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Original Research Article

Ameliorative Potential of Ethyl Acetate and Aqueous Fractions of Methanol Leaf Extract of *Combretum micranthum* against Free Radicals

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Abstract

Combretum micranthum (CM) is well known for its ethno-medicinal uses in the northwest of Nigeria with little or no scientific basis. Thus, the aim of this research is to evaluate the methanol leaf extract fractions of *Combretum micranthum* for possible antioxidant compounds. The results of DPPH free radical scavenging showed that ethyl acetate fraction has a higher radical scavenging activity then followed by aqueous fraction of 87.913±3.927 and 84.718±0.605 respectively, compared with ascorbic acid reference standard 80.351±0.732 at 2500ug/m1 concentration. In FRAP, the result showed that ethyl acetate has a higher antioxidant property of 0.818±0.035 followed by aqueous which is 0.800± 0.002 compared to ascorbic acid which is 0.426±0.000. The LC-MS profile revealed the presences of several compounds as follows: 3,4,5-trihydroxy-6-(3-hydroxy-2-(2-hydroxypropan-2-yl)-7-oxo- 2H, 3H, 7H- furo (3,2-g) chromen -9-yl)oxy)oxane-2-carboxylic acid and (5- (1E) -3- (6- (3,4- dihydroxy -2,5- bis (hydroxymethyl) oxolan -2-yl) oxy) -3,4,5-trihydroxyoxan -2-yl) methoxy) -3- oxoprop -1- en -1- yl) -2-hydroxyphenyl) oxidane sulfonic acid in ethyl acetate fraction and Atavaquone, Fenarimol, 12-tricosanol, Myricetin, Histidylasparagine and Homocycteine thiolactone in aqueous fraction. The result of the docking revealed some potential and antioxidant activities of the fractions with significant binding interactions between compounds and alpha amylase & alpha glucosidase. Therefore based on the compounds identified by LCMS analysis coupled to the *in-vitro* antioxidant studies it can be concluded that these fractions of *Combretum micranthum* can be used against free radicals and potential in drug management.

Keywords: *Combretum micranthum*, ethyl-acetate fraction, aqueous fraction, antioxidant.

INTRODUCTION

Combretum micranthum, Fam. Combretaceae is an undomesticated shrub species of western Africa and is one of the most popular traditional bush teas of Senegal. The herbal beverage is traditionally used for weight loss, digestion, as a diuretic and mild antibiotic, and to relieve pain, and the fresh leaves are used to treat malarial fever. *Combretum micranthum*, specifically, was so widely used in West Africa as a general panacea that the name kinkéliba has become a word synonymous with "medicine" in some languages (Eloff, *et al*., 2008). Additionally, *C. micranthum* was selected as one of the 50 most important

Modern scientific research indicates that leaf extracts exhibit a number of pharmacological activities. For example, a kinkeliba decoction was shown to inhibit both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum in vitro*, indicating its potential for the treatment of malaria disease (Benoit *et al*., 1996). Methanol leaf extracts was reported to significantly inhibit the production of carrageenan induced oedema in rats, suggesting its potential use for anti-inflammatory applications (Olajide *et al*., 2003). Udoh *et al*., (2012) reported that the methanol and water extract of kinkeliba leaves have effective anti-microbial activities against both Gram-positive and Gram-negative isolates including *Pseudomonas aeruginosa* and *Staphylococcus aureaus* (Udoh *et al*., 2012). By studying kinkeliba leaves and extending the investigation to the bark, roots and branches of the plant, the mode of therapeutic action that is utilized in the tea can be elucidated, developed, and ultimately employed in foods, beverages and, if therapeutically valid, modern health care or medicine.

In a normal cell, there is appropriate oxidant: antioxidant balance. However, this balance can be shifted, when production of oxygen species is increased or when levels of antioxidants are diminished. This stage is called oxidative stress (Vinson *et al*., 1998; Cuvelier *et al*., 1992). Oxidative stress results in the damage of biopolymers including nucleic acids, proteins, poly-unsaturated fatty acids and carbohydrates.

Oxidative stress causes serious cell damage leading to a variety of human diseases like Alzheimer's disease, Parkinson's disease, atherosclerosis, cancer, arthritis, immunological incompetence and neurodegenerative disorders, etc. Nutritional antioxidant deficiency also leads to oxidative stress which signifies the identification of natural antioxidative agents present in diets consumed by human population (Cao *et al*., 1966). Plant extracts rich in phenol acids exhibit strong antioxidant and antiradical activity *in vitro* (Mary *et al*., 2003) and *in vivo* (Rajlakshmi *et al*., 2003). The synthetic antioxidants, such as EDTA and BHT, are typically cheaper and can be easier to process than natural antioxidants. However, restrictions in the use of synthetic antioxidants have been enforced because of their health risks and toxicity (Linderschmidt *et al*., 1986). Therefore the need to investigate the potentiality of the different fractions of this plant cannot be overemphasis, this study will determine the free radical Scavenging and antioxidant properties.

