Combinatorial Evaluation of Antiviral Activity of some Nigerian Medicinal Plants on SARS-CoV-2

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ABSTRACT

The coronavirus disease COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2(SARS-CoV-2) has presented unprecedented challenges to the healthcare systems in the world. There are no definite effective therapeutic agents or vaccines against the virus currently. However clinical management of the infection includes prevention, control measures, supportive care and repurposed drug therapy based on pathophysiology of the virus and manifestation of the disease condition thereby using antiviral agents such as remdesivir, lopinavir and favipiravir. Herbal preparations are being promoted for the management of Covid-19. Some selected Nigerian medicinal plants are hereby investigated by *In-silico* studies of the plant constituents. When compared with the listed therapeutic agents, the phytochemical constituents of the selected plants have better binding affinity to several Covid-19 viral target proteins. Also they were found to be safe for human use with LD_{50} of >2000 mg/Kg for the plant extracts. Some of the plants also contained phytochemicals that can be employed for the symptoms of covid-19.

Keywords: COVID-19; SAR-CoV-2; medicinal plants; phytochemical components; remdesivir; lopinavir; favipiravir.

1. INTRODUCTION

The novel coronavirus disease 2019 (COVID-19), caused by the Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) [1], has led to worldwide panic and global health concern since December 2019. It has since then been declared a pandemic by the world Health organization. The situation has continued to rapidly escalate and by the second week of June 2020, close to eight million cases of infection and more than 400,000 deaths have been reported [2] with hospitals and health systems overwhelmed in many countries. Given this scenario, there is an urgent need for drugs that can stop the novel coronavirus, save the lives of severely ill patients, protect health care workers and others at high risk of infection, and reduce hospitalization times [3]. But the novel corona virus has continued to present novel challenges: there are currently no proven effective vaccines or therapeutic agents against the virus [4]. Approaches have tended to focus on repurposing drugs currently being used for other indications [5]. The alternative is for research to develop new drugs and approaches. However, this is impracticable in the circumstance given the cost and time required to present a new drug of de novo design to the market by the traditional chemical synthesis [6]. Thus medicinal plants and herbal based drugs have been recently deployed. There are even reports that more than 85% of SARS-CoV-2-infected patients in China had received some forms of Traditional Chinese Medicine (TCM) treatments [7]. This is not surprising. A huge chemical diversity can be found amongst natural products, such as fungi, marine fauna and flora, bacteria and plants. But

because of the urgency in finding effective treatments for COVID-19, computational drug repositioning, a strategy that has low side effect risk, is low on investment and short R&D cycle has been adopted.

The SARS-CoV-2 Main Protease (Mpro) structure consists of protomers with three domains. These are domain I (Residues 1-99), domain II (100-184), domain (201- 303) and a long loop region (185-200) connecting domains II and III. His41 and Cys145 forms a catalytic dyad and its active site is located in a cleft between domains I and II [8].

The study evaluated the combinatorial efficacy of phytochemical compounds in the listed plants as potential inhibitors of SARS-CoV-2 main protease (Mpro) using *In Silico* methodology.

2. METHODS

2.1 Protein and Ligand Library Preparation

The crystal structure of the SARS- CoV-2 main protease target (7BUY.pdb) was downloaded from the protein data bank (https://www.rcsb.org/). Its original ligands (carmofur) and water were eliminated using Discovery Studio 4.5 Visualizer [9]. There dimensional (3D) structures of fifty nine (59) phytochemical constituents (Table 1) contained in the selected plants were downloaded from PubChem [10] and optimized in Discovery Studio 4.5 Visualizer [9]. Three therapeutic compounds currently in use for the treatment of covid-19 [4] were also downloaded and included in the ligand

library to serve as control. The downloaded therapeutic compounds were Lopinavir, Remdesivir and Favipiravir.

2.2 Virtual Screening

Ligands and Protein target for molecular docking were prepared in Autodock Tools using PyRx 0.8 package [11]. A grid box of dimensions x = - 26.467, y = 12.6894 and z = 58.7203 was employed. Docking simulations of bioactive conformations was done using Autodock Vina [12]. The ligands were inputted as Standard Database Files (.sdf files) while the target protein was inputted as a Protein Database File (.pdb file) then converted to the acceptable Protein Data Bank, Partial Charge (Q), & Atom Type (T) format. (.pdbqt file format) for Autodock Vina. Results obtained were analysed using PyMol [13] and Discovery Studio 4.5 Visualizer [9]. Pharmacokinetic and toxicity parameters of the phytochemical compounds were predicted using the vNN method [14].

