Ethnobotanical Appraisal and *In-silico* Investigation of Plants Used for the Management of COVID-19 in Southwestern Nigeria

Ogunyemi O. Oderinlo[1]; Olapeju A. Adenekan[2]; Taye T. Alawode[1]; Paul M. Osamudiamen[3]; Bolaji B. Oluremi[4]; Oluwatoba E. Oyeneyin[5]; Mpho P. Ngoepe[6].

[1]*Department of Chemistry, Federal University Otuoke*
[2]*National Centre for Technology Management, Lagos,*
[3]*Department of Chemical Sciences, Bells University of Technology,*
[4]*Department of Pharmaceutical Microbiology, University of Ibadan*
[5]*Department of Chemical Sciences, Adekunle Ajasin University, Akungba-Akoko,* [6]*Department of Oral Biology, University of the Witwatersrand,*

Abstract: The continued spread of 2019 novel coronavirus disease is a serious threat to global health. Currently, no approved vaccines or antiviral agents for treatment exist, but, there are many anecdotal accounts of the use of herbal concoctions for the management of this disease in many low-income settings, including Nigeria abound. Using a combination of a web-based survey, and face-to-face interviews with local herbal sellers, this study documents the herbal flora used in the management of COVID-19 in Southwestern Nigeria, and also probes their potential as inhibitors of SARS-CoV-2 proteins. The preliminary *in-silico* studies identified taraxerol, vernonioside D2, nimbolide and mangiferoleanone as potential hit candidates for COVID-19. The current findings highlight that nature can be a source for the discovery of medicinal agents active against COVID-19. These preliminary results necessitate further *in-vitro* and *in-vivo* experimental evidence.

Key words: COVID-19; Herbal Flora; *In-silico* Docking; Phytochemicals; SARS-CoV-2; Southwestern Nigeria

Background to the Study:

The 2019 novel coronavirus (COVID-19) disease outbreak caused by Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) is an ongoing global health crisis. Since the outbreak of COVID-19 late last year in Wuhan, China, the virus has so far infected approximately 58 million people with mortality surpassing 1.3 million(WHO, 2020).

Corresponding Author: Ogunyemi O. Oderinlo, +2347031188902, oderinlooo@fuotuoke.edu.ng

The Africa continent is not spared of the impact of the virus even though the virus appeared late on the continent. As of this writing, the continent has recorded over 2 million confirmed cases of COVID-19(AfricaCDC, 2020) and the number might be much higher but for the low capacity for testing and issues with data in most African countries. South Africa, Morocco, Egypt, Ethiopia, Kenya and Nigeria are amongst the most impacted countries on the continent(AfricaCDC, 2020).

There are currently no officially approved vaccines or antiviral drugs for COVID-19; the recommendation is to provide supportive management based on each patient's symptoms(Tobaiqy et al., 2020). Anecdotal accounts abound on the use of herbal concoctions for the prevention and treatment of COVID-19 in many low-income countries(Kapepula et al., 2020). Presently, concerted efforts are ongoing in finding effective herbs and herbal compounds for the management COVID-19 disease(Hordofa & Kiros, 2020). However, as a new disease, there is dearth of information on herbs that are utilized for this purpose. More importantly, there are no evidence-based studies that have established the effectiveness and safety of these herbal medicines.

Nigeria has a rich cultural heritage and the Southwestern region of the country is endowed with vast botanical diversity. The use of medicinal plants is well-known among the Yorubas of Southwestern Nigeria(Lawal et al., 2020) and a significant proportion of her population are knowledgeable in the treatment of infections and diseases using herbs(Oladunmoye & Kehinde, 2011). Ethnomedicinal inquiries on traditional folk remedies undoubtedly play a significant role in proper identification, documentation and evaluation of herbs with distinct pharmacological activities(Süntar, 2020). This approach has the prospect of delivering candidate drugs for the treatment of various human ailments. With the advances in information and communication technologies, nowadays, online platforms are continually gaining popularity as a valuable tool for assessing people’s perceptions and knowledge of plants used for the treatment of an infectious disease.

There is therefore a need for study and documentation of herbal flora used in Southwestern Nigeria in the management of COVID-19. This study would lay a pivotal foundation for successive scientific studies in establishing the effectiveness and safety of these medicinal plants in the management of COVID-19.

Methods:

Study Design and Setting:

An online based survey which was titled "Documentation of Medicinal Plants used in Prevention or Treatment of COVID-19 Infection, in South Western Nigeria" was conducted from July to August 2020 using a structured, self-administered questionnaire. The questionnaire consisting of 25 questions was developed using the Google Docs application. A pilot study was first conducted among a group of 15 volunteers to ensure the appropriateness of the questions and to avoid ambiguity. We then revised the questionnaires based on the suggestions received from volunteers. The questionnaire was delivered electronically to participants via different social media platforms such as WhatsApp©, Facebook©, Telegram© etc. The first 9 questions seek demographic information of the participants which include gender, age, highest level of education etc. The next five questions solicited information on the participant's knowledge and perceptions of COVID-19. The last set of questions were used to obtain information on the awareness and the use of herbal medicine in the management of the disease by the participants.

