ANTIOXIDANT AND HYPOLYCAEMIC ACTIVITIES OF THE ETHANOL EXTRACT OF SENECIO BIAFRAE LEAVES


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Abstract
Diabetes mellitus is among the most common disorder in developed and developing countries, it has been characterized by increased levels of oxidative stress and hyperglycaemia which are implicated in the development of diabetic complications. Therefore, the aim of this study is to investigate the antioxidant and hypoglycaemic effect of the ethanol leaf extract of Senecio biafrae Oliv & Hier Compositae, using DPPH and alpha amylase inhibition methods respectively. S. biafrae is known for its therapeutic virtues, notably in Nigeria where it is used in the treatment of diabetes or pulmonary defects. The plant extract displayed appreciable level of antioxidant activities by standard assay methods: The DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay showed percentage inhibition range from 44.26±0.069% – 49.96±0.075% for Senecio biafrae leaf extract, 95.83±0.023% – 98.88±0.002% for ascorbic acid (standard) and 54.63±0.038% – 58.99±0.033% for alpha-tocopherol (standard), with IC₅₀ 136.95 µg/ml, 6.65 µg/ml and 17.14 µg/ml respectively at concentration ranging from 20 to 100 µg/ml. The Ferric reducing antioxidant power assay (FRAP) of the extract was 147.3 µM Fe²⁺/g dry extract while the positive control ascorbic acid had a value of 265.4 µMFe²⁺/g dry material. The hypoglycaemic activity using α-amylase inhibition assay revealed inhibition from 21.78±0.002% - 74.67±0.0003% for Senecio biafrae leaf extract and 6.6±0.026% – 48.89±0.00% for acarbose (standard drug), with IC₅₀ 86.20 µg/ml and 513.5 µg/ml respectively at concentration ranging from 20 - 500 µg/ml. Therefore, the results indicated that the extract of Senecio biafrae possess significant anti diabetic and in-vitro antioxidant activities.

Keywords: Hypoglycaemic, antioxidant, α-amylase, Senecio biafrae leaf and phytochemicals.

Introduction
Recent estimates indicate there were about 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 366 million by 2030 [1]. In recent times, Nigeria was rated as a country in Africa with the highest number of people (1.7 million) diagnosed with diabetes between the age of 20 and 79 years [2]. Diabetes is a condition primarily defined by the level of hyperglycaemia giving rise to risk of microvascular damage (retinopathy, nephropathy and neuropathy). It is associated with reduced life expectancy, significant morbidity due to specific diabetes related microvascular complications, increased risk of macrovascular complications (ischemic heart disease, stroke and peripheral vascular disease), and diminished quality of life. Currently available drugs and insulin used in managing the disease are associated with several undesirable side effects [3, 4], and its high cost has led to search for plants with normal glyceric properties in the management of diabetes [5, 6]. Several species of medicinal plants used in traditional treatment and management of diabetes worldwide have been evaluated [7]. The hypoglycaemic properties of plants used in management of diabetes are reported to due to their content of flavonoids, glycosides, alkaloids terpenoids, plant polysaccharides and other bioactive compounds [8]. One of the therapeutic targets currently introduced in the management of type 2 diabetes mellitus is inhibition of α-glucosidase and α-amylase to decrease the reabsorption of glucose in the intestine [9].