MATERIALS AND METHOD

Chemicals/ Equipment

Liquid chromatography-mass spectroscopy (LC Water e2695 separation module with W2998 PDA and couple to ACQ-QDA MS). UV-VIS Spectrometer, Centrifuge, Weighing balance, water bath, and pH meter. Chemicals/reagents used are of analytical grade and were purchased from sigma Aldrich.

Plant Collection and Identification

The leaves of *Combretum micranthum were* collected from Filin Shagari, Bauchi State, Nigeria. It was identified at the herbarium unit of Biological sciences department, Bayero University Kano and voucher specimen no. BUKHAN0349 was deposited for future references. The leaves were shade dried and ground into smooth powder and kept in clean polythene nylon as described by Ibrahim *et al*., (2017).

Preparation of Extract and Fractions

The *Combretum micranthum* leaves was extracted as reported by Ibrahim *et al*., (2017). Briefly: The forty grams (40g) of leaves powder was dissolved in 200 cm^3 of methanol. The container was covered, after 48 hours, the mixture was filtered using a nylon sieve into the small container and the residue spread on a wide plastic plate and allowed to dry. The residue was re-extracted twice with fresh 200 cm^3 of methanol for 24 hours. 5g of the dried extract was soaked in 150 cm^3 of water in separating funnel with equal volume of ethyl acetate added, the mixture was well shaken and allowed to stand until two clear layers were formed. The upper layer being the ethyl acetate fraction was carefully separated from the aqueous layer at the bottom.

Sample Preparation

Stock solution of the fractions (1% w/v) were prepared by dissolving 0.1g of the extract in 10 cm^3 of methanol each as reported by Ibrahim *et al*., (2017). Ascorbic acid as the reference was also prepared in the same manner. The stock solutions were centrifuged at 4000 rpm and the supernatant carefully removed and stored for further analysis.

DPPH Spectrophotometric Assay

The free radical scavenging activity of ethyl acetate and aqueous fraction of *Combretum micranthum* methanol leaf extract was evaluated using 2, 2-diphenyl-2-picryl-hydrazyl (DPPH) according to the method reported by Shen *et al*. (2010) with slight modification. 0.1mM solution of DPPH in methanol was prepared and 2cm^3 of this solution was added to 1cm^3 of various concentration of the extract fraction prepared by serial dilution from the stock (0.16, 0.3125, 0.62, 1.25 and 2.50 mg/cm^3).The mixtures were shaken vigorously and allowed to incubate at room temperature for 30 minutes. Methanol was used as a blank and DPPH in methanol without the plant extract was used as positive control. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer. Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability of scavenging the DPPH radical was calculated by using the following formula; DPPH scavenging effect (% inhibition) = {(A0 –A1)/A0)*100}.

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicate. The IC_{50} value was determine as the half-maximum concentration of the extract (in mg/mL) that scavenge 50% of DPPH.

Ferric Reducing Power Spectrophotometric Assay

The method is based on the reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}), in the presence of antioxidants. Substances having a reduction potential react with potassium ferricyanide forming potassium ferrocyanide which further reacts with $FeCl_3$ to form an intense Prussian blue complex having maximum absorbance at 700 nm. The amount of complex formed is directly proportional to the reducing power of test sample (Kumar *et al*., 2013).

The reducing power of the ethyl acetate and aqueous fraction of *Combretum micranthum was* measured using the method described by Ferreira *et al*. (2007) with slight modification. 2.5 cm^3 of extracts fractions at different concentrations (0.1625, 0.625, 0.3125, 1.25 1.25 and 2.50 mg/cm^3) were mixed with 2.5 cm^3 phosphate buffer (0.2 M, pH 6.6) and 2.5 cm^3 of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minute, and then rapidly cooled, mixed with 2.5 ml of 10% trichloroactic acid and centrifuged at 6500 rpm for 10 minute. The supernatant (2.5 cm^3) was mixed with distilled water (2.5 cm^3) and then ferric chloride (0.5 cm^3, 0.1%) was added and allowed to stand for 10 minute. The absorbance was read spectrophotometrically at 700 nm. Ascorbic acid was used as standard. 2.5 cm^3 of all the reagent in distilled water was used as blank.

Liquid Chromatography Mass Spectrometer Analysis

The samples were analyzed using liquid chromatography (LC) tandem mass spectrophotometer (MS) as described by Piovesana *et al*., (2018) with some modifications. The extracted samples were reconstituted in Methanol and filtered through polytetrafluoroethylene (PTFE) membrane filter with 0.45 µm size. After filtration, the filtrate (10.0 µl) was injected into the LC system and allowed to separate on Sunfire C18 5.0µm 4.6mm x 150 mm column. The run was carried out at a flow rate of 1.0 mL/min, Sample and Column temperature at 25°C. The mobile phase consists of 0.1% formic acid in water (solvent A) and 0.1% formic acid in Acetonitrile (solvent B) with a gradient as below.

