



Antioxidant and inhibitory properties of *Clerodendrum volubile* leaf extracts on key enzymes relevant to non-insulin dependent diabetes mellitus and hypertension

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Abstract

Objective: To characterize the interaction of phenolic (free and bound) extracts from white butterfly (*Clerodendrum volubile* P. Beauv) leaves with key enzymes relevant to non-insulin dependent diabetes mellitus (α -amylase and α -glucosidase) and hypertension (Angiotensin-I converting enzyme) and their antioxidant properties *in vitro*.

Methods: The effects of the extracts on enzymes [α -amylase, α -glucosidase, Angiotensin-I converting enzyme (ACE)], some pro-oxidant (Fe^{2+} - and sodium nitroprusside-induced lipid peroxidation in the pancreas (*in vitro*)) and antioxidant properties and HPLC analysis of the extracts were investigated.

Results: The phenolic extracts inhibited α -amylase, α -glucosidase, ACE and some pro-oxidants (Fe^{2+} - and sodium nitroprusside-induced lipid peroxidation in the pancreas (*in vitro*)). Bound phenolics had significantly higher ($P < 0.05$) α -glucosidase inhibitory effects compared to free phenolics and acarbose (a standard drug). However, captopril, a synthetic ACE inhibitor, showed a higher ACE inhibitory activity compared with both the free and bound phenolics, whereas there was no significant difference ($P > 0.05$) in their α -amylase inhibitory activities. The stronger inhibition of α -glucosidase and ACE compared to α -amylase by both extracts may be of pharmacological relevance. Reversed-phase HPLC analysis of the extracts revealed the presence of quercetin, rutin, kaempferol, and chlorogenic and caffeic acids as the main components of both the free and bound extracts.

Conclusion: The inhibitory properties of phenolic rich extracts on α -amylase, α -glucosidase, ACE, and Fe^{2+} - and sodium nitroprusside-induced lipid peroxidation in the pancreas could be attributed to the antioxidant properties of the extracts and their phenolic composition. The stronger action of the bound phenolic extract on α -glucosidase may provide the possible bioactivity at the brush border end of the intestinal wall. This study may thus suggest that leaves represent a functional food and nutraceutical in the management of non-insulin dependent diabetes mellitus and hypertension.

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Keywords: White butterfly leaves; α -Amylase; α -Glucosidase; Angiotensin-I converting enzyme

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1. Introduction

Reports by the World Health Organisation have shown that non-insulin dependent diabetes mellitus (NIDDM) and its complications are increasing in sub-Saharan Africa and the prevalence of the disease is increasing annually [1]. Central to the aetiology and progression of NIDDM is hyperglycemia. Inhibition of key enzymes (α -glucosidase and α -amylase) involved in starch digestion is considered to be one of the practical strategies for the management of hyperglycaemia. Drugs, such as acarbose, miglitol, voglibose, nojirimycin and 1-deoxynojirimycin, are currently in use; however, they are quite expensive and their side effects are unbearable for patients. In recent years, the potential sources of glucosidase inhibitors from plants have been extensively studied and have resulted in the discovery and development of several natural inhibitors with anti-diabetic effects.

Hypertension or high blood pressure is a common cardiovascular disease, which has become a worldwide problem of epidemic proportions and affects 15–20% of all adults with ailments such as arteriosclerosis, stroke, myocardial infarction and end-stage renal disease [2]. It is regarded as one of the long-term complications of NIDDM. These two diseases (hypertension and NIDDM) are interrelated metabolic disorders with persistent hypertension serving as a risk factor for stroke and the leading cause of chronic renal failure [3]. Angiotensin-I converting enzyme (ACE) is the key enzyme responsible for the regulation of blood pressure. It converts angiotensin I to angiotensin II, which is a potent vasoconstrictor. The inhibition of ACE activity may provide an antihypertensive potential by the concurrent lowering of the blood pressure in diabetic and non-diabetic patients [4]. Drugs, such as lisinopril, captopril and enalapril, are currently in use as ACE inhibitors and have been reported to be safe and effective. In the continued search for ACE inhibitors, dietary phenolic phytochemicals have been shown to have promising potential [5].

In recent decades, the consumption of vegetables has attracted growing interest because many experimental and epidemiological studies have consistently demonstrated a positive correlation between the intake of these natural food products and reduced risks of several degenerative diseases, including NIDDM [5,6]. The protection provided by the intake of vegetables and vegetable-rich foods against these debilitating diseases has been attributed to the presence of several antioxidants, especially antioxidative vitamins, including ascorbic acid (vitamin C), α -tocopherol (vitamin E) and β -carotene

(provitamin A). Nevertheless, recent studies seem to indicate that polyphenolic substances are the main phytochemicals, with higher antioxidant properties found in plants [5,6].

Phenolics in vegetables may be present in free or aglycone and bound or glycoside forms [6]. The free phenolics are more readily absorbed and thus may exert beneficial bioactivities during food digestion. However, the significance of bound phenolics to human health is not well understood [7], and it is possible that different plant foods with different amounts of bound phenolics can be digested and absorbed at different sites of the gastrointestinal tract where they perform their unique health roles. Bound phenolics are mainly found in β -glycosides, and they are not digestible by human enzymes and thus could survive stomach and small intestine digestion to reach the colon. In the colon they are digested by bacteria flora to release phytochemicals locally and result in health benefits.

White butterfly (*Clerodendrum volubile* P. Beauv) leaf is a climbing shrub that is commonly grown in deciduous forests across Africa and belongs to the Family *Verbenaceae* [8]. In the southern part of Nigeria, which is highly dominated by the Ijaws, Urhobos and Itsekiris, it is well-known as a delicious green leafy vegetable that is commonly consumed as food and medicine in folklore for the management of arthritis, rheumatism, dropsy, swellings, oedema, and gout and is also used as an anti-abortifacient and sedative [8,9]. The high nutritional qualities of green leafy vegetables were reported by Erukainure et al. [9]. In another study by Fred-Jaiyesimi and Adekoya [10], the presence of various phytochemicals, such as alkaloids, flavonoids, saponins, anthraquinone and cardiac glycoside, were reported. The anti-inflammatory effects of the leaf extracts were also documented [10]. Moreover, there is a dearth of information on the antioxidant, antidiabetic and antihypertensive effects of the green leafy vegetable. Therefore, this study was planned to investigate the antioxidant effect and possible inhibition against key enzymes (α -amylase and α -glucosidase) relevant to NIDDM and hypertension (Angiotensin-I converting enzyme).

2. Materials and methods

Sample collection: A fresh sample of white butterfly (*C. volubile* P. Beauv) leaf was purchased from the Erekesan market in Akure metropolis, Nigeria. Authentication of the leaves was conducted by Dr. A.A. Sorungbe (a taxonomist) in the Department of Biology, Federal University of Technology, Akure, Nigeria and assigned a voucher number (FUTA/BIO/0121). The vegetable was

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