PRELIMINARY INVESTIGATION INTO THE ALPHA-AMYLASE INHIBITORY ACTIVITIES OF AGERATUM CONYZOIDES (LINN.) LEAF EXTRACTS

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Abstract
Ageratum conyzoides (Linn.) is a plant that has been scientifically proven to have anti-hyperglycaemic effect amongst other medicinal uses. We therefore set out to investigate the leaves for possible alpha-amylase inhibitory activity. At 1 mg/mL, the crude extracts (hexane, ethyl acetate and methanol) gave 50.23%, 45.54% and 26.03% inhibition respectively which were all significantly different from the control at p < 0.05 and comparable to acarbose which gave an inhibition of 50.97%.
The results of this study show that the leaves of A. conyzoides possess alpha-amylase inhibitory potential which is beneficial in treating type 2 diabetes as lowering postprandial hyperglycaemia is one of the treatment goals.

Keywords: Ageratum conyzoides, alpha-amylase inhibition, hyperglycaemia, type 2 diabetes.

Introduction
Diabetes projected as the World’s main disabler and killer in the next 25 years [1], is a heterogeneous type of disorder ranging from insulin resistance to insulin deficiency with both a genetic and important non-genetic components [2, 3]. Current estimates indicate a 69% increase in the number of adults that would be affected by the disease in developing countries between 2010 and 2030, compared to 20% for developed countries [4] with type 2 diabetes (Non-insulin-dependent diabetes), accounting for more than 85% of cases worldwide [5]. All the existing synthetic therapies however have limited efficacy, limited tolerability and/or significant mechanism based side effects [6]. A therapeutic approach for treating type 2 diabetes is to decrease postprandial hyperglycaemia through the inhibition of carbohydrate hydrolyzing enzymes such as alphaglycosidase and alpha-amylase [7, 8]. Amylase inhibitors are known as starch blockers because they prevent dietary starches from being digested and absorbed by the body which could be useful for treating obesity and diabetes mellitus [9]. The use of herbal medicines (medicinal plants or phytotherapy) has recently gained popularity all over the world for their efficacy in type 2 diabetes mellitus and some plants have minor side effects when given in large doses [10]. With, the increasing incidence of diabetes mellitus in rural population throughout the world and due to adverse effects of synthetic medicine, there is a clear need for the development of indigenous, inexpensive botanical sources for anti-diabetic crude or purified drugs [11].

Ageratum conyzoides belongs to the Asteraceae family. It is commonly known as Billy goat weed or White weed in English while traditionally it is called “utfi opioko” and “otogo” by the Igedes in Benue state, Nigeria [12, 13] and in South-western Nigeria, it is known as “Imi esu” [14]. The plant has been used in various parts of Africa, Asia and South America for curing various diseases. The mature plant is used for its haemostatic, anti-inflammatory, anti-spasmodic, anti-asthmatic properties, cardiovascular depressant activity, for the treatment of wounds and in bacterial infections [15, 16]. The plant extract is found to have, hypoglycaemic and anti-hyperglycaemic activities [17, 18]. Though many compounds have been isolated from this plant such as triterpenes including major sterols like β-sitosterol and stigmasterol, a rare sterol-stigmast-7-en-3β-ol from the leaves, two isomeric pyrrolizidine alkaloids; the only alkaloids isolated from this plant [19, 20], and some polyoxygenated flavonoids [21], none of these compounds have been linked to the anti-hyperglycaemic activity of the plant. Thus, the aim of this current work is to investigate this plant for probable alpha-amylase inhibitory activity based on its hypoglycaemic and anti-hyperglycaemic activity.

Materials and Methods
Plant collection
Ageratum conyzoides plant was collected at Oke-Sopin, Ijebu-Igbo in the Ijebu North local government of Ogun state. The plant was identified and authenticated at the Forest Herbarium of the Forestry Research Institute of Nigeria (FRIN) where a voucher specimen with voucher number 110136 was deposited. After collection, the leaves were separated from the stem, air-dried powdered and stored in plastic bags until ready for extraction.

Extraction
The powdered leaf sample of A. conyzoides weighing 76 g was extracted successively by cold maceration with n-hexane, ethyl acetate and methanol at room temperature. The leaves were soaked for 72 hours for each solvent used, with daily stirring at an interval of six hours. The extract was decanted every 24 hours and fresh solvent added. After 72 h the pooled extract was then filtered and concentrated in vacuo using a Buchi rotary evaporator at 37°C. The resulting crude
extracts were poured into already weighed crucibles, and put in a desiccator for further drying.

