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Partial Purification and Characterization of Angiotensin Converting Enzyme Inhibitory Alkaloids and Flavonoids from the Leaves and Seeds of *Moringa oleifera*

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMA designed the study and wrote the protocol. Author OSA did the laboratory work. Author AMW wrote the first draft of the manuscript and managed the analyses of the study. Author HA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: In the present study, the alkaloid, flavonoid and saponin-rich extracts of the leaves and seeds of *Moringa oleifera* were partially-purified and the ACE inhibitory activities and patterns of inhibition determined.
Methodology: Alkaloids, flavonoids and saponins were extracted from the leaves and seeds of *Moringa oleifera*. They were then partially-purified via thin layer chromatography and column chromatography. The Angiotensin Converting Enzyme (ACE) inhibitory activities of each fraction was determined using Cushman and Cheung method with some modifications on the assay conditions, while the mechanisms of inhibition investigated using Lineweaver-Burk plots.
Results: The alkaloid-rich extracts of the leaves (6.27±0.12 mg/ml) and seeds (2.04±0.95 mg/ml) as well as flavonoid-rich extracts of the leaves (1.20±0.31 mg/ml) and seeds (2.16±0.56 mg/ml) of

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M. oleifera inhibited ACE at concentrations significantly (*P <.05*) lower than the saponin-rich extracts (26.01±1.02 and 24.32±2.31 mg/ml for the leaves and seeds, respectively). Thus, both alkaloid and flavonoid-rich extracts were further purified via thin layer chromatography and column chromatography. Of the four fractions obtained from the alkaloid-rich leaf extract, the ACE inhibitory activity of fraction 2 (0.46±0.01 mg/ml) was significantly higher (*P <.05*), while fraction 1 (0.07±0.01 mg/ml) of the alkaloid-rich seed extract significantly (*P <.05*) inhibited ACE compared to other fractions. For the flavonoid-rich extracts, fractions 3 (0.03±0.01 mg/ml) and 4 (0.42±0.03 mg/ml) obtained from the leaves and seeds, respectively, significantly (*P <.05*) inhibited ACE than other fractions. From the kinetic studies, fractions 1 and 2 exhibited uncompetitive types of inhibition with a decrease in V_{max} and corresponding decrease in K_m at 0.5 mg and 1.0 mg of inhibitor, respectively. Fractions 3 and 4 were competitive inhibitors of ACE, as the K_m increased with increasing concentration of inhibitor, while V_{max} remain unchanged. In conclusion, this study has demonstrated the ACE inhibitory activity of alkaloids and flavonoids from the seeds and leaves of *Moringa oleifera* and their potential as source of ACE inhibitors that may be beneficial for the management of hypertension.

Keywords: Angiotensin converting enzyme; alkaloids; flavonoids; Moringa oleifera.

1. INTRODUCTION

Hypertension is a common progressive disorder leading to chronic diseases like cardiovascular disease (CVD), stroke, renal disease and diabetes. One quarter of the world's adult population is afflicted with hypertension and the numbers are likely to increase by 29% in ten years [1]. Due to its asymptomatic nature, unawareness could lead to fatal conditions such as stroke or heart failure, hence the need for frequent measurement of blood pressure. Hypertension is the most modifiable risk factor in prevention of many chronic diseases; hence both prevention and cure are equally important [2]. It is well documented that hypertension could have many causes, and a prominent factor among all is over-activation of the renin angiotensin aldosterone system (RAAS) [3]. Angiotensin converting enzyme (ACE) plays a key role in RAAS by producing angiotensin II (Ang II), which is a potent vasoconstrictor [4]. Therefore, in the past decade, inhibition of ACE has become a key therapeutic target in the treatment of hypertension. Common ACE inhibitors currently in use include captopril, rampiril, lisinopril, enalapril etc., all of which have unacceptable side effects [3,4]. Thus, the search for natural alternatives that may alleviate hypertension.

