Inhibition of renin-angiotensin system enzymes by leafy vegetables polyphenol extracts related to fertilizer micro-dosing and harvest time

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Abstract

Increase in human population coupled with continuous rise in oxidative stress-related diseases requires availability of healthier foods like vegetables, which are polyphenol-rich sources. Polyphenols are able to inhibit angiotensin-I converting enzyme (ACE) and renin, thereby regulating blood pressure but this ability can be influenced by nutrient management during plant growth. Fertilizer micro-dosing is a valuable nutrient management option, which increases crop productivity with minimal expenditure of resources. This study assessed the effects of fertilizer micro-dosing and harvest times on inhibition of ACE and renin activities by aqueous polyphenol extracts from *Amaranthus cruentus, Ocimum gratissimum* and *Solanum macrocarpon* leaves. Different levels of urea (0, 20, 40 and 60 kg ha^{-1}) with cow manure (5 t ha^{-1}) or without manure (80 kg ha^{-1}) were used to grow the leafy vegetables before they were harvested three times at two (*A. cruentus*) and four weeks intervals (*O. gratissimum* and *S. macrocarpon*). Polyphenols were extracted from leaf powders using water (1:20 ratio) and total phenolic content (TPC), total flavonoids content (TFC), ACE and renin inhibition were assayed according to standard protocols. Two-way analysis of variance and Duncan's test ($p<0.05$) revealed that TPC (605.70±39.51 to 1024.83±102.89 mg g^{-1}; gallic acid equivalent) and TFC (149.02±29.12 to 276.19±63.30 mg g^{-1}; rutin equivalent) were not affected by fertilizer doses and harvest times except TPC from *O. gratissimum*. Inhibitions of ACE (0.25±0.01 to 8.46±0.42 mg mL^{-1}) and renin (-95.41±2.25 to 36.42±0.22%) activities by each specie were significantly affected by fertilizer dose and harvest time. Production with cow manure combined with low or medium doses of urea (20 or 40 kg ha^{-1}) followed by collection at the first two harvests are recommended for polyphenol extracts with potential inhibition of renin-angiotensin system enzymes.

Keywords: *Amaranthus cruentus, Ocimum gratissimum, Solanum macrocarpon,* angiotensin-I converting enzyme, mineral fertilizer

INTRODUCTION

Blood pressure in the human body is mainly regulated by the renin-angiotensin system which depend on activities of angiotensin-I converting enzyme (ACE) and renin (Ajibola et al., 2011; Aluko, 2015). Blood pressure regulation is based on the fact that renin can convert angiotensinogen into inactive angiotensin-I, which can be converted by ACE into active angiotensin-II (Ajibola et al., 2011; Aluko, 2015). Angiotensin-II is a potent vasoconstrictor while ACE promotes the deactivation of bradykinin, a potent vasodilator (Aluko, 2015). Therefore, inhibition of ACE or renin or both could be an effective strategy for blood pressure regulation (Ajibola et al., 2011; Aluko, 2015). There is a growing interest to identify natural compounds that can be used as alternative solutions to synthetic drugs in

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blood pressure regulation since the latter present adverse side effects (Ajibola et al., 2011; Aluko, 2015).

Traditional leafy vegetables are widely used for nutritional and medicinal purposes throughout sub-Saharan Africa (Achigan-Dako et al., 2010; Oboh et al., 2016). In the Republic of Benin, several plant species are valued as traditional leafy vegetables among which *Amaranthus cruentus* (*Amaranthaceae*), *Ocimum gratissimum* (*Lamiaceae*) and *Solanum macrocarpon* (*Solanaceae*) have been recognized as having a great importance in the food and medicinal systems (Achigan-Dako et al., 2010). These leafy vegetables are cheap sources of bioactive compounds including minerals, vitamins, carotenoids and polyphenols (Achigan-Dako et al., 2010; Oboh et al., 2016; Jiménez-Aguilar and Grusak, 2017). These bioactive compounds have been related to nutraceutical benefits and have certainly been responsible for promising results associated with the use of these leafy vegetables in folk medicine. *Amaranthus* species are recognized to be efficient in management of fever, haemorrhage, kidney complaints and anaemia (Nana et al., 2012). *Ocimum gratissimum* leaves were reported to treat upper respiratory tract infection, diarrhoea, skin diseases, pneumonia, cough and conjunctivitis (Mahapatra et al., 2010). *Solanum macrocarpon* exhibited antioxidant potential, thereby offering good protection against oxidative stress-related diseases (Adewale et al., 2014). Polyphenol extracts from traditional leafy vegetables were also able to inhibit the renin-angiotensin system enzymes; suggesting that they could be good candidates for blood pressure regulation (Ajibola et al., 2011; Oboh et al., 2016).

