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Original Research Article

Antioxidant, antidiabetic and cytotoxicity effects of E*uadenia trifoliata* leaves extracts

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Abstract

Purpose: *Euadenia trifoliata plant is used traditionally for the management and treatment of several diseases. The current study investigates in vitro antidiabetic, antioxidant and cytotoxic potential of the plant.*
Methods: *The antioxidant activity of the hexane, ethylacetate, methanol and water extracts of Euadenia trifoliata was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethyl-benzothiazoline-6)-sulphonic acid (ABTS), iron chelation, hydroxyl and nitric oxide radical scavenging capability assays. The antidiabetic activity was evaluated by determining the inhibitory effect of the extracts on the activities of α-amylase and α-glucosidase enzyme. Cytotoxic activity was determined against brine shrimps and MCF-7 cancer cell lines using brine shrimp lethality and MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assays respectively.*
Results: *Methanol and ethyl acetate extracts showed significant ($p \leq 0.05$) antidiabetic activity against α-amylase equally (1.38mg/mL) and α-glucosidase (0.52mg/mL and 0.70 mg/mL) respectively compared to hexane and aqueous extracts. Also, both methanol and ethylacetate extracts showed better antioxidant activity compared to other solvent extracts. The ethylacetate extract was found to be highly cytotoxic and aqueous extract showed no cytotoxicity against brine shrimps. None of the extracts were found to be cytotoxic against MCF-7 breast cancer.*
Conclusion: *Methanol and ethylacetate extracts of E. trifoliata possess better antioxidant, antidiabetics and cytotoxic activities against brine shrimps compared to non-polar (hexane) and polar (aqueous) extracts.*

Keywords: *Euadenia trifoliata, antioxidants, antidiabetics and cytotoxicity*

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INTRODUCTION

Eudenia trifoliata is a member of the Capparaceae Family and is commonly found in the dense forest in Nigeria (especially in Osun and Ogun State), Gabon, Ghana and Cameroon. In Nigeria, it is known as 'Olikan' and 'Osoban' among Edo and 'Ologbokiyan' and 'Ajinjingburigbu' among the Yorubas South west Nigeria and also known as 'Aka-ato' among the Igbos of the South East [1].

Traditionally, *E. trifoliata* is used for the treatment of tuberculosis, arthritis, otalgia, aphrodisiac, rectal prolapse, earache, and inflammation [2, 3]. The leaf decoction is used for managing blood disorder, antiemetic, management of diabetes, and as poison [4]. Cytotoxicity against brine shrimps, antimicrobial and antinociceptive activity have been reported [1, 3].

Diabetes and cancer are metabolic diseases posing a serious threat to humans. These metabolism disorders are mostly due to oxidative stress on the tissues of the human body. Recently, it was observed that incidence of some cancers such as liver, pancreas, colo-rectum, kidney and breasts cancer have been linked to diabetes [5]. It was also reported that women with diabetes have a 15–20% increased risk of breast cancer compared to women without diabetes [6]. Intrigued by these findings and the known cytotoxic activity of *E. trifoliata,* the antidiabetic activity of *E. trifoliata* leaf extract was investigated along with the known antioxidant and cytotoxicity effects.

METHODS

Collection and identification of plant materials

The leaves of *E. trifoliata* were collected from Modakeke, Ile-Ife, Osun State, Nigeria (latitude 7°22'59.99" N and longitude 4°16'0.01" E) and identified by Dr. O. O. Oyebanji of the Botany Department, University of Lagos. Voucher specimen (LUH7399) was prepared and deposited at the University of Lagos Herbarium.

Extraction of *Euadenia trifoliata* leaf

Freshly collected plant materials were rinsed with tap water and air dried until constant weight. The dried plant materials were pulverized into powder and stored in an airtight container. A serial extraction was carried out according to the method described by Ahmed et al. [7], with some modifications. Approximately, 1500 g each of the dried powdered materials was successively macerated in 6 L of hexane, ethylacetate, methanol and distilled water (DW) separately in conical flasks and kept on a Labcon Platform shaker (Labouratory consumables, PTY, Durban, South Africa) for 24 hour. Each extract was filtered off using Whatman No 1 filter paper. The procedure was repeated three (3) times and the filtrates were collected and concentrated, the weight of the extracts was taken and percentage yield was calculated.

Phytochemical Analysis

The presence of alkaloids, anthraquinone, flavonoids, phenols, saponin, terpenes and sterols were determined in the leaves of *Euadenia* trifoliate [7]. Total phenol [9], total flavonoids [10], total proanthocyanidins [11] and total saponin were quantified as previously reported [12].

Antioxidant assays

DPPH radical scavenging activity

The free radical scavenging activity of the extracts was based on the stability of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Exactly 150 µL of the different concentration (1.00, 0.75, 0.50, 0.25 and 0.125 mL) of the plant extracts was added separately to 150 µL of 0.004% methanolic solution of DPPH in a 96-well micro titre plate. The absorbance at 517 nm was taken after 30 mins using a 96-well microplate reader (BIO-RAD, model 680, JAPAN) and the percentage inhibition activity was calculated [10].

ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic) scavenging activity

Fifty (50) mL each of 7 mM of ABTS and 2.45 mM potassium persulphate were left to react in the dark. Twenty (20) µL aliquot was then mixed with 200 µL ABTS solution in 96-well microliter plate and the absorbance was taken at 734 nm [10].

Hydroxyl radical scavenging ability

About 100 µL aliquot, 120 µL 20mM deoxyribose, 400 µL 0.1 M phosphate buffer, 40 µL 20 mM hydrogen peroxide and 40 µL 200 µM ferrous sulphate ($FeSO_4$) were mixed in 2mL Eppendorf tubes. Thereafter 100µL of DW was added and incubated for 30 min at 37°C, 500 µL 2.8% trichloroacetic acid (TCA) and 400 µL 0.6% thiobarbituric acid (TBA) solution to terminate the reaction. From the mixture, 300 µL was transferred into a 96-well micro titre and incubated for 20 min, then the absorbance was taken at 532 nm using microplate reader (BIO-RAD, model 680, JAPAN) [10].

Metal chelating capability

Aliquot (100µL) of the was mixed with 500 µL of 2 mM $FeCl_2$ solution. Afterward 200 µL 5 mM of ferrozine was added to ignite the reaction, subsequently agitated for a while, and allowed to stand for 10 mins at room temperature. The absorbance was taken at 562 nm [13].

Nitric oxide scavenging activity

The nitric oxide scavenging activity was determined by reacting 2 mL of 10 mM sodium nitroprusside, 0.5 mL phosphate buffer saline and 0.5 mL of varying concentration of the extract. The reaction mixture was allowed to incubate at 25ºC for 2.5 hrs. Thereafter, 500 mL of Gress's reagent was added and the absorbance was taken at 546 nm on a microplate reader (BIO-RAD, model 680, JAPAN) [14].

In vitro anti-diabetic activity

α-Amylase (α-AML) inhibitory assay

Extracts/ acarbose (50 µL) and 0.05 mL α-AML solution (0.5 mg/mL in 0.02 mol/dm^3 of sodium phosphate buffer SPB, pH 6.9), the mixture was incubated at 25ºC for 10 mins, with addition of 0.05 mL of 1% starch solution (prepared in SPB). Then the mixture was incubated for the second time at 25ºC for 10 mins and terminated with the introduction 100 µL of dinitro salicylic acid reagent. Finally, the mixture was incubated in boiling water for 5 mins, cooled and diluted with 1 mL DW for absorbance reading at 540 nm. Dimethyl sulfoxide (DMSO) and DW (distilled water) replaced the extract to represent the control following similar procedure. The α-AML inhibitory activity was calculated as percentage [9].

Mechanism of α-Amylase inhibition

The mode of inhibition of α-AML enzyme on extracts was carried out as follows: extracts (0.25 mL of 5 mg/ mL) and α-AML (0.25 mL), the mixture was initially incubated for 10 mins at 25^{0}C in a set of 5 test tubes with a concurrent incubation of phosphate buffer (0.25 ml) with α-AML (0.25 mL) in another set of 5 test tubes. Starch solution (0.25 mL) was added in increasing concentration (0.30-5.00 mg/mL) to all the 5 test-tubest to commence the reaction and terminated after 10 mins incubation with DNS (0.5 mL) reagent. Finally, the mixtures were incubated in boiling water for 5 mins, cooled and diluted with DW (2 mL) and the absorbance was taken at 540 nm. A standard maltose curve was used to determine the amount of reducing sugar released and converted to reaction velocities. The kinetics of inhibition of the enzymes by extracts on α-ML activity was thereafter evaluated using Line weaver and Burk plot [9].

α-Glucosidase inhibitory assay

Extracts (0.05 mL) was initially incubated with α-GCD (0.1 mL of 0.5 mg/mL) in a test tube and *p*NPG (0.05 mL) was added to commence the reaction while further introduction of Na_2CO_3 (2 mL of 0.1 M) terminates the process following incubation at 37^{0}C for 30 mins. The α-GCD activity was determined by measuring the yellow coloured *para*-nitrophenol (*p*NP) released from *p*NPG at 405 nm. Percentage inhibition was determined [9].

Mode of α-Glucosidase *inhibition*

Extract (0.05 mL of 5 mg/mL) reacted with α-GCD (0.10 mL) solution for 10min at 25 °C in one set of 5 test tubes while at the same time α-GCD was mixed with 0.05 mL of Phosphate Buffer (pH 6.9) in another set of 5 tubes. pNPG (50 µL) at increasing concentrations (0.25-2.00 mg/mL) was subsequently added to the two sets of test tubes to initiate the process. The resulting mixtures were allowed to stand at 25 °C following the termination of the process with 0.5 mL of Na_2CO_3 A p-Nitro Phenol standard curve was used to determine spectrophotometrically the amount of reducing sugars released and the kinetics of extracts on α -GCD activity determined by using Michaelis-Menten kinetics [9].