3. RESULTS

3.1 Virtual Screening Result

Analysed virtual screening results of the binding energies (Kcal/mol) of phytochemical compounds contained in the plant constituents of the plants to SARS-CoV-2 molecular target (7BUY.pdb) were ranked and are presented in Table 1. Changes in binding energies (Kcal/mol) of the

phytochemicals present in each of the plants (Table 1) attests to the different binding affinities for SARS-CoV-2 (7BUY.pdb). The standards used in this study were Lopinavir, Remdesivir and Favipiravir with binding energies of - 5.7, - 5.0 and - 4.7 Kcal/mol, respectively.

Several non-covalent bonds such as hydrogen bonding, van der waal's force interactions, pi- pi stacking, alkyl interactions and carbon- hydrogen bonds were observed (Fig. 1). Compounds were seen not to be interacting with known active site residue; CYS145, rather they were seen to be interacting with possible allosteric sites on the target. The compounds were observed to be interacting with amino acid residues mostly in domain II and III and occasionally domain I. Several conformations of the phytochemicals were observed (Fig. 2) with remdesivir (2a) and luteolin-7-glucoside (2c) seen to be buried within the protein while 1,5-dicaffeoyl-quinic acid occupied a different allosteric site.

3.2 In Silico ADMETox Analysis

Toxicity predictions (Table 2) showed phytochemicals with varying degrees of ADMETox parameters such, blood brain barrier permeability, ability to induce liver injury and bioavailability. Caryophyllene and β-caryophyllene oxide were predicted to have the least Maximum Recommended Therapeutic Dose (MRTD) of 0.57 mg/day while 1,5-dicaffeoyl-quinic acid was predicted to be 20,900 mg/day based on a 60 kg adult weight.

Table 1. Docking studies of phytochemical compounds and their binding interactions

S/N	Plant Name	Phytochemical constituents	Binding Affinities (Kcal/mol)	Remarks	References
1.	*Nigella sativa*	Thymoquinone	- 5.0	Bioactive constituents showed better binding affinities than ****Favipiravir for the protein Target.	[15, 16]
		Thymol	- 4.9		
		P-cymene	- 4.7		
		Dithymoquinone	- 6.1		
		Thymohydroquinone	- 4.9		
		Carvacrol	- 5.1		
		α-pinene	- 4.4		
		Terpinene-4-ol	- 4.8		
2.	*Syzygium aromaticum*	Eugenol	- 4.6	Most of its Bioactive constituents had a binding affinity less than the control antivirals	[17, 18]
		Chavicol	- 4.6		
		β-Caryophyllene oxide	- 6.0		
3.	*Cinnamomum verum*	Eugenol	- 4.6	Binding affinities less than control	[19, 20]
		Trans-cinnamaldehyde	- 4.5		

