Original Article

JHSMR Journal of Health Science and Medical Research

In Silico Analysis of Antioxidant Phytochemicals with Potential NADPH Oxidase Inhibitory Effect

Daniel Hassan Mhya, Ph.D.[1], Abdullahi Garba Jakwa, M.Sc.[2], John Agbo, Ph.D.[3]

[1]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Abubakar Tafawa Balewa University, Bauchi 740231, Nigeria.

[2]Department of Biochemistry, Faculty of Sciences, Abubakar Tafawa Balewa University, Bauchi 740231, Nigeria.

[3]Department of Pharmacology and Therapeutics, Faculty of Pre-Clinical Sciences, Abubakar Tafawa Balewa University, Bauchi 740231, Nigeria.

Received 2 August 2022 ● Revised 17 September 2022 ● Accepted 23 September 2022 ● Published online 18 November 2022

Abstract:

Objective: NADPH oxidase (NOX) is known to produce reactive oxygen species (ROS) at physiological concentrations. However, it can be over-activated with some disease conditions and produces excess ROS. Several molecules have shown an ability to suppress the enzyme's over-activity, although some weaknesses have been found. Hence, the attempt to screen phytochemicals, with the aim of finding the most specific and effective NOX inhibitor.

Material and Methods: The study was carried-out via an in-silico approach. First, phytochemicals with antioxidant activity, according to the literature review, were selected and downloaded from the PubChem database in SDF files. NOX with PDB: 2CDU was downloaded from the protein databank. Drug-likeness properties and biological activities were predicted using ADMETMESH and the Predict Activity Spectra of Substances (PASS) software. Phytochemical-NOX interactions were performed via molecular docking, whereas, docked conformations and bond residue amino acids were analyzed using Protein-plus software.

Results: The result of this study predicted 13 phytochemicals with drug-likeness properties, out of which 9 showed NOX-inhibitory activity. Docking results predicted all of the 9 phytochemicals were capable of interacting with NOX, by binding to at least one amino acid. The reference inhibitor (Apocynin, -8.3 kcal/mol) and some phytochemicals (caffeic, eriodictyol, hesperetin, and morin with ΔG -6.1 to -7.7 kcal/mol) were predicted to have bonded to Ser115, via hydrogen bonding. On the other hand, epicatechin gallate and quercetin with ΔG −8.7 and −8.1 kcal/mol did not bind to Ser115, but rather through other amino acids.

Contact: Daniel Hassan Mhya, Ph.D.
Department of Medical Biochemistry, Faculty of Basic Medical Sciences,
Abubakar Tafawa Balewa University, Bauchi 740231, Nigeria.
E-mail: dmhyas@gmail.com

J Health Sci Med Res .....
doi: 10.31584/jhsmr.2022912
www.jhsmr.org

Conclusion: This study has led to the prediction of phytochemicals with NOX-inhibitory effects, which could be considered for further study.

Keywords: free-radicals, inhibition, In-silico, NADPH-oxidase, phytochemicals

Introduction

NADPH oxidase (NOX) is an enzyme complex found in several cells within different organs. It is composed of several sub-units; namely, p22phox, and NOX2 (membrane-associated); p47phox; p67phox; p40phox; and activation of the small GTPase Rac (cytosolic units). The NOX family can be expressed in vasculature terms as: NOX_{1-5}. The enzyme is known to have sole responsibility for reactive oxygen species (ROS) production.[1] It uses NADPH produced from the pentose phosphate pathway as an electron donor for the production of ROS.

ROS production has also been reported within several other enzymes like cytochrome P450 oxidases, lipoxygenases, monoamino oxidases, uncoupled nitric oxide synthase, xanthine oxidase, and the mitochondrial electron transport chain.[2] These enzymes switch into ROS production only if they have been altered by oxygen radicals.[2] High levels of ROS; such as superoxide (O_{2-}) and hydrogen peroxide (H_2O_2) in the cells have been known to create havoc on the cells via different biochemical processes; such as. oxidation and nitration of biomolecules like lipids, nucleic acids, and proteins.[3] ROS is also known to wreck havoc by stimulating cell signaling pathways, which in turn modulate factors like growth and transcriptional factors. This leads to the regulation of cell proliferation, differentiation, and finally apoptosis.[3] However, reports have indicated that ROS at physiological concentrations plays a significant role by facilitating the signal transduction obtained from receptor tyrosine kinases and transcriptional factors; such as NF-E2-related factor-2 (Nrf-2). This results in the expression of genes for antioxidant molecules.[4]

NOX-derived ROS has also been found to propagate several pathological conditions; such as; cancer, lung fibrosis, stroke, heart failure, diabetes and neurodegenerative diseases.[5] For instance, ROS produced by NOX (NOX subtype 1, 2 & 4) in tumor cells was reported to have promoted the proliferation of the cells through regulation of proliferative signaling kinases; such as cell survival factors.[6] Induction of endothelial cell proliferation was also found to be caused by ROS influence on angiogenesis through the release and actions of tumor-derived growth factors.[7] In addition, T-cell auto-reactivity was ascertained as a consequence of ROS cell proliferation on the immune cells.[8] Heart ischemic stroke was found to be induced by NOX_2 and NOX_4-derived ROS.[9]