In addition, face-to-face interviews were conducted for herbalists/local herbal sellers for the reason that these set of people might not be conversant with the use of social media and are generally not literate enough to complete the questionnaire on their own.

Data Analysis:

Basic descriptive statistics such as numbers and percentages were calculated for each variable using the Statistical Package for Social Sciences (SPSS 21) asthe analysis tool.

Computational Analysis:

Protein Preparation

The target proteins, SARS-CoV-2 main protease (5R82), SARS-CoV-2 RNA-dependent RNA polymerase (7BV2) and SARS-CoV-2 papain-like protease (PLPro, 7JRN) were obtained from Protein Data Bank (http://www.pdb.org/). PDB structures of all target proteins were prepared for screening using VegaZZ, removing water molecules, adding missing hydrogens, setting correct bond types, and assigning Gasteiger charges. Only one of the monomers was taken into account for PDB files containing more than one subunit. For each

protein, the residues within the 10Å radius of the ligand molecule were chosen, and the ligand was extracted before AutoGrid was started to build the atom maps (map), grid parameter (gpf) and receptor (pdbqt) file using VegaZZ scripts.

Ligand Preparation

Various phytochemicals from the selected medicinal plants: *Azadirachta indica* (66 molecules), *Mangifera indica* (60 molecules) and *Vernonia amygdalina* (57 molecules) were used in this study. The 2D and 3D structure of pharmacologically active phytochemicals in the selected herbs are searched and extracted from various chemical databases, such as Pubchem database (http:// pubchem.ncbi.nlm.nih.gov/), KEGG ligand database (http://www.genome.jp/ligand/), chemdb (http://cdb.ics.uci.edu/) databases, published articles and thesis. A database of these compounds was generated using OpenBabel and saved in SDF file format after addition of missing-hydrogens. Each respective molecule was 3D structure optimized using Vega ZZ software. Optimization included the elimination of water molecules, the addition of a hydrogen atom, and repair charge by adding a Gasteiger partial charge, giving AutoDock force field, and energy minimization(Pamudi et al., 2017).

Virtual Screening

Computational virtual screening has been shown to be a very effective tool for discovering bioactive inhibitors from a wide pool of compounds(Rajendran, 2011). Virtual screening was conducted using a modified method of Erić and colleagues(Erić et al., 2012). Briefly, GriDock software based on AutoDock 4 and developed for virtual screening utilizing multi-core architectures was used. VegaZZ 2.3.2.38 was used for screening and protein preparation. The ligands sdf database containing all the herbal extracts molecules and pdbqt receptor file for each protein in the screening pool were used to run virtual screening using GriDock. A total of 10 molecules were observed to have high binding energy between the three target proteins. These were selected for optimization prior re-docking.

Geometry Optimization

Density Functional Theory (DFT) calculations were made using the hybrid functional B3LYP along with the double zeta Pople's base set, 6-31 G (d, p), as implemented in the Gaussian 16 package. Gauss View 6.0 was used for analysis and visualization of the optimized structures of the shortlisted compounds. The electronic information that correlate to drug properties

such as energies of the highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), energy gaps ($\Delta E_{LUMO\text{-}HOMO}$), ionization potential (IP), electron affinity (EA), electronegativity (χ), global chemical hardness (η), chemical potential (μ), global chemical softness (σ) and electrophilicity index (ω) were calculated as described by Gupta and colleagues(Gupta et al., 2020).

$$IP = -E_{HOMO} \quad (1)$$

$$EA = -E_{LUMO} \quad (2)$$

$$\chi = (IP + EA/2) \quad (3)$$

$$\mu = -\chi \quad (4)$$

$$\eta = (IP - EA/2) \quad (5)$$

$$\sigma = 1/\eta \quad (6)$$

$$\omega = \mu^2/2\eta \quad (7)$$

Docking

The compounds obtained through virtual screening were used in docking analysis using Autodock 4.2.0 and Autodock tools (ADT). In order to identify the potential conformation of the ligand binding to the protein, Lamarckian genetic algorithm (LGA) implemented in an adaptive local method search was used. The VegaZZ generated AutoGrid data used in virtual screening were also used for re-docking studies of DFT optimized structures. The docking parameters: 100 runs were executed and population size was set at 150, maximum number of evaluations was 2,500,000, maximum number of generations was 27,000, rate of gene mutation 0.02 and cross-over rate 0.8. The final docking pose was decided on the basis of interaction energy and inhibition constant (Ki).

3. Results and Discussion:

3.1 Demographic Characteristics of Participants

A total of 163 individuals completed the online questionnaire, of which only 156 were valid for analysis. Of this number, 75% were males and 25% were females. Although the study by Smith showed that females are more likely to respond to online surveys(Smith, 2008), however, the low response recorded in this present survey could be hinged on the inherent traditional responsibilities (domestic activities) of womenfolk(Anunobi & Mbagwu, 2009), which might limit their participation. On the contrary, the higher participation of males could be rationalised based on their comparative greater knowledge of plants of ethnomedicinal values(Idu & Osemwegie, 2007).

