Potential Inhibitors of SARS-CoV-2 from *Neocarya macrophylla* (Sabine) Prance ex F. White: Chemoinformatic and Molecular Modeling Studies for Three Key Targets

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ABSTRACT

Objectives: The novel coronavirus disease-2019 (COVID-19) that emerged in China, is a highly transmittable and pathogenic viral infection caused by the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2); the disease has been declared by the World Health Organization as a Public Health Emergency of International Concern. The unavailability of approved therapeutic agents or vaccines is of great concern. This study performed molecular docking and absorption, distribution, metabolism, excretion and toxicity (ADMET) analysis of some compounds isolated from *Neocarya macrophylla* (Sabine) Prance ex F. White (Chrysobalanaceae) against three targets of SARS-CoV-2 proteins (3C-like protease, spike protein, and papain-like protease).

Materials and Methods: Phytoconstituents isolated from *N. macrophylla* were screened against key targets of SARS-CoV-2 using Auto Dock Vina, while the ADMET analysis was performed using swiss ADME and pkCSM ADMET descriptors algorithm protocols.

Results: The *in silico* computational studies revealed that the compounds (catechin, catechin-3-rhamnoside, quercetin, and epicatechin) isolated from *N. macrophylla* can effectively bind with high affinity and lower energy values to the three target proteins of SARS-CoV-2. ADMET analysis was used to predict important pharmacokinetic properties of the compounds, such as aqueous solubility, blood-brain barrier, plasma protein binding, CYP2D6 binding, intestinal absorption, and hepatotoxicity.

Conclusion: The findings of this study have shown that *N. macrophylla* contains potential leads for SARS-CoV-2 inhibition and thus, should be studied further for development as therapeutic agents against COVID-19.

Key words: *Neocarya macrophylla*, SARS-CoV-2, flavonoids, ADMET, COVID-19

INTRODUCTION

Coronavirus disease-2019 (COVID-19) is a major public health problem. From December 2019, when it was first presented in Wuhan, China,[1] it rapidly spread to several countries around the world, necessitating the declaration of the disease as a Public Health Emergency of International Concern by World Health Organization on the 30th January 2020.[2] The life cycle

of coronavirus mediates its infection in few typical steps *viz*.; (i) attachment that must do with transmission inside the human body as well as binding of the virus spike protein with angiotensin-converting enzyme-2 (ACE-2) receptors of the cell membrane, (ii) penetration, which involves membrane fusion of the virus through endocytosis and release of the viral genome inside the cell, (iii) biosynthesis, which encompasses the

synthesis of replication-transcription complex and replication of viral RNA, (iv) maturation *i.e.* transcription of subgenomic mRNAs, (v) release that must with the translation of the viral proteins, assembly of new virions, and release of the virion from infected cells *via* exocytosis and infect new healthy cells.[3,4] There is presently no specific drug or vaccine targeted at the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) virus but different classes of drugs, including anti-viral, anti-inflammatory, steroids, or anti-coagulants are employed for the symptomatic treatment. There is thus, an urgent need for research to develop an alternative, effective and safe therapy for managing COVID-19, and natural products have been known historically as a veritable source of medicines.

Few studies have reported the potential of medicinal plants as a novel approach for the effective management of the COVID.[5] There are several anecdotal accounts of the use of plant extracts, including *Artemisia annua* L., *Pyrrosia lingua* (Thunb.) Farw., *Lindera aggregata* (Sims) Kosterm, *Zingiber officinale* Roscoe, *Syzygium aromaticum* (L.) Merr. & L.M.Perry, and *Allium sativum* L., singly or as a combination for managing COVID-19.[6] A recent *in silico* drug repurposing study identified several natural product compounds with excellent binding affinity against the three selected COVID-19 viral protein targets.[6,7] Phytochemicals containing biologically active polyphenols have been reported as effective agents against COVID-19 disease.[8]

Neocarya macrophylla (Sabine) Prance ex F. White is a West African plant species belonging to the Chrysobalanaceae family. It has been used in ethnomedicine to treat different diseases such as pulmonary troubles, inflammations, breathing disorders, internal troubles, and other gastrointestinal tract (GIT) related issues.[9] Chemically, steroids, flavonoids, and glycosides are the major secondary metabolites found in the plant.[10] Based on the anecdotal uses and some observed biological effects[10] of *N. macrophylla*, this has strengthened us to perform molecular docking and absorption, distribution, metabolism, excretion, and toxicity (ADMET) analysis of some compounds isolated from the plant against three targets of SARS-CoV-2 proteins [3C-like protease, spike protein, and papain-like protease (PL^{pro})].