S/N	Plant Name	Phytochemical constituents	Binding Affinities (Kcal/mol)	Remarks	References
4.	*Zingiber officinale*	Zingerone zingiberene Gingerol Shogaol	- 5.2 - 5.0 - 5.3 - 5.5	Binding affinities were observed to be better than ***Remdesivir but less than **Lopinavir	[21, 22]
5.	*Artemisia annua*	Artemisinin Artemisitene Arteanuine B 1,8-cineole	- 6.4 - 6.5 - 6.3 - 4.6	Showed great binding affinities compared to the control antivirals	[23, 24]
6.	*Vernonia amygdalina*	vernodalin, vernolide, hydroxyvernolide 1,5-dicaffeoyl-quinic acid chlorogenic acid luteolin-7-O-glucoside	- 6.3 - 6.2 - 6.1 - 7.5 - 6.6 - 6.7	Had the best predicted binding affinity, with all its' bioactive constituent showing great binding affinities better than the controls.	[25]
7.	*Allium sativum*	alliin allicin quercetin	- 4.3 - 3.6 - 6.4	One of its bioactive contituent showed a high binding affinity to the target.	[26, 27]
8.	*Ocimum gratissimum*	Eugenol Thymol Geraniol Cis- ocimene	- 4.6 - 4.9 - 4.3 - 4.0	This plant showed a poor binding affinity	[28, 29]
9.	*Garcinia kola*	GB1 GB2 GB-1a Kolaflavanone kolanone Garcifuran B	- 5.8 - 6.0 - 6.1 - 6.1 - 5.8 - 6.5	better binding affinities than the controls	[30]
10.	*Securidaca longipedunculata*	securinine presenegenin, Quercetin, Chlorogenic acid, 1,7-dihydroxy-4-methoxyxanthone	- 6.2 - 5.6 - 6.4 - 6.6 - 6.0	Better binding affinities than controls	[31]
11.	*Carissa edulis*	cryptomeridiol, nortrachelogenin, carinol. carissanol, kaempferol, quercetin, rhamnetin, isorhamnetin	- 6.2 - 5.9 - 6.6 - 5.9 - 6.0 - 6.4 - 6.2 - 6.5	Great predicted binding affinities	[32, 33, 34]
12.	*Cucumis metuliferus*	cucurbitacin B	- 6.6	Great predicted binding affinity	[35,36]
13.	*Salvia officinalis*	Chlorogenic acid, rosmarinic acid, luteolin-7-glucoside, Quercetin Rutin, caffeic acid.	- 6.6 - 7.2 - 6.7 - 6.4 - 6.1 - 5.5	With varying degrees of binding affinities, this plant had the second ranking bioactive compound better	[37]

S/N	Plant Name	Phytochemical constituents	Binding Affinities (Kcal/mol)	Remarks	References
		borneol	- 4.7	than the controls.	
		camphor,	- 4.8		
		caryophyllene,	- 6.0		
		cineole,	- 4.6		
		elemene,	- 5.6		
		humulene,	- 6.2		
		ledene,	- 5.6		
		pinene	- 4.6		
		oleanolic acid	- 6.2		
	***	Lopinavir	- 5.7		
		Remdesivir	- 5.0		
		Favipiravir	- 4.7		

***Antivirals currently used in the management of COVID-19 which served as control in the study
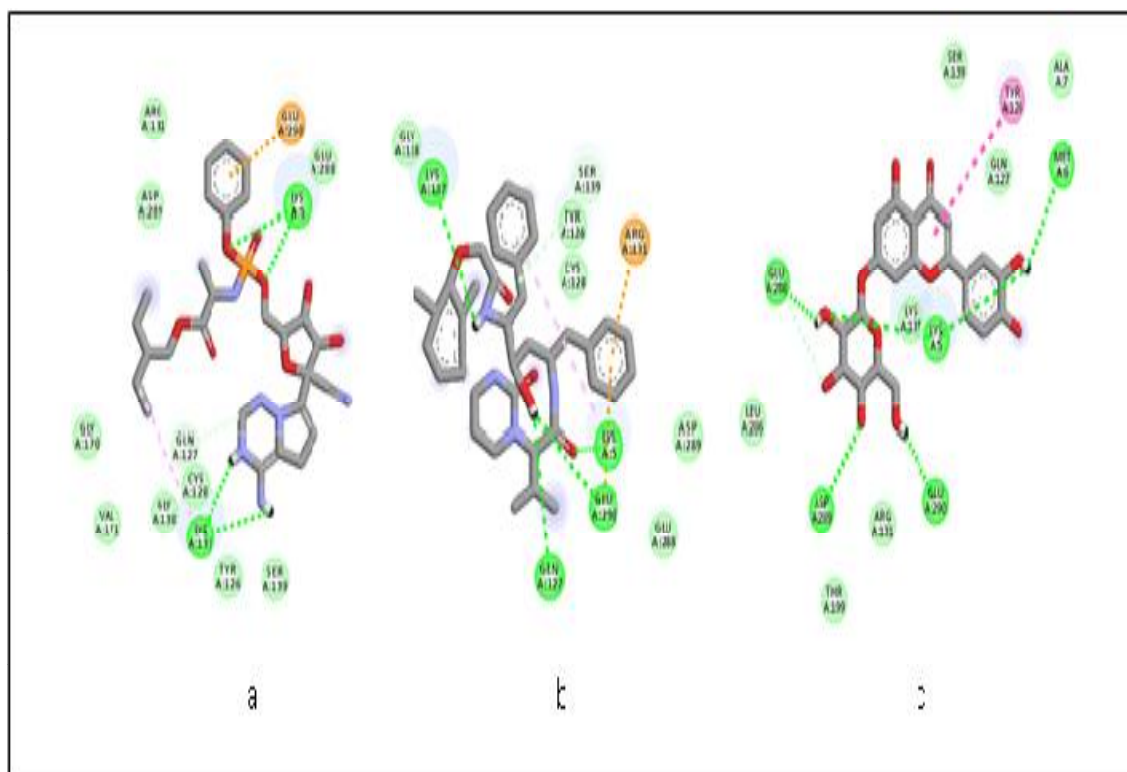