Antioxidants prevent oxidative damage by inhibiting the action of free radicals such as hydroxyl (OH⁻), superoxide (O⁻), nitric oxide (NO⁻), hydrogen disoxide (NO₂⁻), peroxy (ROO⁻) and non-free radical like hydrogen peroxide and singlet oxygen [10]. This had been implicated in the aetiology of these pathological conditions related to cardiovascular diseases, diabetes, inflammatory diseases, cancer, ageing process and perhaps dementia [11, 12, 13]. Senecio biafrae (S. biafrae) also known as English spinach, womowo in Yoruba, is found naturally in the forest zone from Guinea to Uganda 14 [18]. It is cultivated on a small scale, mainly in Nigeria and Cameroon [14]. S. biafrae is known for its therapeutic virtues, notably in Nigeria where it is used in the treatment of diabetes or pulmonary defects [14, 15]. The biological effects of the aqueous leaf extract of Senecio biafrae Oliv & Hier Compositae on hyperglycaemic and haematological parameters of alloxan-induced diabetic rats were studied by Ajiboye and Ojo, [16]. The previous study of free radical scavenging capacity based on DPPH assay for Senecio biafrae root extracts gave percentage inhibition: 17.72±1.29% to 42.15±1.30% for ethanol extract, in the concentration of 0.05-1 mg/ml [17]. The methanol leaves extract of S. biafrae showed percentage inhibition of 64.15% based on DPPH assay at 1 mg/ml [18]. The reducing power of aqueous, ethanol, acetone and petroleum ether extract of Senecio Biafrae root extract were 40, 28 70 and 72 mg/100 mg dry weight [19]. The reducing power of aqueous, ethanol, acetone and petroleum ether extract of Senecio Biafrae root extract were 40, 28 70 and 72 mg/100 mg dry weight respectively [17]. Hence, the aim of this study was to evaluate the antioxidant and hypoglycaemic effect of the ethanol extract of Senecio biafrae leaves.

Materials and Methods
Senecio biafrae leaves were obtained from Ibadan, Southwest Nigeria. Authentication was done in the Department of Botany, University of Lagos, Akoka by Mr O.O. Oyebaiji, where a voucher number LUH 6155 was given. The leaves were then dried using an oven at 40°C for five days, and subsequently pulverised into fine powder.

Chemicals and Drugs used
DPPH (1,1-diphenyl-2-picyrlyl hydrazyl), TPTZ (2,4,6-tripyridyl-s-triazine), alpha amylase, arcabose, ascorbic acid, alpha-tocopherol, 3, 5- dinitrosalicylic acid, and sodium potassium tartrate were purchased from Sigma-Aldrich, USA. All other reagents and chemicals were of analytical grade, obtained from BDH chemicals LTD and May and Baker LTD, England.
Preparation of plant extract

The powdered leaves of *Senecio biafra* (160 g) was subjected to cold maceration in 1500 ml of ethanol for three days. The extracts were filtered over Whatman filter paper No 42 (125 mm). The filtrate was then concentrated using rotary evaporator at 40°C and further lyophilised to remove the remaining solvent. The dried ethanol extract (14 g) was weighed, labelled appropriately and stored in a refrigerator until use.

**Phytochemical Screening**

Phytochemical screenings were carried out on the extract using standard procedures to identify the chemical constituents as described by Tease and Evans [19]. The presence of active constituents such as alkaloids, flavonoids, steroidal nucleus, cardiac glycosides, saponins, tannins, terpenes and reducing sugar were investigated.

**Antioxidant screening**

Two methods were employed for the determination of the in-vitro antioxidant activities of the ethanol extract of *Senecio biafra* leaves.

**DPPH Radical Scavenging Activity**

The ability of the plant extract to scavenge 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radicals was measured by a slightly modified method of Brand–William *et al.* [20]. The following concentrations of extract were prepared 20, 40, 60, 80 and 100 µg/ml. Ascorbic acid and alpha tocopherol were used as standards and the same concentrations were prepared as the test solutions. All of the solutions were prepared with methanol. Two (2) ml of each prepared concentrations were placed into test tubes and 0.5 ml of 1 mM DPPH solution in methanol was added thereafter. The experiments were carried out in triplicates. The test tubes were incubated for 15 min at room temperature and the absorbance was read at 517 nm. A blank solution containing the same amount of 1 mM DPPH solution in methanol was prepared and also measured. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The radical scavenging activity was calculated using the following formula:

\[ \text{DPPH scavenging effect (in %) = } \frac{[\text{AB} - \text{AA}]}{[\text{AA}]} \times 100 \]

\[ \text{Where AB is the absorbance of blank sample and AA is the } \]

\[ \text{absorbance of tested extract solution.} \]