Almost half (44.9%) of the total participants were in the age group of 35 to 44 years and followed by the age group of 25 to 34 years (32.7%). While it is well acknowledged that older age groups are experts and custodians of indigenous flora knowledge(Ouhaddou et al., 2020; Silva et al., 2011; Umair et al., 2017)the relevant explanation for the poor response rates (~2%) recorded from this age group could be attributed to their less patronage of social media.(NOIPolls, 2019)

Majority of the participants, representing 98% had a higher education degree. This is expected as this category of people mostly have access to the internet and are active on social media. A significant proportion (36.5%) of the respondents were from Lagos state, the epicenter of the COVID-19 in Nigeria. Oyo and Ogun were next in line as states with high response rates of 25.0% and 15.4%, respectively. The socio-demographic characteristics of the participants are summarised in **Table 1**.

Table 1: Sociodemographic Characteristics of the Participants

Variable	Subgroup	Frequency	Percentage
Sex	Male	117	75
	Female	39	25
Age Range	21-24 years	23	14.7
	25-34 years	51	32.7
	35-44 years	70	44.9
	45-54 years	9	5.8
	55-64 years	3	1.9
	Above 64	0	0
Education Level	Secondary	2	1.3
	Polytechnic	18	11.5
	University	135	86.5
	Other	1	0.6
State Location	Lagos	57	36.5
	Oyo	39	25.0
	Ogun	24	15.4
	Osun	17	10.9
	Ekiti	10	6.4
	Ondo	9	5.8

3.2 Participants' Perception on COVID-19

With the question "what is your impression about COVID-19"? The majority of the participants (84.6%) indicated that COVID-19 is a viral infection while a notable number of participants (16.7%) gave a religious colouration to the disease; indicating it was a punishment from God and a sign of end time. In addition, about 20% of the participants believed that the disease was made in China. A few numbers (6.3%), however, opined that COVID-19 is a scam, and exaggerated solely for financial and political gains.

More than 65% of the participants agreed that the virus is spread through physical contact with infected people, droplet of body fluid and touching infected surfaces while only a participant indicated mosquito bite as a means of spreading. Difficulty in breathing (94.2%) ranked highest, followed by fever (92.9%) and cough (85.9%) as the most common symptoms of COVID-19 selected by the participants. Others symptoms selected include sore throat (79.5%), fatigue (64.1%) and loss of smell or taste (59.0%).

When asked "how do you protect yourself from contracting COVID-19?" As reflected in **Table 2,** virtually all the participants (>90%) subscribed to wearing facemasks, regular hands washing together with good personal hygiene, avoiding hand shake, hugging and personal contact could mitigate the spread of the virus and confer protection to individuals. Also, a substantial number of the participants (>35%) opined that use of orthodox and herbal medicines will confer protection against the virus. However, more than half (55.1%) of the participants believed that praying to God will protect them against the virus.

Table 2: Respondents Awareness on COVID-19

Variable	Subgroup	Frequency	Percentage
Symptoms	Fever	145	92.9
	Cough	134	85.9
	Fatigue	100	64.1
	Sore throat	124	79.5
	Loss of sense of smell or taste	52	59.0
	Difficulty in breathing	147	94.2
	Flu like symptoms	1	0.6
Protection	Wear mask	152	97.4
	Regularly hand washing and maintaining good hygiene	152	97.4
	Avoid hand shake, hugging and personal contact	144	92.3
	Pray to God	86	55.1
	Use of herbal medicine	58	37.2
	Use of orthodox medicine	56	35.9

3.3 Participants' Awareness on the Use of Herbs

A larger proportion (60.3%) of the participants were aware of the use of herbs in the management of the virus. For the majority of the participants, both social (62.8%) and conventional (60.6%) media outlets serve as their main sources of information. Furthermore, an appreciable number of participants (41.5%) source their information from family and friends while about 5% got informed by traditional healers and local herb sellers (**see Table 3**). Traditionally, knowledge of herbal medicine is commonly acquired through family(Alade et al., 2015) and apprenticeship from traditional healers/herbalists (Adefolaju, 2011). However, with the advent of information technology, the trend is rapidly changing; the use of the print and social media to gain information on herbal medicinal plants is on increase (Peacock et al., 2019). Several social media influencers and very important personalities (VIPs) are promoting the use (and knowledge) of herbal medicines in treating different ailments. In Nigeria for example, during the early phase of COVID-19, two prominent figures; the Ooni of Ife, Adeyeye Eniitan Ogunwusi, a first-class king(TheGuardian, 2020a) and the Governor of Oyo State, Seyi Makinde(TheGuardian, 2020b) used various social media platforms and the conventional media outlets to sensitize the public on different herbal flora they 'effectively deployed for the treatment of the disease'.

