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Potential inhibitors of SARS CoV-2 from *Neocarya macrophylla*: Chemoinformatic and Molecular modeling studies against three key targets

Short title: Constituents of Neocarya macrophylla against coronavirus

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Abstract
Objective: The novel Corona Virus Disease (COVID-19) which emerged in China is a highly transmittable and pathogenic viral infection caused by the 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 aimed to perform molecular docking and ADMET analysis of some compounds isolated from *Neocarya macrophylla* 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 swissADME 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 targets proteins of SARS CoV-2. ADMET analysis was used to predict important pharmacokinetic properties of the compounds such as aqueous solubility, blood-brain barrier (BBB), plasma protein binding, CYP2D6 binding, intestinal absorption, and hepatotoxicity.
Conclusion: The findings of this study have shown that the plant *N. macrophylla* contains potential leads for SARS CoV-2 inhibition and thus, should be studied further for development as therapeutic agents against COVID-19.
Keywords: *Neocarya macrophylla*, SARS-CoV-2, flavonoids, ADME-T

Short title: Constituents of *N. macrophylla* against coronavirus using *in silico* approach

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 of the world necessitating the declaration of the disease as a Public Health Emergency of International Concern (PHEIC) by WHO on the 30th January 2020[2]. The life cycle of coronavirus mediates its infection in few typical steps viz; (i) attachment which has to do with transmission inside the human body as well as binding of the virus spike protein with ACE2 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 RTC and replication of viral RNA, (iv) maturation i.e. transcription of subgenomic mRNAs, (v) release which has to do 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 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 the management of 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 coronavirus disease[5]. There are several anecdotal accounts of the use of plant extracts including *Artemisia annua*, *Pyrrosia lingua*, *Lindera aggregate, Zingiber officinale, Syzygium aromaticum*, and *Allium sativum*, singly or as a combination for the management of COVID-19[6]. A recent *in-silico* drug repurposing studies 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].

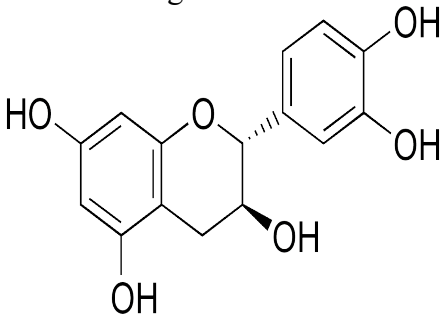
Neocarya macrophylla is a West African plant species that belongs to the Chrysobalanaceae family. It has been used in ethnomedicine to treat different diseases such as pulmonary troubles, inflammations, breathing disorders, internal troubles, and other GIT related issues[9]. Chemically, steroids, flavonoids, 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 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).

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 Sephadex LH-20 column. The structures of the compounds were established using one-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR) 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 PDB format using Chimera 1.14[13].



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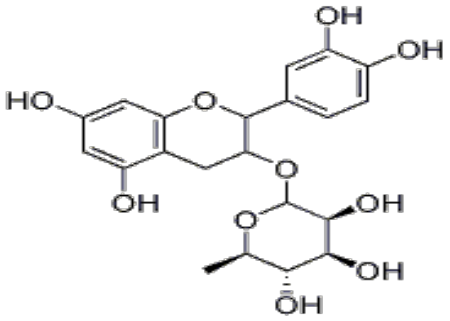

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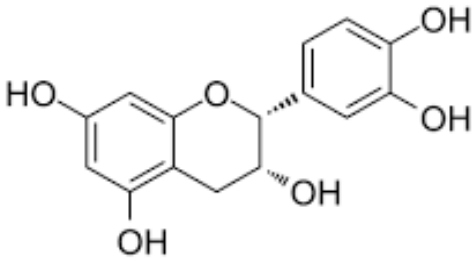

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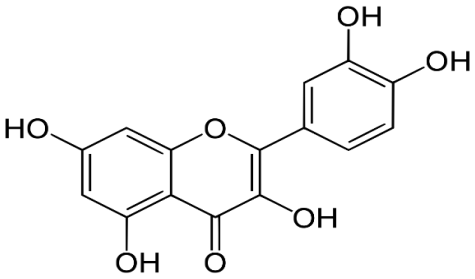

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Figure 1: 2D-Structures of the ligands isolated from *Neocarya macrophylla*

Protein preparation

Crystallography structure of the SARS CoV-2 main protease (PDB: 6LU7), spike protein (PDB ID: 6LZG), and papain-like protease (PDB: 6W9C) were retrieved from the protein data bank (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

The molecular docking studies were conducted to estimate the binding energies of the isolated compounds 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 PDBQT 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].

