Malaysian Journal of Pharmaceutical Sciences
Vol. 21, No. 1, 15–30 (2023)

CHROMATOGRAPHY-SPECTROSCOPIC ISOLATED MO11 (*MORINGA OLEIFERA*) AND MS06 (*MUSA SAPIENTUM*) POSITIVELY IMMUNOMODULATED ACE2 LEVELS IN BLOOD, KIDNEY AND LIVER OF RATS

MUBARAK O. AMEEN[1*] AND ADELAJA A. AKINLOLU[2]
[1]Department of Chemistry, Faculty of Physical Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria
[2]Department of Anatomy, Faculty of Basic Medical Sciences, Federal University of Health Sciences Otukpo, Benue State, Nigeria

Published online: 25 May 2023
To cite this article: AMEEN, M. O. & AKINLOLU, A. A. (2023) Chromatography-spectroscopic isolated MO11 (*Moringa oleifera*) and MS06 (*Musa sapientum*) positively immunomodulated ACE2 levels in blood, kidney and liver of rats, *Malaysian Journal of Pharmaceutical Sciences*, 21(1): 15–30, https://doi.org/10.21315/mjps2023.21.1.2
To link to this article: https://doi.org/10.21315/ mjps2023.21.1.2

ABSTRACT

Moringa oleifera (MO) and Musa sapientum (MS) are ethno-medicinal plants, while cadmium is a carcinogen. SARS-CoV-2 binds to ACE2 for host's cell invasion and infection. This study evaluated the effects of MO11 (isolated from MO leaves) and MS06 (isolated from MS suckers) on ACE2 levels in cadmium chloride (CdCl)-induced toxicity in rats. Twenty-four adult male Wistar rats were randomly divided into six groups (n = 4). Group 1 was control. Groups 2–4 received single 1.5 mg/kg bodyweight of CdCl (i.p.) (Day 1). Groups 3 and 4 were post-treated with MO11 and MO11 + MS06 doses, respectively (Days 1–17). Groups 5 and 6 received only MO11 and olive oil (vehicle), respectively (Days 1–17). MO leaves and MS suckers were subjected to bioassay-guided fractionation and isolation procedures using chromatography and spectroscopic techniques. ACE2 levels (ELISA) were evaluated in blood, kidney and liver of rats. MO11 and MS06 were the most active antioxidant and antimicrobial compounds isolated from MO leaves and MS suckers, respectively. Liquid chromatography-mass spectrometry showed presence of therapeutic compounds and amino-acids in MO11 and MS06, which are part of 14 shared amino-acids used by SARS-CoV and SARS-CoV-2 for ACE2-binding. Post-treatments of CdCl-exposure with MO11 and MS06 showed decreased ACE2 levels in Group 4 (20.63 ± 4.33 ng/mL and 16.11 ± 0.49 ng/mL in blood and kidney samples, respectively), compared with Group 2 (39.39 ± 3.15 ng/mL and 85.39 ± 3.10 ng/mL in blood and kidney samples, respectively). In conclusion, MO11 and MS06 possess significant ethno-medicinal potentials, may compete with SARS-CoV-2 for ACE2 binding, and are recommended for evaluations as anti-SARS-CoV-2 agents.

Keywords: *Moringa oleifera, Musa sapientum,* Chromatography-spectroscopic techniques, Immuno-modulation, ACE2

[*]Corresponding author: moameen@unilorin.edu.ng

INTRODUCTION

Moringa oleifera (MO) and *Musa sapientum* (MS) are ethno-medicinal plants which are well grown in Nigeria (Akinlolu *et al.* 2021). Furthermore, MOF6, which was fractionated from MO leaves using column chromatography methods showed significant antioxidant and neuro-protective potentials against cuprizone-induced cerebellar damage in rats (Omotoso *et al.* 2018), as well as neuro-protective potentials against dysregulated acetylcholinesterase concentrations in sodium arsenite-induced neurotoxicity in rats (Akinlolu *et al.* 2020*)*. MOF6 equally showed hepato-protective, anti-proliferation and anti-drug resistance potentials in 7,12-Dimethylbenz[a]anthracene-induced hepato-toxicity in rats (Akinlolu *et al.* 2021). Similarly, MSF1, which was fractionated from MS sucker using column chromatography methods possesses hepato-protective, anti-proliferation and anti-drug resistance potentials in 7,12-dimethylbenz[a]anthracene-induced hepato-toxicity in rats (Akinlolu *et al.* 2021).

In addition, Aekthammarat *et al.* 2019 reported hypotensive potentials of MO via remediation of vascular dysfunction and reduction of oxidative stress in L-NAME hypertensive rats. Similarly, Dixit *et al.* (2014) reported that MS possessed hypotensive potentials and significantly decreased systolic, diastolic and mean arterial blood pressure as well as decreased angiotensin converting enzyme-2 (ACE2) during cold stress compared to control subjects in humans.

In hypotension, the body system responds by stimulating the renin-angiotensin-aldosterone system (RAAS) and by employing renin (a proteolytic enzyme) to cleave plasma angiotensinogen into angotensin I (Ang I or Ang-1-10). ACE then cleaves angiotensin I into angiotensin II (Michaud *et al.* 2020). The release and increased levels of angiotensin II promote hypertension (Michaud *et al.* 2020). The binding of angiotensin II to type I and type II receptors results in oxidative stress, renal sodium and water reabsorption, inflammation and vasoconstriction. In addition, ACE2 is an ACE-homolog and a membrane-bound carboxypeptidase. Unlike ACE which increases angiotensin II levels, ACE2 decreases angiotensin II levels by converting angiotensin I to Ang-(1-9) instead of angiotensin II, and by converting angiotensin II to a vasodilator Ang-(1-7). Hence, ACE2 has protective regulatory roles on renal and cardiovascular functions. Ang-(1-7) further promotes anti-proliferation, anti-hypertrophic, anti-inflammation and anti-fibrotic effects via Mas-receptors (Michaud *et al.* 2020).