Fig. 1. Binding interactions of Remdesivir (a), 1,5-dicaffeoyl-quinic acid (b) and Luteolin-7-glucoside (c) to residues of SARS- CoV-2 (7BUY.pdb). Ligands are shown in stick forms while amino acid residues are shown in disc forms. Hydrogen- bond interaction with amino acid main chain are indicated by green discontinuous lines, green colored discs shows van der waal's interaction, pink discs shows alkyl interactions while purple discs shows pi- sigma interactions. Discs and lines in red colour represent unfavourable bumps and interactions

3.3 Liver Toxicity

DILI: Drug-induced liver injury (DILI) has been one of the most commonly cited reason for drug withdrawals from the market**.** Yes for if the drug causes drug-induced liver injury and No if not.

Cytotoxicity (HepG2): Cytotoxicity is the degree to which a chemical causes damage to cells.

3.4 Metabolism

HLM: The human liver microsomal (HLM) stability assay is commonly used to identify and exclude compounds that are too rapidly metabolized. For a drug to achieve effective therapeutic concentrations in the body, it cannot be metabolized too rapidly by the liver. Compounds with a half-life of 30 minutes or longer in an HLM assay are considered as stable; otherwise they are considered unstable. Longer half-life= achieve therapeutic conc. in body =Yes. Easily metabolized = less therapeutic conc. in body = No.

Cytochrome P450 enzyme (CYP) inhibition: CYPs constitute a superfamily of proteins that play an important role in the metabolism and detoxification of xenobiotics.

3.5 Membrane Transporters

BBB: The blood-brain barrier (BBB) is a highly selective barrier that separates the circulating blood from the central nervous system. We classified compounds with log BB values of less than –0.3 and greater than +0.3 as BBB non-permeable and permeable. (Yes= crosses BBB, No= does not cross BBB

Pgp Substrates and Inhibitors: P-glycoprotein (Pgp) is an essential cell membrane protein that extracts many foreign substances from the cell. Cancer cells often over-express Pgp, which increases the efflux of chemotherapeutic agents from the cell and prevents treatment by reducing the effective intracellular concentrations of such agents—a phenomenon known as multidrug resistance.

3.6 Others

- **hERG (Cardiotoxicity):** The human ether-à-go-go-related gene (hERG) codes for a potassium ion channel involved in the normal cardiac repolarization activity of the heart. Drug-induced blockade of hERG function can cause long QT syndrome, which may result in arrhythmia and death.
- **MMP (Mitochondrial Toxicity):** Given the fundamental role of mitochondria in cellular energetics and oxidative stress, mitochondrial dysfunction has been implicated in cancer, diabetes, neurodegenerative disorders, and cardiovascular diseases.
- **Mutagenicity (Ames test):** Mutagens are chemicals that cause abnormal genetic mutations leading to cancer. A common way to assess a chemical's mutagenicity is the Ames test.
- **MRTD:** The Maximum Recommended Therapeutic Dose (MRTD) is an estimated upper daily dose that is safe. The predicted MRTD value is reported in mg/day unit based upon an average adult weighing 60 kg.
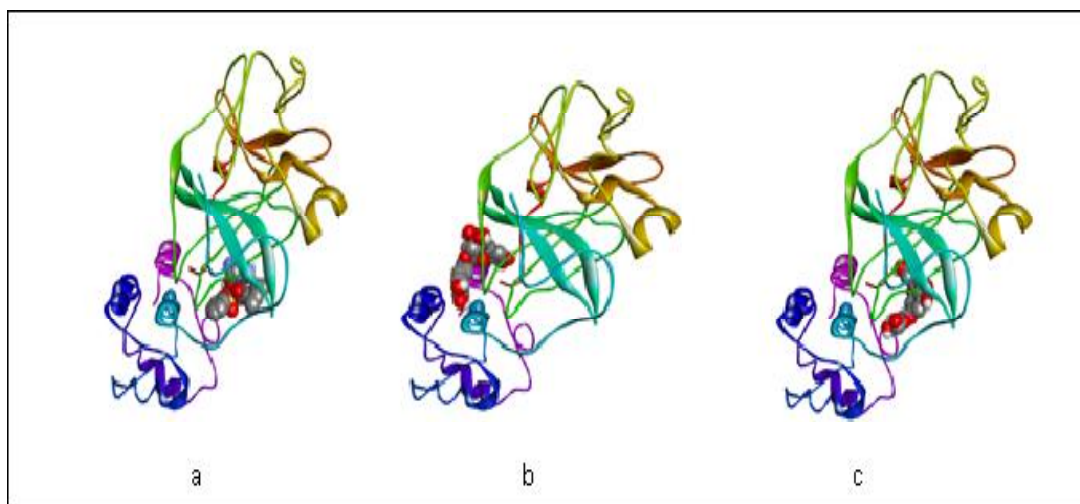