Table 1: Grid box dimension

S/No	Name of protein (PDB 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

2.4 *In silico* ADMET and drug-likeness prediction

The physicochemical properties of the compounds (catechin, catechin-3-rhamnoside, epicatechin, and quercetin) and their ADME (absorption, distribution, metabolism, excretion) and toxicity was conducted using swissADME and pkCSM ADMET descriptors algorithm protocol[15,16]. The drug-likeness properties of the compounds were predicted using Molinspiration Cheminformatics free web services (https://www.molinspiration.com/cgi-bin/properties) by inserting the Canonical SMILES of the compounds.

RESULTS

Molecular docking

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 papain-like protease by conducting a molecular docking analysis using AutoDock Vina tools in PyRx. The docking scores of the four ligands at the active site 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 ranges 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) towards the receptor while epicatechin had the least affinity (Table 2). All the compounds interacted with similar amino acid residues of clinical importance such as TYR 505 (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 TYR 505, ASN501, GLY496 and GLN493. Catechin formed a hydrogen bond with ASN501 and GLY496 while epicatechin interacted (H-bond) with TYR 505 at the active sites (Table 3).

Interaction formed between the ligands and the papain-like protease 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, pi-cation was built between the ligand and ARG166. Two hydrogen bonds were formed with residues ASP302 and ARG166 for catechin, while 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 4,5. The analysis of different parameters including physicochemical properties, absorption, distribution, metabolism and toxicity and drug-likeness was performed.

Table 2: Docking scores of the compounds against three targets proteins of SARS CoV-2

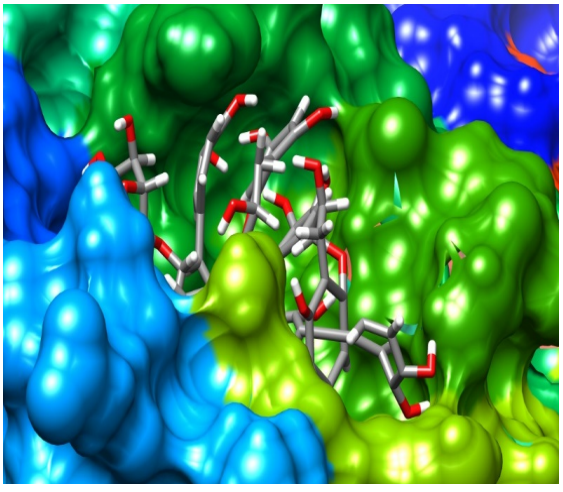
		Docking scores (kcal/mol)		
Compound name	**Compound ID**	**Main protease**	**Spike protein**	**papain-like protease**
Catechin	9064	-7.0	-6.6	-6.4
Catechin-3-rhamnoside	21626704	-8.0	-7.1	-6.9
Epicatechin	72276	-6.9	-6.3	-7.1
Quercetin	528043	-6.9	-6.7	-7.0

Table 3: Interactions of the compounds against the three targets proteins of SARS CoV-2

	Interactions		
Compound name	**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	**H-Bond**: GLN493, GLY496, ASN501, TYR505	**H-Bond**: ARG166

	Others: HIS41, CYS44, MET49, TYR54, CYS145, HIS164, MET165, LEU167, PRO168, ASP187, ARG188, GLN189, THR190, ALA191, GLN192	**Others**: ARG403, TYR453, GLN493, TYR495, SER494, GLN496, PHE497, GLN506	**Others**: LEU162, ASP164, GLY163, MET208, SER245, PRO248, ASN267, TYR268, TYR273, THR301, ASP302