The profile of bioactive compounds has been reported to be influenced by agronomic and environmental factors, especially nutrient supply and harvest times (Sossa-Vihotogbé et al., 2013; Brasileiro et al., 2015; Jiménez-Aguilar and Grusak, 2017). Mineral fertilizers are essential components of agriculture and a micro-dosing technology has been developed to increase crops productivity with substantial decrease in fertilizer usage (Okebalama et al., 2017). However, to the best of our knowledge, effects of fertilizer micro-dosing on nutritional and potential health benefits of crops have not been investigated. Therefore, this study aims at assessing the effects of fertilizer micro-dosing and harvest times on inhibition of the renin-angiotensin system enzymes by polyphenol extracts obtained from *A. cruentus*, *O. gratissimum* and *S. macrocarpon* leaves.

MATERIALS AND METHODS

Plant material

Leafy vegetables were produced by applying mineral fertilizer to respective plants using the micro-dosing technology under a randomized complete block design. Urea (0, 20, 40 and 60 kg ha^{-1}) combined to cow manure (5 t ha^{-1}) and 80 kg ha^{-1} of urea without cow manure were applied to experimental units (6×1 m) in quadruples. Seeds were grown in nursery for four (*A. cruentus*) to six weeks (*O. gratissimum* and *S. macrocarpon*) before the young plants were transplanted. Cow manure was applied one week before transplanting while urea was applied immediately after transplanting. Three harvests were made at 4, 6 and 8 weeks after transplanting for *A. cruentus*; 6, 10 and 14 weeks after transplanting for *S. macrocarpon* and 8, 12 and 16 weeks after transplanting for *O. gratissimum*. The number of harvest and the intervals between harvesting times adopted for each leafy vegetable specie were recommended cultural practices according to National Institute of Agricultural Research of Republic of Benin that provided standard requirements for these crops.

Leaves were collected by cutting stems at 10 cm from collar (except plants located at border lines) and the four replications of one treatment were mixed together to get a homogeneous sample. Samples were washed and oven-dried at 60°C for 24 h or until a constant weight was achieved. Polyphenols were extracted at 60°C using water as solvent (leaf powder to solvent ratio of 1:20) for four hours (twice two hours) for the same sample.

Analysis methods

Total phenolic content (TPC, with gallic acid as standard) and total flavonoids content

(TFC, with rutin as standard) of crude polyphenol extracts were determined according to the method of Fasakin et al. (2011) and Ebrahimzadeh and Bahramian (2009), respectively. Ability of polyphenol extracts (0.30 to 1.25 mg mL^{-1}) and captopril (a synthetic ACE inhibitor, 0.00625 to 0.05 μg mL^{-1}) to inhibit ACE was measured using FAPGG (N-(3-[2-furyl]acryloyl)-l-phenylalanylglycylglycine) as substrate according to the method described by Ajibola et al. (2011) with slight modifications concerning duration (30 minutes rather than 2 minutes) and temperature (37°C instead of room temperature) of reading. Data were used to calculate the half maximal inhibitory concentration (IC_{50}) using GraphPad Prism 6 software. Inhibitory activity against renin was determined at 0.50 mg mL^{-1} using renin inhibitor screening assay kit following the method described by Ajibola et al. (2011) with slight modification regarding reading duration (10 minutes instead of instantaneous reading). Assays were performed in triplicates and data were statistically analysed using R software version 3.02. Factorial analysis of variance on repeated measures were used to determine effects of fertilizer doses and harvest times on investigated variables followed by Duncan's test to reveal significant differences between treatments at 0.05 levels. Pearson's correlation coefficients were calculated for polyphenols composition and inhibitory activities.