Table 3: Respondents Awareness on the use of Herbs

Variable	Subgroup	Frequency	Percentage
Awareness on use of herbs	Yes	94	60.3
	No	62	39.7
Information Source	Family and friends	39	41.5
	Traditional healer	5	5.3
	Local herb sellers	4	4.3
	The media	37	60.6
	Social media	59	62.8
	Personal discovery	1	1.1

Despite the level of awareness demonstrated by the participants, when interrogated if they had ever used herbs to prevent or treat COVID-19, the majority (68.7%) of the participants selected 'No' while a considerable proportion (31.4%) answered in affirmative. Of those that selected 'Yes', 87% attributed their choice for herb to the fact that it is natural and 56.5% subscribed to it because of its easy accessibility. An appreciable number of participants based their choice on the ground that it is cheaper (32.6%) and safer (21.7%). However, 26.1% of the participants opined that it is the only option available.

3.4 Herbs Used, Parts, Modes of Preparation and Administration

From the online survey, a total of 21 botanicals were identified by the participants for the management of COVID-19. *Zingiber officinale*, *Allium sativum* and *Azadirachta indica* top the list of the frequently cited botanical, with citation frequency of 57.1%, 53.1% and 40.8%, respectively. Other frequently cited botanicals include *Citrus limon*, *Curcuma longa*, *Nigella sativa*, *Mangifera indica*, *Vernonia amygdalina*. **Table 4** shows the comprehensive list of the plants cited by the participants.

A higher number (71.4%) of the herbs were cited by the male participants while 28.6% were cited by the female participants. The predominance of male respondents could be attributed to the Yoruba cultural preference of passing down the indigenous folk knowledge to the adult males. This trend mirrored the study by Idu and Osemwegie, where adult males were comparatively more conversant with the plants of ethnobotanical values than their female counterparts(Idu & Osemwegie, 2007). It must be noted however that gender-based knowledge of medicinal plants is heterogeneous, discrepancies exist in various parts of the world and even within specific countries(Torres-Avilez et al., 2016).

The leaves (67.3%) represent the dominant plant parts used followed by the fruits (46.9%), root (44.9%), stembark (20.4%) and seed (14.3%). The majority of the respondents sourced their herbs from the local herbs sellers (40.8%) and nearby bush (34.7%) while less than 10% consulted traditional healers for the herbs to use. Of the different methods of preparations highlighted in the survey, decoction (boiling the herbs in water) was the main method employed by a large number (87.8%) of the respondents. In addition, maceration (soaking in water), expression (squeezing the herb) and grating (grinding the herb to powder) were other methods indicated. Virtually all (~96%) the respondents administered via the oral route

(drinking) and followed by topical (bathing, 2% and rubbing 2%) on the body. When inquired of the possible side effects of these herbs, approximately 70% of the respondents indicated no side effects. However, a few of the respondents complained of stomach upset (16%), stooling (8.2%) and vomiting (6.1%).

3.4 Concurrent Use of Herbs with Orthodox Medicines

Of participants that used herbal remedies in the management of COVID-19, when asked further if they take orthodox medicine together with these herbal concoctions, a minute percentage (6.4%) indicated that they engaged in concurrent use of herbs with orthodox drugs. The orthodox medicines highlighted are Vitamin C (80%) and Chloroquine (10%). But some respondents gave a general categorization of the orthodox medicine used as cough syrup (10%) and antimalarial (10%) without being specific on the brand. Various studies have revealed the increasing prevalence in concomitant use of herbal remedies and orthodox medicines in Nigeria (Akanmu & Odeyemi, 2019; Ghazali et al., 2019). For example, in a recent study by Ghazali *et al*., 39.1% of the outpatients surveyed took herbs concurrently with orthodox medicines(Ghazali et al., 2019). In our study, the lower rate recorded could be premised on the fact that COVID-19 is a new disease and there is virtually no information in public spaces on the possible benefits orside effects that could result from the herbal-drug interactions on the disease.

3.5 Face-to-Face Interview with Herbalist and Local Herbal Sellers

Herbalists/local herbal sellers are great custodians of indigenous knowledge on various plants used to treat infectious diseases. So, in addition to the online survey, we consider it necessary to conduct face-to-face interviews with them in order to fortify the library of plants used in the management of COVID-19. More so, these set of people might not be conversant with the use of social media and are generally not literate enough to complete the questionnaire on their own. The interviews were conducted in four of the Southwestern states; Lagos, Ogun, Oyo and Osun. A total of 14 persons were interviewed, consisting 8 males and 6 females. The age range of interviewee were between 35 and 64 years and some of them have had between 10 to 20 years of experience in herbal medicinal practice. The majority of them (66.7%) were educated up to the secondary school level.

All the interviewees were well aware of COVID-19, its typical symptoms and mode of spreading. However, some of them have the preconceived notion that it is an "advanced form

of malaria". When inquired if their clients have been requesting for herbs to prevent COVID-19, only four of the interviewee answered 'Yes'. But the rest that answered 'No' boasted that they can suggest herbs to prevent or manage the diseases based on its associated symptoms.