A



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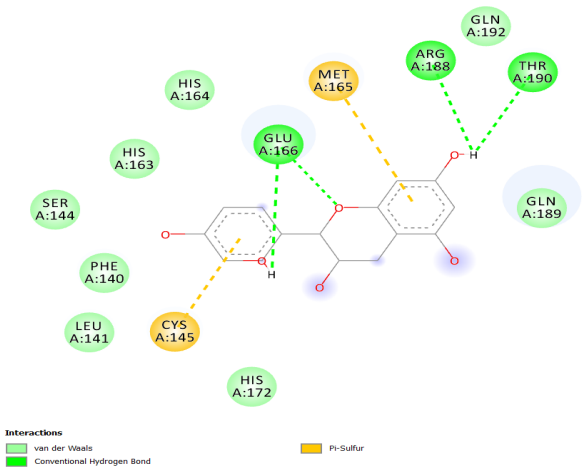

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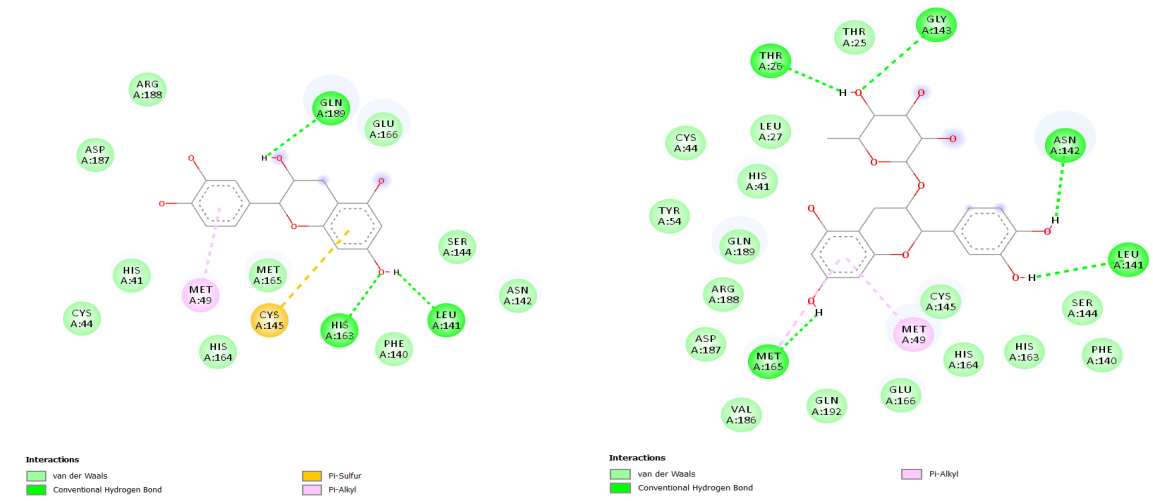

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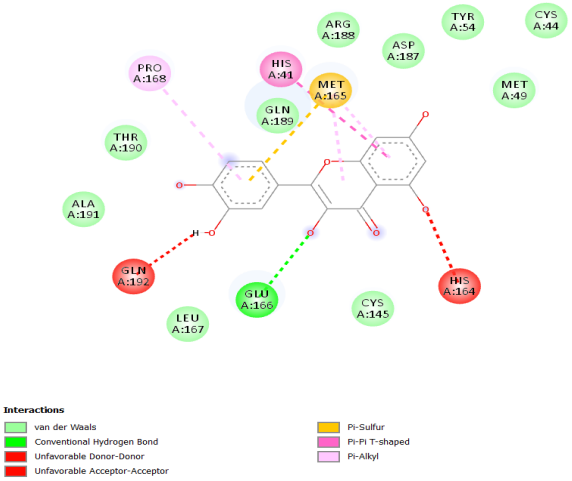
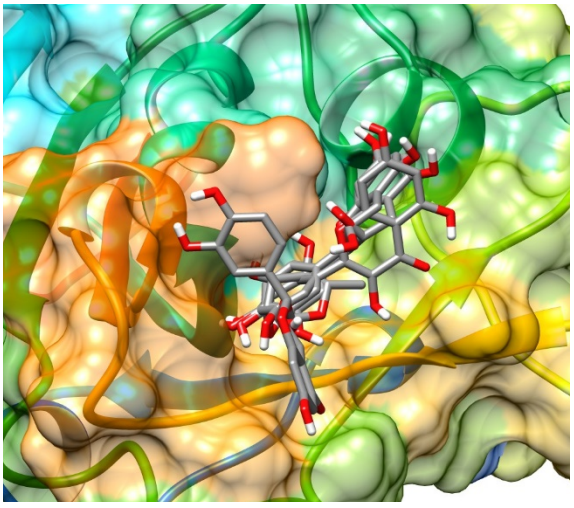