Cytotoxicity assays

Brine shrimps lethality assay

The larvae were hatched by weighing exactly 17 g of brine shrimps (*Artemia salina*) eggs into 500 mL of artificial sea water in a beaker with a partition of dark and light areas. The eggs were introduced to the dark area of the chamber, after 48 hr the matured hatched shrimps moved to the lighter chamber. After hatching, 5 mL of the extracts (0.125- 1.000 mg/mL) were emptied into 10 mL vial and 10 shrimps were introduced into each vial. All the vials were left open under the lamp for 24 hours. All experiments were carried out in triplicate. Negative control was carried out with artificial seawater and distilled water respectively. After 24 hours, the number of surviving shrimps larvae in all the vials were counted and recorded and the percentage mortality calculated [15].

MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assays using mcf-7 cancer cell lines

The anticancer growth inhibitory effects of the samples were tested on the MCF-7 cell line using MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyl-tetrazolium bromide) [16] with some modification.

The MCF-7 cell line was maintained in Minimal Essential Media (MEM), supplemented with 10% heat-inactivated FBS and grown at 37°C in a humidified incubator set at 5% CO_2. The cells were sub-cultured with 0.25% (w/v) trypsin 0.53 mM ethylenediaminetetraacetic acid (EDTA) for a maximum of 15 m in every 2-3 days after they had formed an 80% confluent monolayer.

To analyze the effect of the samples on the cell viability, MCF-7 cells were seeded in 100 µL medium in 96-well microtitre plates at a concentration of 1x1 oscells/ml. Stock solutions of extracts (2mg/ml) were prepared in 10% DMSO. Serial dilutions were made to achieve target concentrations of range 100-0.78125 µg/ml. Subsequently, cells were exposed to the extracts and the control (Doxorubicin), which included vehicle-treated cells exposed to 0.5 % DMSO; cells propagated in growth medium and cells exposed to the positive control, doxorubicin. After the 24 hrs treatment period, the cells were exposed to the MTT reagent (0.5 mg/mL). The colorimetric reaction was measured by means of a plate reader (Multiskan Go, Thermofischer Scientific) at 570 nm wavelength. Colour control blanks were included and utilized to normalize the results and the vehicle control treated cells were regarded as 100% cell viability. The extracts were evaluated in at least three independent experimental repeats and each sample was evaluated in triplicate. The results given are representative of the average percentage inhibition of all the experimental repeats. The fifty percent inhibitory concentrations (IC_{50} values) were determined with the GraphPad Prism.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 statistical package (Graph Pad Software, USA). The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test. All the results were expressed as mean ± standard deviation (SD) of mean of triplicate determinations.

RESULTS

Extract yields

The percentage yields obtained after serial extraction of the leaves using four solvents were 2.19% (32.85g), 1.50% (22.50g), 2.01% (30.15g) and 3.01% (45.15g) for hexane, ethylacetate, methanol and water respectively.

Phytochemical analysis

The results of phytochemical screening of the leaves extracts of *E. trifoliata* are presented in Table 1. It was observed that flavonoids and saponin were present in all the extracts likewise terpenes which was only absent in the water extracts. Alkaloids were found in the hexane and water extracts. Phenol, polysterol and anthraquinone were absent in all the extracts.

In the leaf extracts, the highest total flavonoids (50.43 % QE/g), total saponins (89.46 % DE/g) and total proanthocyanidin (81.91 % CE/g) were observed in the methanol extract while the highest total phenol (41.13% GAE/g) content was in the hexane extract. Proanthocyanidin was completely absent in the aqueous extract as seen in Figure 1. The results of the quantitative analysis of the serial exhaustive extracts of the leaf and were reported in Figure 2.

Antioxidant assay

The antioxidant potential of the extracts of the leaves of *E. trifoliata* is shown in Figure 2 (percentage inhibition of nitric oxide), hydroxyl radical (Figure 3), metal chelation (Figure. 4), DPPH radical (Figure 5) and ABTS (Figure 6).

It was observed that methanol extract and the control quercetin had the best significant ($p \leq 0.05$) half-maximal inhibitory concentration (IC_{50}) activity against DPPH (0.19 mg/mL) and methanol showed the best metal chelation (0.85 mg/mL) and hydroxyl radical (0.64 mg/mL) activity when compared with the other extracts and ascorbic acid. These activities are dose dependent for all the extracts and controls (quercetin and ascorbic acid). Although at 1 mg/mL, quercetin showed the highest percentage inhibition against hydroxyl radical (Figure 3), metal chelation (Figure 4) and DPPH radical (Figure 5) scavenging effect.