A total of 19 herbs covering 13 different plant families were prescribed by the herbalists/local herbal sellers. Some of them include *Cajanus cajan, Enantia chlorantha*, *Lawsonia inermis,* etc. In general, it is noted that plants prescribed by the herbalists/local herbal sellers are uniquely robust from that of participants from the online survey (**Table 4**). The leaves, bark and root are the major plant part dispensed by the interviewee and then followed by the seed. All the interviewees indicated decoction as the most preferred method of preparing these herbs for use. Oral administration (drinking), topical (bathing) and inhalation (steaming**)** are the three most common methods of administration highlighted by the interviewees.

Table 4: Medicinal plants used (or prescribed) for the management of COVID-19 in Southwestern Nigeria.

Variable	Plant Used	Family	PP	MP	F	CF (%)
	Garcinia kola	Clusiaceae	L,S	Decoction Chewing	3	6.1
	Vernonia amygdalina	Compositae	L	Maceration	3	6.1
	Nigella sativa	Ranunculaceae	S	Decoction Maceration	5	10.2
Online	*Anacardium occidentale*	Anacardiaceae	L	Decoction	1	2.0
	Allium sativum	Amaryllidaceae	B, L	Decoction Maceration	26	53.1
	Zingiber officinale	Zingiberaceae	R	Decoction Grating Maceration	28	57.1
	Citrus paradisi	Rutaceae	F	Decoction	1	2.0
	Psidium guajava	Myrtaceae	L	Maceration	2	4.1
	Citrus limon	Rutaceae	F, L	Decoction Expression	12	24.5
	Cymbopogon citratus	Poaceae	L	Decoction	3	6.1
	Citrus aurantifolia	Rutaceae	L	Decoction	3	6.1
	Mangifera indica	Anacardiaceae	L, SB	Decoction Maceration	4	8.2
	Moringa oleifera	Moringaceae	L	Decoction	1	2.0
	Azadirachta indica	Meliaceae	L, SB	Decoction Maceration	20	40.8
	Allium cepa	Amaryllidaceae	B	Maceration	1	2.0
	Citrus aurantium	Rutaceae	F	Decoction	1	2.0
	Ananas comosus	Bromeliaceae	P	Maceration	2	4.1

	Ocimum gratissimum	Lamiaceae	L	Decoction	3	6.1
	Curcuma longa	Zingiberaceae	R	Decoction Grating Maceration	12	24.5
Face-to-face	*Croton penduliflorus*	Euphorbiaceae	S	Grating	2	14.3
	Cajanus cajan	Leguminosae	L,S	Decoction	2	14.3
	Azadirachta indica	Meliaceae	L	Decoction	5	35.7
	Enantia chlorantha	Annonaceae	R	Decoction	3	21.4
	Sorghum bicolor	Poaceae	L	Decoction Maceration	1	7.1
	Nigella sativa	Ranunculaceae	S	Decoction Chewing Maceration	2	14.3
	Nauclea latifolia	Rubiaceae	R, SB	Decoction	3	21.4
	Cymbopogon citratus	Poaceae	L	Decoction	1	7.1
	Khaya ivorensis	Meliaceae	SB	Decoction	1	7.1
	Alstonia boonei	Apocynacea	SB	Decoction Maceration	1	7.1
	Lawsonia inermis	Lythraceae	L	Decoction	1	7.1
	Kalanchoe crenata	Crassulaceae	L	Decoction	1	7.1
	Newbouldia laevis	Bignoniaceae	L, SB	Decoction	2	14.3
	Xylopia aethiopica	Annonaceae	F	Decoction	1	7.1
	Aloe barteri	Liliaceae	L	Decoction	1	7.1
	Ocimum basilicum	Lamiaceae	L	Decoction	3	21.4
	Mangifera indica	Anacardiaceae	L, SB	Decoction	4	28.6
	Anacardium occidentale	Anacardiaceae	SB, R	Decoction Grating	1	7.1
	Tetrapleura tetraptera	Fabaceae	F, SB	Decoction Grating	1	7.1

(**CF**): Citation Frequency: It is obtained by dividing the number of citations for a plant with the total number of respondents; (**F**): Frequency; (**MP**): Method of preparation; (**PP**): Plant part used; (**B**): Bulb; (**F**): Fruits; (**L**): Leaves; (**R**): Roots/ rhizomes; (**P**): Peels; (**SB**): Stem/ stembark; (**S**): Seeds.