ACE2 is the cell receptor used by SARS-CoV-2 which causes Coronavirus disease 2019 (COVID-19) to invade human cells. COVID-19 reportedly infected thousands of individuals across the world in 2019 causing several nations to impose lockdown of social and economic activities (Michaud *et al.* 2020). Significant increase in angiotensin II levels correlated positively with viral load and lung injury, resulting in lung vascular permeability, inflammation and vasoconstriction (Michaud *et al.* 2020). ACE2 is ubiquitously expressed in the body, but specifically more expressed in the kidney, testes, oesophagus, colon, enterocytes of the small intestine, lungs, bladder, cholangiocytes, heart, arterial and venous endothelial cells. This explains the potential multiple organ failure or damage due to SARS-CoV-2 infection. ACE2 is equally expressed in the plasma. However, the membrane bound ACE2 is proteolytically cleaved by a disintegrin and metalloprotease 17 (ADAM17) into soluble ACE2 which reveres the protective effects of tissue bound ACE2. SARS-CoV viruses activate ADAM17 resulting in increased plasma and soluble ACE2 in SARS and COVID-19 cases (Michaud *et al.* 2020).

In addition, Lan *et al.* (2020) reported that no differences in RAAS activity and levels of plasma or circulating ACE2, aldosterone and angiotensin II were observed between SARS-CoV-2 positive and SARS-CoV-2 negative individuals. These observations indicate that COVID-19 has no direct influence on RAAS activity, re-enforcing no need for

ACE2 inhibitors as anti-COVID-19 agents. In addition, no study reported clear evidence that the use of ACE2 inhibitors and angiotensin receptor blockers negatively impact on survival rate in COVID-19 (Ciaglia *et al.* 2020; Lan *et al.* 2020; Michaud *et al.* 2020; Ulrich and Ulrich 2021). Some research efforts were, therefore, directed at sourcing for agents that compete with SARS-CoV-2 in binding to ACE2 preventing host-entry and invasion of cells. Other research efforts employed the usage of increased shed concentrations of soluble or circulating ACE2 levels via the fusion of recombinant ACE2 to immunoglobulin Fc domain or direct administration of recombinant ACE2 in-order to have enough SARS-CoV-2 engaged and consequently distract or prevent viral binding to lung cells and other body cell types (Ciaglia *et al.* 2020; Lan *et al.* 2020; Michaud *et al.* 2020; Ulrich and Ulrich 2021). The association between SARS-CoV-2 and ACE2 makes it significant to examine the biology of ACE2 in animal models employing chemical agents such as Cadmium (Cd) which can alter ACE2 levels.

Cd is a carcinogen and one of the 10 chemicals of concern for health according to the World Health Organization (Huff *et al.* 2007; Wang and Du 2013; Andjelkovic *et al.* 2019). Cd is commercially used in television screens, lasers, batteries, paint pigments, cosmetics, in galvanising steel and as a barrier in nuclear fission (Bernhoft 2013). Approximately 30% of Cd deposits in the liver and 30% in the kidneys, with the rest distributed throughout the body, thus resulting in systemic dysfunctions such as neurotoxicity (Wang and Du 2013; Batool *et al.* 2019), skin alopecia and ulceration (Lansdown *et al.* 2001), inflammation and hepato-toxicity (Bernhoft 2013; Wang and Du 2013; Andjelkovic *et al.* 2019).

Cd induces toxicity via induction of increased oxidative stress and alterations of metalloproteases such as ACE in body organs. Dose-dependent Cd-exposure resulted in decreased ACE levels in the serum (Puri and Saha 2003; Broseghini-Filho *et al.* 2015) and lungs (Broseghini-Filho *et al.* 2015) of rats. However, Cd reportedly had no effects on ACE levels in the aorta and kidney of rats (Broseghini-Filho *et al.* 2015). Cd generally exists as a divalent cation, complexed with other elements, such as cadmium chloride (CdCl) (Bernhoft 2013; Andjelkovic *et al.* 2019). Furthermore, since Cd has been reported to adversely alter ACE2 levels, it is pertinent to examine the effects of plant's candidates on ACE2 levels in Cd-induced toxicity.

In this study, the most active antioxidant and antimicrobial cyto-toxic compounds were isolated from MO leaves (MO11) and MS suckers (MS06), respectively, using column chromatography and spectroscopic analyses. There are paucity of studies which isolated and evaluated the active drug candidates from MO leaves and MS sucker, and no previous study examined the effects of MO leaves and MS suckers on ACE2-immunomodulations. Therefore, this study evaluated the list of compounds present in MO11 and MS06, and equally examined the effects of MO11 and MS06 on ACE2 levels in the blood, kidney and liver samples of rats in CDCl-induced toxicity.

MATERIALS AND METHODS

Ethical Approval

Ethical approval for this study was sought and received from the Ethical Review Committee of the University of Ilorin, Nigeria. Appropriate measures were observed to ensure minimal pain or discomfort of rats used in this study. The ethical approval number is UERC/ASN/2018/1161. Furthermore, this research study was conducted in accordance with the internationally accepted principles for laboratory animal use and care as provided in the European Community guidelines (EEC Directive of 1986; 86/609/EEC), the Directive

2010/63/Eu of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes and the Guidelines of the U.S. Public Health Service and NIH regarding the care and use of animals for experimentation (NIH publication #85-23, revised in 985).

Collection, Authentication and Deposition of MO11 and MS06

Freshly cut leaves of MO11 and MS06 were obtained locally from forest reserves in Ilorin and samples identified and authenticated by a Pharmaceutical Botanist of the Department of Botany, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria. Samples of MO11 and MS06 were deposited at the herbarium of the Department of Botany, Faculty of Life Sciences, University of Ilorin, and assigned Herbarium Identification Numbers UILH/001/1249 and UILH/002/1182 respectively.

Extraction and Partitioning of Fractions of MO11

MO11 were air-dried, grinded, weighed and stored in an air tight container until further analysis. MO leaves weighing 4.0 kg were powdered, extracted with distilled ethanol and concentrated on a rotary evaporator. The crude extract (210.2 g) was successively partitioned into n-hexane (NH), dichloromethane (DCM), ethyl acetate (EA) and methanol (MeOH) soluble fractions in an increasing order of polarity to afford 12 fractions (MO1–MO12).

