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In vitro Antidiabetic Studies of Ethanol Extract of *Emilia sonchifolia* Linn. (Asteraceae)

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Abstract

The prevalence of diabetes worldwide has reached epidemic proportions hence urgent need to invest in research of novel therapies for the treatment and management. Scientific investigation of medicinal plants is considered the best alternative and effective strategy for the discovery of novel therapeutic agents for this condition. This work was then designed to establish the anti-diabetic activity of ethanol extract of *Emilia sonchifolia*, a prominent plant in folklore medicine, using various in vitro techniques. The plant extract was assayed using—Haemoglobin glycosylation, Glucose uptake in yeast cells, α-amylase inhibition assay and α-glucosidase inhibition activity in comparison with the standard for each method. The plant extract was found to contain significant amount of total phenol (5.6%) and flavonoid (7.2%). The plant extracts significantly inhibited the haemoglobin glycosylation and the treatment of the yeast cells with the ethanol plant extracts, the glucose uptake by the yeast was found to increase in a dose-dependent manner. Also α-amylase and α-glucosidase activities were inhibited by the extract in a dose dependent manner with IC50 of 97.10±0.50 and 95.35±0.60 as compared to 46.10±0.25 and 45.33±0.68 for the standard (acarbose) respectively. These results confirmed that the extract of *Emilia sonchifolia* contained antidiabetic activity.

Keywords: *Diabetes mellitus, Emilia sonchifolia, Haemoglobin glycosylation, Glucose uptake, α-amylase α-glucosidase*

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycaemia or high blood glucose levels, deranged carbohydrates, fats and proteins metabolism as result of absolute or relative lack of insulin secretion or insulin resistance by peripheral tissues mainly the liver, skeletal and adipose tissues or all. If left

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untreated, the long-term effects of diabetes may include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and / or neuropathy with risk of foot ulcers, amputation and features of autonomic dysfunction, including sexual dysfunction. The global prevalence of diabetes has reached epidemic proportions, and with worldwide incidence projected to increase from 424 million in 2017 to 629 million by 2045 (IDF, 2017) the global economic burden of the disease can be expected to reach a crescendo. The management of DM through oral hypoglycemic agents and insulin as well as the combination of both which are currently being used is bedeviled with toxic side effect and ineffectiveness (Paul and Majumdar, 2021). In view of these associated side effects and limitations of current drugs, continuous research on natural sources which are believed to be safer, is being conducted to develop new formulations for effective management of DM and the related complications (Chattopadhyay, 1999). Plant bioactive compounds have shown digestive enzyme inhibition abilities with their capability to bind to enzyme protein (Paul and Majumdar, 2021). Besides, dietary fibers and their gelatinous polysaccharides have been proved to play a major role in the reduction of postprandial plasma glucose levels in DM. Different researches conducted have also shown that appropriate glycemic control reduced the prevalence of retinopathy, nephropathy, and neuropathy (Pennathur and Heinecke, 2004; Mayur *et al.,* 2010; Gurudeeban *et al.,* 2012; Thilagam *et al.,* 2013). Hence, herbal sources are considered an alternative for effective management of DM and its associated complications. In this work the anti-diabetic potentials of *Emilia Sonchifilia* Linn. *(*Asteraceae) were investigated using different models. *Emilia Sonchifolia* (Linn.) is a bushy annual herb distributed mainly in Asian countries (Zani *et al.,* 1995). The plant is currently attracting the attention in herbal medicine in view of its promising pharmacological activities such as, hepatoprotective, anti-anxiety, anticataract and anticonvulsant (Dash *et al.,* 2015). It is known traditionally as an important medicinal plant in most tropical and subtropical countries, especially in Akwa Ibom State of the South-South region of Nigeria (Pruski and Robinson, 2018). It is commonly called in English as: Red tassel flower, Cupid's sharing brush, Flora's paint brush, purple sow and Utimense in Ibibio of Akwa Ibom State of Nigeria. A tea made from the leaves of *E. sonchifolia* is used in the treatment of dysentery. The juice of the leaves is used to treat eye inflammations, night blindness, cuts, wounds and sore ears. The plant is astringent, depurative, diuretic, expectorant, febrifuge and sudorific. It is used in the treatment of infantile tympharites and bowel complaints. The juice of the root is used in the treatment of diarrhea. The flower heads are chewed and kept in the mouth for about 10 min to protect teeth from decay. The anti-metastatic potential of this medicinal herb justifies its conventional use in the traditional medicine (George *et al.,* 2002). Extracts of *Emilia sonchifolia* in its preliminary photochemical screening revealed the presence of several metabolites, including flavonoids, terpenes, tannins, saponins and alkaloids (Essien *et al.,* 2009). The plant is documented in ethno-medicine to possess medicinal benefits in treating diarrhea, night blindness and some heat rashes (Fatima *et al.,* 2012), measles, inflammatory diseases, eye and ear ailments, liver diseases, eye inflammation, ear ache and chest pain (American Diabetes Association, 2008). The plant has been documented in the Nigeria folk medicine for the treatment of epilepsy in infants. *Emilia Sonchifolia* is also employed by our local herbalists to treat sugar diabetic patients. It is on this basis that the study was carried out to determine the anti -diabetic property of the plant. This work was also intended to provide scientific proof of the use of *Emilia sonchifolia* in our local community to lower blood glucose and hence confirms the anti-diabetic activity of the plant.