RESULTS

Total phenolic and flavonoids contents

The TPC (gallic acid equivalent) of *A. cruentus* (947.61±29.83 to 1024.83±102.89 mg g^{-1}) were significantly ($p<0.05$) higher than those of *O. gratissimum* (605.70±39.51 to 823.93±114.68 mg g^{-1}) and *S. macrocarpon* (608.89±9.80 to 706.42± 77.39 mg g^{-1}). However, the TFC (rutin equivalent) of *A. cruentus* (154.17±29.46 to 181.25±55.98 mg g^{-1}) and *O. gratissimum* (149.02±29.12 to 265.69±88.73 mg g^{-1}) were each significantly ($p<0.05$) lower than that of *S. macrocarpon* (191.43±24.24 to 276.19±63.30 mg g^{-1}). The analysis of variance did not reveal any significant ($p>0.05$) effect of fertilizer doses and harvest times within the range of values studied on the TPC of *A. cruentus* and *S. macrocarpon* or on the TFC of each of the three leafy vegetables species (Table 1). The TPC of *O. gratissimum* however, was significantly ($p<0.05$) affected by fertilizer dose, harvest time and their interactions (Table 1). The mean scores of fertilizer doses, based on TPC of *O. gratissimum* were significantly higher when low or medium doses of urea (20 or 40 kg ha^{-1}) were combined with cow manure (Table 2). Significantly ($p<0.05$) higher mean scores of TPC, based on harvest time, were recorded at first and second harvest of *O. gratissimum* (Table 3).

Table 1. Effect of fertilizer dose (FD) and harvest time (HT) on total phenolic content (TPC), total flavonoids content (TFC) and inhibition of ACE and renin activities.

	Amaranthus cruentus			*Ocimum gratissimum*			*Solanum macrocarpon*		
	FD	HT	FDxHT	FD	HT	FDxHT	FD	HT	FDxHT
TPC[1]	7.29 [a] ns	1.03[ns]	3.11[ns]	20.19*	34.42*	21.92*	5.23[ns]	3.67[ns]	11.90[ns]
TFC[1]	20.57[ns]	2.81[ns]	2.97[ns]	4.84[ns]	12.91[ns]	10.29[ns]	6.07[ns]	7.17[ns]	9.58[ns]
ACE[2]	32.60**	434.92***	370.91**	376.33**	44.47*	117.99***	7.76[ns]	1246.02**	158.77***
Renin[3]				147.78**	107.13**	225.05**			

Units: [1]: mg g^{-1}, [2]: IC_{50}, mg mL^{-1} and [3]: %.

[a]: F-value

Significance: *$p<0.05$, **$p<0.01$, ***$p<0.001$, ns = not significant and $p\geq0.05$.

Table 2. Effect of fertilizer dose on total phenolic content (TPC), total flavonoids content (TFC) and inhibition of ACE and renin activities.

