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Antioxidant and Angiotensin Converting Enzyme Inhibitory Activity of Fractionated Extract of *Combretum Micranthum* Leaves

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Authors' Contribution-
Rukaiyat Lawal Mashi: Designed the work and carried out the experiment; Michael Sunday Abu: Carried out the analysis; Auwalu Jalo: interpreted the data; Jamila Yahaya Lawal: prepared the manuscript.

Conflict of Interest-
The Authors declared no conflict of interest

ABSTRACT

Background and Objective: Angiotensin Converting Enzyme (ACE) is a glycoprotein with peptidyl dipeptide hydrolase activity that converts Angiotensin I to Angiotensin II which is a powerful vasoconstrictor that stimulates the synthesis of aldosterone. This study evaluated the *in vitro* ACE inhibitory activity and antioxidant potentials of aqueous extract of *Combretum micranthum* leaves and its fractions. **Materials and Methods:** The aqueous extract of *Combretum micranthum* leaves was subjected to chromatographic fractionation where the fractions obtained were assayed for *in vitro* inhibitory activity against rabbit ACE and their possible antioxidant potentials. **Results:** Aqueous extract of *Combretum micranthum* leaves produced 59.43±4.00 % inhibitory activity comparable to captopril which produced 83.02±2.67% activity. The chromatographic fractions A, B, C and D were able to inhibit the *in vitro* activity of rabbit ACE with the inhibitory percentages of 8.21±41.19, 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 possibly 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* where found to be Megastigmatrienone, 3,5-Dimethoxy-4-hydroxyphenylacetic acid and Estra-1,3,5(0)-trien-17β-ol. **Conclusion**: Hence, the results demonstrated that aqueous extract of *Combretum micranthum* has shown 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: *Combretum micranthum*, angiotensin, antioxidant, hypertension, ACE

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 vasoconstrictive 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 to provide hypertensive preventive health benefits, and probably avoids adverse effects associated with the synthetic ones in use[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. Stefano *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 angiotensin converting enzyme.

MATERIALS AND METHODS

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 Kastina State, Nigeria. The Plant sample was identified and authenticated in Herbarium unit of the Department of Botany, Ahmadu Bello University, Zaria where a voucher sample was deposited (Voucher Number 900257).

Methods

Preparation of aqueous extract

The *Combretum micranthum* leaves were washed and air dried at room temperature. Dried samples was pulverised using pestle and mortar. Exactly 1 Litre of distilled water was added to 500g powdered leaves and soaked for 24 hours. The filtrate was then concentrated by evaporation using a water bath at 40^0C. After which the aqueous extract obtained was then stored inside a container and kept at room temperature until required.

Determination of ACE inhibitory effect of aqueous extract of *Combretum micranthum* leaves and its fractions

The assay for ACE inhibitory activity was carried out using the Cushman and Cheung[8] method with some modifications on the assay conditions. Briefly, 50 µl of ACE solution (100 mU/ml) was added to 50 µl of sample solution (0.5 mg/ml) and incubated at 37^oC for 10 minutes. Substrate (15 0µl) solution (8.3 mM Hip-His-Leu in Borate buffer) was then added to the reaction mixture and then incubated for 1hr 20 minutes at 37^oC. 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 air flow at 37^oC; the residual Hippuric Acid (HA) was then dissolved in 1 ml of deionized water and absorbance of the solution taken at 228 nm to determine the hippuric acid concentration. The sample blank was prepared in the same way above, with change in the order in which the

reagents were added, HCl was added before enzyme. The reaction blank was prepared in the same way as the sample blank, replacing the volume of tested sample with buffer. Captopril was used as the standard drug. The percentage inhibition was then calculated from the equation:

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

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 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 ethylacetate 9:1, 8:2, 7:3, ethylacetate 100 %, ethylacetate 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 minutes, the solvent system ethylacetate and methanol 8:2 gave the best separation.

Column chromatography of aqueous extract of C. *micranthum*

The column was packed with 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 profile were pooled together to give four pooled fractions (A-D).The fractions were tested against Angiotensin converting enzyme in order to ascertain their inhibitory potentials.

Determination of total phenolic content of the aqueous extract fractions of *Combretum micranthum* leavess

Total phenolic content of the fractions were determined using the method of McDonald *et al*.[9] with slight modifications.