Anti-diabetic activity

The results for the inhibitory activities of hexane, ethylacetate, methanol and aqueous extracts of *E. trifoliata* against α-amylase and α-glucosidase enzymes are presented in Table 2. The extracts were found to be dose dependent for both). enzyme. The (IC_{50}) values were significantly ($P \leq 0.05$) different from one another, however, the IC_{50} values (Table 2) reveals that both ethylacetate and methanol extracts showed the best α-amylase inhibitory value of 1.38 mg/mL which was significantly ($P \leq 0.05$) lower than the standard acarbose (1.97 mg/mL). Likewise, the

Table 1: Qualitative phytochemical analysis

Extracts	Phenols	Terpenes	Saponin	Polysteroids	Flavonoids	Anthraquinone	Alkaloids
LH	-	+	+	-	+	-	+
LE	-	+	+	-	+	-	-
LM	-	+	+	-	+	-	-
LW	-	-	+	-	+	-	+

(-)= Not detected ; (+)= Detected ; LH= Hexane extract; LE= Ethylacetate extract; LM= methanolic extract; LW= water extract

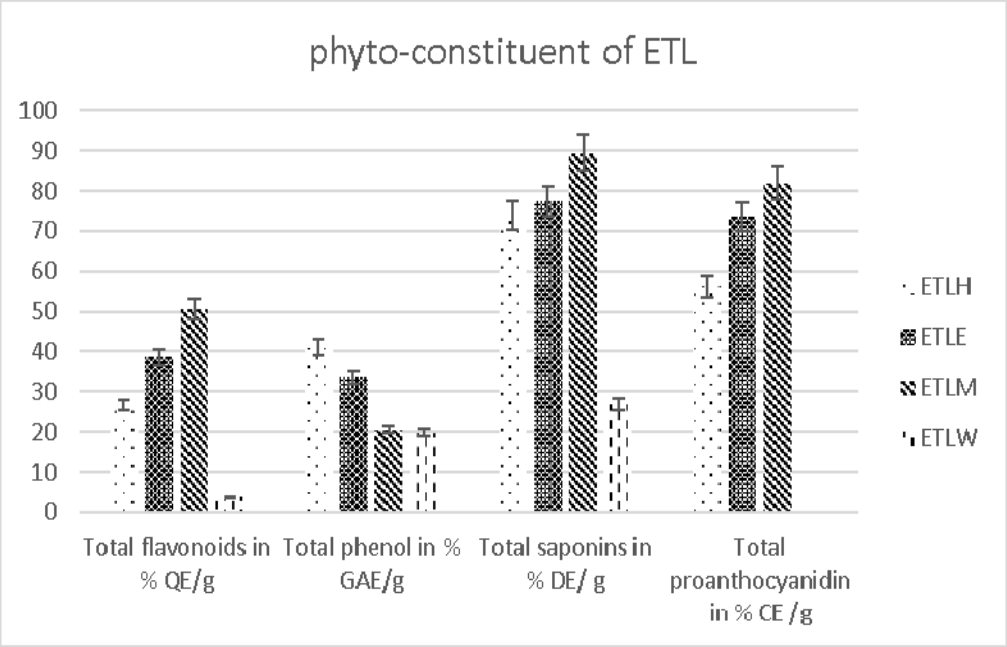

Figure 1: Quantitative determination of phytochemicals

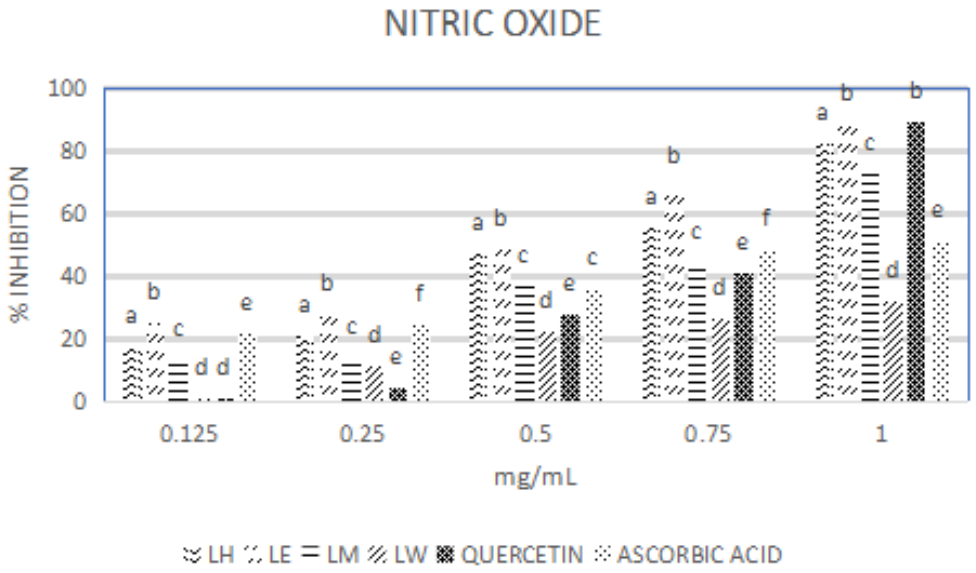

Figure 2: Nitric oxide scavenging effect of *E. trifoliata* leaves. The values are expressed as mean ± SD of triplicate determinations. Bars not sharing a common letter at the same concentration are significantly different ($p < 0.05$). LH= Hexane extract; LE= Ethylacetate extract; LM= methanolic extract; LW= water extract