These alternatives include medicinal plants known to be of great importance to the health of individuals and communities, many of which are used as spices and food plants [5]. They contain bioactive constituents such as alkaloids, tannins, flavonoids, saponins and phenolics that produce definite physiological actions in the human body [6]. Alkaloids are bitter-tasting nitrogen containing compounds found in plants and animals, although most of it belongs to the plant kingdom. They have been shown to possess allelopathic and hypotensive properties [7]. Alkaloids from several plants have been reported to reduce blood pressure and some are used clinically to treat hypertension, e.g. reserpine from *Raulwolfia serpentina* [8], stepharine, from *Stephania glabra*, naucline, from *Nauclea officinalis* [9] and rhynchophylline from *Uncaria rhynchophylla* [10]. Flavonoids, on the other hand have been shown to possess several biological activities, such as anticancer, anti-inflammatory and antimicrobial activities [11], and have been reported to have beneficial effects in treatment of cardiovascular diseases due to their antioxidant, anti-thrombic, anti-ischemic and antihypertensive properties [12]. Saponins play important roles in plants defense [13]. Some species of saponin are used as drugs with extensive medical importance, such as an expectorant and diuretics [14] and also in the treatment of hepatitis, gastritis and bronchitis [15]. Recent research has established saponins as active components in many herbal medicines [16,17]. They possess anticancer properties, triggering tumor cell death by activating death receptors [18], targeting mitochondria and inducing oxidative stress [19]. They have also been shown to have antihypertensive properties [20,21].

Moringa oleifera (Moringaceae) is an important medicinal plant that is a native to Asia and Africa but cultivated around the world. The leaves and seed pods are nutritious and widely consumed as food, while the bark and the roots are used in folk remedies [22]. Products derived from the

herb are used to treat a variety of conditions including asthma, diabetes, ulcer infections and cancer [23]. *In vivo* and *in vitro* studies indicate that the leaves, seeds and root extracts of *Moringa oleifera* possess anti-inflammatory, anti-bacterial, anti-fungal and anti-hypertensive properties. They also lower cholesterol and promote wound healing [24]. *M. oleifera* contain constituents such as carotenoids, glucosinate, glycosides, isothiocynates e.t.c. Also, previous phytochemical studies on *M. oleifera* have reported the presence of saponins, free anthraquinones, alkaloids, flavonoids and phenolics [25]. The seed extracts of *Moringa oleifera* contains alkaloids which have been reported to possess hypotensive and bradycardiac effects [25]. Although, the leaves and pods of *Moringa oleifera* has been shown to reduce blood pressure [26], very little has been documented on the effect of saponins, alkaloids and flavonoids present in the seeds and leaves on the activity of ACE. Therefore, the present work is aimed at determining the ACE inhibitory activities of the alkaloid, flavonoid and saponin-rich fractions of the seeds and leaves of *Moringa oleifera*.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals and reagents

Hippuryl-Histidyl-Leucine (HHL), ACE from rabbit lung, Silica gel (Sigma Chemical Co., St. Louis, MO, USA), Hydrochloric Acid, Chloroform, Acetic Acid, Ammonium Hydroxide, Ethyl Acetate, Ethanol and all other chemicals used were of analytical grade.

2.1.2 Plant material

The seeds and leaves of *Moringa oleifera* were obtained from Samaru Market, Zaria, Kaduna State, Nigeria. They were identified and authenticated by the Mallam U.S. Gallah at the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria. They were air dried, made into powder by grinding and stored in air tight containers until required.

2.2 METHODS

2.2.1 Determination of phytochemical content of seeds and leaves of *Moringa oleifera*

Phytochemical analysis for the detection of alkaloids, flavonoids, tannins and saponins, terpenoids and cardiac glycosides present in the aqueous extract of the leaves and seeds of *Moringa oleifera* were assayed by standard methods [27,28].

2.2.2 Extraction of alkaloids from seeds and leaves of *M. oleifera*

The dried seeds and leaves of *Moringa oleifera* were made into powder and the alkaloid-rich fraction was extracted as described by Harbone [29]. Briefly, 150 g of powdered seeds was weighed and dispersed into 1000 ml of 10% acetic acid solution in 90% ethanol. The mixture was swirled thoroughly and allowed to stand for four hours before filtering using a Whatman's filter paper (No.1). The filtrate was evaporated to one quarter of its original volume and concentrated ammonium hydroxide added drop-wise to precipitate the alkaloid.

2.2.3 Extraction of flavonoids from seeds and leaves of *M. oleifera*

Flavonoids were extracted from the seeds and leaves of *Moringa oleifera* as described by Subramanian and Nagarajan [30]. Briefly, about 100g of each powder was soaked in 80% methanol separately for 48 h. The extract was then filtered using a Whatman's filter paper (No.1) and the filtrate dried by placing in a water bath at 40℃. The filtrate of each sample was subsequently extracted in petroleum ether, diethyl ether and ethyl acetate. Petroleum ether fraction was discarded due to its being rich in fatty substances. Ether fraction was used for free flavonoids whereas ethyl acetate fraction for bound flavonoids. The ethyl acetate fraction was hydrolyzed further with 7% H_2SO_4 for 24 hrs and was then re-extracted with ethyl acetate. The fraction obtained was repeatedly washed with distilled water to neutrality, dried and weighed.