Extraction and Partitioning of Fractions of MS06

MS06 were air-dried, grinded, weighed and stored in an air tight container until further analysis. MS06 weighing 5.2 kg were powdered, extracted with distilled ethanol and concentrated on a rotary evaporator. The crude extract (159.32 g) was successively partitioned into n-hexane (NH), dichloromethane (DCM), ethyl acetate (EA) and methanol (MeOH) soluble fractions in an increasing order of polarity to afford 8 fractions (MS01–MS08). Phytochemical screening of MS showed the presence of saponins, saponin glycosides, tannins, alkaloids and indole alkaloids.

Column Chromatography

Column chromatography of the MO and MS fractions was carried out on silica gel (70–230 and 240–300 mesh size, Merck, Germany), Merck alumina (70–230 mesh ASTM). Thin layer chromatography (TLC) was carried out on pre-coated silica gel 60 F_{254} aluminium foil (Merck, Germany) for the establishment of the purity of isolates. Spots on TLC were examined with an ultraviolet lamp operating at a wavelength of 366 nm for fluorescence and at 254 nm for fluorescence quenching spots.

Evaluations of Antioxidant and Antimicrobial Activities of MO and MS Fractions

Antioxidant activities of plants' extracts were evaluated using modified 2,2- diphenyl-1-picrylhydrazyl method as previously described by Chaves et al. (2020). In addition, antimicrobial activities of plants' extracts were evaluated by testing the cyto-toxic potentials of each fraction against the growths of *Escherichia coli* and *Salmonella tiphimurium* as previously described by Elisha et al. (2017).

Purification of MO Fractions

MO8 and MO11 fractions which had the best antioxidant and antimicrobial potentials were selected and further purified on a silica gel open column, using NH, DCM, EA and MeOH in an increasing order of polarity until the most active antioxidant and anti-microbial isolates ($MO11_{8,3}$ and $MO11_{8,4}$) were obtained. Phytochemical screenings of $MO11_{8,3}$ and $MO11_{8,4}$ showed the presence of flavonoids, saponin, tannins, alkaloids, glycosides and steroids. The resulting grams of $MO11_{8,3}$ and $MO11_{8,4}$ were mixed together as 1.43 g of MO11, which was further tested for its ACE2-effects in this study.

Purification of MS Fractions

The antioxidant and antimicrobial activities of the obtained eight MS fractions (MS01–MS08) were determined. MS06 which had the best antioxidant and antimicrobial potential was selected and further purified on a silica gel open column, using NH, DCM, EA and MeOH in an increasing order of polarity to afford seven fractions ($MS06_{1–7}$). $MS06_5$ showed the best antioxidant and antimicrobial potential. The resulting 1.24 g of $MS06_5$ was further tested for its ACE2-effects in this study.

Liquid Chromatography-Mass Spectrometry (LC-MS) Analyses of $MO11_{8,3}$, $MO11_{8,4}$ and $MS06_5$

The MO isolates ($MO11_{8,3}$ and $MO11_{8,4}$) and MS isolate ($MS06_5$) were submitted for LC-MS analysis at the Chemical Purification Analysis and Screening Core Facility, University of South Florida, Tampa, Florida, USA. Low resolution mass spectra were recorded on an Agilent Technologies LC/MSD VL electrospray ionisation mass spectrometer. High resolution mass spectra were recorded on an Agilent Technologies LC/MSD TOF electrospray ionisation spectrometer.

Animal Care and Feeding

Twenty-four adult male Wistar rats (average weight of 155 g and 2 months old of age) were purchased from a colony breed at Badagry in Lagos state, Nigeria. The rats were randomly divided into six groups with four rats per group. The rats were acclimatised for a week at the animal house of the Faculty of Pharmacy of Olabisi Onabanjo University, Sagamu Campus, Ogun State, Nigeria before the beginning of experimental procedures. The rats were kept under standard conditions and allowed free access to food and drinking water ad libitum. The experimental procedure lasted for 18 days.

Grouping of Rats and Extracts/Drugs Administration

MO11 and MS11 were dissolved in olive oil (vehicle). Rats of Control Group 1 (baseline control) received physiological saline only for 17 days (Days 1–17). Each rat of Experimental Groups 2–4 received single intra-peritoneal administration of 1.5 mg/kg bodyweight CdCl on Day 1. Rats of Group 2 (negative control) were left untreated throughout experimental procedure for 17 days (Days 1–17). Thereafter, rats of Group 3 were post-treated with 15 mg/kg bodyweight of MO11 for 17 days (Days 1–17). Rats of Group 4 were post-treated with combined mixture of 15 mg/kg bodyweight of MO11 and 7 mg/kg bodyweight of MS06

for 17 days (Days 1–17). Rats of Group 5 received only 15 mg/kg bodyweight of MO11 for 17 days (Days 1–17). Rats of Group 6 received only 1 mL/kg bodyweight of vegetable oil (vehicle) for 17 days (Days 1–17).

Completion of Experimental Procedures and Collections of Sera and Tissue Samples

Twenty-four hours after the last day of administration of drugs and extracts on Day 17, the experimental procedures were completed following animal sacrifice of rats on Day 18. Thereafter, the thoracic and abdominal cavities of each rat were opened, blood samples were obtained by cardiac puncture and the kidney and liver excised and removed.

Evaluations of ACE2 in Blood Samples and Homogenates of Kidney and Liver in Rats Using Enzyme Linked Immunosorbent Assay (ELISA)

Blood samples were centrifuged at 10,000 revolutions per min (rpm) for 20 min. The supernatant was used for ELISA analyses of ACE2 levels. In addition, excised kidney and liver samples from each rat were subjected to thorough homogenisation using porcelain mortar and pestle in ice-cold 0.25 M sucrose, in the proportion of 1 g to 4 mL of 0.25 M sucrose solution. The tissue homogenates were filled up to 5 mL with additional sucrose and collected in a 5 mL serum bottle. Homogenates were centrifuged at 3,000 rpm for 15 min using a centrifuge (Model 90-1). The supernatant was collected with pasteur pipettes and placed in a freezer at –4°C, and thereafter assayed for levels of ACE2 in homogenates of the kidney and liver of all rats using ELISA technique as previously described by Akinlolu *et al.* (2021).