	Fertilizer dose (kg ha^{-1})				
	0	20	40	60	80
			Amaranthus cruentus		
TPC (mg g^{-1})	964.09±23.07^{a}	960.76±20.37^{a}	977.06±25.89^{a}	992.98±32.88^{a}	989.09±24.55^{a}
TFC (mg g^{-1})	164.58±31.25^{a}	168.06±30.56^{a}	169.79±32.60^{a}	166.67±30.56^{a}	170.49±31.60^{a}
ACE (IC_{50}, mg mL^{-1})	0.42±0.01^{a}	0.36±0.00^{a}	0.32±0.00^{b}	0.34±0.00^{b}	0.38±0.00^{a}
			Ocimum gratissimum		
TPC (mg g^{-1})	694.21±39.37^{b}	707.12±40.05^{a}	714.91±43.69^{a}	671.04±36.19^{c}	688.51±34.22^{ab}
TFC (mg g^{-1})	194.44±34.64^{a}	205.88±41.50^{a}	233.33±52.61^{a}	183.01±30.39^{a}	192.16±33.66^{a}
ACE (IC_{50}, mg mL^{-1})	1.70±0.04^{c}	1.76±0.08^{c}	3.68±0.04^{b}	4.32±0.14^{b}	6.48±0.12^{a}
Renin (%)	23.46±0.31^{c}	23.08±0.28^{c}	30.28±0.27^{a}	25.28±0.19^{b}	23.50±0.57^{bc}
			Solanum macrocarpon		
TPC (mg g^{-1})	650.86±19.14^{a}	649.22±20.38^{a}	656.42±23.24^{a}	661.98±22.45^{a}	647.37±17.47^{a}
TFC (mg g^{-1})	222.86±28.25^{a}	227.30±30.16^{a}	226.35±29.21^{a}	239.37±33.97^{a}	223.17±26.67^{a}
ACE (IC_{50}, mg mL^{-1})	0.73±0.03^{a}	0.68±0.01^{a}	0.68±0.01^{a}	0.57±0.00^{a}	0.79±0.03^{a}

Mean scores ± standard error within each variable with different alphabets within each row are significantly different ($p<0.05$).

Table 3. Effect of harvest time on total phenolic content (TPC), total flavonoids content (TFC) and inhibition of ACE and renin activities.

	Harvest time		
	First	Second	Third
		Amaranthus cruentus	
TPC (mg g^{-1})	981.50±30.79^{a}	968.83±22.99^{a}	980.06±22.88^{a}
TFC (mg g^{-1})	160.21±26.46^{a}	170.42±32.92^{a}	173.13±33.96^{a}
ACE (IC_{50}, mg mL^{-1})	0.31±0.00^{c}	0.40±0.01^{a}	0.38±0.00^{b}
		Ocimum gratissimum	
TPC (mg g^{-1})	724.10±44.00^{a}	706.55±39.38^{a}	654.81±32.87^{b}
TFC (mg g^{-1})	186.08±34.51^{a}	214.51±42.55^{a}	204.71±38.63^{a}
ACE (IC_{50}, mg mL^{-1})	4.13±0.03^{a}	3.63±0.06^{b}	3.00±0.10^{b}
Renin (%)	26.91±0.45^{a}	26.21±0.35^{b}	22.23±0.07^{c}
		Solanum macrocarpon	
TPC (mg g^{-1})	676.67±34.16^{a}	633.70±13.04^{a}	649.14±14.35^{a}
TFC (mg g^{-1})	251.43±38.67^{a}	211.43±24.57^{a}	220.57±25.71^{a}
ACE (IC_{50}, mg mL^{-1})	1.04±0.02^{a}	0.49±0.00^{c}	0.54±0.00^{b}

Mean scores ± standard error within each variable with different alphabets within each row are significantly different ($p<0.05$).

Inhibition of angiotensin-I converting enzyme

Inhibition of ACE by polyphenol extracts and captopril (expressed as IC_{50} values) indicated that captopril (0.01±0.00 μg mL^{-1}) had the lowest value when compared to *A. cruentus* (0.25±0.01 to 0.69±0.04 mg mL^{-1}), *O. gratissimum* (1.06±0.01 to 8.46±0.42 mg mL^{-1}) and *S. macrocarpon* (0.37±0.04 to 1.55±0.12 mg mL^{-1}). Analysis of variance revealed significant ($p<0.05$) independent and interactive effects of fertilizer doses and harvest times on the inhibition of ACE by *A. cruentus* and *O. gratissimum* (Table 1). Fertilizer dose did not have any significant effect on the inhibition of ACE activity by *S. macrocarpon* contrary to harvest time and interaction of both factors (Table 1). However, the mean scores of fertilizer dose showed a significantly ($p<0.05$) lower IC_{50} for *A. cruentus* when medium or high doses of urea (40 or 60 kg ha^{-1}) were combined with cow manure during crop production (Table 2). On the other hand, *O. gratissimum* presented significantly ($p<0.05$) lower IC_{50} (Table 2) when cow manure was applied alone (0 kg ha^{-1}) or combined with low dose of urea (20 kg ha^{-1}). Based on harvest times (Table 3) mean scores of IC_{50} were significantly ($p<0.05$) low at different harvest times for *A. cruentus* (first), *O. gratissimum* (second and third) and *S. macrocarpon* (second).