3.6 Virtual Screening

Compounds from medicinal plants *A. indica* (neem), *M. indica* (mango) and *V. amygdalina* (bitter leaf) were evaluated as potential lead compounds against COVID-19. It was observed that α-copaene from neem had a binding energy of -7.59kcal/mol (2.72µM), mangiferoleanone from mango had a binding energy of -7.84kcal/mol (1.79µM) and Vernonioside A3 from bitter leaf has binding energy of -7.4 kcal/mol (3.77µM) against **7JRN**. Against **7BV2**, pinocarvone from bitter leaf had a binding energy of -5.65kcal/mol (72.1µM), mangiferoleanone from mango had a binding energy of -6.43kcal/mol (19.2µM) and 28-deoxonimbolide from neem had a binding energy of -6.61kcal/mol (14.2µM). Vernonioside D2 from bitter leaf had better activity of -5.76kcal/mol (59.8µM), mangiferoleanone from mango had a binding energy of -6.49kcal/mol (17.6µM) and limocinol from neem had a binding energy of -5.67kcal/mol (69.4µM) from **5R82**. Overall, mangiferoleanone from mango appears to bind to all proteins. This is followed by vernonioside derivatives from bitter leaf. Studies have shown that mango possesses antidiabetic, anti-oxidant, anti-viral, cardiotonic, hypotensive, anti-inflammatory properties(Parvez, 2016). Natural compounds such as glycyrrhizin, catechin, cepharanthine, isoquinoline, theaflavin-3'-gallate and theaflavin-3,3'-digallate from black tea and tannic acid have been shown to have antiviral activity against SARS-CoV (Chattopadhyay & Naik, 2007). This highlights that nature can be a source of discovering lead compounds against COVID-19.

3.7 Optimization

Density Functional Theory (DFT) was used for structure optimization of potential lead compounds against COVID-19 proteins. The electronegativity (χ), global chemical hardness (η), chemical potential (µ), global chemical softness (σ) and electrophilicity index (ω) values were calculated from HOMO (electron donating) and LUMO (electron withdrawing) energies (**Table 5**). These can enable for the prediction of defining the reactivity and stability of chemical constituents. It has been shown in literature that the binding ability of chemical compounds is directly related to the energy of HOMO and LUMO energy, whereby binding affinity of a compound will be increased if there are more HOMO and less LUMO energies(Patial & Cannoo, 2020).

Table 5: Quantum Chemical Parameters for Phytochemicals with anti-COVID-19 properties

Compound				μ	χ	ω	η	δ
Ascaridole	-0.212	0.004	0.215	-0.104	0.104	0.050	0.108	4.645
α-Copaene	-0.202	0.009	0.212	-0.097	0.097	0.044	0.106	4.727
Humulen	-0.213	0.020	0.234	-0.096	0.096	0.040	0.117	4.278
Limocinol	-0.221	0.027	0.249	-0.097	0.097	0.038	0.124	4.019
Mangiferoleanone	-0.216	-0.006	0.210	-0.111	0.111	0.059	0.105	4.762
Nimbolide	-0.224	-0.062	0.162	-0.143	0.143	0.126	0.081	6.187
β-sitosterol	-0.225	0.029	0.254	-0.098	0.098	0.038	0.127	3.940
Stigmasta-7,24(28)-dien-3-ol	-0.221	0.030	0.251	-0.096	0.096	0.036	0.126	3.978
Taraxerol	-0.225	0.032	0.257	-0.097	0.097	0.036	0.128	3.897
Vernonioside D2	-0.202	-0.009	0.193	-0.106	0.106	0.058	0.097	5.181

In terms of reactivity and stability, this can increase and decrease respectively depending on the decrease of energy gap (**Figure 1**). Thus, large HOMO-LUMO gap relates to high stability and low chemical reactivity, whilst small HOMO-LUMO gap indicates low stability but higher chemical reactivity for electron donation/acceptance(Uzzaman & Hoque, 2018). The energy gap can also be linked to the chemical hardness and reactivity, whereby a large energy gap signifies large chemical hardness and reduced reactivity. From this study, nimbolide had the lowest energy gap (0.162eV), which correlate to low chemical hardness of 0.081Ev. This was followed by vernonioside D2, with energy gap of 0.193eV and chemical hardness value of 0.097eV. This is contrary to taraxerol with energy gap of 0.257. The electrophilicity (opposite of chemical potential (μ)) which indicates the level of compound stability and reactivity, where high electrophilicity.