Table 1: LC-MS Solvent gradient

Time	% A	% B
0	95	5
1	95	5
13	5	95
15	5	95
17	95	5
19	95	5
20	95	5

From ratio of A/B 95:5 this ratio was maintained for further 1 min, then A/B 5:95 for 13min, to 15min. then A/B 95:5 to 17min, 19min and finally 20min. the PDA detector was set at 210-400nm with resolution of 1.2nm and sampling rate at 10 points/sec. The mass spectra were acquired with a scan range from m/z 100 – 1250 after ensuring the following settings: ESI source in positive and negative ion modes; capillary voltage 0.8kv (positive) and 0.8kv (negative); probe temperature 600°C; flow rate 10 mL/min; nebulizer gas, 45 psi. MS set in automatic mode applying fragmentation voltage of 125 V. The data was processed with Empower 3. The compounds were identified on the basis of the following information, elution order, and retention time (Rt), fragmentation pattern, and Base m/z.

Molecular Docking Protocol

Ligand Structures

All ligand structures (obtained from pubchem site https://pubchem.ncbi.nhm.gov) were optimized by using Merck Molecular Force Field (MMFF) and the Chemistry at Harvard Macromolecular Mechanics (CHARMm) force field, both of which are implemented in Discovery studio visualiser (version 3.5, BIOVIA Software, http://www.3dsbiovia.com/product/collaborative-science/biovia-discovery-studio/) in order to remove all strain from the molecular structure. In addition this will ensure a well-defined conformer relationship among compounds of the study (Viswanadhan *et. al.*, 1989). From the setup calculation option on discovery studio visualise 3.5, the calculation was set to equilibrium geometry at the ground state using density functional theory at B3LYP (Becke88 three-parameter hybrid exchange potentials with Lee-Yang-Parr correlation potential) level of theory and 6-311G (d) basis set for the geometrical optimization of the cleansed structures i.e. B3LYP/6-311G (d) level of theory. After optimization, Discovery studio visualiser descriptors were obtained from the display-output and display-properties option on Discovery studio visualiser 3.5. The fully optimized 3D structure without symmetry restrictions, were saved as PDB (Protein Data Bank) file through the file option on the Discovery studio visualiser 3.5.

Enzyme Structure

The X-ray crystal structure of alpha-amylase with PDB ID: 4w93 and resolution of 1.9Å, and alpha-glucosidase with PDB ID: 3wy2 and resolution of 1.47Å were downloaded from RCSB Database (http://www.rcsb.org/pdb). Receptors were optimised, the energy was minimised and also, water was removed using discovery studio visualise 3.5 and Pymol version 2.2.0 respectively and saved the receptors as PDB file.

Docking Simulations

All protein preparation and minimization was done using tools and protocols in the Discovery studio visualiser 3.5. Chemistry at Harvard Macromolecular Mechanics (CHARMm) force field was used to optimize the structure. While using the protein preparation protocol, hydrogen atoms was added to the complex, after which water molecules are removed and the pH of the protein was set to almost neutral value. A sphere binding site with a nine Armstrong A˚ radius was defined around the bonded ligand to identify the binding site of the protein structure. The PDB files of the ligands were then imported into PyRx-virtual screening tool and they were used to dock the prepared receptors. The ligands are scored based on the biased probability Monte Carlo (BPMC) procedure, which

randomly selects a conformation in the internal coordinate space and then makes a step to a new random position independent of the previous one but according to a predefined continuous probability distribution. The results of the best scored and binding energy of all the ligands were reported on a table.

Statistical Analysis

Data were analyzed using Microsoft excel 2013. The results were expressed as mean ± standard deviation (SD) of triplicates determination and IC_{50} for percentage inhibition was calculate.

RESULTS AND DISCUSSION

The results of the DPPH free radical scavenging activity of ethyl acetate and aqueous are presented in Table 2. The result revealed that ethyl acetate fraction shows an appreciable antioxidant activity than that of the aqueous fraction. This also reflect from the IC_{50} value which is negatively related to the antioxidant activity with the ethyl acetate fraction of the plant having an IC_{50} value of 1.001 ± 0.049 which is lower than aqueous fraction.

The results of the FRAP scavenging activity of ethyl acetate and aqueous are presented in Table 3. The result revealed that ethyl acetate fraction has the highest percentage inhibition (87.913 ± 3.927) than the aqueous fraction (80.351 ± 0.732). Therefore, increase in reducing power indicates increase in scavenging activity.