Figure 2: A) Docking pose at the active site of the main protease of SAR CoV-2 for the compounds. 2D animated poses between the compounds and main protease of coronavirus **B)** catechin, **C)** epicatechin, **D)** catechin-3-rhamnoside, **E)** Quercetin

A



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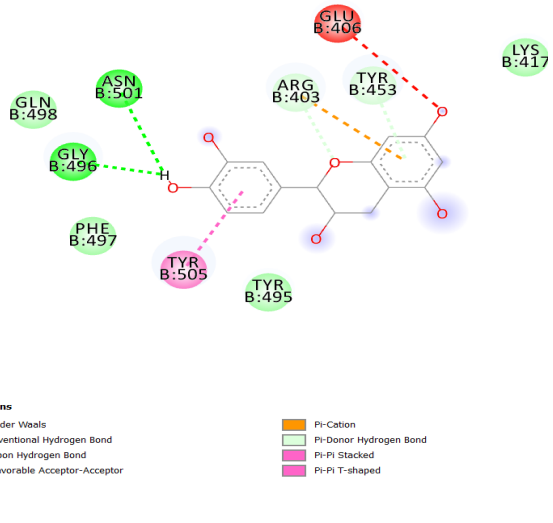

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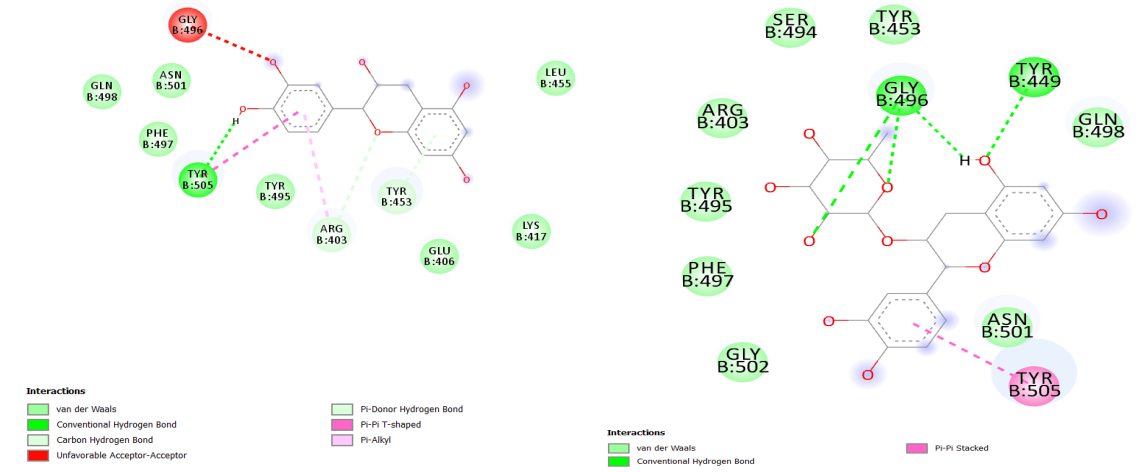

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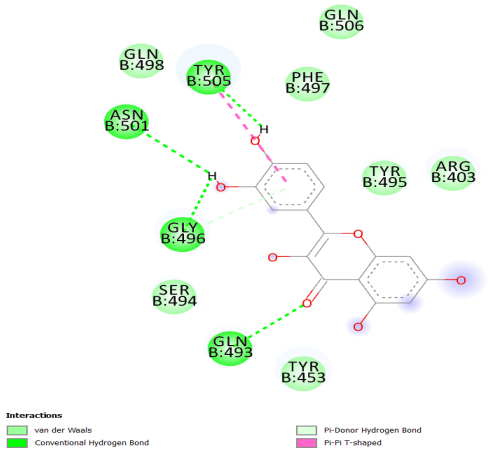

Figure 3: A) Docking pose at the active site of the main spike protein of SAR CoV-2 for the compounds. 2D animated poses between the compounds and spike protein of coronavirus **B)** catechin, **C)** epicatechin, **D)** catechin-3-rhamnoside, **E)** Quercetin