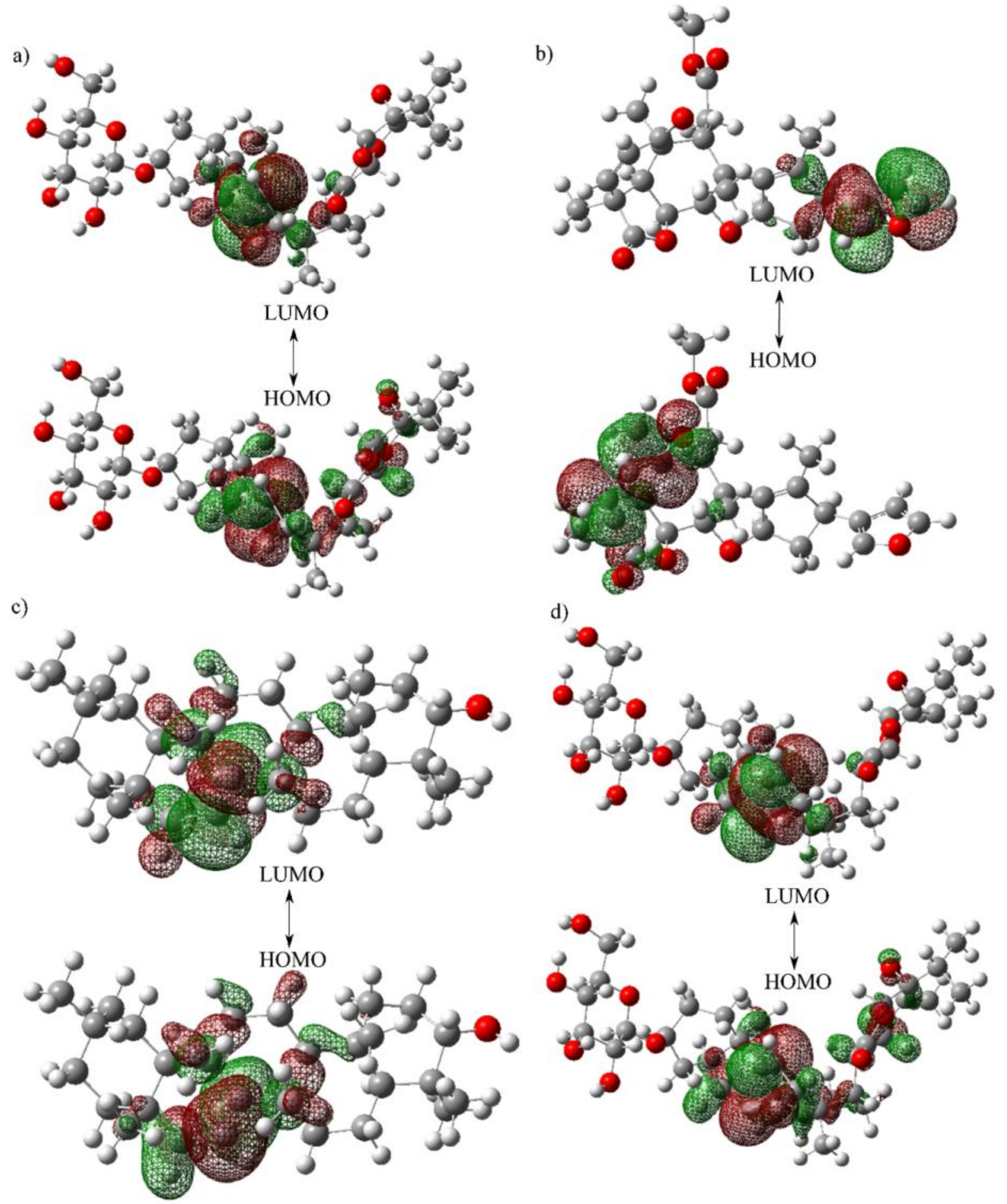

Figure 1: DFT images of (a) Mangiferoleanone, (b) Nimbolide, (c) Taraxerol and (d) Vernonioside-D2

3.7 Docking

Interaction of phytochemicals against SARS-CoV-2 main protease (5R82), SARS-CoV-2 RNA-dependent RNA polymerase (7BV2) and SARS-CoV-2 papain-like protease (PLPro, 7JRN) was conducted using AutoDock (**Table 6**). Taraxerol showed a high affinity for **5R82** with a binding energy of -7.94kcal/mol and inhibition constant of 1.52µM compared to ascaridole with binding affinity of -4.46kcal/mol and inhibition constant of 540.95µM. Against **7BV2**, nimbolide had a high affinity of -8.43kcal/mol and inhibition constant of 0.660µM. Ascaridole also had low affinity with -4.91kcal/mol and inhibition constant of 253.14µM against **7BV2**. High binding affinity against **7JRN** was observed with Vernonioside-D2 due to binding energy of -8.98kcal/mol and inhibition constant of 0.26µM. Mangiferoleanone was observed to have better activity across all proteins with binding affinity ≥-7kcal/mol and inhibition constant of ≤7µM.

Table 6: Docking against SARS-CoV-2 proteins

Compound	5R82		7BV2		7JRN	
	(kcal/mol)	Ki (µM)	(kcal/mol)	Ki (µM)	(kcal/mol)	Ki (µM)
α-Copaene	-4.9	233.72	-5.72	63.97	-6.46	18.45
Ascaridole	-4.46	540.95	-4.91	253.14	-6.74	11.50
β-sitosterol	-6.32	23.28	-6.85	9.56	-6.20	28.56
Humulene	-5.52	89.43	-6.04	37.62	-6.25	26.34
Limocinol	-6.41	19.89	-7.58	2.78	-7.02	7.10
Mangiferoleanone	-7.29	4.50	-7.42	3.65	-7.03	7.00
Nimbolide	-6.71	12.07	-8.43	0.660	-6.65	13.40
Stigmasta-7,24(28)-dien-3-ol	-5.80	55.81	-6.39	20.58	-7.96	1.45
Taraxerol	-7.94	1.52	-7.69	2.29	-7.62	2.59
Vernonioside-D2	-5.31	127.12	-6.13	32.36	-8.98	0.26

Taraxerol was observed to form hydrogen bonds with **5R82**, where the hydroxyl group interacted with methionine as a hydrogen acceptor (**Figure 2(a)**). Nimbolide interacted with **7BV2** thorough formation of 4 hydrogen bonds where hydroxyl groups interacting with lysine (593), glycine (590) and adenine (13) acted as hydrogen acceptors and against uracil (12) acted as hydrogen donor. Vernonioside-D2 interacted with **7JRN** through formation of 2 hydrogen bonds with proline (248) and 1 with glutamine (269).