Fig. 2. Three dimensional view of binding conformations of Remdesivir (a), 1,5-dicaffeoyl-quinic acid (b) and Luteolin-7- glucoside (c) to residues of SARS- CoV-2 (7BUY.pdb). Ligands are shown in spheres while target (7BV2.pdb) is shown in solid ribbons

Table 2. *In silico* ADMETox analysis

	DILI	Cyto-toxicity	HLM	Cyp1A2 inhibitor	Cyp3A4 inhibitor	Cyp2D6 inhibitor	Cyp2C9 inhibitor	Cyp2C19 inhibitor	BBB	P-gp Inhibitor	P-gp Substrate	hERG Blocker	MMP	AMES	MRTD (mg/day)
Thymoquinone	No	No	Yes	No	No	No	No	No	Yes	No	No	No	No	No	58
Thymol	No	No	No	No	No	No	No	No	Yes	No	No	Yes	Yes	No	100
P-cymene	Yes	No	No	No	No	No	No	No	Yes	No	No	No	No	No	112
Dithymoquinone	Yes	No	Yes	No	No	No	No	No	Yes	No	No	No	No	No	94
Thymohydroquinone	No	No	Yes	No	No	No	No	No	Yes	No	No	No	Yes	No	54
Carvacrol	No	No	Yes	No	No	No	No	No	Yes	No	No	No	No	No	59
α-pinene	No	No	Yes	No	No	No	No	No	No	No	No	No	No	No	44
Terpinene-4-ol	No	No	Yes	No	No	No	No	No	Yes	No	Yes	No	No	No	15
Eugenol	No	No	Yes	No	No	No	No	No	No	No	No	No	No	No	319
Chavicol	Yes	No	Yes	No	No	No	No	No	No	No	No	No	No	No	279
β-Caryophyllene oxide	No	No	Yes	No	Yes	No	No	No	No	No	No	No	No	No	0.57
Trans-cinnamaldehyde	Yes	No	Yes	No	No	No	No	No	Yes	Yes	Yes	No	No	Yes	90
Zingerone	No	No	Yes	No	No	No	No	No	No	No	No	No	No	No	597
zingiberene	Yes	No	Yes	No	No	No	No	No	Yes	No	No	No	No	No	55
Gingerol	No	No	Yes	No	Yes	No	No	No	No	No	No	No	No	No	1178
Shogaol	No	No	Yes	No	Yes	No	No	No	No	No	No	No	No	No	576
Artemisinin	Yes	No	Yes	Yes	No	No	No	No	Yes	No	No	No	No	No	139
Artemisitene	No	No	Yes	Yes	No	No	No	No	Yes	No	No	No	No	No	112
Arteanuine B	No	No	Yes	No	No	No	No	No	Yes	No	No	No	No	No	83
1,8-cineole	Yes	No	Yes	No	No	No	No	No	Yes	No	No	No	No	No	106
vernodalin,	No	No	Yes	No	No	No	No	No	No	No	Yes	No	No	No	102
vernolide,	No	No	Yes	No	No	No	No	No	No	Yes	Yes	No	No	No	85
hydroxyvernolide	No	No	Yes	No	No	No	No	No	No	Yes	Yes	No	No	No	99
1,5-dicaffeoyl-quinic acid,	Yes	No	Yes	No	No	No	No	No	No	No	No	No	No	No	20900
chlorogenic acid	Yes	No	Yes	No	No	No	No	No	No	No	No	No	No	No	2605
luteolin-7-O-glucoside	No	No	Yes	No	No	No	No	No	No	No	No	No	No	Yes	620
alliin	No	No	Yes	No	No	No	No	No	No	No	No	No	No	No	7833
allicin	Yes	No	Yes	No	No	No	No	No	Yes	No	No	No	No	No	95
quercetin	No	No	Yes	Yes	No	No	Yes	No	No	No	Yes	No	Yes	Yes	1694
Geraniol	Yes	No	Yes	No	No	No	No	No	Yes	No	No	No	No	No	93
Cis- ocimene	Yes	No	Yes	No	No	No	No	No	Yes	No	No	No	No	No	89