IC_{50} value of α-glucosidase inhibitory activity of the extracts is significantly lower than the standard acarbose (1.30 mg/mL) except for the water extract (1.52 mg/mL

Kinetic studies carried out to determine the mode of inhibition by lineweaver-burke plot (Figure 7 and 8), using most active (extract with lowest IC_{50}) inhibitor; ethylacetate extract exhibited competitive inhibitory activity and a non-competitive (mixed) activity against α-amylase and α-glucosidase enzymes respectively.

Cytotoxicity assays

The degree of percent lethality of the extracts as shown in Table 3 was directly proportional to the concentration of the extracts. Maximum mortality was observed in the ethylacetate extract, it killed the larva at almost all concentrations compared with other extracts. The least cytotoxic is the aqueous extract wit LC_{50} >1000 µL

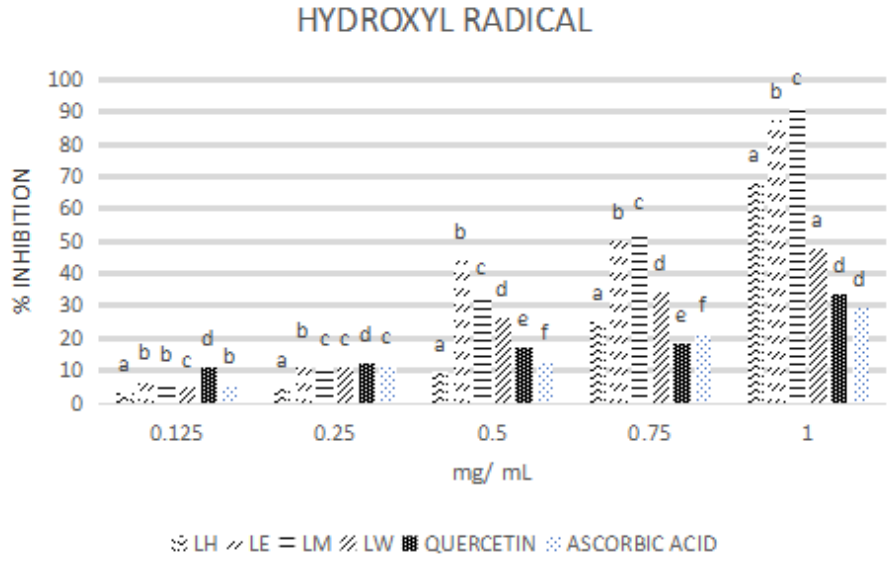

Figure 3: Hydroxyl radical scavenging effect of *E. trifoliata* leaves. The values are expressed as mean ± SD of triplicate determinations. Bars not sharing a common letter at the same concentration are significantly different ($p< 0.05$). LH= Hexane extract; LE= Ethylacetate extract; LM= methanolic extract; LW= water extract

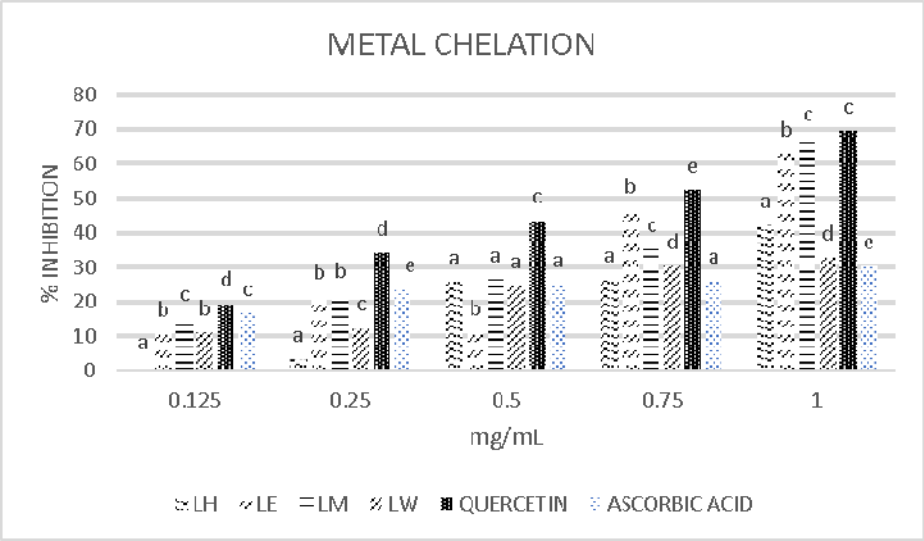

Figure 4: Metal chelation effect of *E. trifoliata* leaves. The values are expressed as mean ± SD of triplicate determinations. Bars not sharing a common letter at the same concentration are significantly different ($p< 0.05$). LH= Hexane extract; LE= Ethylacetate extract; LM= methanolic extract; LW= water extract