**Ferric Reducing Antioxidant Power (FRAP)**

A modified method of Benzie and Strain [21] was adopted for the FRAP assay. The principle involved relies on the ability of the sample to reduce the ferric tripyridyltriazine (Fe (III)-TPTZ) complex to ferrous tripyridyltriazine (Fe (II)-TPTZ) at low pH. Fe (II)-TPTZ has an intensive blue colour which can be read at 593 nm. 1.5 ml of freshly prepared FRAP solution, containing 25 ml of 300 mM TPTZ in 40 mM HCL and 2.5 ml of 20 mM ferric chloride FeCl3.6H2O solution was mixed with 1 ml of the extracts and absorbance was read at 593 nm. Calibration curve was linear between 100 µM and 500 µM, prepared with FeSO4.7H2O. Results are expressed in µM Fe (II)/µg dry plant material and compared with that of ascorbic acid.

**Hypoglycaemic activity**

Preparation of 3, 5 – dinitrosalicylic acid (coloured reagent): 3.5-dinitrosalicylic acid, 1g was weighed in a beaker containing 50 ml of distilled water, followed by the gradual addition of 30 g of sodium potassium tartrate and 20 ml of 2N NaOH.

**Inhibition of α- amylose enzyme**

α- amylose was prepared by weighing 25 mg of the enzyme in a beaker and gradually adding 0.2 mM phosphate buffer solution. The following concentrations were prepared for the test sample (*S. biafra*) 20, 50, 100, 200, 500 µg/ml each were added to 0.5 ml of 0.2 mM phosphate buffer (pH 6.9) containing α- amylose enzyme (0.5 mg/ml) solution and were incubated at 25°C for 10 min. This was followed by 0.5 ml of a 1% starch in 0.02 M sodium phosphate buffer (p H 6.9) that was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5- dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixtures were then diluted by adding 10 ml distilled water and absorbance was measured at 540 nm. For the blank solution (control), 10 ml of distilled water was added instead of enzyme and acarbose, a widely used clinical antidiabetic drug, was used as positive standard. The percentage inhibition (I%) was calculated using (I%) = (Ac – As)/Ac × 100. Where Ac is the absorbance of the control and As is the absorbance of the sample/standard [22].

**Results and Discussion**

Most medicinal plants contain secondary metabolites responsible for observed biological activities. Phytochemical screening of the ethanol extract of *Senecio biafra* leaves shows the presence of alkaloids, saponins, flavonoids, tannins, glycoside and steroids, while terpenes and reducing sugars were absent as shown in (Table 1). Previous studies had shown that qualitative phytochemical constituents of the leaves of *S. biafra* consumed in Ekiti State, Nigeria showed the presence of alkaloids, tannins, phlobatannins, phenols, saponins, terpenes, steroids, flavonoids, glycosides and chalcones [23].

**Table 1: Phytochemical composition of Senecio biafra leaves extract**

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th><em>Senecio biafra</em> leaf</th>
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<tr>
<td>Alkaloids</td>
<td>+++</td>
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<tr>
<td>Reducing sugar</td>
<td>-</td>
</tr>
<tr>
<td>Terpenes</td>
<td>+++</td>
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<tr>
<td>Saponin</td>
<td>++</td>
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<tr>
<td>Flavonoid</td>
<td>+</td>
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<tr>
<td>Cardiac glycoside</td>
<td>+</td>
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<tr>
<td>Steroidal nucleus</td>
<td>++</td>
</tr>
<tr>
<td>Tannin</td>
<td>-</td>
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</table>