Calibration curve was prepared by mixing ethanol solution of Garlic acid (1 ml; 0.025-0.400 mg/ml) with 5 ml 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 (1 ml) of each of the solution of fraction in methanol (5 g/L) was also mixed with the reagents above and after 30 minutes the absorbance was measured. All determinations were carried out in triplicate. The total phenolics components in the fractions in Garlic Acid Equivalent (GAE) were calculated by the formula;

T= C.V/M; where T is the total phenolic contents, milligram per gram of sample fraction in Gallic Acid Equivalent ; C is the concentration of Garlic acid established from the calibration curve, mg/ml; V is the volume of fraction, millilitre; M is the 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 described by Karadag *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). The solution was rapidly mixed and incubated in dark at 37 ^{o}C for 20 minutes. The decrease in absorbance of each solution was measured at 517 nm using 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:

% Free Radical Scavenging Activity = $A_c - A_s/A_c$ x 100

Where, A_c = Absorbance of control at 517 nm

A_s = Absorbance of 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 power of the aqueous extract fractions of *Combretum micranthum* leaves

This was determined according to the method of Oyaizu[11].

The fractions and standard (1ml) of various concentrations (100, 200, 300 ug/ml) were mixed with phosphate buffer (pH 6.6, 0.2M, 2.5ml) and potassium ferricyanide (1 %, 2.5ml).The mixture was incubated at 50 ^{0}C for 20minutes.Trichloroacetic acid (10 %, 2.5 ml) was added to the mixture. A portion of the resulting mixture was mixed with $FeCl_3$ (0.1 %, 0.5ml) and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated 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 Prieto *et al.*[12].

Into a test tubes, 0. 3 mililitre of various concentrations of fractions (100, 200, 300 ug/ml) were combined with 3ml reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4Mm 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 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 x 0.25 mm i.d., x 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 s^{-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 split less 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 analysed by the one way analysis of variance (one-way ANOVA) using SPSS program (version 20 SPSS Inc., Chicago, IL, USA). The differences in parameters were

compared usingBonferroni multiple comparison test (a post-hoc test). The results were expressed as mean ± standard deviation (SD). P value less than 0.05 was considered as significant ($P< 0.05$). Results were presented in table, charts and graphs using MICROSOFT WORD and EXCEL.

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) 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

The four 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 each and repeated three times to obtain the mean value as reported. The result in 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 GAE) as compared to fractions C (118.30 ± 1.27 mg/g GAE) and D (55.73 ±2.56 mg/g GAE).

There was an inverse variation between the amount of phenolic content and the free radical scavenging activity IC_{50} as depicted in Figure 1. The 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) indicating its high free radical scavenging activity as compared to fractions C and D.

Table 1: Percentage Inhibition of ACE activity by the Aqueous Extract of *C.micranthum* Leaves

Sample	% Inhibition
Aqueous	59.43±4.00[b]
Captopril	83.02±2.67[b]

One-way ANOVA, Values with different superscript down the column differs significantly at P<0.05.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

Fraction	% 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. Values with different superscript down the column differ significantly at P≤0.05.

Table 3: Total Phenolic Content and Free Radical Scavenging Activity IC_{50} of Column Chromatographic Fractions of *C .micranthum* Leaves

Fraction	TP (mg/g) GAE	IC_{50} (µg/ml)
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 superscript down the column differ significantly at P≤0.05. IC_{50} = Inhibitory Concentration at 50%.GAE = Gallic Acid Equivalent

