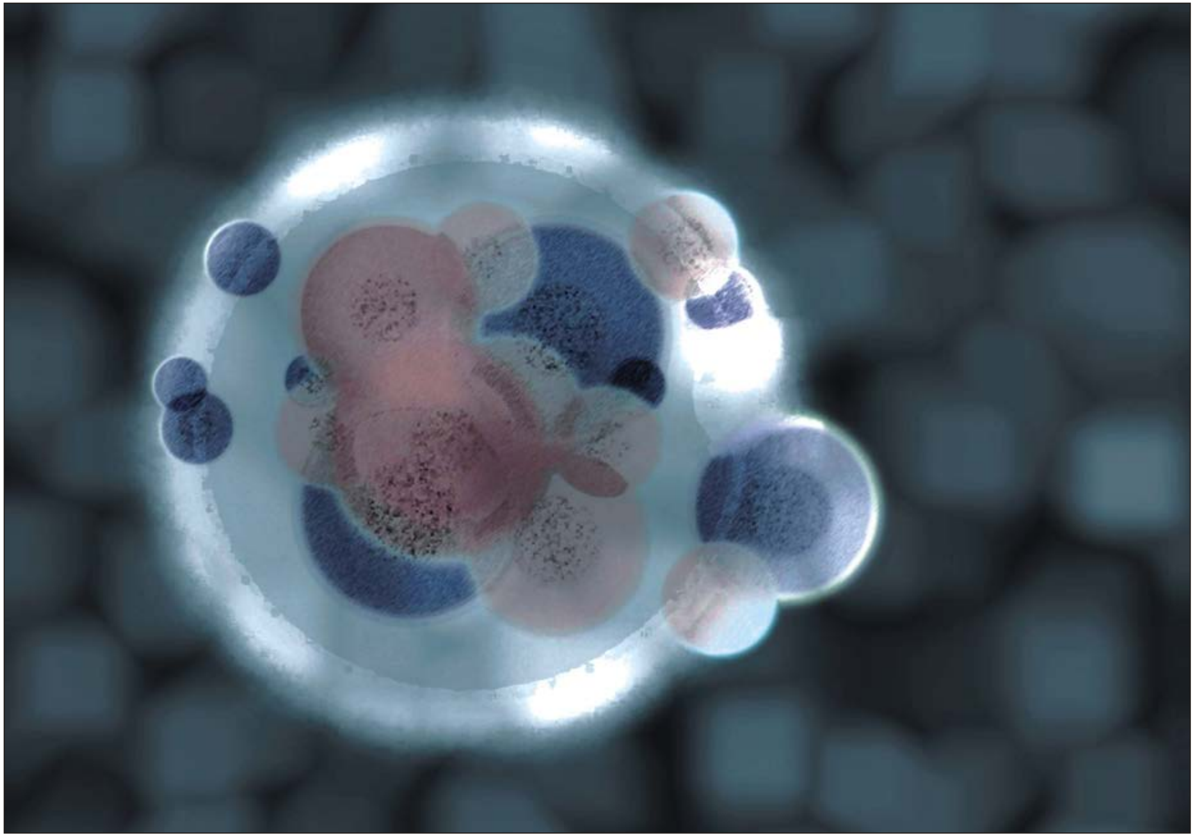

Journal of Biological Sciences

ISSN 1727-3048

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Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2022.57.64

Research Article

Antioxidant and Angiotensin-Converting Enzyme Inhibitory Activities of Fractionated Extract of *Combretum micranthum* Leaves

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Abstract

Background and Objective: Traditional and alternative medicine has been of immense help to developing countries as the source of therapy for many diseases. Recently many drugs are sourced from natural sources because of afford ability and ease of accessibility. This study was carried out to evaluate the *in vitro* ACE inhibitory activity and antioxidant potentials of aqueous extract of *Combretum micranthum* leaves and its fractions. **Materials and Methods:** Aqueous extract of *Combretum micranthum* leaves was subjected to chromatographic fractionation and the fractions obtained were assayed for *in vitro* inhibitory activity against rabbit ACE followed by their possible antioxidant potentials. **Results:** Aqueous extract of *Combretum micranthum* leaves produced 59.43±4% inhibitory activity comparable to captopril which produced 83.02±2.67% activity. The fractions A, B, C and D were able to inhibit the *in vitro* activity of rabbit ACE with the inhibitory percentages of 8.21±1.19, 97.69±8.57, 78.32±7.14 and 98.32±2.66%, respectively. The antioxidant potency of these fractions was evident in their free radical scavenging IC_{50}, reducing power ability and total antioxidant capacity which all showed that fraction B may exhibit the highest antioxidant potency as compared to fractions C and D. The bioactive phytochemical constituents from the GC-MS analysis of fraction B of the aqueous extract of *Combretum micranthum* was found to be Megastigmatrienone, 3,5-Dimethoxy-4-hydroxyphenyl acetic acid and Estra-1,3,5(0)-trien-17β-ol. **Conclusion:** It was obvious from the findings that aqueous extract of *Combretum micranthum* has showed the tendency to inhibit the *in vitro* activity of ACE which could be attributed to its antioxidant activity demonstrated by the reduction power and the total antioxidant capacity of the various fractions.