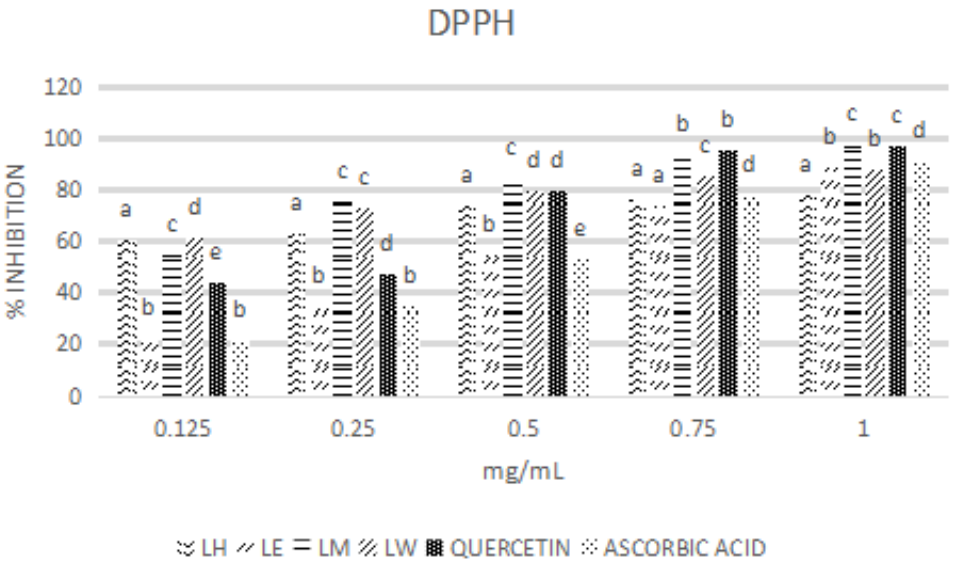

Figure 5: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging effect of *E. trifoliata* leaves. The values are expressed as mean ± SD of triplicate determinations. Bars not sharing a common letter at the same concentration are significantly different ($p< 0.05$). LH= Hexane extract; LE= Ethylacetate extract; LM= methanolic extract; LW= water extract

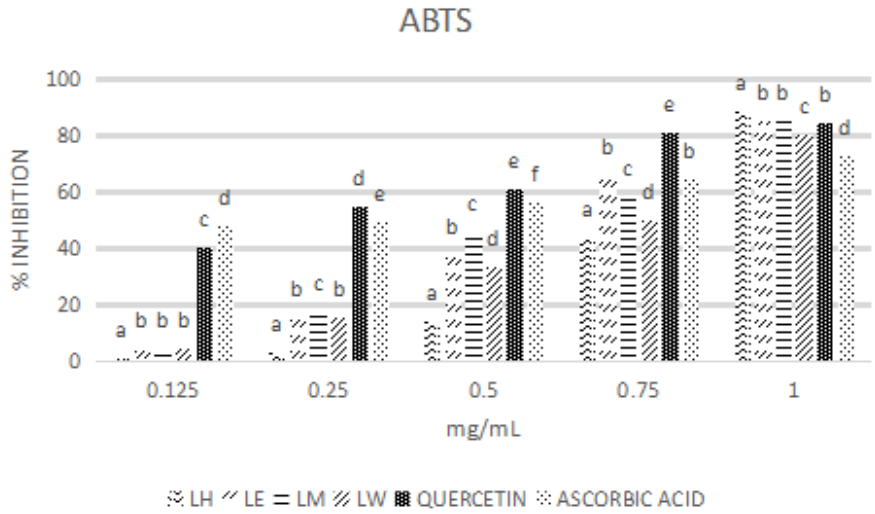

Figure 6: 2, 2-Azino-bis (3-ethylbenzothiazoline)-6-sulphonic acid (ABTS) radical scavenging effect of *E. trifoliata* leaves. The values are expressed as mean ± SD of triplicate determinations. Bars not sharing a common letter at the same concentration are significantly different ($p< 0.05$). LH= Hexane extract; LE= Ethylacetate extract; LM= methanolic extract; LW= water extract

Table 2: IC_{50} values for the inhibitory activity of serial exhaustive extracts of *E. trifoliata* leaf against α-amylase and α-glucosidase enzymes

	IC_{50} in mg/mL (mean±sd)	
	α- Amylase	**α-Glucosidase**
LH	2.70 ± 0.00^{a}	0.92 ± 0.00^{a}
LE	1.38 ± 0.01^{b}	0.52 ± 0.00^{b}
LM	1.38 ± 0.00^{b}	0.70 ± 0.00^{c}
LW	1.61 ± 0.00^{c}	1.52 ± 0.00^{d}
Acarbose	1.97 ± 0.00^{d}	1.30 ± 0.01^{e}

LH= Hexane extract; LE= Ethylacetate extract; LM= methanolic extract; LW= water extract.