MATERIALS AND METHODS

Ligand selection and preparation
Catechin, catechin-3-rhamnoside, epicatechin, and quercetin (Figure 1) were previously isolated from the stem bark and leaves of *N. macrophylla* using a combination of silica gel and a Sephadex LH-20 column. The structures of the compounds were established using one-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance spectroscopic analysis and by direct comparison of data obtained with those reported in the literature.[11,12] The SDF files of catechin (CID: 9064), catechin-3-rhamnoside (CID: 21626704), epicatechin (CID: 72276), and quercetin (5280343) were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/). The structures were prepared and converted to protein data bank (PDB) format using Chimera 1.14.[13]

Protein preparation
Crystallography structures of the SARS-CoV-2 main protease (PDB: 6LU7), spike protein (PDB ID: 6LZG), and PL^{pro} (PDB: 6W9C) were retrieved from the PDB (https://www.rcsb.org). The 3D structures of the proteins were prepared by removing all water molecules and non-standard residues to alleviate errors and as a cleanup of a PDB file retaining only ATOM and TER records and it was modified by adding hydrogens and minimized.[13]

Molecular docking
Molecular docking studies were conducted to estimate the binding energies of the compounds isolated from *N. macrophylla* against the three protein targets of SARS-CoV-2 using AutoDock Vina software.[14] The prepared proteins and the ligands were converted into PDB partial charge, and atom type format using AutoDock tools. The grid box dimensions for each protein were noted as indicated in Table 1. Molecular docking was performed using AutoDock tools in PyRx software and post docking analysis was conducted using the BIOVIA Discovery studio visualizer 2020 and Chimera 1.14.[13]

In silico ADMET and drug-likeness prediction
The physicochemical properties of the compounds (catechin, catechin-3-rhamnoside, epicatechin, and quercetin) and their ADME and toxicity were determined using swiss ADME and pkCSM ADMET descriptors algorithm protocol.[15,16] The drug-likeness properties of the compounds were predicted using the Molinspiration Cheminformatics free web service (https://www.molinspiration.com/cgi-bin/properties) by inserting the Canonical SMILES of the compounds.

No statistical data were used for this study.

RESULTS

Molecular docking data
The four compounds (catechin, catechin-3-rhamnoside, epicatechin, and quercetin) isolated from *N. macrophylla* were screened against three important protein targets of SARS-CoV-2, including main protease, spike protein, and PLpro by conducting a molecular docking analysis using AutoDock Vina
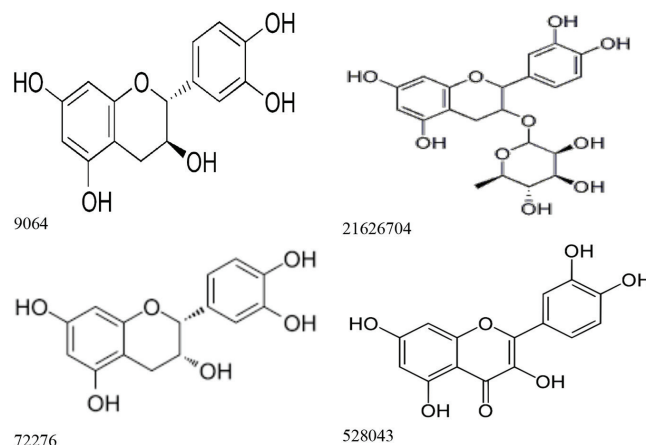

Figure 1. 2D-structures of the ligands isolated from *Neocarya macrophylla*

tools in PyRx. The docking scores of the four ligands at the active sites of the three proteins are shown in Table 2.

Based on the analysis of the docking results (Figure 2), interactions between the ligands (*i.e.* catechin, catechin-3-rhamnoside, epicatechin, and quercetin) and the binding sites of the main protease of the SARS-CoV-2 were consistent; the binding energies for the best pose against the SARS-CoV-2 main protease ranged from -8.0 to -6.9 kcal/mol with catechin-3-rhamnoside having the highest docking score. All the ligands interacted with key active site residues such as HIS41 and CYS145. Catechin formed five conventional hydrogen bonds with GLU166, ARG188, and THR190. Quercetin formed a hydrogen bond with GLU166 only and catechin-3-rhamnoside formed hydrogen bonds with THR26, LEU141, ASN142, GLY143, and MET165, while epicatechin interacted with LEU141, HIS163, and GLN189 *via* H-bond (Table 3).