Key words: Antioxidant, angiotensin-converting enzyme, fractionated, aqueous extract, *Combretum micranthum*, hypertension, *in vitro* activity

Citation: Mashi, R.L., M.S. Abu, O.E. Yakubu, T.V. Silas and M.H. Mayel, 2022. Antioxidant and angiotensin-converting enzyme inhibitory activities of fractionated extract of *Combretum micranthum* leaves. J. Biol. Sci., 22: 57-64.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Angiotensin I Converting Enzyme (ACE) is a glycoprotein with peptidyl dipeptide hydrolase activity which cleaves Angiotensin I to produce Angiotensin II in the blood. The powerful vasoconstriction action of Angiotensin II and its stimulatory action on the synthesis and release of aldosterone favours retention of sodium and water[1]. It also hydrolyses and inactivates bradykinin, a peptide with a powerful vasodilatory action[2]. The utilization of synthetic ACE inhibitors, such as the well-known captopril, provides definitive positive health effects and is considered an important therapeutic approach in the treatment of high blood pressure, though the use of these pharmacological drugs is not advisable in healthy or low-risk populations[3].

The evidence that certain flavonoid-rich natural products can induce reductions in blood pressure and inhibit ACE activity opens the possibility that their consumption may mimic synthetic ACE inhibitors and provide preventive health benefits probably avoiding adverse effects associated with the synthetic ones in current usage[4]. If the formation of angiotensin II and the activation of vasodilatory kinins are suppressed by selective ACE inhibitors, there will be a lowering of blood pressure. Some plant products and substances isolated from plants previously have shown promising inhibitory effects on ACE[5].

Combretum micranthum has a number of uses, traditionally it is used as an antihypertensive, diuretic, anti-diarrhoeal, anti-syphilis, antimalarial agent and to treat hepatitis, jaundice and bronchitis. Banfi *et al.*[6] reported the antimicrobial potency of the leaf extract. Phytochemical studies carried out in the genus *Combretum* including *Combretum micranthum* have demonstrated the occurrence of many classes of constituents, including triterpenes, flavonoids, lignans and non-protein amino acids, among others[7]. The aim of this study was to investigate the antioxidant properties of aqueous extract of *Combretum micranthum* leaves and their effects on the angiotensin-converting enzyme.

MATERIALS AND METHODS

Study area: This research work was carried out from April to November, 2019. *Combretum micranthum* plant was collected during the dry season from Malumfashi Local Government Area of Katsina State, Nigeria and the study was carried out in Ahmadu Bello University, Zaria, Nigeria.

Materials

Chemical and reagents: Angiotensin-converting enzyme as a lyophilized powder from rabbit lung and hippuryl-L-histidyl-L-leucine (HHL) were purchased from Sigma Chemical Co. (Germany). All solvents and other chemicals were purchased from Haddis international Samaru Zaria.

Plant sample collection and identification: *Combretum micranthum* plant was collected during the dry season from Malumfashi Local Government Area of Katsina State, Nigeria. The Plant sample was identified and authenticated in the Herbarium unit of the Department of Botany, Ahmadu Bello University, Zaria where a voucher sample was deposited (Voucher Number: 900257). This work was initiated in June, 2016 and concluded by April, 2017, hence, it lasted for about 10 months.

Methods

Preparation of aqueous extract: The *Combretum micranthum* leaves were washed and air-dried at room temperature. Dried samples were pulverised using pestle and mortar. Exactly 1 L of distilled water was added to 500 g powdered leaves and soaked for 24 hrs. The filtrate was then concentrated by evaporation using a water bath at 40°C. After which the aqueous extract obtained was then stored inside a container and kept at room temperature until required.