2. Materials and Methods

2.1 Materials

2.1.1 Analytical Equipment and Reagents

UV was run on Spectro UV-Vis 2700 Dual beam (200–1100 nm) Labomed, Inc. USA. All reagents used were of analytical grade obtained from Sigma Aldrich. Alpha amylase (from *Aspergillus oryzae*; CAS No 9001-19-8, Enz No. 3-2-1.1) and alpha glucosidase (from Baker's yeast; CAS No. 9001-42-47), 4-Nitrophenyl-α-D-glucopyranoside (CAS No. 3767-28-0) were obtained from Sigma-Aldrich (USA).

2.1.2 Biological Materials

The fresh whole plants of *Emilia sonchifolia* were collected from Itak Ikot Akap in Ikono Local Government Area Akwa Ibom State and authenticated by Professor (Mrs.) Margret Bassey of the Department of Botany and

Ecological Studies University of Uyo with the voucher's number UUPH10(e) and was kept in the Pharmacognosy Herbarium for further experimental procedures.

Blood samples were collected by venipuncture via the cubital vein from eight Wister rats and transferred into a blood bottle containing Ethylene diamine tetraacetic acid (EDTA) as anticoagulant (Williams, 1976).

2.2 Methods

2.2.1 Extraction Procedure

The whole plant parts were cut into size and dried under shed for 21 days. The dried plant was then powdered using a mortar and pestle, the powdery sample weighed with the aid of an electric weighing balance, then packed into suitable bags and labeled appropriately. The dried plant (763.2 g) sample obtained was soaked in 6 L of 70% aqueous ethanol and intermittently shaken for 72 h. The mixture was later filtered through Whatman No.42 filter paper and the filtrate (extract) evaporated to dryness at 40 °C in a rotary evaporator.

2.2.2. Preparation of Haemoglobin

Haemolysate was prepared using the method of James *et al.* (2011). Briefly, the red blood collected was washed three times with 0.14 M NaCl solution. The red blood cells suspension was lysed with 0.01 M phosphate buffer, pH 7.4 and carbon tetrachloride in the ratio 1:2:0.5 (v / v). The suspension was centrifuged at 2300 rpm for 15 min at room temperature. The upper layer (haemoglobin rich fraction) was decanted and transferred into sample bottle and stored in the refrigerator until required for use.

2.2.3. Phytochemical Screening

Phytochemical screening was carried out to determine the chemical constituents in the extract. It was performed using standard procedures (Harbone, 1998; Trease and Evans, 1985; Sofowora and Odebiyi, 2008).

2.2.4. Estimation of Total Phenol Content (TPC)

The extract and fractions (1 mL each) was pre-incubated differently with 1.5 mL of Folin Ciocalteu (FC) reagent for 15 min then 2 mL Na_2CO_3 (7.5%) was added and incubated in dark for 30 min. Absorbance was read at 765 nm, using Gallic acid as standard (Thengya *et al.*, 2019).

2.2.5. Estimation of Total Flavonoid Content (TFC)

Total Flavonoid Content was determined by adding 0.5 mL of $NaNO_3$ (5%) to 0.5 mL of the extract and fractions (1 mg / mL each), incubated for 5 min followed by addition of $AlCl_3$ (10%) and absorbance was read at 430 nm using quercetin was used as standard (Thengya *et al.*, 2019; Gurudeeban *et al.*, 2012).

2.2 6 Test for Anti-diabetic Activity

2.2.6.1. Haemoglobin Glycosylation

One mL each of haemoglobin was transferred into three test tubes, each containing 1ml solution of different concentrations (10 µg / mL, 20 mg / mL and 30 µg / mL of glucose prepared in 0.01 M phosphate buffer at pH 7.4. The test sample (1 mg) was added to each of the test tubes containing different glucose concentrations. The contents were incubated at room temperature for 72 h. A blank preparation without the glucose samples was used as control. The degree of glycosylation of haemoglobin was estimated by taking UV absorbance at 540 nm at different incubation periods of 24 h, 48 hand 72 h (Adisa *et al.*, 2004).