GB1	Yes	No	Yes	No	Yes	No	No	No	No	Yes	No	No	Yes	No	1183
GB2	Yes	No	Yes	No	Yes	No	No	No	No	Yes	No	No	Yes	No	1693
GB-1a	No	Yes	Yes	Yes	Yes	No	No	Yes	No	No	No	No	Yes	No	2298
Kolaflavanone	No	No	Yes	No	Yes	No	No	No	No	Yes	No	No	No	No	855
kolanone	Yes	No	Yes	No	No	No	No	No	Yes	No	No	No	No	No	237
Garcifuran B	No	No	Yes	Yes	Yes	No	No	Yes	Yes	No	No	No	No	No	156
securinine	No	No	Yes	No	No	No	No	No	Yes	No	No	Yes	No	No	72
presenegenin,	No	No	Yes	No	No	No	No	No	No	No	No	No	No	No	640
1,7-dihydroxy-4-methoxyxanthone	No	No	Yes	Yes	No	No	No	Yes	No	Yes	Yes	No	No	Yes	1397
cryptomeridiol,	No	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No	No	508
nortrachelogenin	No	No	Yes	No	No	No	No	No	No	Yes	No	Yes	No	Yes	1392
carinol	No	No	Yes	No	No	No	No	No	No	No	No	No	No	Yes	791
carissanol	No	No	Yes	No	No	No	No	No	No	No	No	Yes	No	Yes	2327
kaempferol	No	No	Yes	Yes	No	No	Yes	No	No	No	Yes	No	Yes	No	1977
rhamnetin	No	No	Yes	Yes	No	No	Yes	No	No	No	Yes	No	Yes	Yes	4901
isorhamnetin	No	No	Yes	Yes	No	No	Yes	No	No	Yes	Yes	No	Yes	Yes	393
cucurbitacin B	No	No	Yes	No	Yes	No	No	No	No	Yes	Yes	No	No	No	115

	DILI	Cyto-toxicity	HLM	Cyp1A2 inhibitor	Cyp3A4 inhibitor	Cyp2D6 inhibitor	Cyp2C9 inhibitor	Cyp2C19 inhibitor	BBB	P-gp Inhibitor	P-gp Substrate	hERG Blocker	MMP	AMES	MRTD (mg/day)
rosmarinic acid	No	No	Yes	No	No	No	No	No	No	No	No	No	No	No	2957
Rutin	Yes	No	Yes	No	No	No	No	No	No	No	Yes	No	No	Yes	1200
caffeic acid	Yes	No	Yes	No	No	No	No	No	No	No	No	No	No	No	875
borneol	No	No	Yes	No	No	No	No	No	Yes	Yes	No	No	No	No	83
camphor	Yes	No	Yes	No	No	No	No	No	No	Yes	No	No	No	No	163
caryophyllene	No	No	Yes	No	Yes	No	No	No	No	No	No	No	No	No	0.57
elemene	No	No	Yes	No	No	No	No	No	Yes	Yes	No	No	No	No	29
humulene	Yes	No	Yes	No	No	No	No	No	No	No	No	No	No	No	62
ledene	No	No	Yes	No	No	No	No	No	Yes	Yes	No	No	No	No	28
pinene	No	No	Yes	No	No	No	No	No	No	No	No	No	No	No	44
oleanolic acid	No	No	No	No	No	No	No	No	No	No	No	No	No	No	568

4. DISCUSSION

Current clinical management includes antiviral agents (remdesivir, lopinavir, umifenovir, favipiravir and oseltamivir), anti-oxidants and micro nutrients (Zinc and selenium). Though these drugs are still undergoing clinical trial in different countries, there are reported scientific backings for their use. This is therefore the bases for their use as reference molecules in the comparison and *In-silico* study.