DISCUSSION

The results of this study provide evidence that *E. trifoliata* possesses antidiabetic activity in addition to its cytotoxic and antioxidant activities. Methanol and ethyl acetate extracts of the plant have shown better antioxidant, antidiabetic and cytotoxic activities against brine shrimps compared to the hexane and aqueous extracts. With the growing interest in the search for alternative remedies for the treatment of diseases such as diabetes and cancer, it is

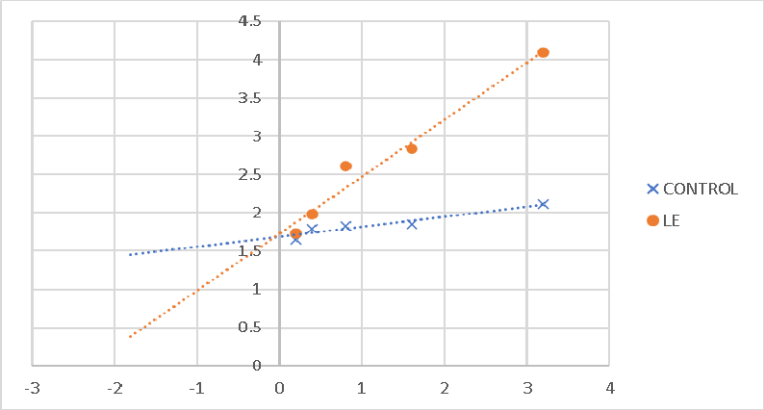

Figure 7: Lineweaver–Burk plot of *E. trifoliata* ethylacetate extract eliciting competitive inhibition on α-amylase.

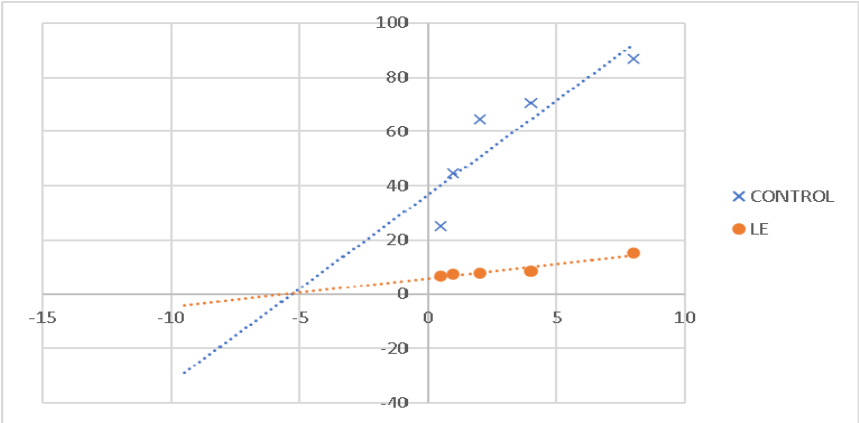

Figure 8: Lineweaver–Burk plot of *E. trifoliata* ethylacetate extract eliciting mixed non-competitive inhibition on α-glucosidase

Table 3: Percent lethality and LC_{50} value of the brine shrimp obtained after chronic exposure of vary concentration of serial exhausted extract of *E. trifoliata*

Extracts	*Concentration (µg/mL)*	*% lethality mean*±SD	*LC_{50} (µg/mL)*
LH	1000	90.00± 0.0	43.56
	750	80.33±5.6	
	500	66.67± 4.6	
	250	60.00± 8.2	
	125	53.33± 4.7	
LE	1000	100.00± 0.0	< 0.00
	750	100.00± 0.0	
	500	100.00± 0.0	
	250	100.00± 0.0	
	125	90.33± 5.0	
LM	1000	100.00± 0.0	25.64
	750	83.33± 4.7	
	500	73.33± 4.7	
	250	66.67± 4.7	
	125	53.33± 4.7	
LW	1000	40.00±0.00	>1000
	750	36.67±4.7	
	500	30.00± 8.1	
	250	16.67± 4.7	
	125	3.33± 4.7	

The percentage lethality was calculated from the mean survival larvae of extracts treated tubes and control. LC50 values were obtained by best-fit line method

evident that phytochemicals have become very promising in proffering solution to this effect. *Euadenia trifoliata* leaves was also found to contain flavonoids and polyphenols, saponins and alkaloids. These phytochemicals are probably responsible for its numerous ethno-pharmacological uses. Interestingly, some of these secondary metabolites or chemical compounds have been found to be active against numerous health-related disorders [17]. For instance, naturally occurring alkaloids and their synthetic derivative have potentials for the elimination and reduction of human cancer cell lines [18], saponins reduce cholesterol levels in the intestinal tract and inhibiting the proliferation of cancer cells, therefore, help in mitigating against obesity and antimutagenicity [19]. Also, flavonoids possess cytotoxic, antimicrobial, antioxidant, antidiabetic properties [14].

