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RESEARCH ARTICLE

In vitro and In vivo effect of aqueous extract of wild lettuce and African eggplant leave extract on key biomolecules Linked to hypertension

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Abstract

The study seeks to assess the underlying antihypertensive property of some of the commonly consumed vegetables, wild lettuce (WL), and African eggplant (AE) leaves, using in vitro and in vivo experimental approaches. Thereafter, aqueous extract from WL and AE leaves was lyophilized into powdery form. Then, the resulting lyophilized power was reconstituted in water (1 g/100 ml) maintained at 4°C for biochemical analysis. The antihypertensive effect of the aqueous extract of WL and AE leaves was assessed via their interaction with the activity of angiotensin-1 converting enzyme (ACE), arginase, and acetylcholinesterase (AChE) in vitro. Thereafter, the modulatory effect of WL and AE leaves extract on purinergic enzymes was assessed in L-NAME-induced hypertensive rats. The experimental design is as follows with each group having six rats. Normotensive control rats: hypertensive rats (L-induced rats) along with hypertensive rats administered with captopril (10 mg/kg/day), WL (250 and 500 mg/kg/day), and AE (250 and 500 mg/kg/day) separately. The experiment lasted for 14 days. Obtained results revealed that WL and AE extract exhibited antioxidant property and an inhibitory effect on activity of ACE, arginase and AChE in vitro. Also, administration of an aqueous extract of WL and AE restored altered purinergic enzymes in L-NAME induced hypertensive rats. Interestingly, WL and AE exhibited anti-hypertensive properties in vitro and in vivo. Nevertheless, WL vegetables seems better than AE. Meanwhile, consumption of these vegetables could be a veritable dietary approach in actualizing healthy status in hypertensive and non-hypertensive individuals.

Keywords: African eggplant, Wild lettuce, Antioxidant, Anti-hypertensive, Bioactive compounds

Introduction

Hypertension is a pathological condition and a key predisposing factor for hypertensive heart disease and chronic kidney disease.[1] It should be on note that sedentary lifestyle, low potassium diet, age and high salt intake Factors contribute significantly to the progression of hypertension.[2] Also, studies have shown that enzymatic activity of ACE,[3] cholinesterase,[4] arginase[5] and adenosine deaminase (ADA)[6] contribute greatly to the pathogenesis and development of hypertension. It is noteworthy that reducing elevated blood pressure can be achieved holistically by regulating the activity of implicated enzymes responsible for the pathology alongside enhanced antioxidant status. Interestingly, ACE, arginase, cholinesterase and ADA contribute to the development of hypertension via different mechanisms as previously reported.[3] Also, oxidative stress contributes to the manifestation of hypertension as its causes an upset in redox status. Study has shown that bioavailability of nitric oxide; a potent vasodilator depends on the cell redox status.[7] Hence, minimization of ROS by antioxidant agents limits NO deficiency and prevents oxidative stress-induced hypertension.

Nevertheless. studies have shown that various natural antihypertensive agents from functional foods and plant-based medicines have been identified and they have little or no side effect common associated with the use of synthetic drugs.[8]

Wild lettuce (Launaea taraxacifolia) is commonly used as leafy vegetable, soup and sauces. Wild lettuce leaves commonly used for the management of liver diseases, dyslipidemia, diabetes.[9-11] Wild lettuce leaves has numerous macro and micronutrients which make its good choice of vegetable for healthy living.

Solanum macrocarpon (African eggplant) leaves is an erect herbaceous plant of the family Solanaceae. Medicinal properties of each part of the plant have been reported (Famuwagun et al). Their health benefit in folklore ranges from weight reduction to treatment of disorders.[12] Although, these two vegetables have been shown to have several nutritional and health benefits. Nevertheless, there are dearth of information on the effect of these locally consumed vegetables on the activity of ACE, AChE, arginase and purinergic enzymes linked to the pathogenesis of hypertension. Thus, this study therefore sought to determine the underlying mechanism through which these vegetables exhibit anti-hypertensive properties as well as to quantify presence of bioactive compounds in wild lettuce leaves and African eggplant leaves by GC-MS.

Materials and Methods

Sample collection and preparation

Wild lettuce leaves and African eggplant leaves were obtained from a farm settlement in Akure, Ondo state. The vegetables were identified, leafy part was removed, rinsed, air dried, pulverized and sieved into powdery form. Aqueous extract was prepared and lyophilised to obtained powdered form of the samples. Then, 1g of each powdered sample was weighed into 100ml of distilled water and mixed. The resulting solutions were stored in the refrigerator for biochemical analysis.

Chemicals and reagents

Used chemicals are of analytical grade purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Experimental animals

Adult male Albino Wistar rats were purchased from Animal colony, the University of Ibadan. Institutional and Department of Biochemistry ethical approval for the utilization of experimental rats was approved prior to their use with ethical number FUTA/ETH/21/02

In vitro ACE inhibition assays

The ability of wild lettuce and African eggplant leaves extract to inhibit ACE activity was evaluated as described by Cushman & Cheung. ACE solution and sample (wild lettuce and African eggplants) preincubated at 37°C for 10 min. Then, enzyme substrate 8.33 mM of HHL was added started the reaction. Then, the mixture was incubated and the reaction was stopped after 30 min upon introduction of 1 M HCl. Then cleaved Hippuric acid from HHL was obtained by adding ethyl acetate, centrifuged and uppermost layer of ethyl acetate was transfer into another clean test tube. The content in the test tube was heat to dryness. Then, distilled water was added to the dried test tube to redissolve the dried hippuric acid residue and the absorbance was read at 228 nm. Result was expressed as percentage (%) inhibition.