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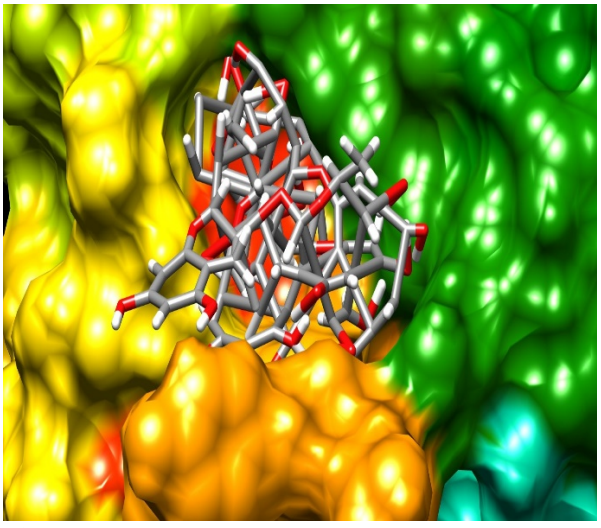

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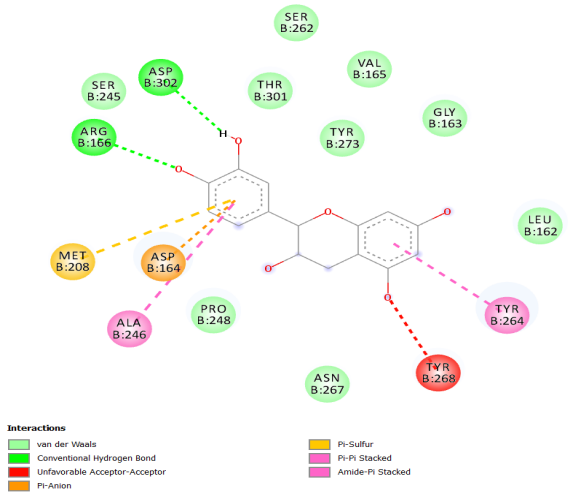

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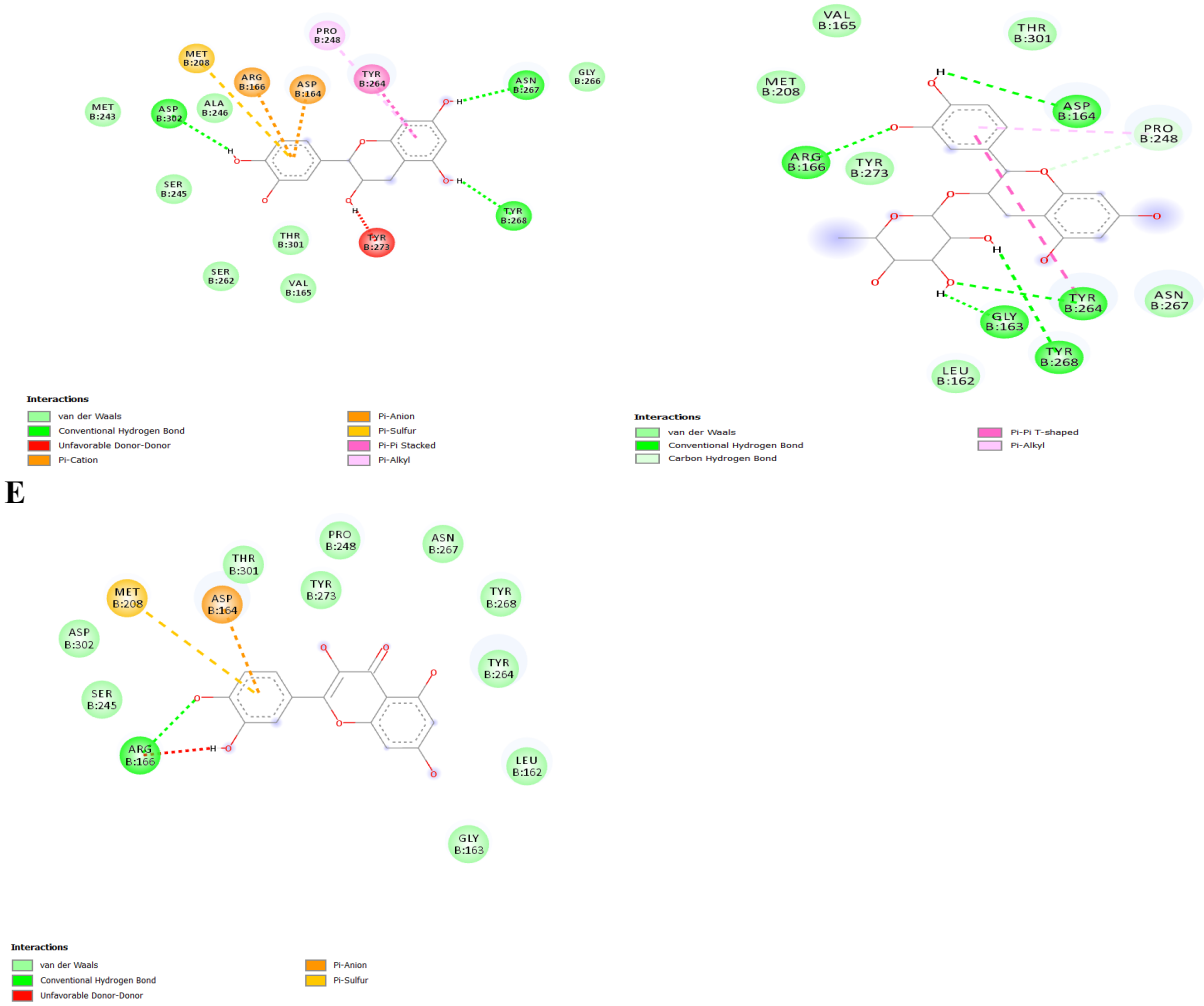