Statistical Analyses

Statistical analyses were conducted using the 2019 Statistical Package for the Social Science software version 23.0. Computed data of ACE2 levels were expressed as arithmetic means ± standard error of mean and were subjected to statistical analyses using One-way Analysis of Variance to test for significant difference amongst Groups 1–6. Degree of freedom (*df*) between groups and within groups, and *F*-values were computed. Significant difference was confirmed at 95% confidence interval with associated *p*-value of less than 0.05 ($p \leq 0.05$). In addition, Scheffe post-hoc analysis was used for separation of mean values amongst Groups 1–6. The statistical comparison of the ACE2 levels between two groups was considered significant only at $p \leq 0.05$.

RESULTS

LC-MS Analyses of $MO11_{8,3}$, $MO11_{8,4}$ and $MS06_5$ Isolates

The list of compounds present in each of $MO11_{8,3}$ (57 compounds), $MO11_{8,4}$ (12 compounds) and $MS06_5$ (39 compounds) isolates are as presented in Tables 1–3.

Table 1: List of compounds present in isolate MOF11.

S/N	Compound	Formula
1.	Glucosamine	$C_6H_{13}NNaO_5$
2.	L-2-aminoadipic acid	$C_6H_{12}NO_4$
3.	Dyphylline	$C_{10}H_{15}N_4O_4$
4.	Idebenone metabolite	$C_{15}H_{22}NaO_6$
5.	Ala Pro	$C_8H_{15}N_2O_3$
6.	Cytarabine	$C_9H_{14}N_3O_5$
7.	Cytosin	$C_4H_6N_3O$
8.	Isoamyl nitrite	$C_5H_{12}NO_2$
9.	Artemisinin	$C_{15}H_{22}NaO_5$
10.	Adenine	$C_5H_6N_5$
11.	4-(diaminomethyllideneamino) butanoic acid	$C_5H_{12}N_3O_2$
12.	Pyroglutamic acid	$C_5H_8NO_3$
13.	Leucine	$C_6H_{14}NO_2$
14.	p-Coumaric acid	$C_9H_9O_3$
15.	N-acetylarylamine	$C_8H_{10}NO$
16.	6-Methoxy-2-naphthylacetic acid	$C_{13}H_{13}O_3$
17.	Indanone	C_9H_9O
18.	1-Indanone	C_9H_9O
19.	p-Hydroxycinnamaldehyde	$C_9H_9O_2$
20.	Perindoprilat glucuronide	$C_{23}H_{37}N_2O_{11}$
21.	6-Methoxy-2-naphthylacetic acid	$C_{13}H_{13}O_3$
22.	Glu Phe His	$C_{20}H_{26}N_5O_6$
23.	N-acrylylglycine methyl ester	$C_6H_{10}NO_3$
24.	Zidovudine	$C_{10}H_{14}N_5O_4$
25.	2-amino-4-hydoxy-propiophenone	$C_9H_{12}NO_2$
26.	Guanosine	$C_{10}H_{14}N_5O_5$
27.	Guanine	$C_5H_6N_5O$
28.	Terbutaline-1-glucuronide	$C_{18}H_{28}NO_9$
29.	Tranexamic acid	$C_8H_{16}NO_2$
30.	17-Epiestriol	$C_{18}H_{24}NaO_3$
31.	Leu Pro	$C_{11}H_{21}N_2O_3$
32.	L-Glutamic acid n-butyl ester	$C_9H_{18}NO_4$
33.	Methylprednisolone acetate	$C_{24}H_{32}NaO_6$
34.	Pro His Tyr	$C_{20}H_{26}N_5O_5$
35.	2-Amino-8-oxo-9,10-epoxy-decanoic acid	$C_{10}H_{18}NO_4$
36.	N-heptanoyl-homoserine lactone	$C_{11}H_{20}NO_3$
37.	2R-aminoheptanoic acid	$C_7H_{16}NO_2$
38.	Lunarine	$C_{25}H_{31}N_3NaO_4$
39.	Cys Leu Thr	$C_{26}H_{50}N_6NaO_{10}S_2$
40.	Indoramin	$C_{22}H_{25}N_3NaO$
41.	Phenacetine	$C_{10}H_{14}NO_2$
42.	Trp Arg Asp	$C_{21}H_{29}N_7NaO_6$
43.	Protoporphyrin IX	$C_{34}H_{36}N_4NaO_4$

(continued on next page)

Table 1: (*continued*)

S/N	Compound	Formula
44.	Norharman	$C_{11}H_9N_2$
45.	Swietenine	$C_{32}H_{40}NaO_9$
46.	Azobenzene	$C_{12}H_{11}N_2$
47.	Aceclidine	$C_9H_{16}NO_2$
48.	Levobunolol	$C_{17}H_{26}NO_3$
49.	Tetradecylamine	$C_{14}H_{32}N$
50.	Lys Cys His	$C_{15}H_{27}N_6O_4S$
51.	1-Hexadecylamine	$C_{16}H_{36}N$
52.	Penbutolol	$C_{18}H_{30}NO_2$
53.	Linoleamide	$C_{18}H_{34}NO$
54.	N-methyl-gabapentin	$C_{10}H_{20}NO_2$
55.	9,1,14-Octadecatrienoic acid	$C_{18}H_{31}NO_2$
56.	(Z)-N-(2-hydroxyethyl)hexadec-7-enamide	$C_{18}H_{36}NO_2$
57.	Oleamide	$C_{18}H_{36}NO$

Table 2: List of compounds present in isolate MOF11.

S/N	Compound	Formula
1.	Isoamyl nitrite	$C_5H_{12}NO_2$
2.	Tropine	$C_8H_{16}NO$
3.	Acetilidine	$C_9H_{16}O_2$
4.	Gabapentin	$C_9H_{18}NO_2$
5.	2-Chlorophenyl	$C_{19}H_{16}Cl$
6.	Tetradecylamine	$C_{14}H_{32}N$
7.	Penbutolol	$C_{18}H_{30}NO_2$
8.	1-Hexadecylamine	$C_{16}H_{36}N$
9.	Linoleamide	$C_{18}H_{34}NO$
10.	N-methyl-Gebapentin	$C_{10}H_{20}NO_2$
11.	9,12,14-Octadecatrienoic acid	$C_{18}H_{31}O_2$
12.	(+/-) 14,15-EpETrE	$C_{40}H_{64}NaO_6$

Table 3: List of compounds present in isolate MS06.