2.2.4 Extraction of saponins from seeds and leaves of *M. oleifera*

Saponins were extracted from the leaves and seeds of *Moringa oleifera* as described by Morris and Hussey [31]. Briefly, 100 g of the powdered leaves was suspended in 600 ml of n-hexane and allowed to stay for 24hrs, after which the mixture was filtered using double layered white handkerchief, and the residue suspended in 600 ml of 70% ethanol. It was left overnight, filtered through double layered white handkerchief followed by a filter paper and the filtrate concentrated at 50℃ in water bath. About 100

ml of 0.1% HCl was added and placed in a water bath to evaporate. The extract treated with HCl directly yields a precipitate of crude saponin.

2.2.5 Partial-purification of alkaloid and flavonoid-rich extracts of seeds and leaves of *M. oleifera*

The alkaloid and flavonoid-rich extracts of the seeds and leaves of *M. oleifera* were partially-purified by column chromatography after thin layer chromatographic separation. All columns were run with the chosen solvent and fractions collected at a flow rate of 0.2 ml/min.

2.2.6 Determination of angiotensin-converting enzyme inhibitory activity

Angiotensin converting enzyme converts hippuryl-histidyl-leucine (HHL) to hippuric acid and Histidyl-leucine. The concentration of hippuric acid formed is measured spectrophotometrically and determines the activity of the enzyme. The assay for ACE inhibitory activity was determined using the Cushman and Cheung method [32] with some modifications on the assay conditions. Briefly, the inhibitor solution (alkaloid, flavonoid and saponin-rich fractions) was added to a test-tube containing 0.1 M potassium phosphate buffer (pH 8.3), 5 mM hippuryl-histidyl-leucine (HHL), 0.1 M potassium phosphate and 0.3 M NaCl (pH 8.3). Then the enzyme, ACE was added to the mixture and incubated at 37ºC for 30 minutes. The reaction was terminated by adding 0.25 ml of 1 M HCl, and then 1.5 ml ethyl acetate also added to extract the hippuric acid formed by the action of ACE. The ethyl acetate was removed by heat evaporation, residual hippuric acid (HA) dissolved in 1 ml of deionized water, and absorbance of the solution was taken at 228 nm to determine the hippuric acid concentration. The inhibitory activity is the amount of inhibitor solution needed to inhibit the original ACE activity by 50% (IC_{50}).

2.2.7 Determination of inhibitory pattern and measurements of kinetic constants

To investigate the inhibitory pattern of the partially-purified alkaloid and flavonoid-rich fractions from *Moringa oleifera* leaves and seeds, different concentrations of the substrate, hippuryl histidine leucine (HHL) (0, 1, 2, 3, 4, and 5 mM) were tested in the assay system. The ACE activity was determined both in the absence and presence of the inhibitor. The Michaelis-Menten constant, K_m, the inhibition constant, K_i and the V_{max} were obtained from the Lineweaver–Burk plots.

2.3 Statistical Analysis

All data obtained were expressed as mean ± standard deviation (mean ± SD) and analyzed using SPSS 20. The significance was determined by one way analysis of variance and LSD post hoc test was applied for multiple comparisons. Values of P <.05 were regarded as statistically significant.

3. RESULTS

3.1 Phytochemical Analysis of the Leaves and Seeds of *Moringa oleifera*

The result of the qualitative phytochemical analysis of the leaves and seeds of *M. oleifera* shows the presence of alkaloids, flavonoids, saponins, terpenoids and cardiac glycosides (Table 1).

Table 1. Phytochemical composition of aqueous extract of the leaves and seeds of *M. oleifera*

Phytochemicals	Leaves	Seeds
Alkaloids	+	+
Flavonoids	++	+
Saponins	+	+
Tannins	+	++
Terpenoids	+	+
Cardiac glycoside	+	+

(+), (++) and (+++) represent intensity observed

3.2 Yield of Extracts and Fractions Obtained From the Leaves and Seeds of *M. oleifera*

The percentage yields of extracts of *M. oleifera* are presented in Table 2. The yields of alkaloid, flavonoid and saponin-rich extracts from 100 g of the leaves were 17, 36 and 37 g respectively, while that from the seeds were 15, 34 and 31 g, respectively.