Inhibition of renin enzyme

Amaranthus cruentus samples (-95.41±2.25 to -16.10±1.49%) and most of *S. macrocarpon* samples (-33.03±0.28 to -1.09±1.92%) were not able to inhibit renin activity when compared to all *O. gratissimum* samples (12.26±0.68 to 36.42±0.22%) which exhibited higher inhibition than the remaining samples of *S. macrocarpon* (2.89±0.56 to 19.10±1.23%). Only five samples of *S. macrocarpon* were able to inhibit renin activity; the results indicate that the inhibitory activity of this vegetable against renin enzyme was dependent on fertilizer dose and harvest time. The active samples were those produced with high doses of urea (60 or 80 kg ha^{-1}) with or without cow manure and collected at first harvest as well as those produced with cow manure alone or combined with low doses of urea (0 or 20 kg ha^{-1}) and harvested the third time. Samples cultivated with cow manure alone (0 kg ha^{-1}) and collected at second harvest also exhibited renin inhibitory activity. Analysis of variance performed on *O. gratissimum* showed significant ($p<0.05$) independent and interactive effects of fertilizer dose and harvest time (Table 1). Mean scores of fertilizer dose were significantly ($p<0.05$) higher when medium dose of urea (40 kg ha^{-1}) was combined with cow manure (Table 2). Depending on harvest time, all mean scores were significantly ($p<0.05$) different and decreased with increase in harvest time (Table 3).

Relationship between total polyphenol content and enzymes inhibition

Pearson correlation test indicated significantly ($p<0.05$) positive correlations (Table 4) between TPC and TFC of *A. cruentus* (r=0.92, p=0.00), *O. gratissimum* (r=0.97, p=0.00) and *Solanum macrocarpon* (r=0.94, p=0.00). Inhibition of ACE by *O. gratissimum* was significantly and positively correlated with TPC (r=0.59, p=0.02) and TFC (r=0.66, p=0.01) while the inhibitory activities against ACE (*A. cruentus* and *S. macrocarpon*) and renin (*O. gratissimum*) were not correlated to TPC or TFC.

Table 4. Pearson correlation coefficient (r) between total phenolic content (TPC), total flavonoids content (TFC), inhibition of ACE and renin activities.

	Amaranthus cruentus			*Ocimum gratissimum*				*Solanum macrocarpon*		
	TPC	TFC	ACE	TPC	TFC	ACE	Renin	TPC	TFC	ACE
TPC	1.00			1.00				1.00		
TFC	0.92**	1.00		0.97**	1.00			0.95**	1.00	
ACE	-0.01ns	-0.06ns	1.00	0.59*	0.66**	1.00		-0.17ns	-0.19ns	1.00
Renin				-0.09ns	-0.13ns	0.30ns	1.00			

Significance: *$p<0.05$, **$p<0.01$, ns = not significant (2-tailed).

DISCUSSION

The TPC values (gallic acid equivalent) obtained in this study are higher than those reported for *A. cruentus* (2.20-3.20 mg g^{-1}) by Jiménez-Aguilar and Grusak (2017) and for *Amaranthus hybridus* (4.61 mg g^{-1}), *Talinum triangulare* (3.31 mg g^{-1}), *Telferia occidentalis* (5.06 mg g^{-1}) and *Vernonia amygdalina* (5.11 mg g^{-1}) by Oboh et al. (2016). Lower TPC values have also been reported by Sossa-Vihotogbé et al. (2013) on *Ceratotheca sesamoïdes* (3.00-13.3 mg g^{-1}), *Justicia tenella* (6.60-8.60 mg g^{-1}) and *Sesamum radiatum* (2.40-2.80 mg g^{-1}). Similarly, the TFC recorded in this study are higher than those reported by Jiménez-Aguilar and Grusak (2017) for *A. cruentus* (1.00 mg g^{-1}) in catechin equivalent and by Oboh et al. (2016) for *A. hybridus* (0.70 mg g^{-1}), *T. triangulare* (0.56 mg g^{-1}), *T. occidentalis* (0.89 mg g^{-1}) and *V. amygdalina* (1.30 mg g^{-1}) in quercetin equivalent. These reports suggest that fertilizer micro-dosing could have increased levels of total phenolic and flavonoids but specificities of analytical procedures should be considered since different standards were used for TFC determination. Higher total phenolic content have been related to conversion of bound phenolic from complex to hydrolysable forms; increasing their reactivity (Sultana et al., 2008). However, lower contents could be related to changes in structure of polyphenol compounds, vegetables species, environmental or agronomic factors (Sultana et al., 2008; Steffensen et al., 2011). Polyphenols levels have been reported to vary according to genotype and growing conditions (Steffensen et al., 2011).