In vitro arginase inhibition assay

In vitro effect of wild lettuce and African eggplant on activity of arginase was assessed as reported by Kaysen & Strecker. The reaction mixture consists of arginase solution (enzyme), sample (wild lettuce and African eggplant extract), Tris-HCl (50 mmol/l, pH 7.5) containing 10 mmol/l MnCl2. L-arginine was added to the mixture to start the reaction and incubated at room temperature. Then, Erhlich

reagent (2500 µl) was added to halted the process and incubated at 37 °C for 20 minutes. Absorbance was taken at 450 nm. Result was expressed as percentage (%) inhibition.

In vitro ACHE inhibition assay

In vitro effect of wild lettuce and African eggplant plant leaves on AChE activity as illustrated by Ellman et al. The reacting mixture consist of samples (wild lettuce and African eggplant leaves), phosphate buffer pH 7.4 and DTNB. Then, AChE solution was added to the mixture followed by introduction of AChE substate [acetylthiocholine iodide (AcSCh)]. The rate of 5, 5′-dithio-bis-acid-nitrobenzoic liberation was observed at absorbance of 412 nm for 3 min. The result was presented as (%) inhibition (%) AChE activity.

DPPH radical scavenging assay

Effect of wild lettuce and African eggplant leaves extract on DPPH solution was evaluated as described by Gyamfi et al. The reaction mixture consists of 500µL of samples (wild lettuce and African eggplants) and 500µL of DPPH solution. The mixture was incubated and the absorbance was read at 516 nm in a UV-visible spectrophotometer. The result was presented as a percentage (%) DPPH radical scavenging ability.

Determination of Total Antioxidant Capacity

The ABTS radical scavenging ability of wild lettuce and African eggplant leaves extract was evaluated as described by Re et al. The reacting mixture consists of 200 µL of the sample (wild lettuce and African eggplant leaves) and 200 µL of ABTS solution. The mixture was incubated and the absorbance was read at 734 nm after 15 min. the result was reported as Trolox equivalent antioxidant capacity (TEAC) was subsequently calculated using Trolox as the standard.

Lipid peroxidation inhibition assay

The effect wild lettuce and African eggplant leaves extract on $Fe2^{+}$ induced lipid peroxidation was done as described by Ohkawa, Ohishi, and Yagi. The reaction mixture consists of tissue homogenate, 0.1 M Tris–HCl buffer (pH 7.4), samples (wild lettuce and African eggplant leave extract) and 500 mM freshly prepared $Fe2^{+}$ solution and distilled water was added, incubated for 60 min at 37°C. Then, TBA (0.8%), acetic acid/HCl solution and 0.1 ml of SDS were added. Then incubated at 100°C for 90 min. The mixture was cool, and absorbance was read at 532 nm. The TBARS produced was reported as MDA equivalent.

In vivo antihypertensive effect of wild lettuce and African eggplant leaves

The experimental rats (200-220 gram) were procured to the Animal colony, the University of Ibadan. Thereafter, ethical approval for the use of experimental rats was sought and approved by the university ethical committee with ethical number FUTA/ETH/21/02. Thereafter, acclimatized rats were administered with 40 mg/kg BW L-NAME for the induction of hypertension. L-NAME administration lasted for 14 days. Animal grouping and experimental design as follow:

Group 1: Control rats (Normotensive) + basal diet;

Group 2: Hypertensive (Hyp) rats + basal diet;

Group 3: Hyp rats + captopril orally [10 mg/kg body weight (BW)] + basal diet;

Group 4: Hyp rats + 250mg/kg BW wild lettuce;

Group 5: Hyp rats + 500mg/kg BW wild lettuce;

Group 6: Hyp rats + 250mg/kg BW African eggplant;

Group 7: Hyp rats + 500mg/kg BW African eggplant

Thereafter, the rats were sacrificed and necessary organs were isolated for homogenate preparation (Belle et al. 2004) the resultant homogenates were used for biochemical evaluations

Determination of e-NTPDase activity

Heart and kidney e-NTPDase activity was evaluated as determined as illustrated by Schetinger et al. Tissue homogenate (heart and kidney) was introduced to mixture of KCl, mM CaCl2, mM EDTA, mM glucose, sucrose and Tris–HCl buffer, pH 8.0 and incubated at room temperature for 10 min. Followed by addition of ATP and incubated for 20 mins. The reaction was halted by addition 10% TCA and cooled on ice for 10 min. The free Pi was determined as described by Chan et al. using malachite green as a colorimetric reagent. Enzyme activities are reported as nmol Pi released/min/mg of protein.

Determination of 5'-nucleotidase (5' NT) activity

5'-nucleotidase activity in heart and kidney was evaluated using Heymann et al. method. Tissue homogenate was added to mixture of 10 mM $MgSO_4$ and 100 mM Tris–HCl buffer pH 7.5, incubated for 10 min at 37 ºC. Then, AMP was introduced to the medium to start the reaction for duration of 20 mins.

Then, the rate of reaction was halted by introduction of 10% TCA. The free Pi was determined as described by Chan et al. using malachite green as a colorimetric reagent. Results are reported as nmol Pi released/min/mg of protein.

Evaluation of ADA activity

Heart and kidney ADA activity was evaluated according to Guisti and Galanti. Tissue homogenate was incubated at 37 ºC for 1 hr. with 21 mmol/l of adenosine, NaOH, phenol and hypochlorite. After incubation at room temperature for 45 min, absorbance was read at 625 nm. Results were expressed in units per liter (U/l).