2.2.6.2. Estimation of Haemoglobin Glycosylation (G+Hb)

A modified method of Parker *et al.* (1981) and Adisa *et al.* (2004) for estimating non-enzymic glycosylation of haemoglobin was used for this determination. Briefly, 1 mL each of haemoglobin was transferred into three test tubes, each containing 1 mL solution of different concentrations (10 µg / mL, 20 µg / mL and 30 µg / mL of glucose prepared in 0.01 M phosphate buffer at pH 7.4. The contents were incubated at room temperature for 72 h. A blank solution in which the addition of glucose solution was omitted was used as control. The UV absorbance of advanced glycated end products released were taken at different incubation periods of 24 h, 48 h and 72 h which corresponded to the degree of glycosylation at 540 nm.

2.2.6.3. Effect of Extract at Physiological Glucose Concentration

One mL of haemoglobin solution, 1 mL of 20 mg / mL glucose solution (the concentration of glucose where maximum glycosylation is supposed to take place) and 5 μL of gentamycin in 0.01 M phosphate buffer pH 7.4 were mixed and incubated in the dark at room temperature in the presence of 10 μg, 20 μg or 30 μg / mL of *E. sonchifolia* extract. The UV absorbance of advanced glycated end products released was taken at different incubation periods of 24 h, 48 h and 72 h which corresponded to the degree of glycosylation at 540 nm. This procedure was repeated using Gallic acid (10 μg, 20 μg or 30 μg / mL) as the standard. The assay was carried out in triplicate (Adisa *et al.*, 2004).

2.2.6.4. Glucose Uptake in Yeast Cells

Baker's commercial yeast (20 g) was dissolved in 200mL distilled water and centrifuged (3,000 rpm for 5 min) until the supernatant was clear. A 10% v / v of the supernatant was prepared in distilled water. Glucose (1 g) was dissolved in 100 mL of distilled water and 1mL each of the solution at different concentrations (20 mg / mL, 40 mg / mL, 60 mg / mL, 80 mg / mL and 100mg / mL) were transferred to various test tubes containing 4 mL 10%v / v suspension of yeast solution, vortexed and incubated in a dark cupboard for 60 min at 37 °C. 1mL of the extract was then transferred to the test tubes containing glucose and yeast solution mixture, vortexed and further incubated at 37 °C for 60 min. The UV absorbance readings of the different solutions were taken at 340 nm. This was compared with a standard drug (metformin). The percentage increase in glucose uptake by yeast cells was calculated using the formula (Paul and Majumdar, 2021):

$$\text{Increase in glucose uptake (\%)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

$Abs_{control}$ is absorbance of the control reaction (containing all reagents except the test sample)

$Abs_{control}$ is absorbance of the test sample.

2.2.6.5. α-Amylase Inhibition Assay

The method reported by Johnson *et al.* (2016) was used to determine the α-amylase inhibition activity with slight modification (Gurudeeban *et al.*, 2012). The assay mixture comprising of 200 μL of 0.02 M sodium phosphate buffer, 20 μL of enzyme and the *E. sonchifolia* extract, prepared in the concentrations of 20, 40, 60, 80 and 100 μg / mL was incubated for 10 min at room temperature followed by addition of 200 μL of 1% starch (1.0 g of starch in 100 mL of sodium phosphate buffer (20 mM), pH, 6.8) in all the test tubes. The reaction was terminated with addition of 400 μL of 3, 5-dinitrosalicylic acid (DNSA) and placed in boiling water for 5 min, cooled and diluted with 15 mL of distilled water. The absorbance was measured at 540 nm. Acarbose was used as positive control.

$$\%\text{inhibition} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

2.2.6.6. α-Glucosidase Inhibition Activity

The assay was carried out using the method reported by Johnson *et al.* (2016). The á-glucosidase was dissolved in 100 mM phosphate buffer (pH 6.8) and used as the enzyme extract. p-Nitrophenyl-α-D-glucopyranoside (PNPG) was used as the enzyme substrate. Different concentrations of test samples (as in 2.2.6.5) were mixed with 320 μL of 100 mM phosphate buffer pH 6.8 at 30 °C for 5 min. 3 mL of 50 mM sodium hydroxide was added to the mixture and the absorbance was read at 410 nm. Acarbose was used as the positive control. The % inhibition was calculated using the formula.

$$\%\text{inhibition} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

2.3 Statistical Analysis

Results are expressed using multiple comparisons of Mean ± SEM by one-way analysis of variance (ANOVA), followed by the *Tukey-Kramer* multiple comparisons test. Results were considered statistically significant at $p<0.05$.