This study showed that fertilizer dose and harvest time did not affect TPC and TFC of the studied leafy vegetables except for *O. gratissimum*, which exhibited significantly ($p<0.05$) higher TPC when low or medium doses of urea (20 or 40 kg ha^{-1}) were combined with cow manure during crop production. In agreement with this observation, beneficial role of concurrent use of mineral and organic fertilizer on synthesis of polyphenols has been reported (Ibrahim et al., 2013; Sossa-Vihotogbé et al., 2013). TPC of *O. gratissimum* was higher at first and second harvest; indicating that synthesis of these secondary metabolites could be related to maturity stage. Sossa-Vihotogbé et al. (2013) reported higher TPC of *C. sesamoïdes*, *J. tenella* and *S. radiatum* at second harvest when NPK and cow manure were combined. Mature leaves of *Amaranthus* species have been reported to exhibit higher TFC (Steffensen et al., 2011) while TPC of *T. triangulare* have been reported to be higher during growing period (Brasileiro et al., 2015). Synthesis of polyphenols has been related to defense strategy of young leaves during normal ontogeny to explain higher TPC observed during growing period of *T. triangulare* (Brasileiro et al., 2015).

Inhibition of ACE activity by *Amaranthus cruentus* was similar to those reported by Ajibola et al. (2011) for *Gongronema latifolium* (0.41 mg mL^{-1}) and *V. amygdalina* (0.21 mg mL^{-1}) while they were lower than those recorded for *Ocimum gratissimum* and *Solanum macrocarpon*. Oboh et al. (2016) recorded lower inhibition of ACE activity by *A. hybridus* (53.44 µg mL^{-1}), *T. triangulare* (63.13 µg mL^{-1}), *T. occidentalis* (44.59 µg mL^{-1}) and *V. amygdalina* (28.46 µg mL^{-1}).

Inhibition of renin activity by *G. latifolium* (12.50-17.50%) and *V. amygdalina* (17.50-30.00%) reported by Ajibola et al. (2011) are consistent with those found in this study for *O. gratissimum*. Conversely, reports of Ajibola et al. (2011) on inhibition of renin activity are higher than those recorded for *S. macrocarpon* in our study. Inhibitions of ACE and renin activities have been reported to involve competitive, uncompetitive or mixed-type interactions between enzyme, inhibitor and substrate, thereby inducing loss of enzymatic activity by changing conformation of active site on the enzyme (Aluko, 2015). Higher inhibitory properties could indicate a mixed-type inhibition (enzyme-inhibitor and enzyme-substrate-inhibitor complexes) as reported by Ajibola et al. (2011) for higher ACE and renin inhibition by *V. amygdalina*. Inversely, lower ACE inhibition by *O. gratissimum* could be related to an uncompetitive inhibition binding enzyme in its substrate-bound site (enzyme-substrate-inhibitor complexes).