Assessment of SOD activity

Heart and kidney SOD activity was evaluated using method of Misra and Frivorich. Tissue homogenate was added to epinephrine. The entire reaction processes were based on the formation of adrenochrome which was detected at 480 nm after addition of sample for 180 sec.

Assessment of Catalase (CAT) activity

Heart and kidney activity determined using method of Nelson & Kiesow 1972. Tissue homogenate was introduced to mixture containing 0.1 M PO42- buffer (pH 7.4), H2O2. Absorbance was taken at 240 nm for 120 seconds due to degradation of H2O2.. CAT activity was expressed in units/mg protein.

Reduced glutathione (GSH)

Heart and kidney GSH level was assessed by the method of Ellman. Tissue homogenate was added to 500 µl of Ellman's reagent and 0.2 M phosphate buffer (pH 8.0). The absorbance was read at 412nm in spectrophotometer.

Characterization of bioactive components of wild lettuce and African eggplant leaves

Bioactive compounds in wild lettuce and Africa eggplant leaves were characterized according to the method of Chipiti et al.

Data Analysis

The result of replicate experiments (n = 6) was pooled and expressed as mean ± standard deviation (SD). The means were analyzed using one-way analysis of variance (ANOVA) Significance was accepted at $P < 0.05$.

Results

Figure 1 (A-C) represents in vitro anti-hypertensive properties of wild lettuce and African eggplant leaves adjudged by their effect on angiotensin-1-converting enzyme (ACE), arginase and acetylcholinesterase. Wild lettuce and African eggplant leaves aqueous extract inhibited ACE, arginase and AChE activity significantly. ($p < 0.05$) in a dose-dependent manner. However, as revealed by their IC50, wild lettuce inhibited ACE (IC50 = 0.3915 mg/ml) and arginase (IC50 = 0.6690 mg/ml) higher than African eggplant [ACE (IC50 = 0.4638 mg/ml) and arginase (IC50 = 0.6939 mg/ml). Conversely, African eggplant leaves (IC50 = 0.4954 mg/ml) had higher AChE inhibitory effect when compared with wild lettuce (IC50 = 0.6697 mg/ml).

Furthermore, Figure 2 (A-C) depicts in vitro antioxidant properties of wild lettuce and African eggplant leaves aqueous extract adjudged by their radical scavenging ability (DPPH, ABTS) and ability to inhibit Fe2+ induced lipid peroxidation. Wild lettuce and African eggplant leaves aqueous extract scavenged DPPH radical, ABTS radical and prevent Fe2+ stimulated lipid oxidation in a dose-related manner. However, as revealed by their IC50, wild lettuce had higher scavenging effect on DPPH* (0.6250mg/ml), ABTS* (0.033 µmol.TEAC/100100mg) and reduced malondialdehyde (MDA) level (IC50 = 11.41 mg/ml) than African eggplant leaves [DPPH* (0.7144mg/ml), ABTS* (0.028µmol.TEAC/100100mg) and reduced malondialdehyde (MDA) level (IC50 = 12.35 mg/ml).

Figure 3(A-F) depicts effect of wild lettuce and African eggplant leaves extract on heart and kidney activity of e-NTPDase, 5'NT and ADA in L-NAME induced hypertensive rats. L-NAME administration significantly increase activity of e-NTPDase in untreated hypertensive rats (negative control group) in relation to the non-hypertensive rats (normotensive rats). Meanwhile, captopril and aqueous extract of wild lettuce and African eggplant leaves administration respectively caused a dose response (250 & 500 mg/kg BW) lower e-NTPDase activity when compared with the negative control rats as represented in Figure 3(A-B) respectively. Comparatively, wild lettuce reduced e-NTPDase activity less than African eggplant extract in treated hypertensive rats. Also, Figure 3(C-D) depicts heart and kidney 5'NT activity in the of normotensive, untreated hypertensive rats, treated hypertensive rats. L-NAME administration significantly reduced activity 5'NT in comparison with the rats in the normal group (normotensive rats). However,

Table 1: Bioactive compound constituent of Wild lettuce and African eggplant leaves

S/N	Compounds Detected	Molecular Formula	Phenolic content (mg/g)	
			Wild lettuce	African eggplant
1	1,2,3-Benzenetriol	$C_6H_6O_3$	0.04	0.02
2	Gentisic acid	$C_5H_{11}NO_2$	0.03	0.02
3	Benzoic acid, 4-hydroxy-	$C_7H_6O_3$	0.36	0.38
4	Cinnamic acid	$C_{12}H_{16}O_2Si$	1.22	1.31
5	Syringic acid	$C_{15}H_{26}O_5Si_2$	1.07	1.03
6	Protocathecolic acid	$C_{16}H_{30}O_4Si_3$	0.32	0.49
7	Kaempferol	$C_{27}H_{44}O_6Si_4$	0.91	0.62
8	Quercetin	$C_{15}H_{10}O_7$	1.18	1.14
9	3- Caffeoyl quinic acid	$C_{34}H_{66}O_9Si_6$	0.41	0.54
10	Cathecol	$C_{12}H_{22}O_2Si_2$	0.44	0.54
11	Tyrosol	$C_{14}H_{26}O_2Si_2$	0.01	0.02
12	P- coumaric acid	$C_9H_8O_3$	1.31	1.41