The interaction of the spike protein with the compounds is shown in Figure 3. The ligands exhibited lower binding energies ranging from -7.1 to -6.3 kcal/mol. Catechin-3-rhamnoside indicated the highest affinity (-7.1) toward the receptor, while epicatechin had the least affinity (Table 2). All the compounds interacted with similar amino acid residues of clinical importance such as TYR505 (active site residue). Catechin-3-rhamnoside formed a conventional hydrogen bond with TYR449, and GLY496. Quercetin was found to form four conventional hydrogen bonds with TYR505, ASN501, GLY496 and GLN493. Catechin formed a hydrogen bond with ASN501 and GLY496, while epicatechin interacted (H-bond) with TYR505 at the active sites (Table 3).

The interaction formed between the ligands and the PL[pro] of SARS-CoV-2 coronavirus is shown in Figure 4. Epicatechin had the lowest docking score and highest affinity while catechin was the least. Catechin-3-rhamnoside formed five hydrogen bonds with residues ARG166, ASP164, TYR264, TYR268, and GLY163. Epicatechin formed three hydrogen bonds with ASP302, ASN267, TYR264, and TYR268. Also, pication was built between the ligand and ARG166. Two hydrogen bonds were formed with residues ASP302 and ARG166 for catechin, whereas quercetin formed only one hydrogen bond with ARG166 (Table 3).

In silico ADMET and drug-likeness evaluation

The results of the *in silico* ADMET screening and drug-likeness of the compounds are presented in Tables 4 and 5. The analysis of different parameters, including physicochemical properties, ADMET and drug-likeness was performed.
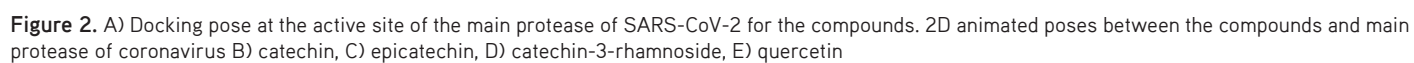
DISCUSSION

COVID-19, a highly transmissible disease, has rapidly spread all over the world.[17,18] This necessitated the need for research to develop effective and safe therapy for managing the disease in the absence of therapeutic drug(s) and vaccine. Natural products, either singly or in combination, have proven to be effective in the management of COVID-19.[5,19] *N. macrophylla* has been used traditionally to treat pulmonary troubles, inflammations, breathing disorders, internal issues, and other GIT related issues.[9] In this study, we selected three important coronavirus protein targets

i.e. the main protease, spike protein, and PL[pro], which were docked with four compounds isolated from *N. macrophylla* (catechin, catechin-3-rhamnoside, epicatechin, and quercetin) as ligands.[9-12] The spike glycoprotein (SGp) of coronavirus attaches to ACE-2 receptor, thereby allowing viral entry.[20] Viral genome replication will thereafter set in by RNA-dependent RNA polymerase *(RdRP)* gene.[21] The main proteinase (3CL[pro]) and PL[pro] facilitates the process of proteolysis of the viral polyprotein into functional units.[4] In order words, the proteins SGp, ACE-2, 3CLpro, PLpro, and RdRP are directly involved in either the establishment of the disease, translation, and replication or facilitate the proliferation of the virus in the host cell.[20]

The docking scores of the compounds against the individual proteins revealed binding energies ranging from -6.3 to -8.0 kcal/mol, which was higher compared to the docking scores reported for remdesivir, hydroxychloroquine, ribavirin, and arbidol that were used as control.[17] All the ligands interacted with key active site residues of the SARS-CoV-2 main protease, such as HIS41 and CYS145.[22] Shah et al.[23] reported that the OH group of lopinavir interacted with GLU166 and HIS41. Besides, remdesivir and methisazone have also been reported to form H-bond with GLU166, ASN142, and THR190 for the main protease. Peterson[16] also reported a higher docking score (-7.7 kcal/mol) for quercetin. The main protease plays a vital role in viral replication. Thus, inhibiting the activity of the main protease could block the replication of the coronavirus inside infected cells. Hence, based on the results of our study, phytoconstituents of *N. macrophylla* may be potential inhibitors of the main protease of SARS-CoV-2.