S/N	Compound	Formula
1.	Dimethylglycine	$C_4H_9NO_2$
2.	Pyroglutamic Acid	$C_5H_7NO_3$
3.	O-acetylserine	$C_5H_9NO_4$
4.	Isoarmyl nitrate	$C_5H_{11}NO_2$
5.	5-Oxo-7-decynoic acid	$C_{10}H_{14}O_3$
6.	Jasmonic acid	$C_{12}H_{14}O_3$

(continued on next page)

Table 3: (*continued*)

S/N	Compound	Formula
7.	5-Methyl cytosine	$C_5H_7N_3$
8.	Gabapentin	$C_9H_{17}NO_2$
9.	Ethoxyquin	$C_{14}H_{19}NO$
10.	5-(4-Hydroxy-2,5-dimethyl phenoxy)-2,2 dimethyl-pentanoic acid	$C_{15}H_{22}O_4$
11.	O-desmethylvenlafaxine	$C_{15}H_{25}NO_2$
12.	Dyclonine	$C_{18}H_{27}NO_2$
13.	Betaxolol	$C_{18}H_{29}NO_3$
14.	Penbutolol	$C_{18}H_{29}NO_2$
15.	Phe.His	$C_{15}H_{18}N_4O_3$
16.	Hydroxytolbutamide	$C_{12}H_{18}N_2O_4S$
17.	Tetradecylamine	$C_{14}H_{32}N$
18.	Androota-4-dien-3, 17 dione	$C_{19}H_{24}O_2$
19.	Descaboethoxyloratadine	$C_{19}H_{19}ClN_2$
20.	Phytosphingosine	$C_{18}H_{39}NO_3$
21.	Estrone Hemisuccinate	$C_{22}H_{26}O_5$
22.	1-Hexadecylamine	$C_{16}H_{35}N$
S/N	**Compound**	**Formula**
23.	10-Pentadecenal	$C_{15}H_{28}O$
24.	Linoleamide	$C_{18}H_{33}NO$
25.	Dodecaramide	$C_{12}H_{25}NO$
26.	6-(3)-ladderane-1-hexanol	$C_{18}H_{30}O$
27.	N-methyl-galapentin	$C_{30}H_{19}NO_2$
28.	12-Oxo-9-octadecynoic acid	$C_{18}H_{30}O_3$
29.	(Z)-N-(2,-hydroxyl ethyl) hexades-7-enamide	$C_{18}H_{35}NO_2$
30.	7-Hydroxyl-10E, 16-heptade cadien-8-ynoic acid	$C_{17}H_{26}O_3$
31.	N-acetylsphigosine	$C_{20}H_{39}NO_3$
32.	Ergata-5,7,22,24(28)-tettaene-3 beta-ol	$C_{28}H_{42}O$
33.	Oleamide	$C_{18}H_{35}NO$
34.	9,12-Octadecadienal	$C_{18}H_{32}O$
35.	3,3,5,5-Tetra-tert-4,4-dihydroxybiphenyl	$C_{28}H_{42}O_2$
36.	Strearamide	$C_{18}H_{37}NO$
37.	12-Beta-hydroxyl-3-oxo-5 beta-cholan-24-oic acid	$C_{24}H_{38}O_4$
38.	C_{16}OH sulfatide	$C_{40}H_{77}NO_{12}S$
39.	Leu leu pro	$C_{17}H_{31}N_3O_4$

Table 4: ACE2 levels (ng/mL) in blood, kidney and liver of rats.

Drug/Extract administered	Blood	p-value: Group 1 versus Groups 2–6	Kidney	*p*-value: Group 1 versus Groups 2–6	Liver	*p*-value: Group 1 versus Groups 2–6
Normal saline	21.12 ± 2.02^{b}		83.10 ± 5.17^{ab}		32.12 ± 0.41^{b}	
CdCl only	39.39 ± 3.15^{ab}	0.02*	85.39 ± 3.10^{a}	0.99	28.34 ± 5.07^{c}	0.99
CdCl-exposure + MO11 post-treated group	42.57 ± 0.71^{a}	0.01*	61.29 ± 3.59^{c}	0.02*	11.58 ± 3.87^{d}	0.17
CdCl-exposure + MO11 + MS06 post-treated group	20.63 ± 4.33^{c}	0.99	16.11 ± 0.49^{e}	< 0.001*	34.70 ± 6.37^{b}	0.99
MO11 only	21.84 ± 1.26^{b}	0.99	37.17 ± 1.52^{d}	< 0.001*	38.43 ± 2.93^{ab}	0.96
Olive oil only	11.52 ± 4.01^{d}	0.46	28.46 ± 2.79^{d}	< 0.001*	107.31 ± 9.71^{a}	< 0.001*

Note: Results of one-way ANOVA from Days 1–17; Mean ± SEM across the columns between groups are significantly different with a > ab > b > c > d > e (n = 4 per group).

Sera Levels of ACE2

Results showed statistically significant higher levels ($p \leq 0.05$) of ACE2 in sera samples of rats of Group 2 (df = 5, 11; F = 17.97; p = 0.02), when compared with Control Group 1 (Table 4 and Figure 1). In addition, results showed statistically significant higher levels ($p \leq 0.05$) of ACE2 in sera samples of rats of Group 3 (df = 5, 11; F = 17.97; p = 0.01), when compared with Control Group 1 (Table 4 and Figure 1). However, results showed statistically non-significant lower levels ($p \geq 0.05$) of ACE2 in rats of Group 4 (df = 5, 11; F = 17.97; p = 0.99), when compared with Control Group 1 (Table 4 and Figure 1). Furthermore, results showed statistically non-significant similar levels ($p \geq 0.05$) of ACE2 in sera samples of rats of Group 5 (df = 5, 11; F = 17.97; p = 0.99), when compared with Control Group 1 (Table 4 and Figure 1). In addition, results showed statistically non-significant lower levels ($p \geq 0.05$) of ACE2 in sera samples of rats of Group 6 (df = 5, 11; F = 17.97; p = 0.46), when compared with Control Group 1 (Table 4 and Figure 1).