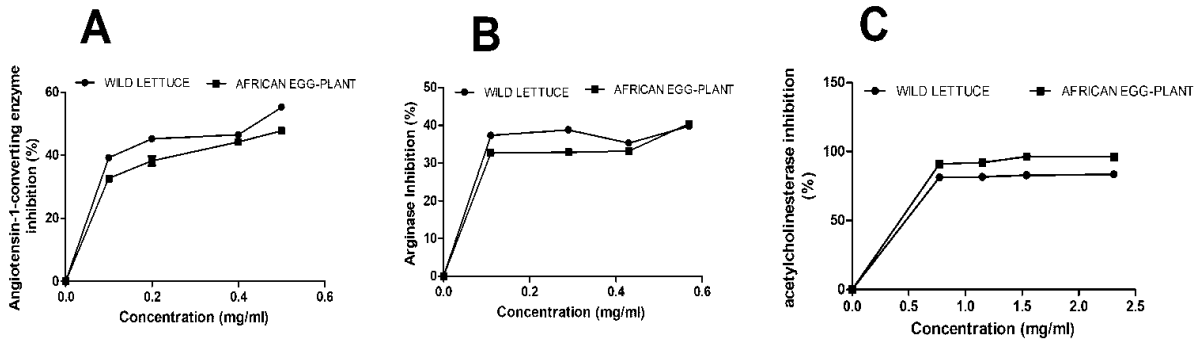

Figure 1 (A-C): Effect of wild lettuce and African eggplant on activity of angiotensin-1-converting enzyme (A), on activity of arginase (B) and on activity of acetylcholinesterase. Values represent mean ± SD (n = 3).

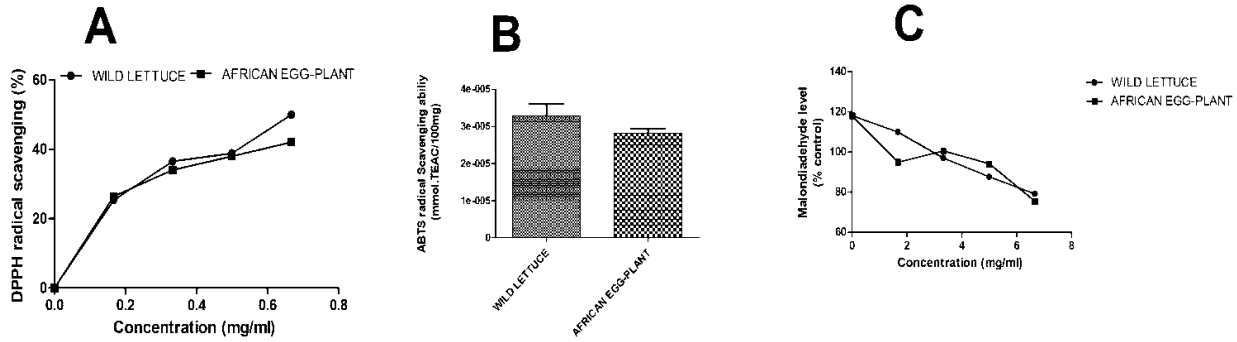

Figure 2 (A-C): Effect of wild lettuce and African eggplant leaves extract on DPPH radical (A), ABTS radical (B) and on Fe^{2+} induced lipid peroxidation (C). Values represent mean ± SD (n = 3).