SGp of SARS-CoV-2 plays an important role in facilitating the viral attachment, fusion, and viral entry into the host cells.[20] Phytoconstituents with lower binding energy toward the receptor could serve as a potential drug for further studies. The ligands demonstrated lower binding energy and have a higher affinity for the spike glycoprotein. Non-covalent interactions of the compounds detected by AutoDock Vina tools in PyRx revealed that all compounds interacted with catalytic residue TYR505 (active site) of the spike protein. Chikhale et al.[24] reported similar docking results and interactions for quercetin-3-O-galactosyl-rhamnosylglucoside, hydroxychloroquine, and lopinavir. However, the binding affinities of the tested ligands (catechin, catechin-3-rhamnoside, epicatechin, and quercetin) were higher than those of hydroxychloroquine (-3.57 kcal/mol), remdesivir (-4.41 kcal/mol), and lopinavir (-4.22 kcal/mol).[24] Pandey et al.[25] also reported a lower binding affinity of -5. 6 for hydroxychloroquine.

The PL[pro] plays a vital role in processing viral polyproteins to generate a functional replicase complex and enable viral spread[26,27] and it is also implicated in cleaving proteinaceous post-translational modifications on host proteins as an evasion mechanism against host antiviral immune responses.[28-30] The selected ligands could dock into an entirely different binding pocket of PL[pro] with lower binding energy compared to the standard inhibitor, α-ketoamide 13 b (-8.24 kcal/mol) as reported by Gurung et al.[19]

A

B

C

D

E

Figure 2. A) Docking pose at the active site of the main protease of SARS-CoV-2 for the compounds. 2D animated poses between the compounds and main protease of coronavirus B) catechin, C) epicatechin, D) catechin-3-rhamnoside, E) quercetin

The ADMET analysis of catechin, catechin-3-rhamnoside, epicatechin, and quercetin was predicted by Swiss ADME and pkCSM ADMET descriptors algorithm protocol.[15,16] The important parameters related to ADMET properties such as Lipinski's[31] rule of five, the solubility of drug, pharmacokinetic properties, molar refractivity, and drug likeliness were evaluated. According to the rule, molecules should have molecular weight ≤500, hydrogen bond donors ≤5 and acceptors ≤10, calculated octanol-water and -partition coefficient, and log *p*≤5 possess good membrane permeability and molar refractivity should be between 40-130.[31] The ADMET and drug-likeness results indicated that all compounds have satisfied the limitations and drug-likeness. The study assisted in screening the best compound(s) with drug-likeness in a biological system and the compounds were considered to be well absorbed based on the predicted values of intestinal absorption, which were >30% and Caco2 permeability values were also normal. Human VDss

of the compounds ranged from -1.559 to 2.001 L/kg that was within the range; thus, values of 0.71 L/kg are considered low and 2.81 L/kg as high. Blood-brain barrier (BBB) permeability log BB of <-1 is considered as poorly absorbed, while a value

Table 1. Grid box dimensions

S/no	Name of the protein (PDB[a] ID)	Grid box center	Grid dimension
1	Main protease (PDB: 6LU7)	-25.8 x 13.3 x 56.2	54 x 69 x 64
2	Spike protein (PDB ID: 6LZG)	-32.1 x 28.0 x 21.2	44 x 54 x 61
3	Papain-like protease (PDB: 6W9C)	-22.3 x -2.84 x 24.3	44 x 74 x 77

[a]PDB: Protein data bank

Table 2. Docking scores of the compounds against three target proteins SARS-CoV-2

Compound name	Compound ID	Docking scores (kcal/mol)		
		Main protease	Spike protein	Papain-like protease
Catechin	9064	-7.0	-6.6	-6.4
Catechin-3-rhamnoside	21626704	-8.0	-7.1	-6.9
Epicatechin	72276	-6.9	-6.3	-7.1
Quercetin	528043	-6.9	-6.7	-7.0

Table 3. Interactions of the compounds against the three target proteins of SARS-CoV-2