Determination of ACE inhibitor effect of aqueous extract of *Combretum micranthum* leaves and its fractions: The assay for ACE inhibitor activity and its numerical estimation was carried out using the method and equation described by Murray and FitzGerald[8] with some modifications on the assay conditions. Briefly, 50 μL of ACE solution (100 mU mL^{-1}) was added to 50 μL of sample solution (0.5 mg mL^{-1}) and incubated at 37°C for 10 min. Substrate (150 μL) solution (8.3mM Hip-His-Leu in Borate buffer) was then added to the reaction mixture and then incubated for 1 hr 20 min at 37°C. The reaction was terminated by adding 250 μL of 1 M HCl and then 1.5 mL ethyl acetate was added to extract the hippuric acid formed by the action of ACE. Ethyl acetate was then evaporated under airflow at 37°C, the residual Hippuric Acid (HA) was then dissolved in 1 mL of deionized water and the absorbance of the solution was taken at 228 nm to determine the hippuric acid concentration. The sample blank was prepared in the same way above, with a change in the order in which the reagents were added, HCl was added before the enzyme. The reaction blank was prepared in the same way as

the sample blank, replacing the volume of the tested sample with buffer. Captopril was used as the standard drug. The percentage inhibition was then calculated from the equation:

$$\text{IACE (\%)} = \frac{(A - B) - (C - D)}{A - B} \times 100$$

A represents absorbance in the presence of ACE, B absorbance of the reaction blank, C absorbance in the presence of ACE and inhibitor and D absorbance of the sample blank. All determinations were carried out in triplicate.

Thin layer chromatography (TLC): Thin layer chromatography was carried out to determine the best solvent system for the column chromatography. A thin layer chromatographic plate pre-coated with silica gel was used. The crude extract was dissolved in the solvent and applied to the plate. The plates were placed in chromatographic tanks with a mixture of different solvent systems. The different solvent systems used include n-hexane 100%, n-hexane and ethyl acetate 9:1, 8:2 and 7:3, ethyl acetate 100%, ethyl acetate and methanol 8:2, chloroform and methanol 9:1 and chloroform 100%. Thereafter, the plates were removed, sprayed with p-anisaldehyde and followed by heating at 110°C for 5 min, the solvent system ethyl acetate and methanol 8:2 gave the best separation.

Column chromatography of aqueous extract of *Combretum micranthum*: The column was packed with a slurry of 150 g of silica gel (60-120 mesh) in 350 mL of ethyl acetate. After the column has settled, 5 g of the crude aqueous extract was loaded and eluted with 500 mL ethyl acetate 100%, ethyl acetate and methanol (3:2, 2:3, 1:4, each 500 mL) and 500 mL 100% methanol. After collecting 75 Aliquots (40 mL each), TLC was carried out and those aliquots with similar TLC profiles were pooled together to give four pooled fractions (A-D). The fractions were tested against the Angiotensin-converting enzyme to ascertain their inhibitory potentials.

Determination of total phenolic content of the aqueous extract fractions of *Combretum micranthum* leaves: The total phenolic content of the fractions was determined using the method of McDonald *et al.*[9] with slight modifications.

The calibration curve was prepared by mixing ethanol solution of garlic acid (1 mL, 0.025-0.400 mg mL^{-1}) with 5 mL^{-1} Folin-ciocalteu reagent (diluted tenfold) and sodium carbonate (4 mL, 0.7 M). Absorbance values were measured at 765 nm using a UV-VIS spectrophotometer (Uvmini-1240, Shimadzu Corporation, Kyoto, Japan) and the standard curve was plotted. One millilitre of each of the solution of fraction in methanol (5 g L^{-1}) was also mixed with the reagents above and after 30 min the absorbance was measured. All determinations were carried out in triplicate. The total phenolics components in the fractions in gallic acid equivalent (GAE) were calculated by the formula:

$$T = \frac{C.V}{M}$$

Where:

- T = Total phenolic contents, milligram per gram of sample fraction in gallic acid equivalent
- C = Concentration of garlic acid established from the calibration curve (mg mL^{-1})
- V = Volume of fraction (mL)
- M = Weight of sample fraction (g)

Determination of antioxidant activity using 2, 2-diphenyl-1-picrylhydrazyl free radical activity of the aqueous extract fractions of *Combretum micranthum* leaves: The antioxidant activity of fractions of aqueous extract of the plant was assayed by the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method and formula described by Yakubu *et al.*[10].