An antioxidant is a chemical that prevents the oxidation of other chemicals. They protect the key cell components by neutralizing the damaging effects of free radicals, which are natural by-products of cell metabolism (Miller *et al*., 2000). The oxidative stress (OS) induced by reactive oxygen species (ROS) can be described as a dynamic imbalance between the amounts of free radicals generated in the body and levels of antioxidants to quench and/or scavenge them and protect the body against their deleterious effects (Shirwaikar *et al*., 2006).

Excessive amounts of ROS may be harmful because they can initiate bimolecular oxidations which lead to cell injury and death, and create oxidative stress which results to numerous diseases and disorders such as aging, cancer, atherosclerosis, cirrhosis and cataracts (Halliwell and Gutteridge, 2000). The result revealed that the ethyl acetate fraction of *Combretum micranthum* exhibited the highest DPPH radical scavenging activity (which is slightly above to the value of Ascorbic acid) Aqueous have the radical scavenging activity which is slightly lower than the activity of the ascorbic acid.

In other words, FRAP is one of the most rapid tests and very useful for routine analysis. The antioxidative activity is estimated by measuring the increase in absorbance caused by the formation of ferrous ions from FRAP reagent. The result revealed that the ethyl acetate fraction of *Combreteum micranthum* exhibited the highest antioxidant activity, with lower activity in aqueous fraction compared to ascorbic acid reference.

Table 2: DPPH scavenging activity of *Combretum micranthum* leaf extract in ethyl acetate and aqueous fractions using ascorbic acid as standard

Concentration of the extract (Mg/ml).	Antioxidant inhibition (%)	Antioxidant inhibition (%)	Antioxidant inhibition (%)
	Ascorbic acid	Ethyl acetate	Aqueous
2,5	84.718 ± 0.605	87.913 ± 3.927	80.351 ± 0.732
1,25	82.481 ± 1.359	85.676 ± 4.935	80.032 ± 0.889
0,625	80.405 ± 2.567	84.239 ± 4.772	78.594 ± 1.365
0,3125	78.754 ± 3.503	79.925 ±5.272	76.358 ± 0.319
0,15625	9.861 ± 14.279	75.985 ± 5.706	68.424 ± 2.272
IC 50	1.438 ± 0.688	1.001 ± 0.049	1.491 ± 0.716

Results are expressed in mean ± standard deviation

Table 4 and 5 shows the compounds identified in LC-MS analysis. Eight (8) compounds were identified; two (2) compounds in ethyl acetate fraction and six (6) compounds in aqueous fraction which are 3,4,5- trihydroxy -6- (3-hydroxy-2 -(2-hydroxypropan-2-yl) -7-oxo-2H,3H,7H-furo(3,2-g)chromen-9-yl)oxy)oxane-2-carboxylic acid and (5- (1E) -3- (6-(3,4- dihydroxy -2,5- bis (hydroxymethyl) oxolan -2-yl) oxy) -3,4,5- trihydroxyoxan -2-yl) methoxy)-3-oxoprop-1-en-1-yl)-2-hydroxyphenyl)oxidanesulfonic acid, Atavaquone, Fenarimol, 12-tricosanol, Myricetin, Histidylasparagine and Homocycteine thiolactone, which were detected and tentatively identified using the human metabolomic database (HMDB).

Table 3: FRAP scavenging activity of *Combretum micranthum* leaf extract in ethyl acetate and aqueous fractions using ascorbic acid as standard

Concentration of the extract (Mg/ml).	Antioxidant inhibition (%)	Antioxidant inhibition (%)	Antioxidant inhibition (%)
	Ascorbic acid	Ethyl acetate	Aqueous
2,5	0.426 ± 0.000	0.818 ± 0.035	0.80 0± 0.002
1,25	0.425 ± 0.001	0.73 2± 0.047	0.359 ± 0.001
0,625	0.364 ± 0.045	0.701 ± 0.001	0.35 1± 0.001
0,3125	0.281 ± 0.001	0.411 ± 0.249	0.31 3± 0.001
0,15625	0.167 ± 0.001	0.256 ± 0.006	0.204 ± 0.001

Results are expressed in mean ± standard deviation

The result from the docking of the compounds obtained from LC-MS analysis of the ethyl acetate fraction of CM against the target protein α- amylase showed that the compound oxane-2-carboxylic acid has the highest binding energy (-7.8) which can be compared to the reference drug L-acarbose (-9.9) and α-glucosidase showed that the compound oxane-2-carboxylic acid has the same binding energy (-9.5) which can be used to replace the reference drug L-acarbose (-9.5) as shown in Table 5 .