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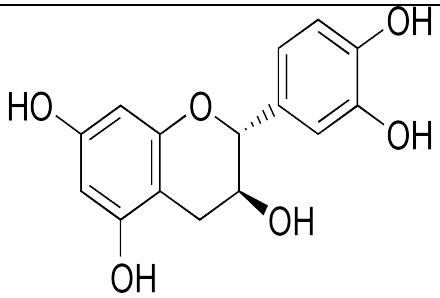
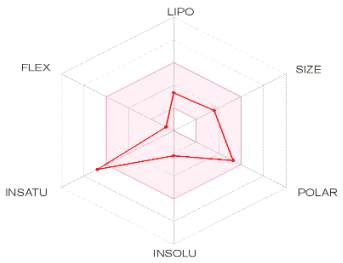
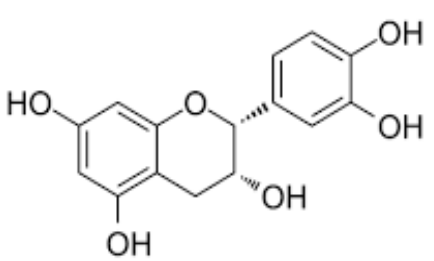
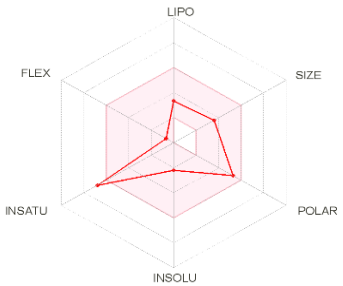
Figure 4: A) Docking pose at the active site of the papain-like protease of SAR CoV-2 for the compounds. 2D animated poses between the compounds and spike protein of coronavirus **B)** catechin, **C)** epicatechin, **D)** catechin-3-rhamnoside, **E)** Quercetin