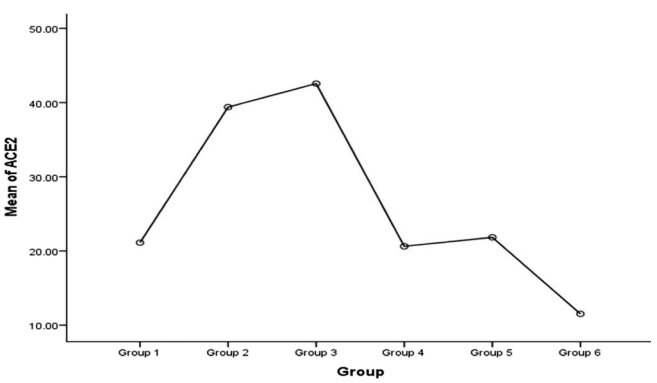

Figure 1: ACE2 levels (ng/mL) in blood of rats.

ACE2 Levels in Kidney Homogenates

Results showed statistically non-significant higher levels ($p \geq 0.05$) of ACE2 in kidney homogenates of rats of Group 2 (df = 5, 11; F = 64.67; p = 0.99), when compared with Control Group 1 (Table 4 and Figure 2). In addition, results showed statistically significant lower levels ($p \leq 0.05$) of ACE2 in kidney homogenates of rats of Group 3 (df = 5, 10; F = 64.67; p = 0.02) and Group 4 (df = 5, 10; F = 64.67; $p < 0.001$), when compared with Control Group 1 (Table 4 and Figure 2). Furthermore, results showed statistically significant lower levels ($p \leq 0.05$) of ACE2 in kidney homogenates of rats of Group 5 (df = 5, 10; F = 64.67; $p < 0.001$) and Group 6 (df = 5, 10; F = 64.67; $p < 0.001$), when compared with Control Group 1 (Table 4 and Figure 2).

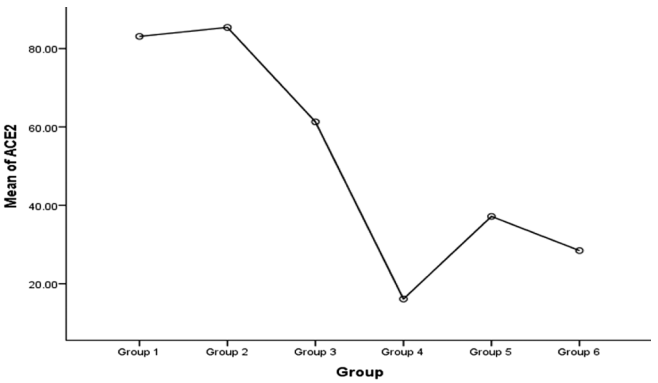

Figure 2: ACE2 levels (ng/mL) in kidney of rats.

ACE2 Levels in Liver Homogenates

Results showed statistically non-significant lower levels ($p \geq 0.05$) of ACE2 in liver homogenates of rats of Group 2 (df = 5, 11; F = 36.58; p = 0.99), when compared with Control Group 1 (Table 4 and Figure 3). In addition, results showed statistically non-significant lower levels ($p \geq 0.05$) of ACE2 in liver homogenates of rats in rats of Group 3 (df = 5, 11; F = 36.58; p = 0.17), when compared with Control Group 1 (Table 4 and Figure 3). However, results showed statistically non-significant higher levels ($p \geq 0.05$) of ACE2 in liver homogenates of rats of Group 4 (df = 5,11; F = 36.58; p = 0.99), when compared with Control Group 1 (Table 4 and Figure 3). Furthermore, results showed statistically non-significant higher levels ($p \geq 0.05$) of ACE2 in liver homogenates of rats of Group 5 (df = 5, 11; F = 36.58; p = 0.96), but significant higher levels ($p \leq 0.05$) in liver homogenates of rats of Group 6 (df = 5, 11; F = 36.58; $p < 0.001$), when compared with Control Group 1 (Table 4 and Figure 3).

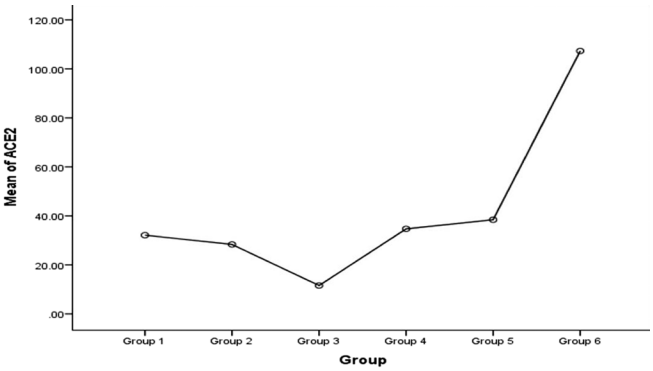

Figure 3: ACE2 levels (ng/mL) in liver of rats.

DISCUSSION

LC-MS analyses showed the presence of therapeutic agents in MO11 and MS06 such as Artemisinin (antimalarial) (Akinlolu *et al.* 2012), Glutamic acid (antioxidant, anti-cancer and vasodilator) (Dutta *et al.* 2013), Guanine (anticancer) (Dasari and Tchounwou 2014) and several others (Tables 1–3). These plants' derived constituents confirm the potential therapeutic capabilities of MO11 and MS06 in the treatments of many diseases such as cancers, neurodegenerative diseases, malaria, Sickle Cell Anemia and many others which are associated with oxidative stress, inflammation, vaso-occlusion and hyperplasia.

Previous studies reported shared 14 amino acids positions employed by the receptor-binding motif (RBM) of each of SARS-CoV-2 and SARS-CoV to bind to ACE2 (Lan *et al.* 2020; Ulrich and Ulrich 2021). Of these 14 amino acids positions, 1 amino acid residues location is at the Gln498/Tyr484 location, where Tyr484 of SARS-CoV and Gln498 of SARS-CoV-2 both interact with Asp38, Gln42, Leu45, Lys353 and Tyr41 of ACE2. Eight of the fourteen amino acids positions, have similar residues between the two receptor binding domains (RBDs) viz: Gly496/Gly482, Tyr449/Tyr436, Tyr453/Tyr440, Tyr489/Tyr475, Tyr505/Tyr491, Asn487/Asn473, Thr500/Thr486 and Gly502/Gly488. Five of the different 14 amino acids positions (Asn501/Thr487, Gln493/Asn479, Leu455/Tyr442, Phe456/Leu443 and Phe486/Leu472), though possess different side chains, still possess similar biochemical properties. Four of the six amino acids positions with dissimilar residues are SARS-CoV residues (Asn479, Leu472, Thr487 and Tyr442), which are relevant to ACE2-binding (Ulrich and Ulrich, 2021).

