Polyphenolic compositions and *in vitro* angiotensin-I-converting enzyme inhibitory properties of common green leafy vegetables: A comparative study

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**Abstract**

This study compared the phenolic compositions of common green leafy vegetable extracts from *Vernonia amygdalina* (VA), *Telfairia occidentalis* (TO), *Talinum triangulare* (TT), and *Amaranthus hybridus* (AH) and their effects on the angiotensin-I-converting enzyme (ACE) and cisplatin-induced malonylaldehyde (MDA) production in an isolated rat kidney homogenate. HPLC confirmed the presence of phenolic compounds in the extracts. Furthermore, all extracts inhibited ACE activity dose-dependently; however, the extract from VA exhibited the highest ACE activity while TT exhibited the least. Incubation of the kidney homogenate with 1mM cisplatin caused an increase in MDA production; however, all the extracts inhibited the level of MDA produced. Nevertheless, VA extract exhibited the highest inhibition. These activities of the vegetable extracts could be attributed to their phenolic compositions and may suggest some possible mechanism of the actions. However, VA appeared to be the most potent among the vegetables tested.

**Keywords**: vegetables, polyphenolic, angiotensin-I-converting enzyme, oxidative stress, renal damage

**Introduction**

The renin-angiotensin system is a key factor in the pathophysiology of hypertension and kidney dysfunction (1,2). Renin catalyzes the conversion of angiotensinogen to active angiotensin-I, which could be converted to angiotensin-II by the action of angiotensin-I-converting enzyme (ACE). Angiotensin-II is a potent vasoconstrictor and its production deactivates bradykinin (vasodilator), which plays a vital role in hypertension and kidney diseases. Hence, the regulation of angiotensin-II formation could be of great physiological relevance in the management of hypertension and oxidative stress-induced renal injury. Therefore, reduction in the level of angiotensin-II production via inhibition of ACE activity could be an effective strategy for the prevention and management of hypertension and kidney damage (1,2). Although several ACE inhibitors such as captopril, enalapril, lisinopril, and temocapril are already in use, reports have shown that they also inflict several side effects. Consequently, bioactive compounds such as phenolic acids and flavonoids from plant materials, including fruits and vegetables, have been reported to act as excellent ACE inhibitors (1,2).

Chronic kidney disease (CKD), a condition in which the kidneys are damaged, has been reported to be one of the leading health challenges among the adult population throughout the world because of increasing incidences of hypertension (3,4). Furthermore, CKD can be induced by oxidative stress because of the continuous excessive production of free radicals such as reactive oxygen and nitrogen species in the body either during normal cellular metabolism and/or because of exposure to environmental factors such as tobacco smoke, alcohol, radiation, and drugs (5). These radicals or their metabolites are capable of inducing oxidative stress and damage to biomolecules such as lipids, proteins, and DNA. This could lead to the development and progression of several chronic diseases, including CKD and other oxidative stress-induced metabolic disorders such as hypertension (1,2). Thus, combating oxidative stress in the body could be one of the therapeutic ways of ensuring holistic treatment and management of hypertension and CKD.
Cisplatin (CP), an antineoplastic drug, is used in the treatment of various forms of cancer. Though effective, it also induces some side effects such as kidney dysfunction via inhibition of antioxidant enzyme activities and initiation of lipid peroxidation. This could result in oxidative stress in the renal tissue (1,6). These deleterious effects may be linked to the fact that kidneys, which are the major route of excretion, can bio-accumulate CP to a greater degree, which could induce CKD. Nowadays, the need for new therapeutic modalities for CKD treatment and oxidative stress management has become a major research focus. Hence, inhibition of ACE has been suggested to be a good therapeutic approach for the management and prevention of CKD, and dietary phytochemicals have promising potential in similar applications (1,7).

From time immemorial, green leafy vegetables have been utilized either for culinary purposes or as spices in human diets. Also, the use of their infusion or extracts to treat or manage several human ailments in traditional medicine is a major practice in folklore (8). Interestingly, a report has logically linked the nutraceutical values of consuming vegetable-rich foods and/or extracts for the management of several human diseases to their antioxidative properties and constituent phytochemicals, such as vitamins C, α-tocopherol, β-carotene, and polyphenols (9).