4.1 Predicting Binding Affinity from Docking Scores

The lower the energy released when a ligand forms a bond with a protein, mostly translates to a possible higher affinity for that protein target and it is usually negative. Binding pattern analysis (Fig. 1), of the compounds showed numerous hydrogen- bond and van der waals interactions. In as much as the unfavourable interactions could most likely result from induced fit nature of binding of the ligand to the receptor, there is the possibility of affecting functionality of the drug but this can only be concluded after *in-vitro* testing. Three dimensional (3D) view of the binding in Fig. 2a is similar to that of Fig. 2c as they are both seen to occupy similar active site pockets. This could possibly be attributed to bulkiness of the compounds and shows a conformation that suggests being buried in a much deeper pocket in the protein target. Xue et al., [7] describes the importance of the N-finger (N-terminal residues 1-7 of domain I) of SARS-CoV-2 Mpro, with Zhong, et al., [38] also noting how critical they are to the formation of the right quaternary structure, hence the binding of some phytochemicals in this study such as Luteolin-7-glucoside (Fig. 1c) to those residues could result in an inactive SARS-CoV-2 Mpro. Without Its N-Finger, the Main Protease of Severe Acute Respiratory Syndrome Coronavirus Can Form a Novel Dimer through Its C-Terminal Domain [38].

4.2 ADMETox Analysis

The computer based ADME toxicity evaluation of the phytochemicals showed the possible distribution, transport and metabolic sites as well as possible effects on cells, organs and systems. The study considered drug-induced liver injury (DILI), metabolic rate, metabolic enzymes and mutagenicity in the hepatic system. DILI is usually dose dependent and also a critical parameter in determination of doses and also possible withdrawal of drugs from circulation depending on whether the injury is permanent or reversible. Some of the chemical constituents that might cause injury to the liver include the compounds in *Syzygium aromaticum* (such as Chavicol)*, Cinnamon verum* (such as Trans-cinnamaldehyde), *Artemisia annua* (such as artemisin and 1,8 cineole) *Bitter kola* (such as GB1 and GB2) and *Vernonia amygydalina* (such as chlorogenic acid and 1,5-dicaffeoyl-quinic acid), *Salvia officinalis* (such as chlorogenic acid). Similarly agents that may likely have effects on the liver include allicin in *Allium sativum,* geraneiol and cis- ocimene in *Ocimum gratissimum.* Hepatotoxic effects are some of the most important effects for which herbal products may be viewed with suspicion.

However, some of the components of these same plants have hepato-protective and anti-oxidant effects which therefore shield the liver from damage. Agents such as quercertin and rutin are typical examples. Quercetin is abundantly found in *Allium sativum, Securidaca longipedunculata, Carissa edulis* and *Salvia officinalis* while rutin is found in *Salvia officinalis.* Some of these observations are responsible for the notion that if properly combined, some herbal products act better and in a synergistic manner.
Almost all the plants in this evaluation are reported to be non-cytotoxic following computer based modelling. A striking observation has to do with the derivative crytomeridiol, which is present in *Carissa edulis.* However, the explanation with respect to synergism and the ameliorative effects of other phytochemical compounds acting in a complimentary manner when these plants are properly combined will mean that this observation will be taken care of. Also the rate of metabolism allows for fast elimination of these compounds after their therapeutic effects.

The Phytochemical compounds in the plants evaluated score very favourably with respect to the human liver microsomal (HLM) stability assay. This assay is commonly used to identify and exclude compounds that are too rapidly metabolized. For a drug to achieve effective therapeutic concentrations in the body, it cannot be metabolized too rapidly by the liver. Compounds with a half-life of 30 minutes or longer in an HLM assay are considered as stable; otherwise they are considered unstable. The evaluation showed that only three of the phytochemical compounds are likely to be unstable with respect to this assay. These are thymol and p-cymene in *Nigella sativa* as well as oleonolic acid in *Salvia officinalis*. A careful look

at Table 1 will however show that these agents are not the ones that have the best binding affinities in the plants concerned. Therefore the observed instability in the HLM assay will pose no much problem but side effects.