The ACE inhibitory activities of the extracts were presented as the amount of inhibitor (extract) needed to inhibit ACE activity by 50% (IC_{50}). As presented in Table 3, the IC_{50} value of the standard drug, captopril (0.22±0.01 mg/ml) was significantly (P <.05) lower than all the extracts, which shows it had the highest ACE inhibitory

activity. There was no significant difference (P >.05) in the inhibition activity of alkaloid (2.04±0.95 mg/ml) and flavonoid-rich (2.16±0.56 mg/ml) extracts of the seeds. The IC_{50} value of the alkaloid-rich (6.27±0.12 mg/ml) leaf extract, though higher than other alkaloid and flavonoid-rich extracts, was significantly (*P <.05*) lower than that of the saponin-rich extracts from the leaves (26.01±1.02 mg/ml) and seeds (24.32±2.31 mg/ml).

The alkaloid and flavonoid-rich extracts of the leaves and seeds of *M. oleifera* with the highest ACE inhibitory activities (Table 3) were further purified. Thin layer chromatography of the alkaloid-rich extracts was carried out using ethyl acetate: chloroform (2:3) solvent which gave five separate constituents, visible under UV-spectrum. Column chromatography using silica gel was then run with ethyl acetate (100%) and ethyl acetate: chloroform (2:3) and seven fractions were each obtained from the leaves and seeds. The fractions from the leaves and seeds were reduced to four and five, respectively, after thin layer chromatographic separation (Table 4).
For the flavonoid-rich extracts, chloroform: methanol (3:2) used for thin layer chromatography gave four separate constituents, visible under UV-spectrum. Column chromate-graphy using silica gel was then run with chloroform (100%), methanol (100%) and chloroform: methanol (3:2). Ten fractions each were obtained from the leaves and seed extracts. Thin layer chromatographic separation of all fractions was carried out; and the fractions were reduced to four (Table 4).

Table 5 shows the ACE inhibitory activities of alkaloid and flavonoid-rich extracts of *M. oleifera* leaves and seeds. From the results, the inhibition of ACE activity by fractions 1 (0.07±0.01 mg/ml) and 2 (0.46±0.01 mg/ml), obtained from the alkaloid-rich extracts of the seeds and leaves, respectively, were significantly (*P <.05*) higher than that of other fractions. On the other hand, fractions 3 (0.03±0.01 mg/ml) and 4 (0.42±0.03 mg/ml) from the flavonoid-rich extracts of the leaves and seeds, respectively, inhibited ACE at concentrations significantly (*P <.05*) lower than other fractions within the same group.

Table 2. Yields of alkaloid, flavonoid and saponin-rich extracts of *M. oleifera* leaves and seeds

Extracts	Percentage yield (%)	
	Leaves	Seeds
Alkaloid-rich (100 g)	17	15
Flavonoid-rich (100 g)	36	34
Saponin-rich (100 g)	37	31

Table 3. Angiotensin converting enzyme inhibitory activities of alkaloid, flavonoid and saponin-rich fractions of *M. oleifera* leaves and seeds

Extracts	IC_{50} (mg/ml)
Captopril	$0.22±0.01^{a}$
Alkaloid-rich (leaves)	$6.27±0.12^{b}$
Alkaloid-rich (seeds)	$2.04±0.95^{c}$
Flavonoid-rich (leaves)	$1.20±0.31^{d}$
Flavonoid-rich (seeds)	$2.16±0.56^{c}$
Saponin-rich (leaves)	$26.01±1.02^{e}$
Saponin-rich (seeds)	$24.32±2.31^{e}$

Values are mean ± standard deviation; ^{a,b,c,d,e} Values with different superscripts within the same column are significantly different ($p < 0.05$); IC_{50} defined as the concentration which inhibits 50% of the angiotensin converting enzyme activity

3.3 ACE Inhibitory Pattern of Alkaloid and Flavonoid-rich Fractions of *M. oleifera* Leaves and Seeds

The ACE inhibition pattern of fractions 1 (from alkaloid-rich seeds), 2 (from alkaloid-rich leaves), 3 (from flavonoid-rich leaves) and 4 (from flavonoid-rich seeds), having the lowest IC_{50}, and thus highest ACE inhibitory activity were determined using the Lineweaver-Burk's plot.