Methanol extract activities against DPPH (0.19 mg/mL), hydroxyl radical (0.86 mg/mL) and metal chelating (0.64 mg/mL), as well as the activities of ethylacetate extract against ABTs (0.61 mg/mL), hydroxyl radical (0.64 mg/mL) and nitric oxide (0.50 mg/mL) are found to be higher than the controls, ascorbic acid and quercetin. These results support the claims that semi polar solvent extracts a wide range of chemicals and shows more activity than other solvent extracts [20].

Agents that scavenge free radicals have proven to have strong antioxidant activity as well as good α-glucosidase and α-amylase inhibitory properties [21]. Hence, they play significant role in the treatment and prevention of type 2 diabetes and related complications. A reliable therapeutic approach for reducing postprandial hyperglycaemia is to reduce the digestion and absorption of carbohydrates. This can be achieved by inhibiting carbohydrate hydrolyzing enzymes such as; α-amylase and α-glucosidase in the digestive tract [22]. Methanol and ethylacetate extract of *E. trifoliata* leaf exhibited equal and most potent inhibition of α-amylase and ethylacetate exhibited most potent inhibition against α-glucosidase (Table 3). The activity demonstrated by methanol and ethylacetate extracts against α-amylase suggested that the extracts is a potential antidiabetic drug candidate which may not have any physiological side effects such as bloating, abdominal distention and sometimes diarrhea resulting in indigestion of carbohydrate food in the colon as usually observed when strong α-amylase inhibitors like acarbose and miglitol are used [23]. Antidiabetic efficacy displayed by the extracts of *E. trifoliata* leaf extracts may be linked to the presence of phenolic compounds, flavonoids and proanthocyanidins, which might be responsible for mopping up free radicals generated by diabetes-induced hyperglycaemia [21]

The Lineweaver–Burk plot (Figure 8) depicting the mode of inhibition of α-amylase and α-glucosidase by the ethylacetate extract showed competitive and non-competitive (mixed) inhibition, respectively. Observed competitive inhibition of α-amylase by the ethylacetate extract suggest the active components in the extract competed at the active sites of the enzymes with the substrate (acarbose) and this indicates the likelihood to delay the breakdown of starch to disaccharides [24]. Non-competitive (mixed) inhibition of α-glucosidase by the ethylacetate extract also observed in the results suggests that its inhibitory components also bind to a site other than the active site of the enzyme (α-glucosidase) but have different affinities for the free enzyme and enzyme-substrate complex [25].

The *in vitro* brine shrimp lethality test is used as a preliminary bioassay guide for active cytotoxic and anticancer agents [26]. Hexane, ethylacetate and methanol extracts of *E. trifoliata* leaf displayed significant lethality against the brine shrimps nauplii (Table 4) based on a general toxicity test agreement that LC_{50} above 100 µg/mL is non-toxic while that below 100 µg/mL is indicative of high level of toxicity. This signifies that the ethylacetate extract is highly toxic which support the use of the *E. trifoliata* in ethnomedicine as poison [4]. Some researchers have also proven that ethylacetate extract is more cytotoxic than methanol of this same plant extracts [1]. The outcome of the brine shrimp lethality test presents *E. trifoliata* leaf as a promising natural source of anti-cancer agents but the level of toxicity of the leaf needs to be studied.

Based on the encouraging data obtained for brine shrimp lethality test, anticancer assay of the extracts against MCF-7 breast cancer cell lines was carried out, since there is a correlation between brine shrimp cytotoxic assay and anticancer cytotoxic assay [27]. It was surprising to observed that there was no correlation between the brine shrimps cytotoxic assay and anticancer MCF-7 cytotoxic assay. This may suggest that the plant extracts are selective in action and may not have action against breast cancer cells. The IC_{50} value of the anti-proliferation activity of all extracts (hexane, ethylacetate, methanol and water) was greater than 100 µg/mL and that of the control drug, Dexorubicin (49.89 µg/mL). Based on the general assumption, the extracts are non- potent antiproliferative agent for breast cancer.

CONCLUSION

This work has shown that the extracts of *E. trifoliata* possess antidiatetic effects supporting the use of the plant in the traditional system of medicine for the management of diabetes. Its cytoxicity profile against brine shrimp supports its use as poison. Furthermore, the extracts have shown that semi-polar solvents (methanol and ethylacetate) extracts possess the best antioxidant and antidaibetic activity when compared to other solvent extracts used. Further studies are needed to identify the compounds in the extracts responsible for the biological activities reported.

DECLARATIONS

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Conflict of Interest

There is no conflict of interest attached to this work.

Contribution of the authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by us. This work is part of Otunomo I. I Ph. D Research work supervised by Prof. O. T Asekun and Dr. M. O Sofidiya in University of Lagos, Nigeria. The work was done in Prof. T. O Ashafa lab in The University of Free State, Qwaqwa campus, South- Africa. All authors have read the manuscript.

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