Correlations observed between total phenolic and flavonoids content and inhibition of ACE (*O. gratissimum*) linked this inhibitory activity to common sources as polyphenol compounds. The strong correlations observed in this study suggest that this inhibitory activity could be linked to growing conditions (fertilizer micro-dosing) and harvest time since synthesis of polyphenols has been reported to be influenced by agronomic and environmental factors (Steffensen et al., 2011; Ibrahim et al., 2013; Sossa-Vihotogbé et al., 2013; Brasileiro et al., 2015). Highest inhibitory activities recorded after combined use of low or medium doses of urea (20 or 40 kg ha^{-1}) with cow manure followed by leaf collection during the first two harvests are consistent with higher polyphenol contents observed in this work. Lack of correlation for inhibitory activities against ACE (*A. cruentus* and *S. macrocarpon*) and renin (*O. gratissimum*) could be related to differences in structure of polyphenol compounds or contributions from other phytochemicals (chlorophylls or carotenoids) to the observed inhibitory activities. For example, chlorophyll-rich extracts have been reported to be more active ACE and renin inhibitors than crude extracts (Ajibola et al., 2011).

CONCLUSION

Amaranthus cruentus, Ocimum gratissimum and *Solanum macrocarpon* produced under fertilizer micro-dosing and harvested at different times were rich sources of polyphenols with ability to inhibit ACE and renin activities. *Amaranthus cruentus* and *Solanum macrocarpon* were more potent ACE inhibitors while *Ocimum gratissimum* was an effective renin inhibitor. Polyphenol content was not significantly affected by fertilizer dose and harvest time except for *O. gratissimum*. Inhibitions of ACE and renin activities were significantly affected by fertilizer dose and harvest time, except *Solanum macrocarpon* of which ACE inhibition was not significantly affected by fertilizer dose. Application of low or medium doses of urea (20 or 40 kg ha^{-1}) combined with cow manure followed by earlier harvests (first or second) could be recommended for production of these leafy vegetables under fertilizer micro-dosing in order to fully benefit from their health promoting ability, especially ACE and renin inhibitions. However, effect of crop production seasons and fertilizer application after each harvest should be assessed to provide additional information on the roles of fertilizer micro-dosing and harvest time on inhibition of renin-angiotensin system enzymes by polyphenol extracts from these leafy vegetables.

ACKNOWLEDGMENTS

This research was supported by the International Development Research Centre (IDRC) and the Canadian Department of Foreign Affairs, Trade and Development (DFATD), through Canadian International Food Security Research Fund (CIFSRF) Project No.107983. The authors would like to thank PhD and MSc students (Adjogboto André, Likpètè Didier, Dassigli Pierre and Kpadonou Esaï) who worked on the experimental fields that produced the leafy vegetables.

Literature cited

Achigan-Dako, E.G., Pasquini, M.W., Assogba-Komlan, F., N'danikou, S., Yédomonhan, H., Dansi, A., and Ambrose-Oji, B., eds. (2010). Traditional Vegetables in Benin (Institut National des Recherches Agricoles du Bénin, Imprimeries du CENAP), pp.282.

Adewale, O.B., Onasanya, A., Fadaka, O.A., Iwere, H., Anadozie, S.O., Osukoya, A.O., and Olayide, I.I. (2014). In vitro antioxidant effect of aqueous extract of *Solanum macrocarpon* leaves in rat liver and brain. Oxid. Antioxid. Med. Sci. *3* (*3*), 225–229 https://doi.org/10.5455/oams.161214.or079.

Ajibola, C.F., Eleyinmi, A.F., and Aluko, R.E. (2011). Kinetics of the inhibition of renin and angiotensin-I converting enzyme by polar and non-polar polyphenolic extracts of *Vernonia amygdalina* and *Gongronema latifolium* leaves. Plant Foods Hum. Nutr. *66*, 320–327 https://doi.org/10.1007/s11130-011-0257-x.

Aluko, R.E. (2015). Antihypertensive peptides from food proteins. Annu. Rev. Food Sci. Technol. *6*, 235–262 https://doi.org/10.1146/annurev-food-022814-015520.

Brasileiro, B.G., Leite, J.P.V., Casali, V.W.D., Pizziolo, V.R., and Coelho, O.G.L. (2015). The influence of planting and harvesting times on the total phenolic content and antioxidant activity of *Talinum triangulare* (Jacq.). Willd. Acta Sci. *37* (*2*), 249–255 https://doi.org/10.4025/actasciagron.v37i2.19130.