+++ = abundant, ++ = moderate, + = minute, - = absent

The DPPH (1, 1-diphenyl-2-picrylhydrazyl test shows the ability of *S. biafra* leaves to act as a free radical scavenger, the free radical scavenging assay showed percentage inhibition range from 44.26±0.069% – 49.96±0.075% for *Senecio biafra* leaf extract, 95.83±0.023% – 98.88±0.002% for ascorbic acid (standard) and 54.63±0.038% – 58.99±0.033% for alpha-tocopherol (standard), with 136.95 µg/ml, 6.65 µg/ml and 17.14 µg/ml respectively. The ethanol leaf extract was at least twenty times and eight times less potent when compared to ascorbic acid and alpha-tocopherol respectively (see figure 1). The higher the percentage inhibition of DPPH absorbance the higher the free radical scavenging activity. Previous study on the ethanol extract of *S. biafra* leaves showed percentage
inhibition of 64.15% at 1 mg/ml based on DPPH assay [23], whereas the current study revealed the highest inhibition of 49.96% at 100 µg/ml for the of *S. biafrae* extract.

This variation may be attributed to environmental factors such as temperature, UV light, solar radiation, soil nutrient and soil water availability which could have impact on the plant constituents [24]. It is well known that vegetables are rich in various antioxidants, including ascorbic acid, carotenoids, and phenolic and can be considered as sources of natural antioxidant. Many plants including vegetables had been categorized as sources of natural antioxidants that can protect against oxidative stress and thus play an important role in the chemoprevention of diseases that have their aetiology and pathophysiology in reactive oxygen species [25, 26]. FRAP (Ferric reducing antioxidant power) is one of the most rapid test and very useful for routine analysis. The antioxidant activity is estimated by measuring the increase in absorbance caused by the formation of ferrous ions from FRAP reagent. The reducing ability of the extract (147.3 µM Fe²⁺/g) further shows that it is less active when compared to ascorbic acid (265.4 µMFe²⁺/g) table 2.

<table>
<thead>
<tr>
<th>Table 2: Total antioxidant activity (FRAP assay)</th>
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<tr>
<td>Sample/standard</td>
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<tr>
<td><em>Senecio biafra</em> leaves</td>
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<tr>
<td>Ascorbic acid</td>
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This is expected from a crude extract. Isolation of active compounds from the extract may yield compounds with better antioxidant activity. The study revealed that the leaves extract of *Senecio biafrae* caused significant inhibition of α-amylase enzyme, which shows that it was more potent than the clinically used drug acarbose, with α-amylase inhibition from 21.78±0.002% - 74.67±0.0003% for *Senecio biafrae* leaf extract and 6.6±0.026% – 48.89±0.00% for acarbose (figure 2), IC₅₀ 86.20 µg/ml and 513.51 µg/ml respectively.

The inhibition of this enzyme slows the elevation of sugar after a carbohydrate meal, which is one the way to decrease postprandial increase in blood glucose level [9]. This was also corroborated by the study on the root part of the plant by Okoro et al., 18 [22]. It revealed that the aqueous root extracts of *Senecio biafrae* caused significant reduction in the blood glucose level for the extracts tested in Streptozocin-induced diabetic mice, by 10.652±6.36 - 19.858±7.65% within 6 hrs of extracts administration.

**Figure 1**: Percentage inhibition of DPPH against concentration of *Senecio biafrae* leaves extracts and standard drugs.

*Figure 2*: α-amylase percentage inhibition against concentration for *Senecio biafrae* leaves extracts and acarbose.

Also the biological effects of the aqueous leaf extract of *Senecio biafrae* on hyperglycaemic parameters of alloxan-induced diabetic rats were studied by Ajiboye and Ojo 17 [21], the extract showed significant decrease (p<0.05) in the blood glucose, especially at 400mg/kg body weight with a final glucose level of 120 mg/dl when compared to the standard drug (metformin) with 113 mg/dl blood glucose.

**Conclusion**

This study shows that the ethanol extract of *Senecio biafrae* leaves possesses various phytochemical constituents, has antioxidant potential and could be very useful in the management of diabetes with mechanism of action that requires the inhibition of α-amylase enzyme.

**Acknowledgments**

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**References**