While the result obtained from LC-MS analysis of the aqueous fraction of CM against the target protein α- amylase showed that the compound 12-tricosanol has the highest binding energy (-8.5) which can be compared to the reference drug L-acarbose (-9.9) and α-glucosidase showed that the compound Atavaquone has the highest binding energy (-9.6) which can be compared to the reference drug L-acarbose (-9.5) as shown in Table 6.

Table 4: LC-MS analysis of Ethyl acetate fraction of methanolic leaf extract of *Combretum micranthum*

RETENTION TIME (min)	COMPOUNDS NAME	FORMULA	MW	M/Z
6.892	3,4,5-trihydroxy-6-(3-hydroxy-2-(2-hydroxypropan-2-yl)-7-oxo-2H,3H,7H-furo(3,2-g)chromen-9-yl)oxy)oxane-2-carboxylic acid	$C_{20}H_{22}O_{12}$	454.376	455.376
7.172	(5-(1E)-3-(6-(3,4-dihydroxy-2,5-bis(hydroxymethyl)oxolan-2-yl)oxy)-3,4,5trihydroxyoxan-2-yl)methoxy)-3-oxoprop-1-en-1-yl)-2-hydroxyphenyl)oxidanesulfonic acid	$C_{21}H_{28}O_{17}$	584.399	585.399

Table 5: LC-MS analysis of aqueous fraction of methanolic leaf extract of *Combretum micranthum*

RETENTION TIME(min)	COMPOUNDS NAME	CHEMICAL FORMULA	MW	M/Z
12.630	Atavaquone	$C_{22}H_{23}ClO_5$	366.818	367.818
4.467	12-tricosanol	$C_{23}H_{48}O$	340.666	341.666
5.679	Fenarimol	$C_{17}H_{12}Cl_2N_2O$	331.132	332.132
8.131	Myricetin	$C_{15}H_{10}O_8$	318.062	319.062
2.498	Histidylasparagine	C_4H_7NOS	269.262	270.262
5.837	Homocycteine thiolactone	$C_6H_{15}NO$	117.286	118.286

Table 6: Molecular docking scores (Kcal/mol) for Ethyl acetate fraction of methanolic leaf extract of *Combretum micranthum* using alpha-amylase and alpha-glucosidase

Compounds	Alpha-amylase		Alpha-glucosidase	
	Binding Energy	Number of Hydrogen Bond	Binding Energy	Number of Hydrogen Bond
Oxidansulfonicacid	-5.9	1	-8.2	4
oxane-2-carboxylic acid	-7.8	4	-9.5	6
Acarbose (control)	-9.9	6	-9.5	8

Table 7: Molecular docking scores (Kcal/mol) for aqueous fraction of methanolic leaf extract of *Combretum micranthum* using alpha-amylase and alpha-glucosidase

Compounds	Alpha-amylase		Alpha-glucosidase	
	Binding Energy	Number of Hydrogen Bond	Binding Energy	Number of Hydrogen Bond
Atavaquone	-8.0	2	-9.6	2
12-tricosanol	-8.5	1	-8.9	3
Fenarimol	-7.3	2	-8.5	2
Myricetin	-8.2	5	-9.1	3
Histidylasparagine	-6.1	5	-6.7	3
Homocycteinethiolactone	-3.8	3	-4.1	5
Acarbos (control)	-9.9	6	-9.5	8

