industry. The rich phytochemistry and antioxidant properties of the leaf fraction suggest that the plant extracts could also be considered for investigating anticancerous activities. Therefore, further studies on the plant leaves extracts are warranted.

Abbreviations

BChE	Butyrylcholinesterase
GABA-T	γ-Aminobutyric acid transaminase
GAE	Garlic acid equivalent
QE	Quercetin equivalent
SLELF	*Sida linifolia* Ethanolic leaf fraction
TAE	Tannic acid equivalent

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43094-023-00527-8.

Additional file 1: Gas chromatography-flame ionization detector (GC-FID) phytochemical profile and chromatogram of ethanolic leaf fraction *S. linifolia*.

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Author contributions

ECE and FNN conceptualized the project and designed the study, FNN curated the methodology, ECE and FNN performed the study, ECE, DOO, and FNN performed the statistical analysis and data interpretation, ECE wrote the paper, and the final manuscript was proofread and approved by all authors.

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Availability of data and materials

The datasets of this study is available from E.C. Ezeako, upon request.

Declarations

Ethics approval and consent to participate

Ethical clearance certificate was acquired from the research ethics unit of the Department of Biochemistry, University of Nigeria Nsukka, indicating that the study was carried out with utmost meticulousness and void of any potential threat to human or the environment (Approval No:UNN-BCH-9014). The informed consent of the volunteering human blood donor was duly solicited before their participation. Appropriate permission to research on the study plant was duly solicited from the local legislations.

Consent for publication

The authors declare no conflict of interest.

Competing interests

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Author details

[1]Pharmacology and Pharm-Biology Unit, Department of Biochemistry, University of Nigeria, Nsukka, Enugu 410001, Nigeria.

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