The assay mixture contained 2 mL of 1.0 mM DPPH radical solution prepared in methanol and 1 mL of standard or extract solution of different concentrations (10-500 µg mL^{-1}). The solution was rapidly mixed and incubated in dark at 37°C for 20 min. The decrease in absorbance of each solution was measured at 517 nm using a spectrophotometer. Ascorbic acid was used as positive control while 2 mL of 1.0 mM DPPH radical solution with 1 mL ethanol was taken as blank.

The percentage of radical scavenging (%) was calculated by:

$$\text{Free radical scavenging activity (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

Where:

- A_c = Absorbance of control at 517 nm
- A_s = Absorbance of the sample at 517 nm

The concentration of sample required to scavenge 50% of DPPH free radical (IC_{50}) was determined from the curve of percentage inhibitions plotted against the respective concentrations.

Estimation of reducing the power of the aqueous extract fractions of *Combretum micranthum* leaves: This was determined according to the method of Karadag *et al.*[11].

The fractions and standard (1 mL) of various concentrations (100, 200 and 300 μg mL^{-1}) were mixed with phosphate buffer (pH 6.6, 0.2 M, 2.5 mL) and potassium ferricyanide (1%, 2.5 mL).The mixture was incubated at 50°C for 20 min. Trichloroacetic acid (10%, 2.5 mL) was added to the mixture. A portion of the resulting mixture was mixed with $FeCl_3$ (0.1%, 0.5 mL) and the absorbance was measured at 700 nm in a spectrophotometer. The higher absorbance of the reaction mixture indicated the reductive potential of the fractions.

Determination of total antioxidant capacity of the aqueous extract fractions of *Combretum micranthum* leaves: The total antioxidant capacity of the fractions was evaluated by the phosphor-molybdenum method according to the procedure described by Jafri *et al.*[12].

Into a test tube, 0.3 mL of various concentrations of fractions (100, 200 and 300 μg mL^{-1}) were combined with 3 mL reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 Mm ammonium molybdate). The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer against a blank after cooling to room temperature. Methanol (0.3 mL) in place of fractions was used as blank. The total antioxidant activity was expressed as the number of gram equivalents of ascorbic acid.

GC-MS (gas chromatography-mass spectroscopy) analysis of fraction B: Fraction B was further subjected to GC-MS analysis. The analysis was conducted with an Agilent Technologies 68 90 GC coupled with an Agilent 5973 mass selective detector and driven by Agilent Chemstation software (Agilent Technologies, USA). A DB-5SIL MS capillary column was used (30 m×0.25 mm i.d.,×0.25 μm film thickness). The carrier gas was ultra-pure helium at a flow rate of 0.7 mL min^{-1} and a linear velocity of 37 cm sec^{-1}. The injector temperature was set at 250°C. The initial oven temperature was 60°C, which was programmed to 280°C at the rate of 10°C min^{-1} with a hold time of 3 min. Injections of 2 μL were made in the splitless mode with a manual split ratio of 20:1. The mass spectrometer was operated in the electron ionization mode at 70 eV and electron multiplier voltage at 1859 V. Other MS operating parameters were as follows: Ion source temperature 230°C, quadrupole temperature 150°C, solvent delay 4 min and scan range 50-700 amu. Compounds were identified by direct comparison of the retention times and mass fragmentation pattern with those from the National Institute of Standards and Technology (NIST) library.

Statistical analysis: The data were analyzed by the One-way Analysis of Variance (One-way ANOVA) using the SPSS program (version 20 SPSS Inc., Chicago, IL, USA). The differences in parameters were compared using Bonferroni multiple comparison test (a post-doc test). The results were expressed as Mean±Standard Deviation (SD). The p-value less than 0.05 was considered as significant ($p<0.05$).

RESULTS

Percentage inhibition of aqueous extracts of *C. micranthum* leaves against ACE: Table 1 indicates the results of ACE inhibitory activity of aqueous extract of *Combretum micranthum* leaves and a standard antihypertensive synthetic drug of the class of ACEI (captopril). A given concentration (500 μg mL^{-1}) of the sample was used and the result reported was a reflection of the mean values of triplicate performances. It showed that the aqueous extract of *Combretum micranthum* leaves possessed inhibitory activity of 59.43±4.00% whereas, the standard drug (captopril) exhibited an activity of 83.02±2.67% against ACE.