In Nigeria, green leafy vegetables form a major part of the local dishes. Notable among them are Vernonia amygdalina (VA), Telfaria occidentalis (TO), Tolinium triangule (TT), and Amaranthus hybridus (AH). VA, which is generally known as bitter leaf because of its bitterness. These vegetables are cultivated majorly for consumption and various pharmacological purposes (10). TO is another vegetable that is very desirable not only for its nutritive value but also for its medicinal properties. According to Oboh et al. (11) and Akang et al. (12), leaves are rich in vitamins, minerals, protein, fiber, and phytochemicals such as polyphenols. The pharmacological properties of leaves include anxiolytic, sedative, and antidiabetic (10-13). A study reported that according to folklore, TT can increase stamina and prevent hepatic ailments and cancer. It also exhibits immunostimulatory and immunomodulatory properties (14). Studies on its pharmacological, pharmacognostic, and phytochemical properties have also been reported. AH contains polyphenols, steroids, terpenoids, saponins, betalains, and a large amount of squalene (15).

These vegetables are commonly consumed in most rural areas and villages across Nigeria mainly because of their cheapness, accessibility, and palatability. According to the report of Oboh and Rocha (9), consumption of these green leafy vegetables could alleviate the risk of CKD; however, their efficacy in managing/treating this ailment could be different. On the basis of the abovementioned findings, this study aims to determine and compare the phenolic contents and biological properties of common green leafy vegetables extracts from VA Del. (bitter leaf), TO Hook f. (Ugwu leaf), TT (water leaf), and AH (African spinach) via their effects on ACE activity and cisplatin-induced lipid peroxidation in an isolated rat kidney homogenate.

**Materials and Methods**

**Chemicals and reagents** A rabbit’s lung ACE (EC 3.4.15.1) and the substrate [hippuryl-L-histidyl-L-leucine (HHL)] were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cisplatin was purchased from Korea United Pharm. Inc. (Seoul, Korea). All other chemicals and reagents were of analytical grade, and glass-distilled water was used. HPLC with diode array detection (HPLC-DAD) was performed via a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan) equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20AS degasser with a CBM 20A integrator, a SPD-M20A diode array detector, and the LC solution 1.22 SP1 software. A Jenway ultraviolet-visible (UV-Vis) spectro-photometer (Model 6305; Bibby Scientific Limited, Stone, UK) was used to measure the absorbance.

**Sample collection and preparation of extracts** Green leafy vegetables, namely VA, TO, TT, and AH were freshly harvested from a local farmland at Ilara-Mokin, Akure metropolis, Nigeria. Authentication of the vegetables was conducted by A. A. Sorungbe, Department of Biology, Federal University of Technology, Akure, Nigeria, and voucher numbers FUTA/BIO/201, FUTA/BIO/202, FUTA/BIO/203, and FUTA/BIO/204 were assigned to VA, TO, TT, and AH, respectively. The leaves were separated from the stems, washed twice with distilled water, air-dried, and pulverized using a laboratory blender. Each powdered sample (10 g) was extracted with 100 mL of distilled water for 16 h in an orbital shaker. Thereafter, the homogenate was filtered using a muslin cloth and further centrifuged at 360 x g for 10 min to obtain a clear supernatant, which was freeze-dried into powder using a freeze-drier. Each powdered sample (1 g) of dried extract was redissolved in 100 mL of distilled water and stored at 4°C for subsequent analysis. The freeze-dried extracts were used for the HPLC-DAD analysis.

**ACE Inhibition assay** The ability of the extracts to inhibit ACE activity was assayed using the spectrophotometric method described by Cushman and Cheung (16). Different concentrations of the extract (0-100 μL) and 50 μL ACE (EC 3.4.15.1) solution (4 mU/mL) were incubated at 37°C for 15 min. Thereafter, 150 μL of 8.33 mM hippuryl-L-histidyl-L-leucine (HHL) in a 125 mM Tris-HCl buffer (pH 8.3) was added to the reaction mixture and incubated at 37°C for 30 min. The reaction was stopped by adding 250 μL of 1 M HCl. The hippuric acid produced was extracted using 1.5 mL ethyl acetate. Then, the extracted acid was centrifuged. The ethyl acetate layer (1 mL) was transferred to a clean tube and evaporated to dryness. The residue was reconstituted with 1 mL distilled water, and the absorbance was measured at 228 nm. The ACE inhibitory activity of the extract was calculated and expressed as follows:

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\text{% Inhibition} = \frac{(\text{Abs}_{0.5 \mu \text{M}} - \text{Abs}_{0.5 \mu \text{M}} \text{control})}{\text{Abs}_{0.5 \mu \text{M}} \text{control}} \times 100 (1)
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where Abs_{0.5 \mu \text{M}} is the absorbance without the extract and Abs_{0.5 \mu \text{M}} is the absorbance with the extract.