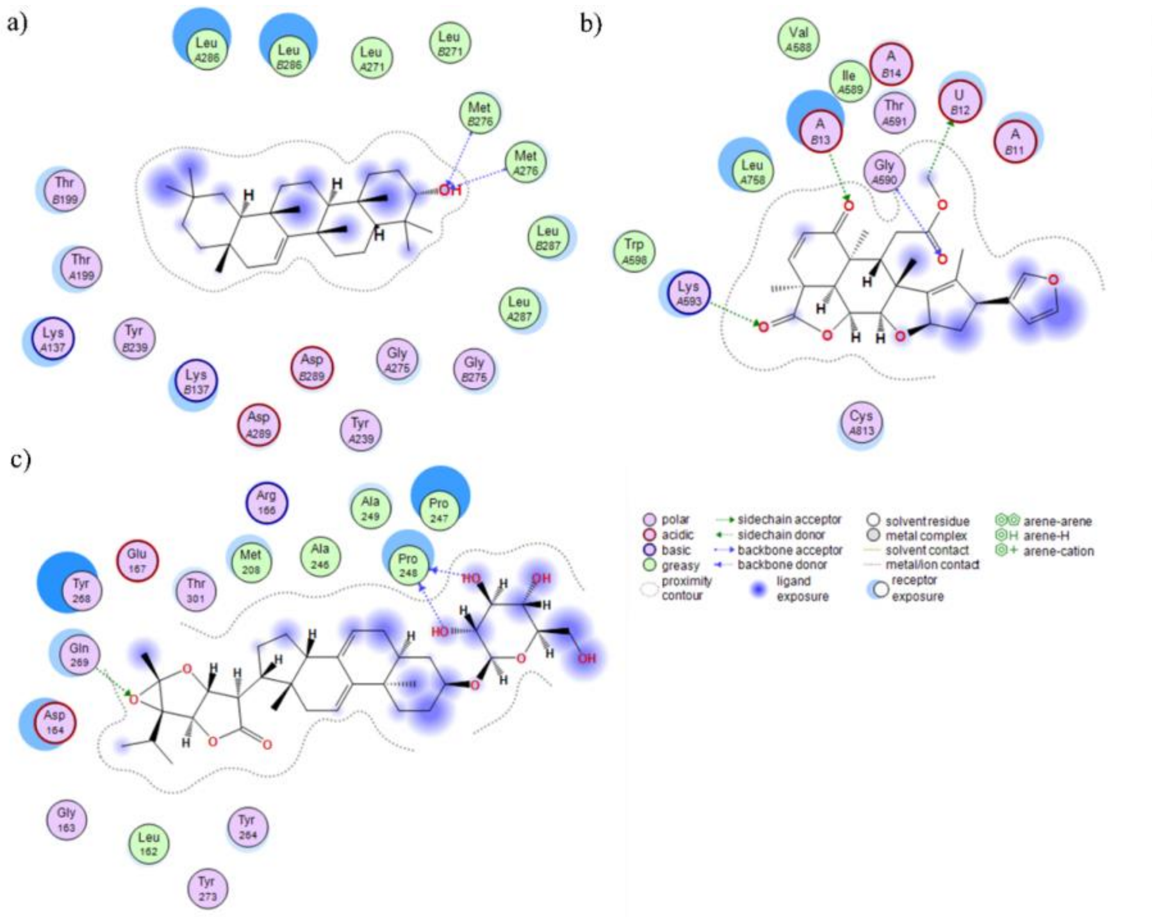

Figure S2: Docking studies of (a) Taraxerol-5R82, (b) Nimbolide-7BV2 and (c) Vernonioside-D2-7JRN complexes.

Conclusion:

Medicinal plants are an important source of hit and lead compounds for drug discovery and development processes. Due to the peculiarity of the ongoing COVID-19 infections, there is renewed interest in the use of medicinal herbs. The present ethnobotanical survey-based investigation identified a total of 32 medicinal plant species belonging to 23 botanical families used in Southwestern Nigeria for the management of the disease. Subsequently, molecular docking studies of the interaction of the phytochemicals present in the selected medicinal plants led to the identification of taraxerol, vernonioside D2, nimbolide and mangiferoleanone. These compounds exhibited high binding energy against the selected COVID-19 proteins. The current findings have necessitated further in-vitro (and in-vivo) efficacy, safety and mechanism of action studies.

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