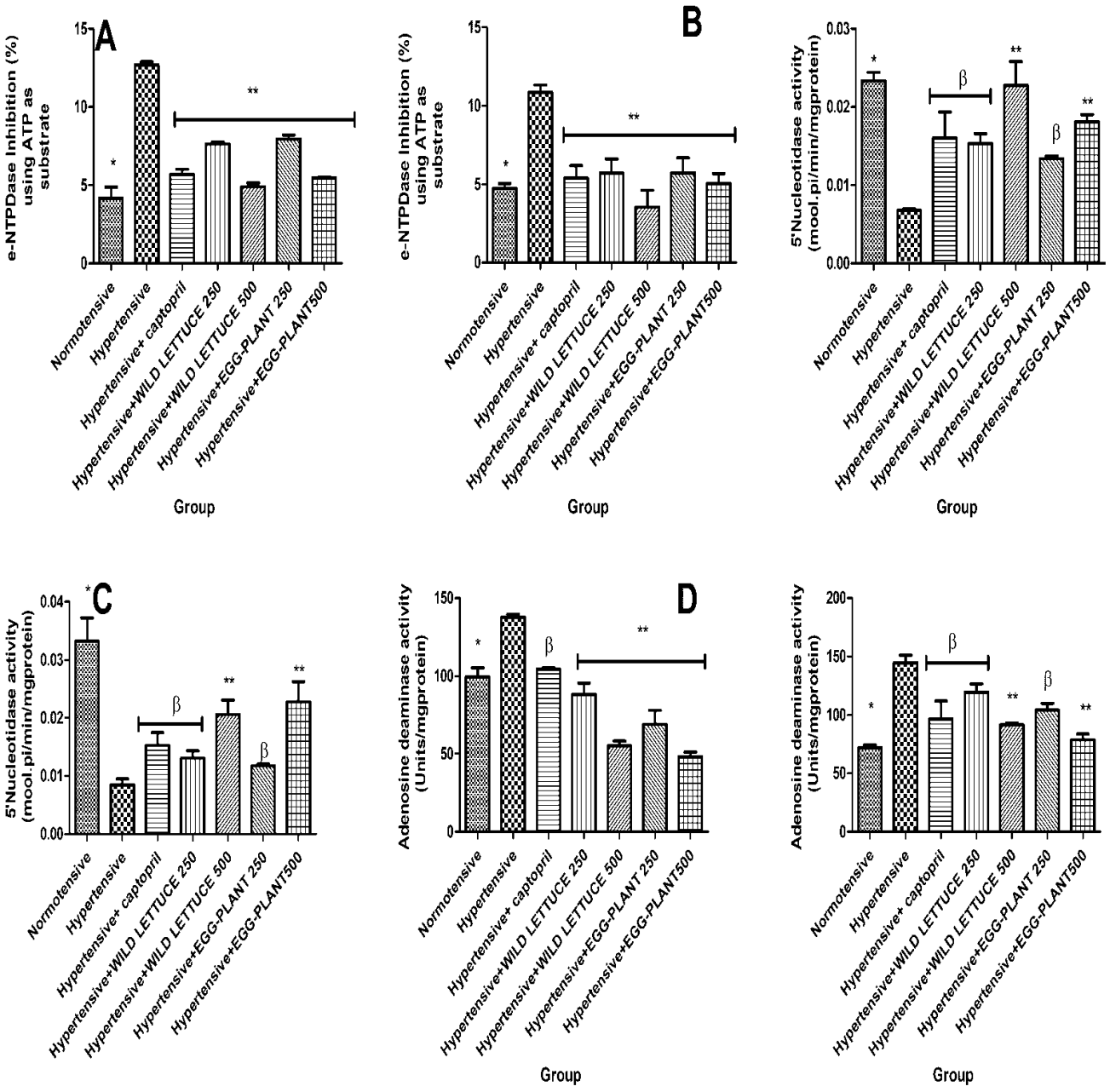

Figure 3 (A-F): Effect of wild lettuce and African eggplant leaves extract on heart (A) and kidney (B) e-NTPDase activity (using ATP as substrate); heart (C) and kidney (D) 5' Nucleotidase activity (using AMP as substrate); heart (D) and kidney (F) adenosine deaminase (ADA) activity in L-NAME induced hypertensive. Values represent mean±standard deviation (n=6). *Significantly different when compared normotensive with hypertensive ($p<0.05$). **Significantly different when compared wild lettuce and African eggplant leaves extract treated hypertensive with hypertensive ($p<0.05$). $^{\beta}$not significantly different when compared wild lettuce and African eggplant leaves extract treated hypertensive with hypertensive.