Compound name	Interactions		
	Main protease	Spike protein	Papain-like protease
Catechin	**H-bond:** GLU166, ARG188, THR190 **Others**: PHE140, LEU141, SER144, LYS145, HIS163, HIS164, MET165, HIS172, GLN189, GLN192	**H-bond:** GLY496, ASN501 **Others**: ARG403, GLU406, LYS417, TYR453, PHE497, TYR495, GLN498, TYR505	**H-bond:** ARG166, ASP302 **Others**: LEU162, GLY163, ASP164, VAL165, MET208, SER245, ALA246, PRO248, SER262, TYR264, ASN267, TYR268, TYR273, THR301
Catechin-3-rhamnoside	**H-bond:** THR26, LEU141, ASN142, GLY143, MET165 **Others:** THR25, LEU27, HIS41, CYS44, MET49, TYR54, PHE140, SER144, CYS145, HIS163, HIS164, GLU166, ASP187, VAL186, ARG188, GLN189, GLN192	**H-bond:** TYRd449, GLY496 **Others**: ARG403, TYR453, SER494, TYR495, PHE497, GLN498, ASN501, GLY502, TYR505	**H-bond:** GLY163, ASP164, ARG166, TYR264, TYR268 **Others**: VAL165, LEU162, MET208, PRO248, TYR273, ASN267, THR301
Epicatechin	**H-bond:** LEU 141, HIS163, GLN189 **Others:** HIS41, CYS44, MET49, PHE140, ASN142, SER144, CYS145, HIS164, MET165, GLU166, ASP187, ARG188,	**H-bond:** TYR505 **Others:** ARG403, GLU406, LYS417, TYR453, LEU455, TYR495, GLY496, PHE497, GLN498, ASN501	**H-bond:** ASN267, TYR268, ASP302 **Others:** VAL165, ASP164, ARG166, MET208, MET243, SER245, ALA246, PRO248, SER262, TYR264, GLY266, TYR273, THR301
Quercetin	**H-bond:** GLU166 **Others:** HIS41, CYS44, MET49, TYR54, CYS145, HIS164, MET165, LEU167, PRO168, ASP187, ARG188, GLN189, THR190, ALA191, GLN192	**H-bond:** GLN493, GLY496, ASN501, TYR505 **Others:** ARG403, TYR453, GLN493, TYR495, SER494, GLN496, PHE497, GLN506	**H-bond:** ARG166 **Others:** LEU162, ASP164, GLY163, MET208, SER245, PRO248, ASN267, TYR268, TYR273, THR301, ASP302