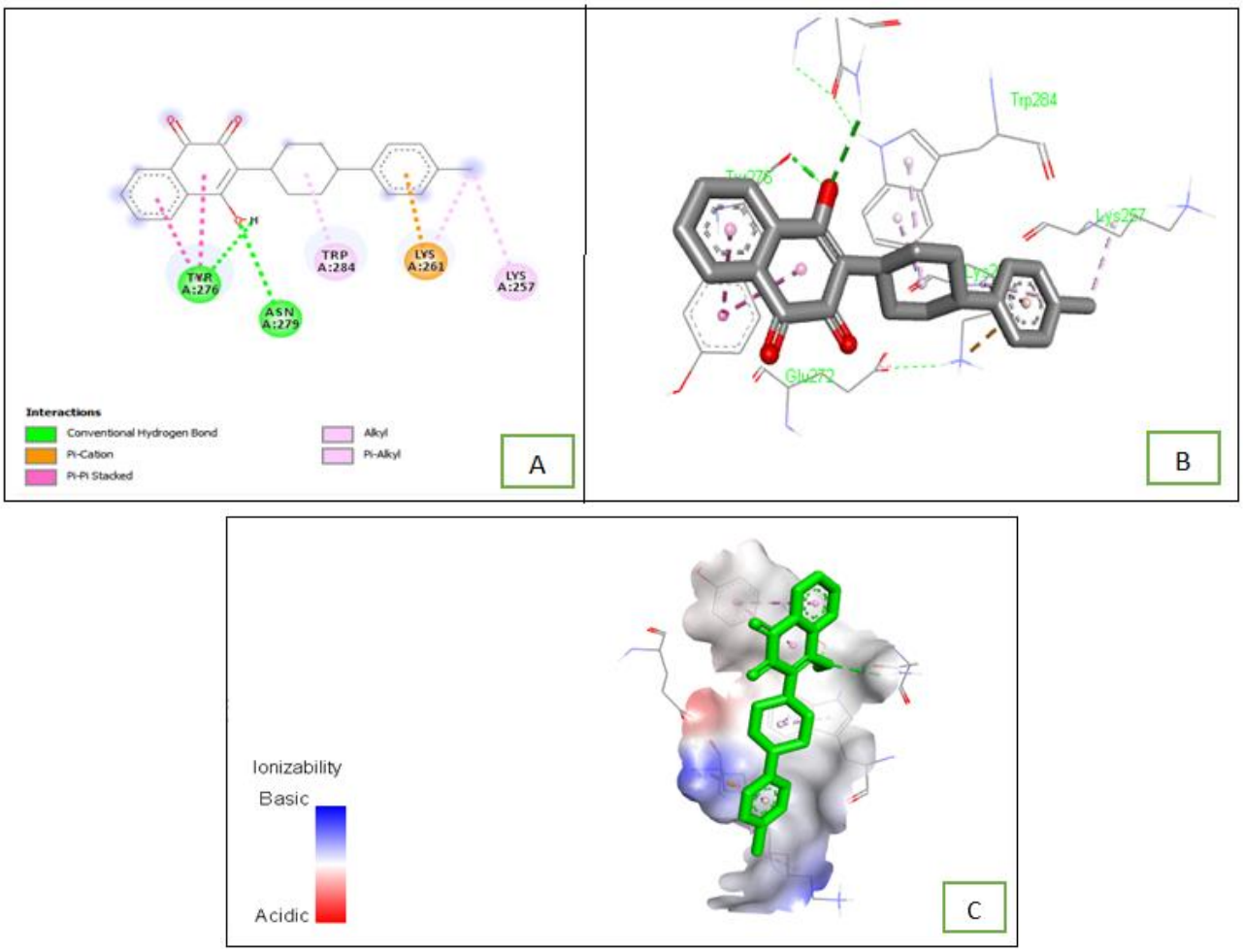


Figure 1: Molecular docking predicted for atovaquone (found in aquoues fraction of CM leaf extract) with amylase (PDB ID 4W93): showing (panel A and B) interactions with amino acid residues of target protein in 3D and 2D respectively. (Panel C) the compound docked in the binding pocket of the protein