Ebrahimzadeh, M.A., and Bahramian, F. (2009). Antioxidant activity of *Crataegus pentaegyna* subsp. *elburensis* fruits extracts used in traditional medicine in Iran. Pak. J. Biol. Sci. *12* (*5*), 413–419.

Fasakin, C.F., Udenigwe, C.C., and Aluko, R.E. (2011). Antioxidant properties of chlorophyll-enriched and chlorophyll-depleted polyphenolic fractions from leaves of *Vernonia amygdalina* and *Gongronema latifolium*. Food Res. Int. *44*, 2435–2441 https://doi.org/10.1016/j.foodres.2010.12.019.

Ibrahim, M.H., Jaafar, H.Z.E., Kharimi, E., and Ghasemzadeh, A. (2013). Impact of organic and inorganic fertilizers application on the phytochemical and antioxidant activity of Kacip Fatimah (*Labisia pimula* Benth). Molecules *18* (*9*), 10973–10988 https://doi.org/10.3390/molecules180910973.

Jiménez-Aguilar, D.M., and Grusak, M.A. (2017). Minerals, vitamin C, phenolics, flavonoids and antioxidant activity of *Amaranthus* leafy vegetables. J. Food Compos. Anal. *58*, 33–39 https://doi.org/10.1016/j.jfca.2017.01.005.

Mahapatra, S.K., Chakraborty, S.P., and Roy, S. (2010). Aqueous extract of *Ocimum gratissimum Linn* and ascorbic acid ameliorate nicotine-induced cellular damage in murine peritoneal macrophage. Asian Pac. J. Trop. Med. *3* (*10*), 775–782 https://doi.org/10.1016/S1995-7645(10)60186-1.

Nana, F.W., Hilou, A., Millogo, J.F., and Nacoulma, O.G. (2012). Phytochemical composition, antioxidant and xanthine oxidase inhibitory activities of *Amaranthus cruentus* L. and *Amaranthus hybridus* L. extracts. Pharmaceuticals *5* (*12*), 613–628 https://doi.org/10.3390/ph5060613.

Oboh, G., Akinyemi, A.J., Adeleye, B., Oyeleye, S.I., Ogunsuyi, O.B., Ademosun, A.O., Ademiluyi, A.O., and Boligon, A.A. (2016). Polyphenolic compositions and in vitro angiotensin-I converting enzyme inhibitory properties of common green leafy vegetables: a comparative study. Food Sci. Biotechnol. *25* (*5*), 1243–1249 https://doi.org/10.1007/s10068-016-0197-1.

Okebalama, B.C., Ibrahim, A., Safo, E.Y., Yeboah, E., Abaidoo, R.C., Logah, V., and Uzoh, I.M. (2017). Fertilizer micro-dosing in West African low-input cereals cropping: Benefits, challenges and improvement strategies. Afr. J. Agric. Res. *12* (*14*), 1169–1176 https://doi.org/10.5897/AJAR2016.11559.

Sossa-Vihotogbé, C.N.A., Anihouvi, B.V., Akissoé, H.N., Amadji, L.G., and Hounhouigan, D.J. (2013). Effect of organic and mineral fertilization on proximate composition of three leafy vegetables harvested at different periods. Int. J. Biol. Chem. Sci. *7* (*1*), 271–286 https://doi.org/10.4314/ijbcs.v7i1.23.

Steffensen, S.K., Pedersen, H.A., Labouriau, R., Mortensen, A.G., Laursen, B., de Troiani, R.M., Noellemeyer, E.J., Janovska, Stavelikova, D.H., Taberner, A., et al. (2011). Variation of polyphenols and betaines in aerial parts of young, field-grown *Amaranthus* genotypes. J. Agric. Food Chem. *59*, 12073–12082 https://doi.org/10.1021/jf202969e.

Sultana, B., Anwar, F., and Iqbal, S. (2008). Effect of different cooking methods on the antioxidant activity of some vegetables from Pakistan. Int. J. Food Sci. Technol. *43* (*3*), 560–567 https://doi.org/10.1111/j.1365-2621.2006.01504.x.