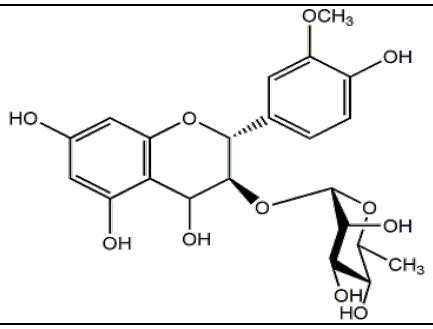
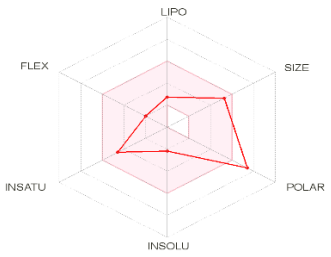
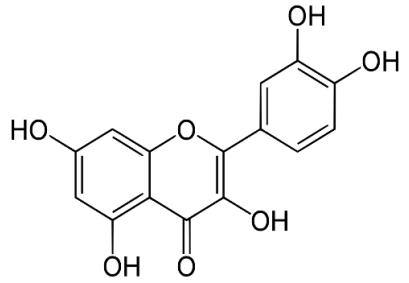
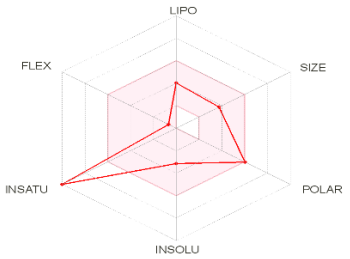
Table 4: *In silico* ADMET properties of compounds from *N. macrophylla*

Properties	Quercetin	Catechin	Epicatechin	Catechin-3-rhmanoside
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 (logBB)	-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				
Max. 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
T.Pyriformis toxicity (log ug/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 *N. macrophylla*

Compounds	2D structure	Boiled Egg model for ADMET/Druglikeness
Catechin		
Epicatechin		

Catechin-3-rhamnoside		
Quercetin		

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 the management of 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* have been used traditionally to treat pulmonary troubles, inflammations, breathing disorders, internal troubles, and other GIT related issues[9]. In this study we selected three important coronavirus protein targets i.e. the main protease, spike protein, and papain-like protease 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 angiotensin-converting enzyme 2 (ACE2) receptor thereby allowing virus entry[20]. Viral genome replication will thereafter set in by RNA-dependent RNA polymerase (RdRP) gene[21]. The main proteinase (3CLpro) and papain-like protease (PLpro) facilitates the process of proteolysis of the viral polyprotein into functional units[4]. In order words, the proteins SGp, ACE2, 3CLpro, PLpro, and RdRP are directly involved in either establishment of the disease, translation, and replication or facilitates 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 residue 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 were also 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 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.

Spike glycoprotein 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 towards the receptor could serve as a potential drug for further studies. The ligands have demonstrated lower binding energy and have a higher affinity for the spike glycoprotein. Noncovalent interactions of the compounds detected by AutoDock Vina tools in PyRx revealed that all the compounds interacted with catalytic residue TYR 505 (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 to 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 papain-like protease 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 were able to dock into an entirely different binding pocket of the papain-like protease with lower binding energy compared to the standard inhibitor, α-ketoamide 13 b (-8.24 kcal/mol) as reported by Gurunga et al.[19].

ADMET analysis of catechin, catechin-3-rhamnoside, epicatechin, and quercetin was predicted by the swissADME and pkCSM ADMET descriptors algorithm protocol[15,16]. The important parameters related to ADMET properties such as Lipinski's rule of five, the solubility of drug, pharmacokinetic properties, molar refractivity, and drug likeliness were evaluated[31]. 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 the 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 ranges from -1.559 to 2.001 L/kg which were within the range; thus, values of 0.71 L/kg are considered as low and 2.81 L/kg as high. BBB permeability logBB of <-1 is considered as poorly absorbed while a value of >0.3 is considered as good. A drug can be able to penetrate CNS when the LogPS is >-2, however, LogPS of <-3 is considered as poor. A compound is considered toxic when the *T. 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 <-0.3Mm[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 the compounds have good absorption. Thus, higher log P values result in poor absorption. Calculated PSA was within the range of 7.0-200.0 Å. One violation of Lipinski's rule (polar surface area, 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 use 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 of the properties 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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CONFLICT OF INTERESTS

None declared.

ABBREVIATIONS

SARS-CoV-2:	Severe acute respiratory syndrome coronavirus 2
WHO:	World Health Organization
ADMET:	Absorption, Distribution, Metabolism and Excretion &Toxicology tests
3C-like protease:	Chymotrypsin-like protease
CYP2D6:	Cytochrome P450 2D6
ACE2:	Angiotensin converting enzyme
RTC:	Replication-transcription complex
RNA:	Ribonucleic acid
GIT:	Gastrointestinal tract
CID:	Compound Identity
PDB:	Protein Data Bank
PDBQT:	Protein Data Bank, Partial Charge (Q), & Atom Type (T)

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