Results of LC-MS analyses of $MOF11_{8,3}$ showed the presence of Pro His Tyr ($C_{20}H_{26}N_5O_5$), N-Acrylylglycine methyl ester ($C_6H_{10}NO_3$), Cys Leu Thr ($C_{26}H_{50}N_6NaO_{10}S_2$), 2-chlorophenyl ($C_{19}H_{16}Cl$), Glu Phe His ($C_{20}H_{26}N_5O_6$), Phe His ($C_{15}H_{18}N_4O_3$), 3,3,5,5-Tetra-tert-4,4-dihydroxybiphenyl ($C_{28}H_{42}O_2$) and Trp Arg Asp ($C_{21}H_{29}N_7NaO_6$) (Table 1). In addition, LC-MS analyses of $MOF11_{8,4}$ showed the presence of Lys Cys His ($C_{15}H_{27}N_6O_4S$) (Table 2), while LC-MS analyses showed the presence of Dimethylglycine ($C_4H_9NO_2$), Leu leu pro ($C_{17}H_{31}N_3O_4$) and Phe His $C_{15}H_{18}N_4O_3$ in $MS06_5$ (Table 3). These results indicate that MO11 and MS06 may have possible potentials to compete with SARS-CoV-2 in binding to ACE2, and may also be able to distract and prevent host-entry and invasion of SARS-CoV-2. However, further evaluations are required to establish the possible anti-SARS-CoV-2-ACE2 binding in *in vitro* and *in vivo* models.

In addition, previous studies noted that increased plasma or soluble ACE2 levels are not beneficial to the body system, and that increased plasma ACE2 levels aid higher COVID-19 mortality rate (Ciaglia *et al.* 2020; Lan *et al.* 2020; Michaud *et al.* 2020; Ulrich and Ulrich 2021). Results of this study showed increased sera ACE2 levels in rats of CdCl-only treated Group 2, when compared with normal saline-only treated Control Group 1 (Table 4). These results indicate that CdCl-exposure altered and increased non-beneficial sera ACE2 levels in rats.

The results of this study showed decreased levels of sera ACE2 in rats treated with MO11 only and rats of CdCl-exposure + MO11 + MS06 post-treated Group 4, when compared with normal saline-only treated Control Group 1 (Table 4). These results indicate that MO11 and MO11 + MS06 could aid decreased plasma ACE2 levels and could be of interest to the ongoing efforts towards the discovery of anti-plasma ACE2 agents.

The findings of this study showed approximately similar ACE2 levels in liver homogenates of rats of CdCl-only treated Group 2, CdCl-exposure + MO11 + MS06 post-treated Group 4 and MO11-only treated Group 5 rats, when compared with Normal saline-only treated Control Group 1 (Table 4). These results indicate that CdCl-exposure, CdCl-exposure + MO11 + MS06 and MO11-only did not significantly alter liver ACE2 levels in rats.

The kidney is the main target organ of the RAAS and Acute kidney injury is intimately related to higher morbidity and mortality in COVID-19. ACE2 and Transmembrane serine protease 2 (TMPRSS2)-genes which aid SARS-CoV-2 cell entry and infection are ubiquitously present in many cell types of the kidney. This explains the increased SARS-CoV-2 load in kidney cell types as well as the association between Acute kidney injury and higher morbidity and mortality in COVID-19 (Lan *et al.* 2020). Hence, the relevance of the emphasis on kidney ACE2 levels in the discovery of possible anti-SARS-CoV-2 agents.

There is paucity of reports on Cd-effects on ACE2 levels. However, Broseghini-Filho *et al*. (2015) reported that Cd had no effects on ACE (not ACE2) levels in the aorta and kidney. Therefore, obtained kidney ACE2 levels in this study probably provide a reference point on the effects of MO11 and MS06 on kidney ACE2 levels. The results of this study showed approximately similar ACE2 levels in kidney homogenates of rats of CdCl-only treated Group 2, when compared with Normal saline-only treated Control Group 1 (Table 4).

Results showed decreased levels of kidney ACE2 in rats treated with CdCl-exposure + MO11 post-treated Group 3, CdCl-exposure + MO11 + MS06 post-treated Group 4 and MO11-only treated Group 5, when compared with Normal saline-only treated Control Group 1 (Table 4). These results indicate that administrations of MO11 and MS06 possibly reduced kidney ACE2 levels in rats.

Generally, it may be that the presence of some amino acids in MO11 and MS06, which are part of the shared 14 amino acids positions used by SARS-CoV and SARS-CoV-2 for ACE2 binding could possibly be responsible for the decreased ACE2-effects of MO11 and MS06. It is, therefore, possible that MO11 and MS06 especially when combined may compete with SARS-CoV and SARS-CoV-2 for ACE2 binding. This is recommended for further evaluations.

CONCLUSION

Overall, this study evaluated the effects of MO11 (isolated from MO leaves) and MS06 (isolated from MS suckers) on ACE2 levels in blood, kidney and liver in Cadmium chloride (CdCl)-induced toxicity in rats. Based on results and discussion of the findings

of this study, MO11 and MS06 possess the potentials to decrease ACE levels. Hence, further evaluations are required and recommended to establish the possible anti-SARS-CoV-2-ACE2 binding in *in vitro* and *in vivo* models.

ACKNOWLEDGEMENTS

The authors acknowledge the technical support of Laboratory staff members of the Department of Chemistry of the University of Ilorin, Nigeria and the Central Research Laboratory, Ilorin, Nigeria.