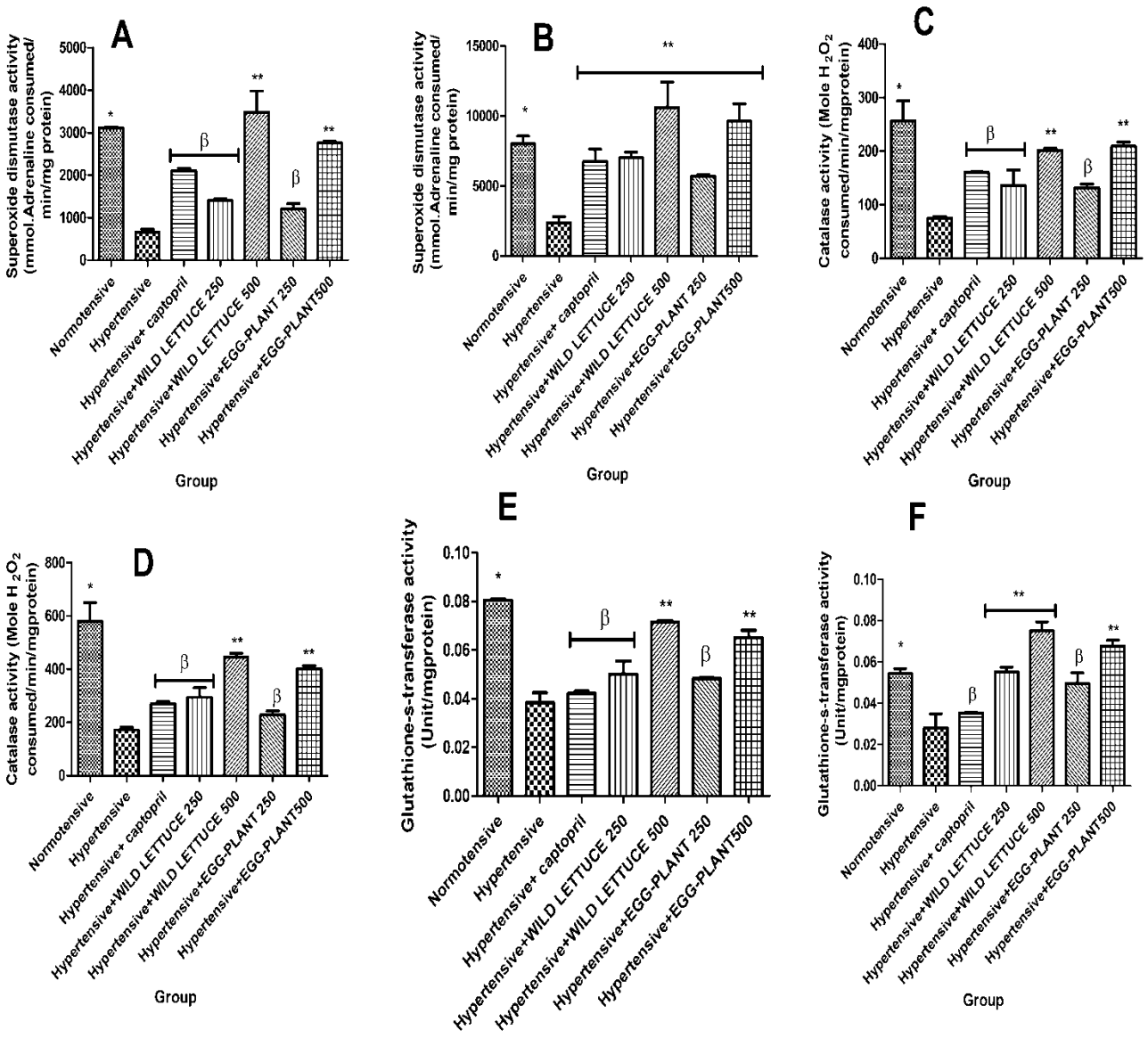

Figure 4 (A-F): Effect of wild lettuce and African eggplant leaves extract on heart (A) and kidney (B) superoxide dismutase activity; heart (C) and kidney (D) catalase activity; heart (D) and kidney (F) reduced glutathione level in L-NAME induced hypertensive. Values represent mean±standard deviation (n=6). *Significantly different when compared normotensive with hypertensive ($p<0.05$). **Significantly different when compared wild lettuce and African eggplant leaves extract treated hypertensive with hypertensive ($p<0.05$). βnot significantly different when compared wild lettuce and African eggplant leaves extract treated hypertensive with hypertensive.

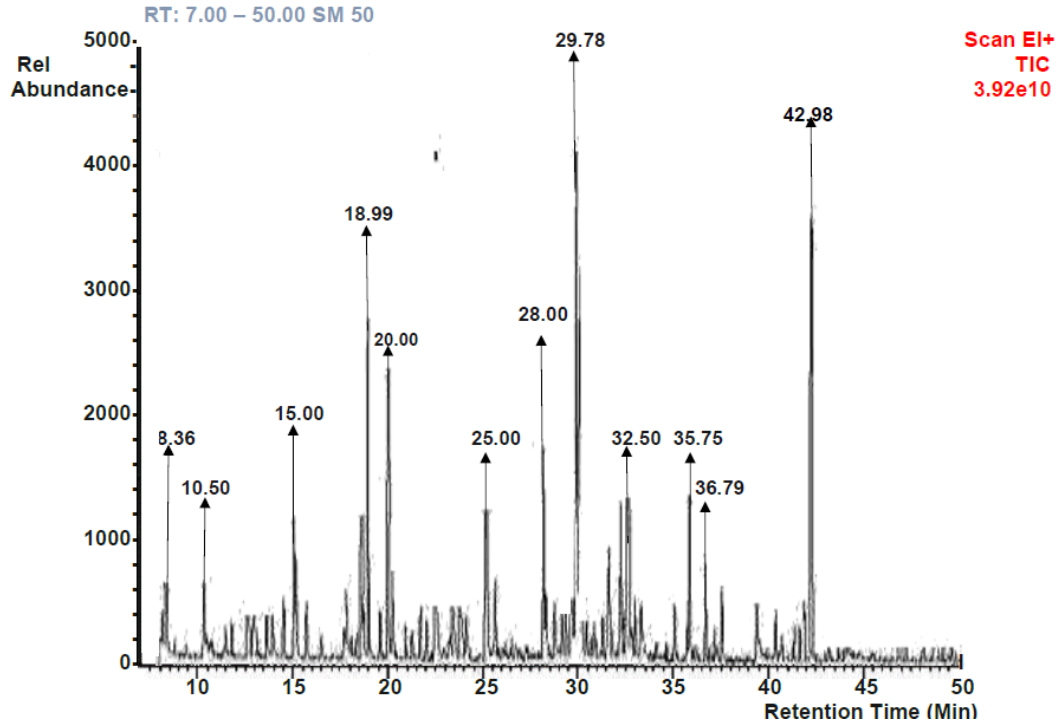

Figure 5(a): GC-MS fingerprint of wild Lettuce (Launaea Taraxacifolia) leavesPhenols constituents.

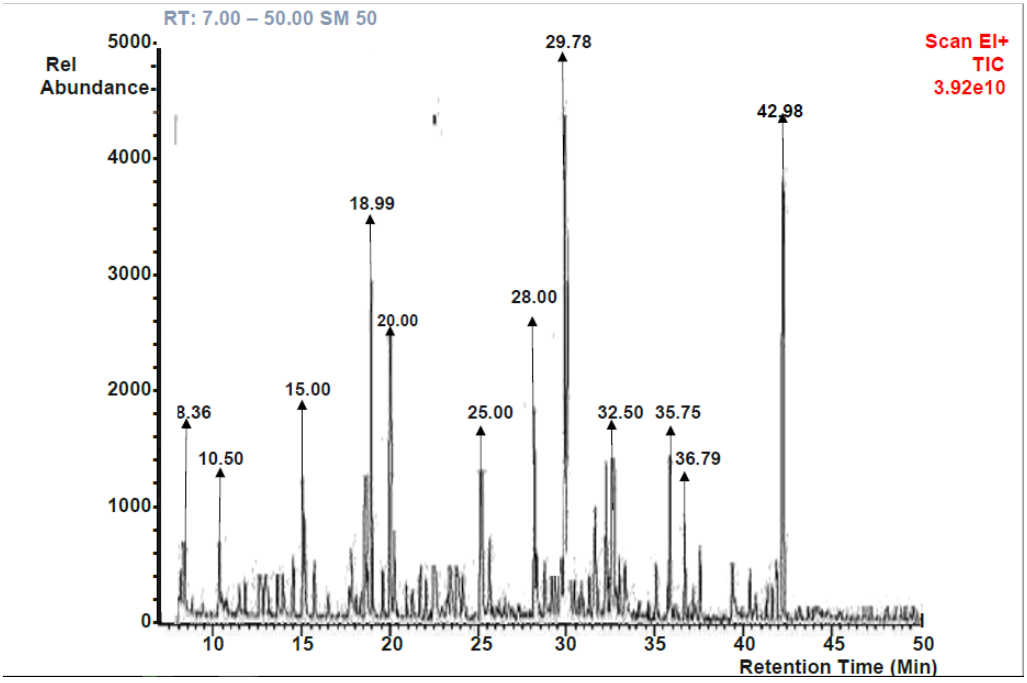

Figure 5(b): GC-MS fingerprint of African eggplant (Solanum macrocarpon) leaves Phenol constituents.