Table 4. Yields of partially-purified alkaloid and flavonoid fractions of leaves and seeds of *M. oleifera*

Fractions	Yield (g)			
	Leaves		Seeds	
	Alkaloid	Flavonoid	Alkaloid	Flavonoid
1	0.60	0.54	1.06	1.53
2	1.00	1.06	1.12	0.88
3	1.15	1.19	0.88	0.74
4	1.90	0.97	0.75	1.15
5			0.52	

Table 5. Angiotensin converting enzyme inhibitory activities (IC_{50}) of partially-purified fractions of alkaloid and flavonoid-rich fractions of *M. oleifera* leaves and seeds

Fractions	IC_{50} (mg/ml)			
	Alkaloids		Flavonoids	
	Leaves	Seeds	Leaves	Seeds
1	4.03±1.16[a]	0.07±0.01[b]	0.19±0.01[c]	1.20±0.02[s]
2	0.46±0.01[b]	0.82±0.04[e]	0.15±0.01[d]	0.88±0.05[d]
3	3.61±2.42[a]	0.18±0.03[d]	0.03±0.01[e]	1.06±0.03[e]
4	4.35±0.60[a]	0.11±0.01[c]	0.12±0.01[f]	0.42±0.03[f]
5		0.25±0.02[g]		

Values are Mean ± SEM; [a-s] = Values with different superscripts in the same column are significantly (P =.05) different; IC_{50} defined as the concentration which inhibits 50% of the angiotensin converting enzyme activity

The alkaloid-rich fraction 1 obtained from the seeds was found to be uncompetitive, with both K_m and V_{max} decreasing with increasing concentration of inhibitor. The K_m decreased from 3.3 mM without inhibitor to 2.0 mM at 0.5 mg and 1.43 mM at 1.0 mg of the inhibitor. Also, the V_{max} decreased from 0.17 µmol/min without inhibitor, to 0.10 µmol/min at 0.5 mg of inhibitor and 0.07 µmol/min when the inhibitor concentration was increased to 1.0 mg. The K_i changed from 0.83 to 0.065 mg/ml at 0.5 and 1.0 mg inhibitor, respectively (Fig. 1a).

Fraction 2 obtained from the alkaloid-rich leaves also exhibited an uncompetitive type of inhibition. The K_m decreased from 12.5 mM without inhibitor, to 5.26 and 3.09 mM, at 0.5 mg and 1.0 mg inhibitor, respectively, while the V_{max} decreased from 0.5 µmol/min without inhibitor to 0.2 and 0.11 µmol/min at 0.5 and 1.0 mg inhibitor, respectively (Fig. 1b).

The most active fractions obtained from the flavonoid-rich seeds (fraction 4) and flavonoid-rich leaves (fraction 3) inhibited ACE competitively (Figs. 1c and 1d). For fraction 4, the K_m increased from 1.1 mM in the absence of the inhibitor to 1.9 mM at 0.5 mg and 2.6 mM at 1mg concentration of the inhibitor while V_{max} of 0.2 µmol/min remain unchanged. The K_i was 2 mg/ml at both 0.5 and 1 mg concentration of the inhibitor. Fraction 3 had a K_m of 10, 5.0 and 3.3 mM at inhibitor concentration of 0, 0.5 and 1.0 mg/ml respectively. The K_i value was 0.046 and 0.048 mg/ml at 0.5 and 1 mg concentration of the inhibitor, while the V_{max} remained unchanged at 0.05 µmol/min (Fig.1d).

4. DISCUSSION

Secondary metabolites of plants, mainly alkaloids, flavonoids and saponins extracted from *Moringa oleifera* were the main target inhibitors for ACE inhibition in this study. The alkaloid, flavonoid and saponin-rich extracts of the seeds and leaves were obtained and their effect on the activity of ACE determined. From the results, the alkaloid and flavonoid-rich extracts were found to contain potent ACE inhibitors than the saponin-rich extract whose inhibitory effect on ACE was significantly (*P <.05*) low (26.01±1.02 mg/ml). These extracts were partially-purified to obtain different fractions that were screened and those with the best ACE inhibitory activity characterized.