3. Results

3.1. Phytochemical Studies

The qualitative phytochemical analysis was conducted on *Emilia sonchifolia* (Linn.) Asteraceae revealed tannins, saponins, flavonoids, alkaloids and phenols as major constituents.

The result of Quantitative Phytochemical studies are shown in Table 1.

Table 1: Total Phenol Content (TPC) and Total Flavonoid Content

Phytochemical	Concentration mg/100g	% Composition
Total Phenol Content	10.85	5.6
Total Flavonoid Content	25. 35	7.20

3.2. Anti-diabetic Studies by Haemoglobin Glycosylation

Table 2: Effect of *Emilia sonchifolia* Leaf Extract on Haemoglobin Glycosylation as Compared with the Standard (Gallic acid)

Conc.(μg/mg)	G+Hb			*Emilia sonchifolia* Extract			Gallic Acid		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
10 μg/mL	0.582 ± 0.20	0.584 ± 0.12	0.592 ± 0.10	0.74 ±0.29	0.84 ± 0.50	0.86 ± 0.50	1.02 ± 0.20	1.32 ± 0.50	1.39 ± 0.52
20 μg/mL	0.618 ± 0.30	0.628 ± 0.4	0.634 ± 0.21	0.83 ±0.32	0.90 ± 0.60	0.99 ± 0.45	1.30 ± 0.30	1.47 ± 0.70	1.49 ± 0.65
30 μg/mL	0.626 ± 0.40	0.635 ± 0.4	0.642 ± 0.40	0.87 ± 0.10	0.91 ± 0.10	.090 ± 0.11	1.32 ± 0.10	1.32 ± 0.40	1.34 ± 0.12

Note: Values are expressed as mean ± SEM; n=3).

Table 3: Absorbance of Glucose Standard Solution at 340 nm

Conc. mg/mL	Absorbance (nm)
20	0.235 ± 0.01
40	0.389 ± 0.01
60	0.589 ± 0.02
80	0.788 ± 0.01
100	0.979 ± 0.03

Note: Values are expressed as mean ± SEM; n=3).

Table 4: Percentage Increase in Glucose Uptake by Yeast Cells Due to the Effect of Ethanol Extract and the Standard (Metformin) at Different Glucose Concentrations

Conc. mg/mL	% G±Y	% ES Ethanol Extract	Standard (Metformin)
20	27	30	49
40	28.5	34	54
60	29.6	42	62
80	31.4	44	67
100	33.1	48	71

4. Discussion

The control of blood glucose levels, is the primary aim of antidiabetic therapy (Gurudeeban *et al.*, 2012). Hence the screening for antidiabetic agents must be directed at the various targets and mechanisms by which blood

Table 5: % Inhibition of α-amylase, α-glucosidase by Extract of *E. sonchifolia* and the Control (acarbose)

% Inhibition of α-Amylase					% Inhibition of α-Glucosidase			
Conc.	ETOH Extract	ETOH Extract IC^{50}	ACARB	ACARB IC^{50}	ETOH Extract	ETOH Extract IC^{50}	ACARB	ACARB IC^{50}
20	35	97.10±0.50*	45	46.10±0.25*	36	95.35±0.60*	32	45.33±0.68*
40	34		49		38		44	
60	39		64		40		63	
80	41		69		43		69	
100	49		74		50		74	

Note: Data are given as mean ± SD (n=3; * p<0.05).

glucose levels can be lowered to normal levels (euglycaemia or normoglycaemia); that is the fasting plasma glucose of < 7.0 mmol / L. In this research work, three common experimental procedures were employed to screen the ethanol extract of the plant *Emilia sonchifolia* for antidiabetic activity namely: inhibition of glycosylation of haemoglobin, glucose uptake in yeast cells, inhibition of glucose hydrolyzing enzymes—α-amylase and α-glucosidase. Quantitative analysis of the phenolic and flavonoid contents of the extract was also carried out to identify the type of metabolite that may be responsible for the activity. The results are as presented in the tables above.

4.1. Quantitative Determination of Total Phenolic Content and Total Flavonoid Content

Quantitative phytochemical analysis showed that *E. sonchifolia* contained higher levels of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC).