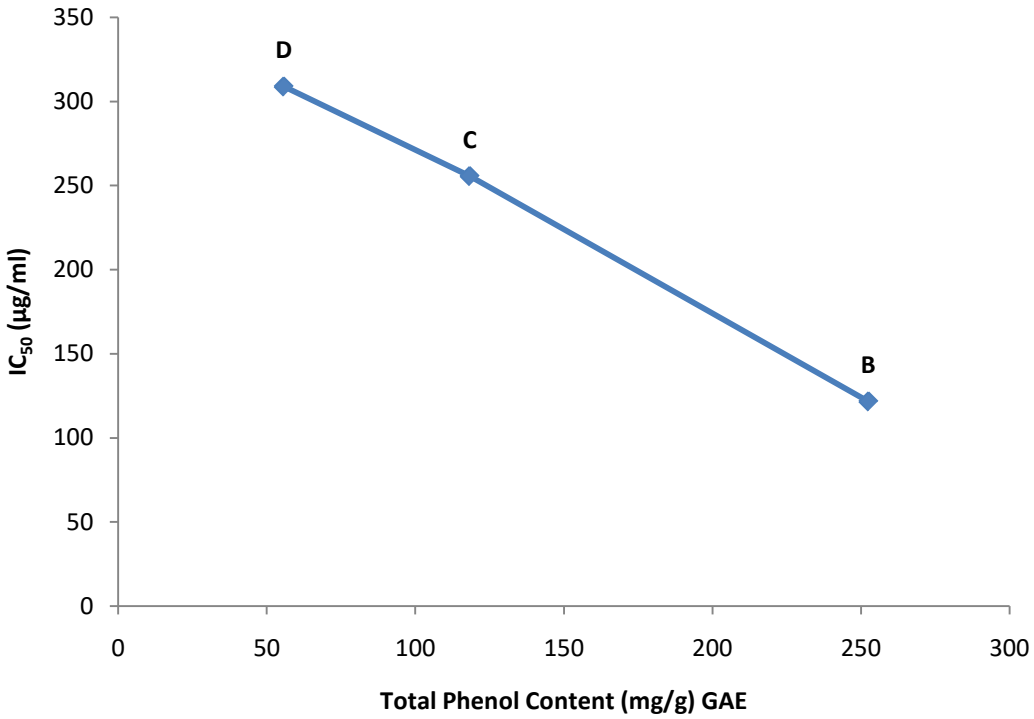

Figure 1: Relationship between the IC_{50} and the TPC of the Fractions

As Total phenol increases, the IC_{50} decreases which implies 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 micrantum* and its column chromatographic fractions is represented in Table 4. Fraction D shows significant ($P<0.05$) low reducing power when compared with fractions B and C at 100µg/ml, 200 µg/ml and 300µg/ml. 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 C. *micranthum*. Hence, fraction B was selected for further analysis using GC-MS to identify the possible active components that were responsible for such 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 on Table 6 revealed the presence of Megastigmatrienone, 3,5-Dimethoxy-4-hydroxyphenylacetic acid and Estra-1,3,5(10)-trien-17β-ol with retention time of 32.745, 38.629 and 39.139 minutes respectively.

Table 4: Reducing power of Fractions from Column Chromatographic Fractions of Aqueous extract of *C.micranthum* leaves

Fraction	100 ug/ml	200 ug/ml	30 0ug/ml
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. Values with different superscript down the column differ significantly at P≤0.05.

Table 5: Total Antioxidant Capacity of Aqueous Extract Column Chromatographic Fractions of *C. Micranthum* Leaves

Fraction	TAC (µg AA/mg 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 superscript down the column differ significantly at P≤0.05. TAC= Total Antioxidant Capacity; AA= Ascorbic Acid

Table 6: Identified Compounds of the Fraction B of the Aqueous Extract of *Combretum micranthum* Leaves by GC-MS

S/No	Compound	Retention Time (min)	% Similarity
1	Megastigmatrienone	32.745	94
2	3,5-Dimethoxy-4-hydroxyphenylacetic acid	38.629	64
3	Estra-1,3,5(10)-trien-17β-ol	39.139	99

DISCUSSION

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]. 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, 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, 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 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 polymorphonuclear 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 antioxidant effect. Hence, *Combretum micranthum* leaves extract and its fractions were investigated for indicators of antioxidant potential such as total phenolic content and antioxidant activity itself in addition to its ACE inhibitory activity.

The 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 Niusha *et al.*[20] where *Rubus Sp, Crataegusmicrophylla 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 Giri *et al.*[21] where extracts from *Terminali achebula, Terminali abellirica* 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 medium such as plant extracts caused 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 micrathum* 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 possibly connected to the presence of reductones.

Also, larger molecules like Megastigmatrienone, 3,5-Dimethoxy-4-hydroxyphenylacetic acid and Estra-1,3,5(10)-trien-17β-ol found in *Combretum micranthum* leaves extract may have provided 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.

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 the traditional medicine. Similarly, the various fractions demonstrated antioxidant activity that would be helpful in management of hypertension that is accompanied with the generation of free radical species.

RECOMMENDATIONS

From the study, it is recommend that the aqueous fraction of *Combretum micranthum* leaves possess *in vitro* antioxidant and ACE inhibitory effect.

More work should be done on the *in vivo* inhibitory effect of aqueous extract of *C. micranthum.*

SIGNIFICANCE STATEMENT

This study discovers that extract of *Combretum micranthum* leaves inhibits angiotensin converting enzyme. 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 anti-hypertensive activity of *Combretum micranthum* leaves may emerge.

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