ACE inhibitors reduce blood pressure by preventing the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor. They also degrade bradykinin, either by binding to the active site as a substrate for the enzyme or as a non-substrate [33], hence, inhibiting the activity of this enzyme ultimately results to low blood pressure [34]. Naturally occurring peptides from animals and plants [35,36] as well as several plant metabolites [33] have been shown to possess ACE inhibitory properties. Green tea, blueberry, *Hibiscus sabdariffa* and *Senecio inaequidens* extracts were reported to inhibit ACE with IC_{50} values of 125 µg/ml, 46 µg/ml, 91 µg/ml and 192 µg/ml, respectively [37-39]. Although hypotensive properties of some plant bioactive constituents have been widely reported [21,40], very little has been done to determine their effect on ACE.

Flavonoids occur naturally in plant-based foods, found commonly in fruits, vegetables, grains and herbs, with several health benefits, due to their wide range pharmacological activities. Experimental evidence suggests that flavonoids exert beneficial effects on blood pressure either by increasing endothelial-derived nitric oxide (NO) via the modulation of endothelial nitric oxide synthase (eNOS) activity and expression [41], or inhibiting ACE [38,42] through the generation of

complexes that chelate zinc ion within the active center of ACE [43]. Flavonoid-rich foods, like wines, chocolates, and teas have been shown to inhibit ACE *in vitro* [44], and results of randomized controlled trials on flavonoids and flavonoid-rich foods provides evidence that some subclasses of flavonoids are associated with a significant reduction in blood pressure [45]. Other plant components such as xanthones [46], terpenoids [47], fatty acids [48], hydrolysable tannins, proanthocyanidins [49] have also been reported to exert ACE inhibition activity.

The hypotensive properties of alkaloids from several plants [8-10] have also been reported and their mechanisms of hypotensive action determined [9,50]. A report by Oh et al. [51], demonstrated the ACE inhibitory activities of verticinone, verticine, and peimisine alkaloids isolated from *Fritillaria ussuriensis.* In another study, An et al. [52] isolated another ACE inhibitory alkaloid puqienine E from *Fritillaria puqiensis*.

Moringa oleifera has numerous medicinal uses, which have long been recognized in the Ayurvedic and Unani systems of medicine [22], but there is paucity of information on the ACE inhibitory effects of its bioactive constituents. This study demonstrates that alkaloid and flavonoid-rich fractions of *Moringa oleifera* leaves and seeds inhibit ACE *in vitro*. It is well established that *in vitro* analysis offers the best opportunity and insight to widen analysis in different directions. Also, investigating the inhibition pattern of potential drugs and their kinetic parameters could generate valuable information for initiating further developments of bioactive molecules as therapeutic targets.