The Cytochrome P_{450} enzyme system constitutes a superfamily of proteins that play an important role in the metabolism and detoxification of xenobiotics. Thus a critical look at this system is important in the evaluation system for any drug product. The simulation study showed majority of the phytochemicals are not inhibitors of the Cytochrome P_{450} enzyme system isoforms (CYP1A2, CYP3A4, CYP2D6, CYP2C9, and CYP2C19) though one or two phytochemicals found in *Zingiber officinale, Syzygium aromaticum*, *Carissa edulis* and *Garcinia kola* are inhibitors of CYP3A4 and CYP2C19. CYP3A4 is the most abundant of the cytochrome enzyme system isoform and is involved in the metabolic activities of xenobiotics. Therefore due consideration must be given to it when formulations of the polyherbal medicine is being undertaken.

The phytochemical compounds in the plants investigated are neither substrates nor inhibitors of P-glycoprotein (Pgp). Pgp is an essential cell membrane protein that extracts many foreign substances from the cell. The over-expression of Pgp increases the efflux of agents from the cell and prevents treatment by reducing the effective intracellular concentrations of such agents. Therefore, the inhibitory activity with respect to Pgp is a desirable property with some agents. This is evident in the evaluation with respect to compounds in *Cinnamon verum, Venonia amygdalina, Carissa edulis, Garcinia kola, Cucumis metuliferus* and *Salvia officinalis.*

Most of the phytochemical compounds evaluated performed favourably with respect to the human ether-à-go-go-related gene (hERG). This gene codes for a potassium ion channel involved in the normal cardiac repolarization activity of the heart. Drug-induced blockade of hERG function can cause long QT syndrome, which may result in arrhythmia and death. Notably most of the agents do not cause this blockade. The only exceptions are agents such thymol in *Nigella sativa*, securinine in *Securidaca longipedunculata* and carisanol as well as nortrachelogenin in *Carissa edulis* which reportedly cause blockade. However, the Maximum Recommended Therapeutic Dose

(MRTD, (Table 2) is the estimated upper daily dose, showed that these are safe. Thus in the normal course of use as contemplated in the formulation of these plants, cardiotoxicity as a result of hERG blockade is not likely.

The effect of the phytochemicals on cells and subcellular systems such as the mitochondrial were also investigated through computer simulation. The mitochondria are directly involved in cellular energetics and oxidative stress. Mitochondrial dysfunction has been implicated in cancer, diabetes, neurodegenerative disorders, and cardiovascular diseases. It is therefore imperative to study the effect of the phytochemicals at the cellular levels. The phytochemical compounds have no mitochondria related toxicity effects except for quercetin. However, at the usual doses of quercetin and also by the computed Maximum Recommended Therapeutic Dose (MRTD), it is safe. The phytochemical compounds were found to be non-mutagenic on the AMES evaluation for mutagenicity except for quercetin and rutin. The two compounds are however being used in humans for ages. The AMES mutagenicity test will have to be considered with respect to the maximum recommended therapeutic dose during formulation for human use.

All the phytochemicals are from plant materials used as food ingredients and condiments in Nigeria and other parts of the world for years, therefore the WHO condition/guideline for selection and approval of herbs for therapeutic considerations is met.

5. CONCLUSION

In silico techniques is an acceptable and fast method of evaluation of chemical activities at specific receptors or ligand sites for selection of chemical leads for laboratory work [39]. The computational simulations in this study has shown that plants contain phytochemical compounds that have favourable binding and inhibitory activity at the active site of SARS-CoV-2 protease responsible for COVID-19. The pharmacokinetic profile of the phytochemicals showed good bioavailability properties and safety.

From the result, all the plants (Save for *Syzygium aromaticum*, *Ocimum gratissimum* and *Cinnamomum verum*- since their binding affinities are less than the control used in the study) can be used for their antiviral properties. The molecular docking has helped us to narrow

down our choices to 10 out of the 13 plants to proceed to *in-vitro* studies.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

DISCLAIMER

The products used for this research are common and predominant in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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