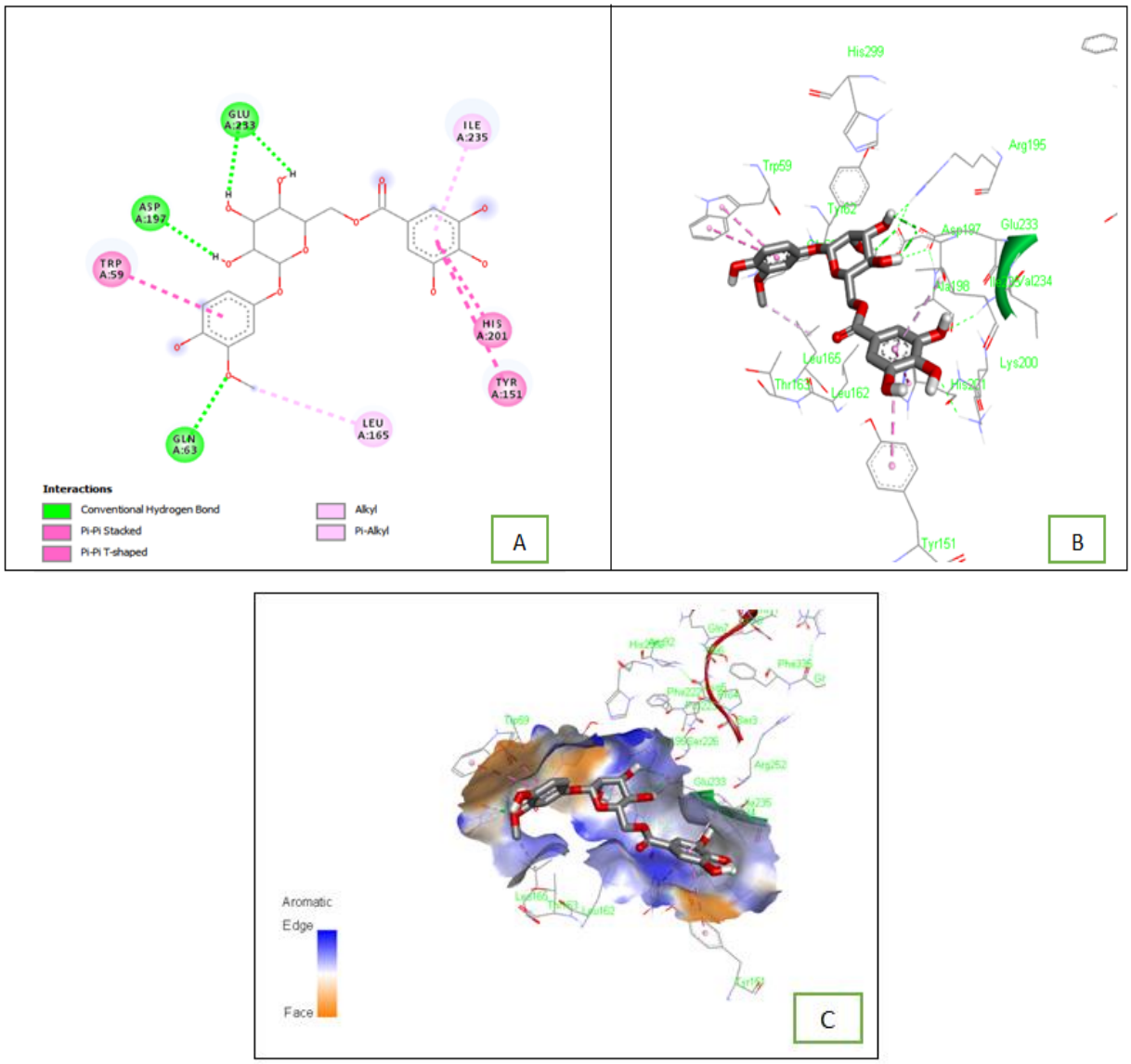
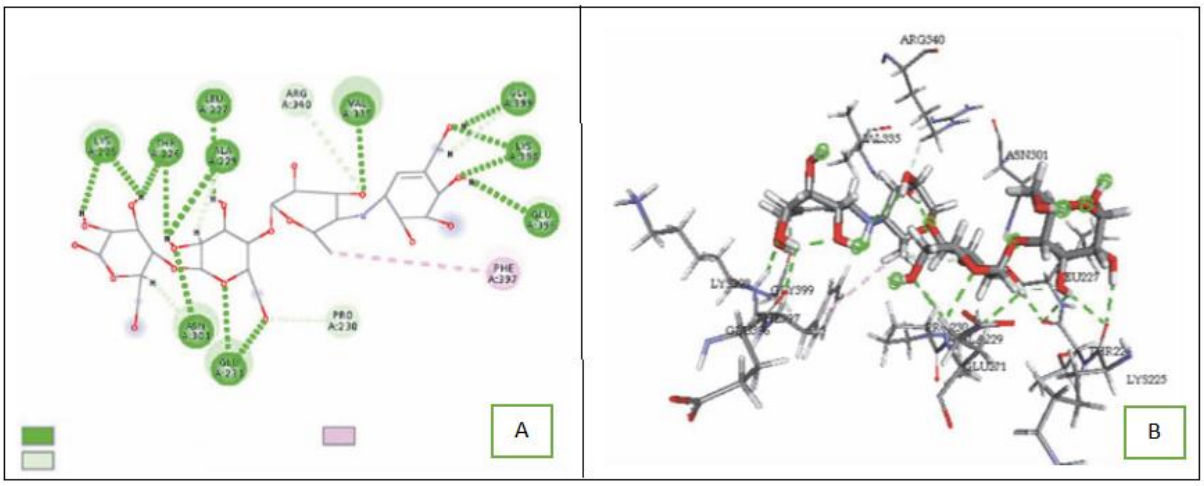


Figure 2: Molecular docking predicted for (5-(1E)-3-(6-(3,4-dihydroxy- 2,5- bis (hydroxymethyl) oxolan-2-yl) oxy) -3,4,5 trihydroxyoxan-2-yl) methoxy)- 3-oxoprop -1- en -1- yl) -2-hydroxyphenyl) oxidanesulfonic acid (found in ethyl acetate fraction of CM leaf extract) with alpha-glucosidase (PDB ID 3WY2) showing (panel A and B) interactions with amino acids residues of target protein in 3D and 2D respectively: panel C, the compound docked in the binding pocket of the protein