SARS-CoV-2: Severe acute respiratory syndrome-coronavirus 2
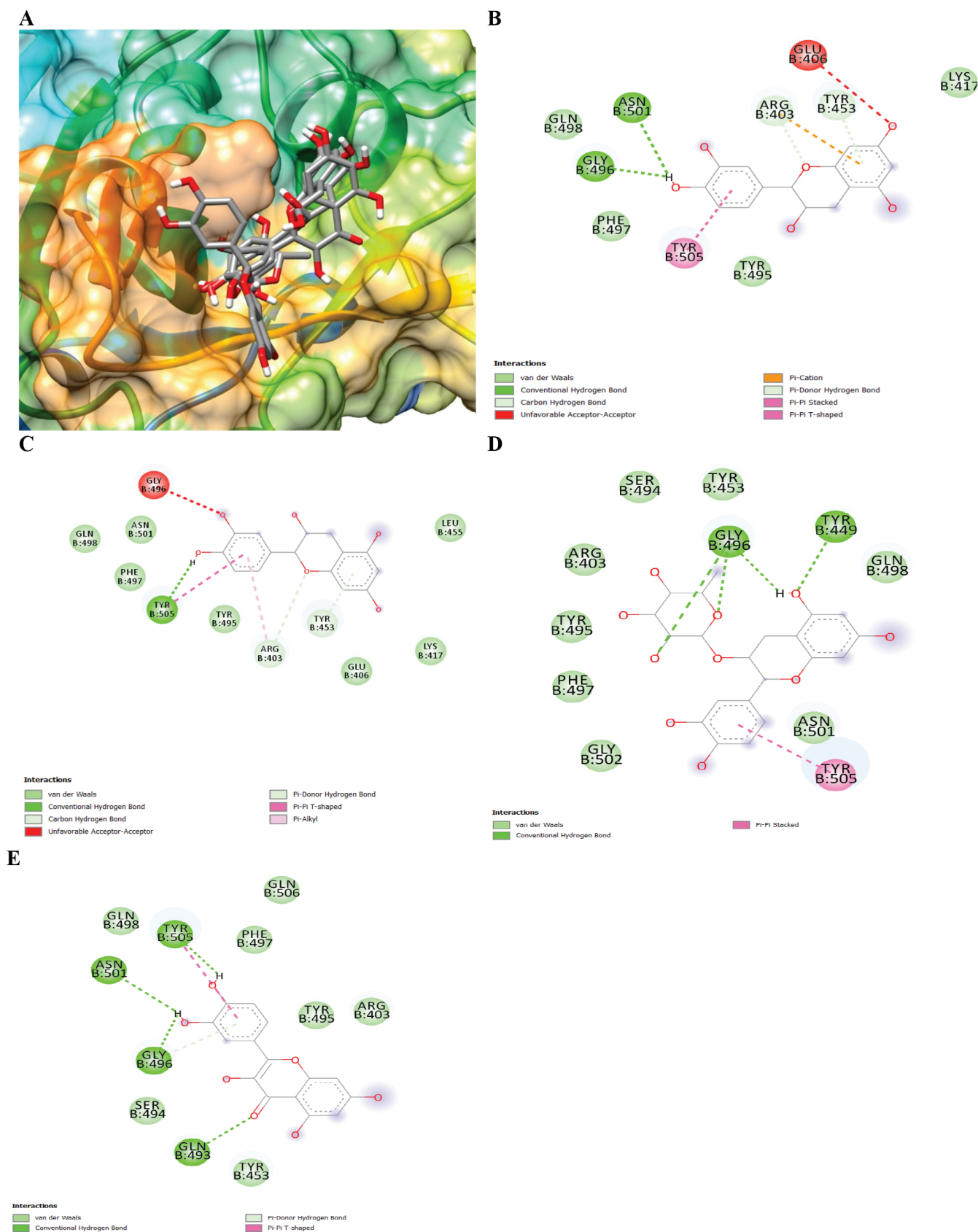
Figure 3. A) Docking pose at the active site of the main spike protein of SARS-CoV-2 for the compounds. 2D animated poses between the compounds and spike protein of coronavirus B) catechin, C) epicatechin, D) catechin-3-rhamnoside, E) quercetin
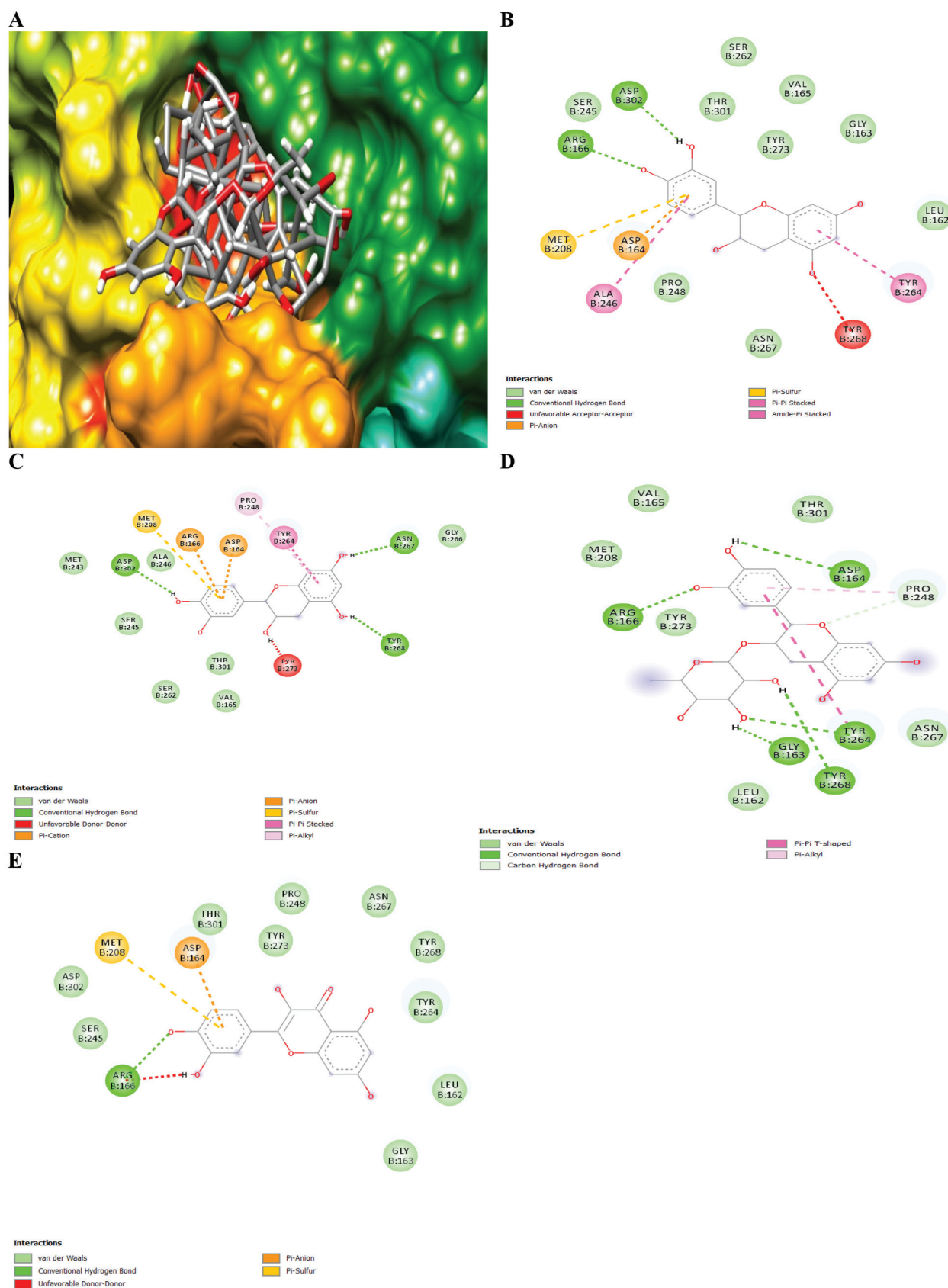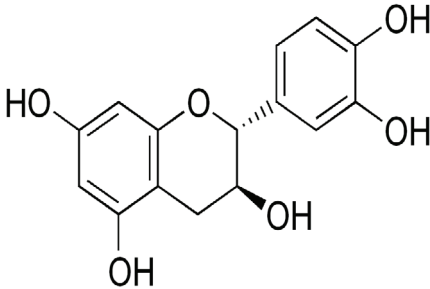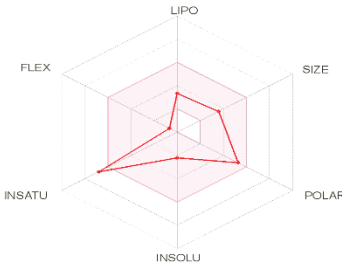