administration of aqueous extract of wild lettuce and African eggplant leaves enhanced 5'NT activity in a dose-response manner in relation to untreated hypertensive rats. Meanwhile, at higher dose of administration 5'NT activity was significantly increased than lower dose. Comparatively, wild lettuce aqueous extract had a higher effect on 5'NT activity than African eggplant in the treated hypertensive rats. Furthermore, Figure 3(e-f) represents activity of ADA in normotensive rats, untreated hypertensive rats and treated rats. Obviously, in untreated hypertensive rats, activity of ADA was significantly higher when compared with normotensive rats. Nevertheless, wild lettuce and African eggplant leaves aqueous extract administration caused a significant reduction in the activity of ADA in the administered hypertensive in comparison with untreated hypertensive rats.

Figure 4(A-F) represents activity of superoxide dismutase (SOD), catalase and reduced glutathione (GSH) level in heart and kidney of normal rats, untreated hypertensive rats and treated hypertensive rats. As presented in Figure 4 (A-F), SOD activity, catalase activity and level of GSH in heart and kidney of hypertensive rats (untreated) was significantly reduced in comparison to normotensive rats. Although, wild lettuce and African eggplants administration caused an elevation in the heart and kidney SOD, catalase and level of GSH which was significant when compared with untreated hypertensive rats. Nevertheless, wild lettuce leaves enhanced endogenous antioxidant status than African eggplant leaves but the difference was not significant when compared statistically.

Figure 5 (A-B) and Table 1 showed the peaks and bioactive compounds constituents of wild lettuce and African eggplant leaves obtained from GC-MS analysis. According to the obtained result, twelve bioactive compounds such as P-coumaric acid (1.31 and 1.41 mg/g), cinnamic acid (1.22 and 1.31 mg/g), Quercetin (1.18 and 1.14 mg/g), Syringic acid (1.07 and 1.03mg/g), Kaempferol (0.91, 0.62 mg/g) and others were quantified from wild lettuce and Africa eggplant leaves respectively as shown in Table 1.

Discussion

Hypertension is one of the most important factors associated with the development of several cardiovascular diseases such as heart failure, renal failure and stroke.[14] It is a multifactorial pathology that has been linked with the activity of some enzymes such as ACE, arginase, cholinergic and purinergic enzymes and oxidative stress as well.[1] The anti-hypertensive properties of wild lettuce and Africa eggplant (vegetables) were assessed via their in vitro effect on ACE, arginase, AChE as well as their effect on purinergic enzymatic cascade in L-NAME induced rats. ACE catalyses the conversion of inactive angiotensin-I to angiotensin-II, a powerful vasopressor peptide[15] (Santos et al). The involvement of ACE in the manifestation of hypertension could be associated with vasoconstrictive effect of the angiotensin II, a peptide produced from ACE enzymatic activity.[15] Interestingly, aqueous extract of wild lettuce and African eggplant leaves exhibited inhibitory effect on activity of ACE in a dose-related manner. Inhibition of ACE activity by wild lettuce and African eggplant leaves aqueous extract is a strong pointer to the anti-hypertensive property of these vegetables and one of the possible mechanisms to support their folklore claim. The observed inhibitory effect of these vegetables (wild lettuce and African eggplant leaves) on ACE activity could be linked to the presence of diverse bioactive compounds present in the leaves which could have exhibited a synergistic inhibitory effect on ACE activity. Also, inhibition of ACE activity promotes the preservation of bradykinin, a vasodilator that has been programmed for degradation by ACE activity.[16,17] Interestingly, characterization of the possible bioactive compounds indicates the presence of p-coumaric acid, cinnamic acid, quercetin, syringic acid Kaempferol in both leaves which are known for various nutraceuticals properties. Research findings have shown that the dietary approach offers a protective effect against hypertension[18] (Alashi et al). Findings from this study agree with previous reports on the inhibitory effect of phenolic from vegetables on ACE activity[19] (Alashi et al). In the same vein, the pathogenesis of hypertension is central to the bioavailability of nitric oxide (NO). Here, arginase and AChE play a vital role in a series of activities that lead to the reduction in the NO level.[1] Studies have revealed that eNOS and arginase struggle for the same substrate, L-arginine. Study has shown that in hypertensive subjects, arginase activity is significantly elevated with down-regulated nitric oxide biosynthesis process by struggling eNOS due to scanty L-arginine. Consistent with this hypothesis, in essential or secondary hypertensive states higher activity/expression of arginase had been reported in various vascular beds. Interestingly, research findings have shown that arginase inhibitors prevent the progression of hypertension and improve aortic endothelial function via a NO-dependent mechanism when administered to pre-hypertensive, young or adult spontaneously hypertensive rats.[20] Wild