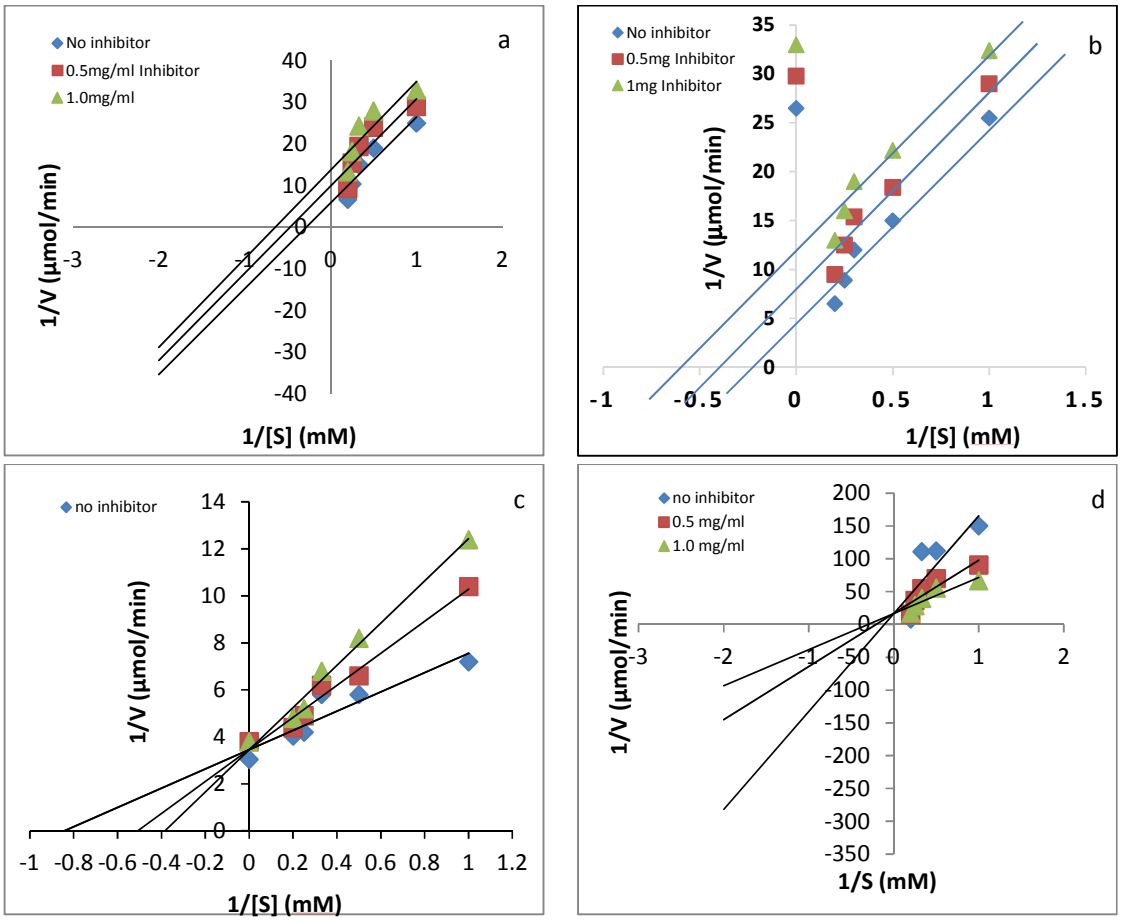

Fig. 1. Lineweaver-Burks' plots for the inhibition of angiotensin-converting enzyme by *M. oleifera* (a) Alkaloid-rich fraction of the seeds, (b) alkaloid-rich fraction of the leaves, (c) flavonoid-rich fraction of the seeds, (d) flavonoid-rich fraction of the leaves

The IC_{50} values of saponin-rich extracts from the seeds (24.32±2.31 mg/ml) and leaves (26.01±1.02 mg/ml) of *M. oleifera* were significantly (*P <.05*) higher than other extracts, indicating lower ACE inhibitory activities. This means that alkaloids and flavonoid-rich extracts of *M. oleifera* may be better and more potent ACE inhibitors than saponins obtained from the same plant. This does not mean saponins are not hypotensive. In fact, studies have demonstrated the antihypertensive properties of saponins from other plants, but their mode of action may be different [20,53-55].

Results after partial-purification showed that fractions 1 (0.07±0.01 mg/ml) and 2 (0.46±0.01 mg/ml), obtained from the alkaloid-rich extracts of the seeds and leaves, respectively, and fractions 3 (0.03±0.01 mg/ml) and 4 (0.42±0.03 mg/ml) from the flavonoid-rich extracts of the leaves and seeds, respectively, inhibited ACE at concentrations significantly lower (*P <.05*) lower than other fractions within the same group. Thus, the kinetic properties of all four fractions were determined.

ACE inhibitors isolated from different plant and animal sources have been shown to exhibit different inhibition patterns [56-60]. In this study, the two alkaloid-rich fractions (1 and 2) exhibited an uncompetitive type of inhibition, with K_m and V_{max} decreasing as concentration of inhibitor increased. This implies that the alkaloid-rich fractions bind at a site away from the substrate binding site and only to the ACE-HHL complex [61]. On the other hand, the flavonoid-rich fractions (3 and 4) acted as competitive inhibitors with respect to the substrate, HHL. This implies that they inhibit ACE activity by competing with the substrate for the active site, exhibiting a dose-dependent inhibitory effect on ACE. Thus, there is an increase in K_m with increase in concentration of inhibitor, while V_{max} remained unchanged. This conforms to reports by Wu and Ding, [62], Hyong Lee et al. [56] and Gouda et al. [57] that most ACE inhibitors exhibit competitive inhibition, though, some may be noncompetitive [58,63-66] or mixed inhibitors [59,60], and very few are uncompetitive inhibitors [63,67].

5. CONCLUSION

This study has shown that alkaloids and flavonoids from the seeds and leaves of *Moringa oleifera* are potent ACE inhibitors, and so may be beneficial as drugs or nutraceuticals for lowering blood pressure. However, further studies are recommended to determine the ACE inhibitory and blood pressure lowering effects of the extracts and fractions *in vivo.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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