Percentage inhibition of column chromatographic fractions of aqueous extract of *C. micranthum* leaves against standard rabbit ACE: Therefore, fractions of the extract obtained from the column chromatographic process were evaluated for ACE inhibitory activity using standard rabbit ACE. Samples were prepared in a concentration of 500 μg mL^{-1} each and repeated three times to obtain the mean value as reported. Table 2 shows the inhibitory activity against ACE of the various column chromatographic fractions obtained from the aqueous extract of *Combretum micranthum*. Fraction A showed a significantly ($p<0.05$) lower inhibitory activity against standard rabbit ACE as compared to fractions B, C and D. However, there was no significant difference ($p>0.05$) among B, C and D fractions.

Total phenolic content and DPPH free radical scavenging activity IC_{50} of column chromatographic fractions of *Combretum micranthum* leaves: The phenolic content and IC_{50} are presented in Table 3. It showed that all the fractions are significantly ($p<0.05$) different from one another with fraction B having the highest phenolic content (252.50±5.62 mg g^{-1} GAE) as compared to fractions C (118.30±1.27 mg g^{-1} GAE) and D (55.73±2.56 mg g^{-1} GAE).

There was an inverse variation between the amount of phenolic content and the free radical scavenging activity IC_{50} as depicted in Fig. 1. Table 3 clearly showed the significant difference ($p<0.05$) among the IC_{50} of the fractions with fraction B having the lowest IC_{50} (121.51±5.23 μg mL^{-1}) indicating its high free radical scavenging activity as compared to fractions C and D.

As total phenol increases, the IC_{50} decreases which imply a positive correlation between the total phenol and free radical scavenging activity.

Reducing power of column chromatographic fractions of *C. micranthum* leaves: The reducing power of aqueous extract of *Combretum micranthum* and its column chromatographic fractions are represented in Table 4. Fraction B increases in reducing power across the concentrations, 0.75±0.04>1.26±0.056>1.72±0.11. Fraction C equally increases as the concentration increases, 0.68±0.05>1.11±0.03>1.42±0.08. Similar attribute was seen in fraction D as well, 0.39±0.04[b]>0.52±0.00>0.67±0.03. However, fraction D shows significant ($p<0.05$) low reducing power when compared with fractions B and C at 100, 200 and 300 μg mL^{-1}. The result has shown clearly that fraction B possesses the highest reducing power as compared to fractions C and D at the various concentrations.

Total antioxidant capacity of column chromatographic fractions of *Combretum micranthum* leaves: The aqueous extract fractions of *Combretum micranthum* leaves showed potent total antioxidant capacity. The result is presented in Table 5 where fraction B demonstrated a significantly ($p>0.05$) higher total antioxidant capacity as compared to fractions C and D.

Phytochemical constituents of column chromatographic fraction B of *Combretum micranthum* leaves: Considering the ACE inhibitory activity, total phenolic content, free radical scavenging activity and total antioxidant capacity assessment of the various fractions, it has been adjudged that fraction B is the most active fraction of all in terms of ACE inhibitory and the antioxidant activity of the aqueous extract of *Combretum micranthum*. Hence, fraction B was selected for further analysis using GC-MS to identify the possible active components that were responsible for much better performance noticed as compared to fraction C and D. The GC-MS phytochemical screening of fraction B of the aqueous extract of *Combretum micranthum* as shown in Table 6 revealed the presence of Megastigmatrienone, 3,5-Dimethoxy-4-hydroxyphenyl acetic acid and Estra-1,3,5(10)-trien-17β-ol with retention time of 32.745, 38.629 and 39.139 min, respectively.

Table 1: Percentage inhibition of ACE activity by the aqueous extract of *Combretum micranthum* leaves

Samples	Inhibition (%)
Aqueous	59.43±4.00[b]
Captopril	83.02±2.67[b]

One-way ANOVA, values with different superscripts down the column differs significantly at $p<0.05$ and data are expressed in Mean±Standard deviation

Table 2: Percentage inhibition of ACE activity by the column chromatographic fractions of aqueous extract of *Combretum micranthum* leaves

Fractions	Inhibition (%)
A	8.21±41.19[a]
B	97.69±8.57[b]
C	78.32±7.14[b]
D	98.32±2.66[b]
Captopril	86.16±5.76[b]

One-way ANOVA, data are expressed as Mean±Standard, n = 3 and values with different superscripts down the column differ significantly at $p\leq0.05$