lettuce and African eggplant leave aqueous extract significantly inhibited arginase activity as presented in Figure 3B. This event will ensure bioavailability of L-arginine for NO production for vasodilatory activities and the prevention of atherosclerosis.[21,22] Also, arginase derived metabolites have been associated with vascular thickening and vascular stiffness.[23] Interestingly, the use of natural products proves therapeutic since their bioactive compounds exhibit multi-therapeutic effects in healthy and disease states. Nevertheless, the comparative advantage of natural products over anti-hypertensive drugs is that natural products have the ability to inhibit other enzymes such as arginase, AChE and modulate purinergic enzymes which play vital role in the progression of hypertension, these attributes are devoid of in main anti-hypertensive drugs which focus on very few out of many mechanisms involved in the pathology.[16] Interestingly, reduction in arginase activity proves therapeutic since it prevents angiotensin II mediated arterial thickening, stiffness, and fibrosis (Bhatta et al). The observed effect on arginase activity could be linked the presence of bioactive compounds in wild lettuce and African eggplant leave as present in Figure 5 (A-B) & table 1 respectively. Furthermore, it is good to note that acetylcholinesterase (AChE) has been reported as one of the enzymes involved in the development of hypertension (Scacchi et al). Acetylcholine (ACh) a neurotransmitter plays a pivotal function in relaxing and vasodilating of smooth muscle.[16] ACh liberated contribute greatly to to smooth muscle relaxation coupled with the eNOS mechanism.[24] However, the effect is truncated through the degradative effect of AChE which breakdown ACh into acetate and choline.[25] Thus, limiting bioavailability of ACh to muscarinic receptor will ensure limited NO level for relaxation. The inhibitory effect wild lettuce and African eggplant leaves extract on the activity of AChE implies that more ACh will be bioavailable for vasodilatory effect and protect ACh from AChE hydrolytic effect of AChE.[25]

Administration of L-NAME (40mg/BW) has been reported to cause NO-deficient hypertension in normal rats (normotensive). The model has been used to study the NO-deficient hypertensive model in rats (Paulis et al). Studies have shown that L-NAME administration alters the purinergic enzymatic cascade with hypertension being the resultant effect.[26] Interestingly, this alteration alters NO availability since extracellular nucleotides ATP, UTP, and ADP induces eNOS phosphorylation.[27] Nucleotide induces phosphorylation modulate eNOS via phosphorylation at Ser-1177 activates eNOS activity.[27] It is worth noting that alteration in the activity of purinergic enzymes causes sequential dephosphorylation of extracellular nucleotide thereby impairing eNOS activation resulting in a reduction in NO production.[27] In the same vein, studies have shown that extracellular nucleotides promote vasodilation. However, this vasodilatory effect of nucleotides is limited by the sequential dephosphorylating activity of purinergic enzymes. Interestingly, administration of aqueous wild lettuce and African eggplant leave extract restores altered purinergic cascade as presented in Figure 4. The effect wild lettuce and African eggplant leave extract on purinergic enzymes [e-NTPDase, 5'NT and ADA] could contribute to their overall anti-hypertensive effect. Therefore, this necessitates the need for modulation in their altered activity. As presented, e-NTPDase and ADA activity were elevated in L-NAME induced hypertensive rats (untreated) while wild lettuce and African eggplant leaves extract counter the effect of L-NAME in the treated hypertensive rats. These events ensure adequate availability of ATP and adenosine for vasodilation, phosphorylation of eNOS. Meanwhile, administration of wild lettuce and African eggplant leaves extract enhance dephosphorylation of AMP to adenosine. This phenomenal will promote more adenosine bioavailability for vasodilatory processes.[1] The overall observations agree with previous reports on the modulatory effect of bioactive compounds on purinergic.[1,26]

Oxidative stress plays vital role in pathogenesis of hypertension (Montezano & Touyz). Interestingly, aside inhibitory effect on the pertinent enzymes linked to the onset of hypertension, a good anti-hypertensive agent must have an antioxidant potential.[1] It worthy to note that wild lettuce and African eggplant leaves exhibit antioxidant properties. Meanwhile, antioxidant properties of wild lettuce and African eggplants leaves were assessed via their ability to scavenged generated free radicals and chelated metal ions capable of initiating free radicals. These were evident by the ability of wild lettuce and African eggplants leaves aqueous extracts to scavenge DPPH, ABTS, and prevent Fe^{2+} induce lipid peroxidation as shown in Figure 1 (A-C). In the same vein, Administration of aqueous extract of wild lettuce and African eggplant leaves significantly enhanced endogenous antioxidant status of the hypertensive rats as grossly adjudged by an increase in the heart and kidney SOD, catalase and GST activity of the treated hypertensive rats as presented in Figure 4 (A-F). The use of medicinal plants with proven antioxidant properties could play a significant role in the management of hypertension.[28] Meanwhile, the anti-radical ability of the wild lettuce and African eggplant leaves typified by ability to scavenge radicals, inhibit lipid peroxidation, and enhance endogenous antioxidant status reveals the

antioxidant properties of these vegetables. Vegetables have been reported to exhibited diverse antioxidant properties as a result of presence of polyphenolic content in the leaves.[29] However, these observed antioxidant properties might be due to the presence of diverse bioactive compounds present in the leaves as shown in Figure 5 and Table 1 respectively.

Conclusion

In conclusion, wild lettuce and African eggplant exhibited in vitro and in vivo antihypertensive and antioxidant properties via their inhibitory effect on relevant enzymes associated with the onset of hypertension and strong antioxidant properties. Nevertheless, wild lettuce leaves had better anti-hypertensive and antioxidant properties than African eggplant leaves. However, the consumption of these vegetables will offer a protective effect against the pathogenesis of hypertension.

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Approval for the use of experimental animals soughed and given by Center for Research and Development of FUTA. During the study animal's rights were obeyed strictly.

Conflict of Interest

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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