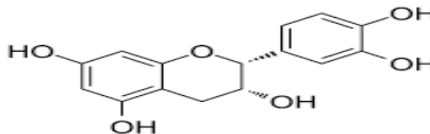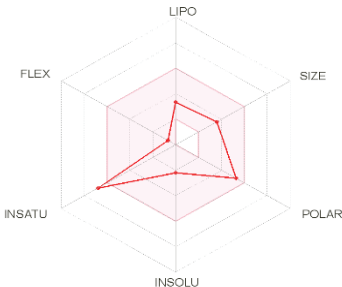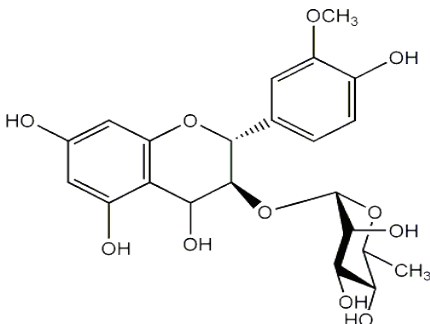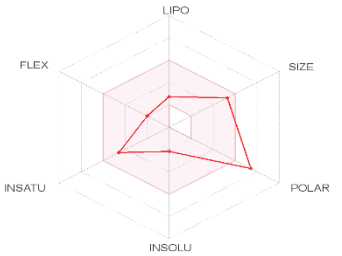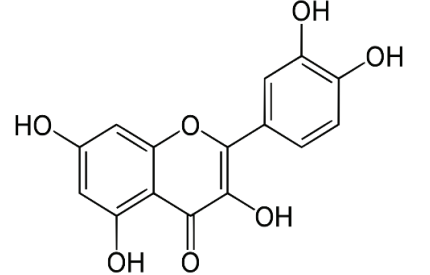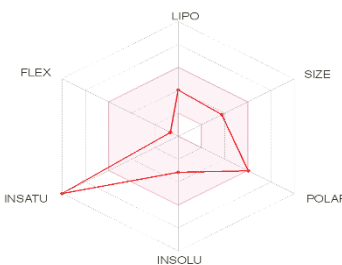
Figure 4. A) Docking pose at the active site of the papain-like protease of SARS-CoV-2 for the compounds. 2D animated poses between the compounds and spike protein of coronavirus B) catechin, C) epicatechin, D) catechin-3-rhamnoside, E) quercetin