Table 3: Total phenolic content and free radical scavenging activity IC_{50} of column chromatographic fractions of *Combretum micranthum* leaves

Fractions	TP (mg g^{-1}) GAE	IC_{50} (μg mL^{-1})
B	252.50±5.62[c]	121.51±5.23[a]
C	118.30±1.27[b]	255.55±19.59[b]
D	55.73±2.56[a]	308.83±14.60[c]

One-way ANOVA, data are expressed as Mean±Standard, n = 3, values with different superscripts down the column differ significantly at $p\leq0.05$, IC_{50}: Inhibitory concentration at 50% and GAE: Gallic acid equivalent

Table 4: Reducing power of fractions from column chromatographic fractions of aqueous extract of *Combretum micranthum* leaves

Fractions	100 (μg mL^{-1})	200 (μg mL^{-1})	300 (μg mL^{-1})
B	0.75±0.04[a]	1.26±0.056[a]	1.72±0.11[a]
C	0.68±0.05[a]	1.11±0.03[b]	1.42±0.08[b]
D	0.39±0.04[b]	0.52±0.00[c]	0.67±0.03[c]

One-way ANOVA, data are expressed as Mean±Standard, n = 3 and values with different superscripts down the column differ significantly at $p\leq0.05$

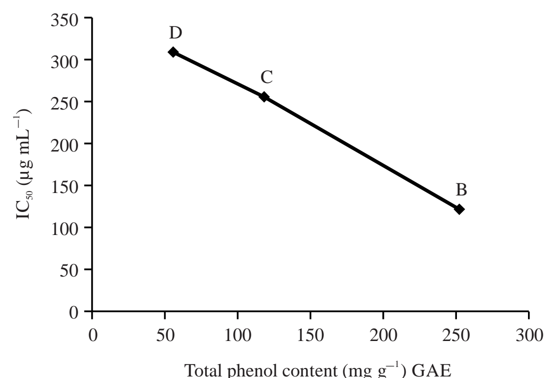

Fig. 1: Relationship between the IC_{50} and the TPC of the fractions

Table 5: Total antioxidant capacity of aqueous extract column chromatographic fractions of *Combretum micranthum* leaves

Fractions	TAC (μg AA mg^{-1} of extract)
B	16.40 ± 0.89^{b}
C	1.10 ± 0.14^{a}
D	2.08 ± 0.32^{a}

One-Way ANOVA, data are expressed as Mean±Standard, n = 3, values with different superscripts down the column differ significantly at $p\leq0.05$, TAC: Total antioxidant capacity and AA: Ascorbic acid

Table 6: Identified compounds of the fraction B of the aqueous extract of *Combretum micranthum* leaves by GC-MS

Compounds	Retention time (min)	Similarity (%)
Megastigmatrienone	32.745	94
3,5-Dimethoxy-4-hydroxyphenylacetic acid	38.629	64
Estra-1,3,5(10)-trien-17β-ol	39.139	99

DISCUSSION

The chromatographic fractions A, B, C and D were able to inhibit the *in vitro* activity of rabbit ACE. The antioxidant potency of these fractions was evident in their free radical scavenging IC_{50}, reducing power ability and total antioxidant capacity. The GC-MS analysis of fraction B of the aqueous extract of *Combretum micranthum* was found to be Megastigmatrienone, 3,5-Dimethoxy-4-hydroxyphenyl acetic acid and Estra-1,3,5(0)-trien-17β-ol.

High blood pressure is a silent killer, causing several serious diseases such as heart failure, kidney failure and stroke[13]. Some of the treatment options using synthetic drugs include diuretics, β-blockers, calcium channel blockers and angiotensin II receptor blockers as well as angiotensin-converting enzyme inhibitors[14]. The angiotensin-converting enzyme is a zinc metallopeptidase that converts angiotensin I (inactive decapeptide) to angiotensin II (a potent vasoconstrictor) and bradykinin (a hypotensive peptide) to inactive components and consequently leading to an increase in the concentration of angiotensin II and decrease in the concentration of bradykinin thereby initiating hypertension[15]. Therefore, the development of agents that inhibit the conversion of angiotensin I to angiotensin II and the breakdown of bradykinin to inactive components began as a therapeutic strategy to treat hypertension. Natural products and active substances derived from medicinal plants could as well be important sources of ACE inhibitors such as captopril, a synthetic antihypertensive drug, which was developed by changing and optimizing the structure of the venom of the Brazilian viper[16].