**In-vitro alpha-amylase assay**

The alpha-amylase inhibitory activity was determined by slightly modifying the 3,5 dinitrosalicylic acid (DNSA) method [22]. The reaction medium for the test samples contained 200 μL of 0.02 M sodium phosphate buffer (pH 6.9), 200 μL of 1% α-amylase enzyme from *Aspergillus oryzae*(E.C.3.2.1.1 30 units/mg, Sigma Aldrich) and 200 μL of plant samples (0.1-1.0 mg/mL) or acarbose (standard reference drug) which was incubated at 37°C for 20 min. 200 μL of 0.02 M sodium phosphate buffer (pH 6.9), 200 μL of 1% soluble starch (Sigma Aldrich) was added and the mixture was further incubated for 20 min at 37°C. The reaction was terminated by the addition of 400 μL of DNSA (Sigma Aldrich) colour reagent, placed in a boiling water bath for 5 min, cooled to room temperature and diluted with 5 mL distilled water. The generation of maltose was quantified by the reduction of 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid. This reaction (corresponding to colour change from orange-yellow to red) was detected at 540 nm. In the presence of an α-amylase inhibitor, less maltose would be produced and the absorbance value which was measured using a T90 V/VIS spectrophotometer would be decreased. The control samples were also prepared accordingly without any plant extracts and were compared with the test samples. All readings were done in triplicate.

The results were expressed as % inhibition calculated using the formula:

\[
\%\text{Inhibition} = \left(\frac{A_{(control)} - A_{(test)}}{A_{(control)}}\right) \times 100
\]

Where \(A_{(control)}\) = Absorbance of Control.

\(A_{(test)}\) = Absorbance of inhibitor (plant extract or acarbose).

**Statistical Analysis**

Alpha-amylase inhibitory activity was expressed as means ± SEM. One-Way ANOVA was used to evaluate the level of significance between the test means and the control mean at p < 0.05.

Results and Discussion

The powdered leaf of *A. conyzoides* weighing 76 g was extracted successively using hexane, ethyl acetate (EtOAc) and Methanol (MeOH). The crude hexane, EtOAc and MeOH extracts weighed 1.71 g, 2.23 g and 5.08 g respectively, representing 2.25%, 2.93% and 6.68% of the powdered sample. The results of the α-amylase inhibitory activity of the crude extracts of *A. conyzoides* tested are shown in Tables 1, 2 and 3, while Fig. 1 shows the percentage α-amylase inhibition of the extracts at 1 mg/mL compared to acarbose the reference drug.

Table 3 shows the reduction in the mean absorbance values of the test samples which were not significantly different from the mean value of the control. Percentage inhibition values was from 3.04%-
26.03% with peak percentage inhibition of 26.03% observed at 0.4 mg/mL. From these results it appears that the stronger α-amylase inhibitory activity resides in the hexane and EtOAc extracts. The hypoglycaemic and anti-hyperglycaemic properties of the aqueous extracts of the leaves of Ageratum conyzoides were evaluated in normoglycemic and in streptozotocin-induced diabetic rats, using aqueous extracts of the plant at the doses of 100, 200 and 300 mg/kg. Of all the doses, the aqueous extracts at 200 and 300 mg/kg showed statistically significant hypoglycaemic and anti-hyperglycaemic activities [17]. Also work by [18] on the leaf extracts of A. conyzoides tested on hyperglycaemic rabbits gave significant reduction in blood glucose levels, showing that the plant demonstrated anti-hyperglycaemic actions and hence confirming its anti-diabetic activity. Many medicinal plants with anti-diabetic properties do so by retarding the absorption of glucose through the inhibition of carbohydrate hydrolysing enzymes [23]. These inhibitors delay carbohydrate digestion and prolong overall carbohydrate digestion time causing reduction in the rate of glucose absorption and consequently reducing the postprandial plasma glucose rise [24]. Synthetic drugs like acarbose which inhibit carbohydrate hydrolysing enzymes are used in conjunction with other antidiabetic drugs, but these inhibitors have been found to possess gastrointestinal side effects like abdominal discomfort, flatulence and diarrhoea [25,26] giving rise to the need for alternative mode of treatment particularly of plant origin due to their little or no side-effect, ready availability and efficacy.

**Conclusion**

The results of this study show that the leaves of A. conyzoides have α-amylase inhibitory activity with the hexane and EtOAc extracts being very promising. This plant could serve as a potential source of α-amylase inhibitor which will be of help in the management of type 2 diabetes. Further work however, should be done to isolate the compound(s) responsible for this activity.

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**References**