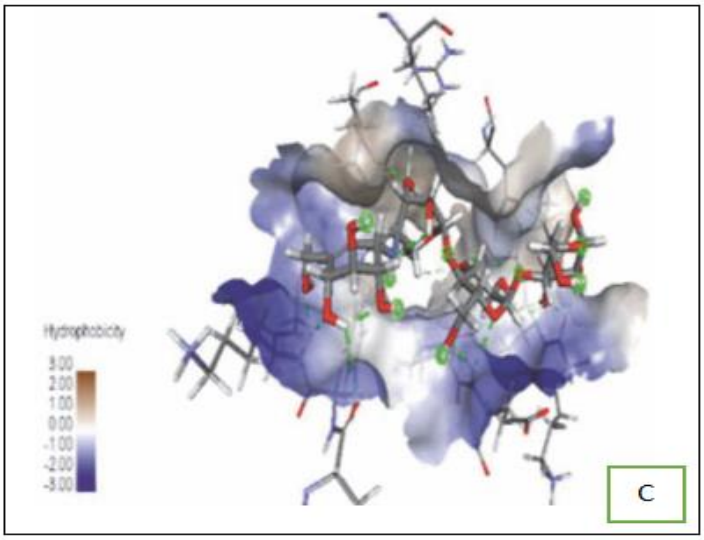


Figure 3: Molecular docking predicted pose for acarbose in alpha-glucosidase and alpha-amylase (PDB ID: 3WY2 and 4W93) showing: (Panel A and B) interactions with amino acid residues of target protein in 3D and 2D respectively; (Panel C) the compound docked in the binding pocket of the protein C

Phenolic compounds are plant metabolites widely spread throughout the plant kingdom. Recent interest in phenolic acids stems from their potential protective role, through ingestion of fruits and vegetables, against oxidative damage diseases (coronary heart disease, stroke, and cancers). Phenolic compounds are essential for the growth and reproduction of plants, and are produced as a response for defending injured plants against pathogens. The importance of antioxidant activities of phenolic compounds and their possible usage in processed foods as a natural antioxidant have reached a new high in recent years. Molecular docking Molecular docking is an *in-silico* modeling technique used to study the interaction between protein targets and small ligands (compounds) (Iheagwam *et al*., 2019). The docking score or binding energy derived from the docking is considered to be a function of the binding affinity of the ligand to the protein target. In the present study, the docking of potentially active compounds of *Combretum micranthum* (Ibrahim *et al*., 2017, 2018) against the target proteins human pancreatic α-amylase, α-glucosidase, was performed. The compounds 3,4,5-trihydroxy-6-(3-hydroxy-2-(2-hydroxypropan-2-yl)-7-oxo-2H,3H,7H-furo(3,2-g)chromen-9-yl)oxy)oxane-2-carboxylic acid and (5-(1E)-3-(6-(3,4-dihydroxy-2,5-bis(hydroxymethyl)oxolan-2-yl)oxy)-3,4,5trihydroxyoxan-2-yl)methoxy)-3-oxoprop-1-en-1-yl)-2-hydroxyphenyl)oxidanesulfonic acid, Atavaquone, Fenarimol, 12-tricosanol, Myricetin, Histidylasparagine, Homocycteinethiolactone showed a relatively high potential to bind and interact with at least one of the three target proteins screened. The observed docking scores (binding energy) were comparable to that of the reference drug L (acarbose). Overall, the docking interaction results showed that most of the compounds with high binding affinity (in terms of docking score) also exhibited many interactions with the active site residues of the target protein. The compound (5-(1E)-3-(6-(3,4-dihydroxy-2,5-bis(hydroxymethyl)oxolan-2-yl) oxy) -3,4,5-trihydroxyoxan-2-yl)methoxy)-3-oxoprop-1-en-1-yl)-2-hydroxyphenyl) oxidanesulfonic acid, showed hydrogen bond interaction with the active site, acarbose, and also with the catalytic residues of α-amylase. This is expected to significantly affect the activity of the target proteins and result in inhibitory modulation of their activity. On the other hand, the interactions between 12-tricosanol, myricetin and atovaquone/ α-amylase were not directly linked to active site residues, but rather to residues outside the active site cleft. However, such interaction is also capable of affecting the activity of the target proteins by disordering the polypeptides that constitute the active site as observed by (William *et al*., 2012) in their study with myricetin and ethyl caffeate. Thus, this molecular docking study corroborates the inhibition of the enzymes α-glucosidase and α-amylase reported earlier by (Ibrahim *et al*., 2017) and further supports the postprandial glucose lowering ability of the plant extract. These compounds possess the ability to scavenge free radicals (generated as a result of hyperglycaemic induced oxidative stress) due to their ability to transfer hydrogen atom or single electron transfer.

CONCLUSION

In conclusion, the use of DPPH assay coupled with other useful method such as FRAP, preferred because it is able to reflect the antioxidant properties more accurately. In both DPPH and FRAP, the results obtained from this study shows that ethyl acetate has higher scavenging activity than aqueous fraction when compared with ascorbic acid as reference standard. These potential of the plant observed may linked to the

phenolic and flavanols identified by the LCMS analysis and coupled to the molecular docking of the compounds prediction. Therefore these fractions of the methanol leaf extraction of *C. micranthum* could be very useful in the free radical scavenging potential, source of therapeutic agent against diabetic and drug management in general.

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