In this study, an aqueous extract of *Combretum micranthum* leaves and its partially purified fractions were found to exhibit ACE inhibitory activity. Similarly, ACE inhibitory activity was previously reported on plants such as *Rubus* sp., *Crataegus microphylla* and *Onopordon acanthium* that were traditionally used in the management of hypertension[17]. Hence, this research revealed that the observed ACE inhibitory activity of *Combretum micranthum* leaves extract could be one of the possible mechanisms while this plant has been effectively utilized for the treatment/management of hypertension in folklore medicine.

In hypertensive patients, angiotensin II increases chronically and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is activated which causes a rise in ROS[18]. Angiotensin II also stimulates the production of superoxide anion and hydrogen peroxide in polymorphonuclears leukocytes which inactivate the vasodilatory endothelial-derived vascular relaxing factor (nitric oxide-NO) and proatacyclins[19] and as a result, it is more beneficial for an antihypertensive drug to have an antioxidant effect. Hence, *Combretum micranthum* leaves extract and its fractions were investigated for indicators of antioxidant potential such as total phenolic content and the antioxidant activity itself in addition to its ACE inhibitory activity.

Aqueous *Combretum micranthum* leaves extract fractions were found to contained phenol and consequently showed free radical scavenging activity as well as total antioxidant activity. These findings were in agreement with the earlier study carried out by Sharifi *et al.*[20], where *Rubus* sp., *Crataegus microphylla* and *Onopordon acanthium* were investigated for both ACE inhibitory and antioxidant activities and were equally found to possess both activities. However, fraction B showed a significantly ($p<0.05$) good performance as compared to fraction C and D in terms of the phenolic content, free radical scavenging activity expressed as IC_{50} and the total antioxidant capacity. Furthermore, it was also found out that there was an inverse relationship between the free radical scavenging activity and the phenolic content indicating that the higher the phenolic content, the lower the IC_{50} but, the better the total antioxidant performance which corresponds to the findings of Genwali *et al.*[21] where extracts from *Terminalia chebula*, *Terminalia bellirica* and *Bergenia ciliate* demonstrated a similar correlation between the total phenolic content and the free radical scavenging activity IC_{50}. The presence of reductones in a medium such as plant extracts caused the reduction of Fe^{3+}/Ferric cyanide complex to ferrous form when monitored spectrophotometrically[22]. Consequently, the three selected fractions (B, C and D) of aqueous extract of *Combretum micranthum* were confirmed for their reducing capacity with the fraction B showing the highest reducing tendency. Hence, the overall performance of the antioxidant activity of the various fractions of the aqueous extract of *Combretum micranthum* leaves may be as well among other antioxidant mechanisms possibly connected to the presence of reductones in them.

The larger molecules like Megastigmatrienone, 3,5-Dimethoxy-4-hydroxyphenyl acetic acid and Estra-1,3,5(10)-trien-17β-ol found in *Combretum micranthum* leaves extract may provide more hydroxyl and heterocyclic oxygen groups for the ACE inhibition as it was experienced with some flavonoids[23], anthocyanins[24] and isoflavones[25] that have proved to be effective in decreasing the ACE activity.

From the study, it can be recommended that the aqueous fraction of *Combretum micranthum* leaves possess *in vitro* antioxidant and ACE inhibitory effects. More work should be done on the *in vivo* inhibitory effect of aqueous extract of *Combretum micranthum*.

CONCLUSION

Aqueous extract of *Combretum micranthum* leaves and its fractions have shown the tendency to inhibit the *in vitro* activity of ACE which may be the mechanism while it has been used in the past for the treatment of hypertension in traditional medicine. Similarly, the various fractions demonstrated antioxidant activity that would be helpful in the management of hypertension that is accompanied by the generation of free radical species.

SIGNIFICANCE STATEMENT

This study discovers that the extract of *Combretum micranthum* leaves inhibits angiotensin-converting enzymes. This study will help researchers to reveal other hypotensive mechanisms such as beta receptor blockers and calcium antagonist activities of this plant that were not assessed. Thus, a new theory on the anti-hypertensive activity of *Combretum micranthum* leaves may emerge.

ACKNOWLEDGMENT

The authors would like to acknowledge the Departments of Biochemistry and pharmacology, Ahmadu Bello University (A.B.U), Zaria for making their facilities available for this study to be done.

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