REFERENCES

AEKTHAMMARAT, D., PANNANGPETCH, P. & TANGSUCHARIT, P. (2019). *Moringa oleifera* leaf extract lowers high blood pressure by alleviating vascular dysfunction and decreasing oxidative stress in L-NAME hypertensive rats, *Phytomedicine*, 54: 9–16. https://doi.org/10.1016/j.phymed.2018.10.023

AKINLOLU, A., KASIM, L. S. & SHOKUNBI, M. T. (2012) Neurotoxic effects of the administration of artemisinin combination therapy (artemether and quinine) and ascorbic acid on the cytoarchitecture of the cerebellum and trapezoid nuclei in adult rats, *African Journal of Medicine and Medical Sciences*, 41(Supplement*)*: 149–155. https://www.ncbi.nlm.nih.gov/pubmed/23678651

AKINLOLU, A. A., AMEEN, M., QUADRI, T., ODUBELA, O., OMOTOSO, G., YAHYA, R. *et al*. (2020) Extraction, isolation and evaluation of anti-toxic principles from *Moringa oleifera* (MOF_6) and *Myristica fragrans* (Trimyristin) upregulated Acetylcholinesterase concentrations in sodium arsenite-induced neurotoxicity in rats, *Journal of Phytomedicine and Therapeutics*, 19(2): 503–519.

AKINLOLU, A. A., OYEWOPO, A. O., KADIR, R. E., LAWAL, A., ADEMILOYE, J., JUBRIL, A. *et al.* (2021) *Moringa oleifera* and *Musa sapientum* ameliorated 7,12-Dimethylbenz[a] anthracene-induced upregulations of Ki67 and Multidrug resistance1 genes in rats. *International Journal of Health Sciences*, 15(3): 26–33.

ANDJELKOVIC, M., BUHA, D. A., ANTONIJEVIC, E., ANTONIJEVIC, B., STANIC, M., KOTUR-STEVULJEVIC. J. *et al*. (2019) Toxic effect of acute Cadmium and Lead exposure in rat blood, liver, and kidney, *International Journal of Environmental Research and Public Health*, 16(2): 274. https://doi.org/10.3390/ijerph16020274

BERNHOFT, R. A. (2013) Cadmium toxicity and treatment, *Scientific World Journal*, Article ID 394652. https://doi.org/10.1155/2013/394652

BATOOL, Z., AGHA, F., TABASSUM, S., BATOOL, T. S., SIDDIQUI, R. A. & HAIDER, S. (2019) Prevention of cadmiuminduced neurotoxicity in rats by essential nutrients present in nuts, *Acta Neurobiologiae Experimentalis*, 79: 169–183.

BROSEGHINI-FILHO, G., ALMENARA, C., VESCOVI, M., FARIA, T., VASSALLO, D., ANGELI, J. *et al*. (2015) Acute Cadmium exposure reduces the local Angiotensin I converting enzyme activity and increases the tissue metal content, *Biological Trace Element Research*, 166: 149–156. https://doi.org/10.1007/s12011-015-0250-6

CHAVES, N., ANTONIO, S. & JUAN C. A. (2020) Quantification of the antioxidant activity of plant extracts: analysis of sensitivity and hierarchization based on the method used, *Antioxidants*, 9(1): 76. https://doi.org/10.3390/antiox9010076.

CIAGLIA, E., VECCHIONE, C. & PUCA, A. A. (2020) COVID-19 infection and circulating ACE2 levels: Protective role in women and children, *Frontiers Pediatrics*, 8: 206. https://doi.org/10.3389/fped.2020.00206

DASARI, S. & TCHOUNWOU, P. B. (2014) Cisplatin in cancer therapy: Molecular mechanisms of action, *European Journal of Pharmacology*, 740: 364–378. https://doi.org/10.1016/j.ejphar.2014.07.025

DIXIT, P., KUMAR, V. & SHUKLA, R. (2014) Therapeutic and medicinal effects of different parts of *Musa sapientum*, *VIVECHAN International Journal of Research*, 5(1): 62–68.

DUTTA, S., RAY, S. & NAGARAJAN, K. (2013) Glutamic acid as anticancer agent: An overview, *Saudi Pharmaceutical Journal*, 21(4): 337–343. https://doi.org/10.1016/j.jsps.2012.12.007

ELISHA, I. L., BOTHA, F. S., MCGAW, L. J. & ELOFF, J. N. (2017) The antibacterial activity of extracts of nine plant species with good activity against *Escherichia coli* against five other bacteria and cytotoxicity of extracts, *BMC Complement Alternative Medicine*, 17(1): 133. https://doi.org/10.1186/s12906-017-1645-z

HUFF, J., LUNN, R. M., WAALKES, M. P., TOMATIS, L. & INFANTE, P. F. (2007) Cadmium-induced cancers in animals and in humans, *International Journal of Occupational Environmental Health*, 13(2): 202–212. https://doi.org/10.1179/oeh.2007.13.2.202

LAN, J., GE, J., YU, J., SHAN, S., ZHOU, H., FAN, S., *et al*. (2020) Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor, *Nature*, 581: 215–220. https://doi.org/10.1038/s41586-020-2180-5

LANSDOWN, A. B., KING, H. & AUBERT, R. E. (2001) Experimental observations in the rat on the influence of cadmium on skin wound repair, *International Journal of Experimental Pathology*, 82 (1): 35–41.

MICHAUD, V., DEODHAR, M., ARWOOD, M., AL RIHANI, S. B., DOW, P. & TURGEON, J. (2020) ACE2 as a therapeutic target for COVID-19; Its role in infectious processes and regulation by modulators of the RAAS system, *Journal of Clinical Medicine*, 9: 2096. https://doi.org/10.3390/jcm9072096.

OMOTOSO, G. O., KADIR, E. R., LEWU, S. F., GBADAMOSI, I. T., AKINLOLU, A. A., ADUNMO, G. O. *et al*. (2018) *Moringa oleifera* ameliorates cuprizone-induced cerebellar damage in adult female rats, *Research Journal of Health Sciences*, 6(1): 13–25.

PURI, V. N. & SAHA, S. (2003) Comparison of acute cardiovascular effects of Cadmium and Captopril in relation to oxidant and Angiotensin converting enzyme activity in rats, *Drug and Chemical Toxicology*, 26(3): 213–218. https://doi.org/10.1081/DCT-120022646

ULRICH, O. W. & ULRICH, K. (2021) ACE2 and SARS-CoV-2: Tissue or plasma, good or bad?, *American Journal of Hypertension*, 34(3): 274–277.

WANG, B. & DU, Y. (2013) Cadmium and its neurotoxic effects, *Oxidative Medicine and Cellular Longevity*, Article ID 898034. https://doi.org/10.1155/2013/898034