Table 4. *In silico* ADMET properties of compounds from *Neocarya macrophylla*

Properties	Quercetin	Catechin	Epicatechin	Catechin-3-rhamnoside
Physicochemical properties				
Formula	$C_{15}H_{10}O_7$	$C_{15}H_{14}O_6$	$C_{15}H_{14}O_6$	$C_{21}H_{24}O_{10}$
Molecular weight (g/mol)	302.24	290.27	290.27	436.41
Fraction Csp3	0.00	0.20	0.20	0.43
H-bond donor	5	5	5	7
H-bond acceptor	7	6	6	10
Molar refractivity	78.03	74.33	74.33	105.56
TPSA	131.36 Å²	110.38 Å²	110.38 Å²	169.30 Å²
Absorption				
Water solubility (log mol/L)	-2.925	-3.117	-3.117	-2.98
Caco2 permeability (log Papp in 10^{-6} cm/s	-0.229	-0.283	-0.283	-0.002
Intestinal absorption (human) (% absorbed)	77.207	68.829	68.829	48.902
Skin permeability (log Kp)	-2.735	-2.735	-2.735	-2.735
P-glycoprotein substrate	Yes	Yes	Yes	Yes
P-glycoprotein inhibitor I	No	No	No	No
P-glycoprotein inhibitor II	No	No	No	No
Distribution				
VDss (human, log L/kg)	-1.559	1.027	1.027	2.001
Fraction unbound human (Fu)	-0.206	0.235	0.235	0.309
BBB permeability (log BB)	-1.098	-1.054	-1.054	-1.345
CNS permeability (log PS)	-3.065	-3.298	-3.298	-3.988
Metabolism				
CYP2D6 substrate	No	No	No	No
CYP3A4 substrate	No	No	No	No
CYP1A2 inhibitor	Yes	No	No	No
CYP2C19 inhibitor	No	No	No	No
CYP2C9 inhibitor	No	No	No	No
CYP2D6 inhibitor	No	No	No	No
CYP3A4 inhibitor	No	No	No	No
Excretion				
Total clearance (log mL/min/kg)	0.407	0.183	0.183	-0.393
Renal OCT2 substrate	No	No	No	No
Toxicity				
Maximum tolerated dose (human) (log mg/kg/day)	0.499	0.438	0.438	0.449
Oral rat acute toxicity (LD_{50}) (mol/kg)	2.471	2.428	2.428	2.446
Oral rat chronic toxicity (LOAEL) (log mg/kg_bw/day)	2.612	2.500	2.500	2.808
Hepatotoxicity	No	No	No	No
Tetrahymena pyriformis toxicity (log µg/L)	0.288	0.347	0.347	0.285
Minnow toxicity (log mM)	3.721	3.585	3.585	6.116
Drug-likeness				
MiLogP	1.68	1.37	1.37	0.69
TPSA	131.35	110.37	110.37	169.30

Table 5. Boiled-egg model for ADMET/drug-likeness of the compounds from *Neocarya macrophylla*

Compounds	2D structure	Boiled egg model for ADMET/drug likeness
Catechin		
Epicatechin		
Catechin-3-rhamnoside		
Quercetin		

of >0.3 is considered as well. A drug can penetrate central nervous system, when the logPS is >-2, however, logPS of <-3 is considered as poor. A compound is considered toxic, when *Tetrahymena pyriformis* value is >-0.5 µg/L and high acute toxicity for compounds can as well be attributed to a minnow toxicity LC_{50} of <-0.3 Mm.[20]

All the compounds had five hydrogen bonds except catechin-3-rhamnoside, which had seven and the hydrogen bond acceptors were within the range. The hydrophilicity of the compounds determined by calculating the log *p* value indicated that the compounds have good absorption. Thus, higher log *p* values result in poor absorption. The calculated polar surface area (PSA) was within the range of 7.0-200.0 Å. One violation of Lipinski's rule (PSA, molecular weight, number of hydrogen donors, and acceptors) was observed for catechin-3-rhamnoside, which indicates the compound's potential as a drug-like molecule.

Boiled-egg for ADMET/drug-likeness is an accurate predictive model used to estimate various stages of drug discovery; it works by computing the lipophilicity and polarity of small

molecules.[32] The pink area represents the optimal range for each property constituting lipophilicity, molecular weight, polarity, solubility, saturation, flexibility among others.

CONCLUSION

In conclusion, we have screened four compounds (catechin, catechin-3-rhamnoside, epicatechin, and quercetin) isolated from *N. macrophylla* using molecular docking, *in silico* ADMET, and drug-likeness prediction. The findings of this study have shown that the plant *N. macrophylla* may contain potential leads for SARS-CoV-2 inhibition and thus, should be studied further for development as effective therapeutic agents against COVID-19.

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Ethics

Ethics Committee Approval: Not applicable.

Informed Consent: Not applicable.

Authorship Contributions

Concept: A.J.Y., Design: A.J.Y., M.I.A., A.M.M., H.A., A.M.A., A.H.N., Data Collection or Processing: A.J.Y., M.I.A., A.M.M., H.A., A.M.A., A.H.N., Analysis or Interpretation: A.J.Y., A.H.N., Literature Search: A.J.Y., M.I.A., A.M.M., H.A., A.M.A., A.H.N., Writing: Amina A.J.Y., H.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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