4.2. Anti-diabetic Activity by Haemoglobin Glycosylation

An increase in the glycosylation was observed on incubation of haemoglobin with increasing concentrations of the glucose (10, 20, 30 µg / mL) with a climax at 20 µg / mL over a period of 72 h. This was indicated by lower values of absorbance for Glucose + haemoglobin (G+Hb) experiment (Table 2), because most of the glucose molecules had been attached to the haemoglobin (low free glucose). However, when the plant extract was added, higher absorbance values were obtained because the sample had detached the glycated glucose thus making more fee glucose available. The *Emilia sonchifolia* significantly inhibited the haemoglobin glycosylation indicated by the presence of increasing concentration of hemoglobin (high absorbance values; Table 2) which were comparable with those of the standard—Gallic acid a typical flavonoid (Table 2). With the high content of total phenol and flavonoid observed in this plant from this work, it is obvious that these phytochemicals may be responsible for the antidiabetic activity of the plant extract. It is an established fact increased concentration of glucose in the blood leads to its binding to haemoglobin which may result in the formation of the reactive oxygen species (American Diabetes Association, 2008). Thus besides inhibitory activity of the plant against advanced glycosylation end products it may also function as a good anti-oxidant agent. Regulation of glucose level in the blood of the diabetic patient can prevent the various complications associated with the disease (Lankatillake *et al.*, 2019). The *in-vitro* assay of the present study indicated that *Emilia sonchifolia* (Linn.) Asteraceae possesses good anti-diabetic activity.

4.3. Glucose Uptake in Yeast Cells

In yeast (*Saccharomyces cerevisiae)*, glucose transport takes place through facilitated diffusion. After the treatment of the yeast cells with the ethanol plant extracts, the glucose uptake was found to increase in a dose-dependent manner. Table 3 shows the Absorbance of Glucose standard solution at 340nm used for calibration curve, while Table 4 depicts the percentage increase of glucose uptake by the yeast cell at different glucose concentrations in the presence of the plant extract compared with the standard drug (metformin). Furthermore, a decrease was noted in the UV absorbance of the glucose and yeast solution containing the plant extract, as

compared to the yeast and glucose solution. This was due to an increased absorption of the glucose solution into the yeast cell (which represents the human cells).

4.4. α-Amylase and ?-glucosidase inhibitions

α-Amylase and α-glucosidase are carbohydrate hydrolyzing enzymes that break α, 1-4 bonds in disaccharides and polysaccharides to liberate glucose (Paul and Majumdar, 2021). Thus postprandial hyperglycaemia can be alleviated by the inhibition of these chief enzymes. From our results the extract of *Emilia sonchifolia* was observed to show satisfactory inhibitory activities on these enzymes. These results were comparable to a previous study on *Aspilia africana* by Johnson *et al.* (2016). Alpha-glucosidase is involved in the digestion of polysaccharides and disaccharides to monosaccharides, which increases the blood glucose level (Paul and Majumdar, 2021; Chattopadhyay, 1999). Thus a delay in digestion of polysaccharides or disaccharide is one of the primary way of controlling hyperglycemia, which is effected by inhibition of α -glucosidase (Eichler *et al.*, 1984).

5. Conclusion

The anti-diabetic properties of plant extract has been evaluated *in-vitro* by three methods namely: glucose uptake, effect on glycosylation of the haemoglobin and inhibition of alpha glycosidase and alpha amylase enzymes. *Emilia Sonchifilia* exhibited high degree of inhibition of glycosylation, increased absorption of the glucose solution into the yeast cell and higher percentage of inhibition of chief carbohydrate hydrolyzing enzymes α-amylase and α-glucosidase compared to their respective standards. Thus the present study has provided evidence that the ethanol extract of *Emilia Sonchifolia* possesses anti-diabetic property. The presence of phenolic and flavonoid compounds in the extract, which has been noted to reduce the uptake of glucose in the gut through intra-luminal physiological interaction may be directly linked to the anti-hyperglaecemic property of the extract.

5.1. Significance of the Study

The significance of this research work is the validation of the use of *Emilia sonchifolia* in the treatment of the various types of diabetes by the traditional herbal medicine practitioners in local our community.

Author Contributions

Conceptualization, ECJ. Methodology, FDE, CMO and CI.; software, ECJ.;. Formal analysis, ECJ and FDE. Investigation, CMO and CI.; resources, ECJ.; writing—original draft preparation, ECJ.; writing—review and editing, ECJ, FDE and PCA. visualization, CMO and CI.; supervision, ECJ and PCA.; project administration, ECJ.

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Conflicts of Interest

The authors declare no conflict of interest.

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