NOX activity is reported to be over-activated in some disease conditions. For instance, it was found that NOX_2 expression was increased in diabetic rats.[9] High blood glucose levels in diabetes are reported to over-activate NOX by stimulating diacylglycerol formation, a physiological activator of protein kinase C, which in turn phosphorylated NOX. COVID-19 was found also to increase ROS generation, via a similar scenario of NOX over-activation. It was reported that once SARS-CoV-2 intrudes upon a cell it alters the glycolytic pathway in favor of glucose-6-phosphate production: an intermediate of the glycolytic pathway that is used in the pentose phosphate pathway for the production of ribose-5-phosphate and NADPH. NOX is over-activated by the high amount of NADPH; thereby producing ROS.[10,11]

Inhibition of NOX have been suggested as part of the best targets, in order to control oxidative stress in

diseases that are associated to it.[12] Several drugs; such as, metformin, rapamycin, statin etc[13–15], as well as some chemical compounds (rutin, triptolides, spironolactone etc[16–18]) were reported to act in part by inhibiting NOX activity. Reports have indicated that several numbers of molecules have been, and are still being used as, direct NOX inhibitors. Although, it has been indicated that many of these inhibitors are unspecific in their inhibition of NOX activity.[19] For instance, diphenylene iodonium (DPI) and apocynin, the most common and frequently used NOX- inhibitors, have been proved to be unspecific. DPI was found to inhibit xanthine oxidase, and proteins of the mitochondrial electron transport chain, apart from NOX, while Apocynin inhibits rho kinases.[20] In sum, most of the currently used NOX-inhibitors have been found to have several challenges, like weaknesses among others. This has triggered the scientific community to undertake research for more potent as well as more specific compounds that could inhibit NOX.

Evaluation of phytochemicals, via in silico methods, is widely utilized by many researchers as both an alternative and reliable approach compared to the conventional method that demand a lot of time, is not cost-effective, and is a long process. Several in silico studies have led to the prediction of novel phytochemicals having robust pharmacological activities. One of these includes a study conducted by Mazumdar et al.[21]; wherein, Gedunin, a tetranortriterpenoid compound isolated from Azadirachta indica, was reported to have suppressed ROS production by influencing NOX activity. The study predicted that, Gedunin disrupted the enzyme stability leading to its retardation. In another development, α-bisabolol and 5-methyl-1,2,3,4-tetrathiane, phyto-constituent from Garlic oil was reported to retard NOX activity.[22] These two volatile components were found to have in part exerted their antioxidant activity by inhibiting NOX.[22] In the silico study by Santos et al.[23] it was found that the caffeic acid-phtalimide hybrid compound was able to effectively inhibit NOX. Also, a study to develop a novel candidate as NOX inhibitor for hypertension treatment, by Laksono et al.[24], has led to the discovery of a compound known as: morindone, among several Indonesian phytochemicals as an effective NOX- inhibitor.

Since inhibition of NOX via natural products is one novel strategy to impede diseases that are associated with ROS escalation different scientific communities have geared toward it.[10] This is because, phytochemicals are presumed to be the leading candidate in the search for potent and more specific agents that could inhibit NOX without influencing the physiological redox reaction. Hence, this study attempted to screen phytochemicals with the aim of finding the most specific and effective NOX-inhibitor.

Material and Methods

This study deployed in silico methods, which were carried out in different stages; as described below.

Selection of phytochemicals with antioxidant activity

Phytochemicals with antioxidant or free radical scavenging activity, according to the literature, were selected for this study. This was conducted by searching through scientific articles using the key terms: phytochemicals, antioxidant, free radical scavengers, and anti-radicals activity. The chemical structures of the phytochemicals were obtained from the PubChem database (https://www.ncbi.nlm.nih.gov/pccompound), and were downloaded in the SDF files. The chemical structure of the reference inhibitor (Apocynin) was likewise searched and downloaded in a similar manner from the PubChem database. Chemical details; such as canonical SMILES and PubChem chemical identification numbers (CID) for each phytochemical were obtained and recorded.

Drug-likeness and activities analysis

The prediction of phytochemicals drug-likeness properties was performed using an ADMETMESH online server from https://admetmesh.scbdd.com. Drug-likeness properties for each phytochemical were predicted following four drug rules, by observing each of the drug rule criteria. The drug rules involved here in this prediction study are: Lipinski rule of five, Pfizer rule, GSK rule and Golden triangle rule. The respective criteria for each are as follows: Lipinski rule of 5; the criteria is that, a compound molecular weight (MW) should be less than or equal to (≤) 500 Daltons (Da), logP (Log of the octanol/water partition coefficient) to be equal or less than 5, hydrogen acceptor should be ≤10; hydrogen donor should be ≤5. If two properties are out of range there is a possibility of poor absorption or permeability; however, one is acceptable.

For Pfizer rule; the logP should be greater than (>) 3; the TPSA (Topological Polar Surface Area) should be less than (<) 75. Compounds with a high log P (that is >3) and low TPSA (that is <75) are not accepted and likely to be toxic. For GSk rule; MW ≤400; logP £ 4n. Compounds that satisfies the GSK rule may have a more favorable ADMET profile. For the Golden triangle; MW should be 200 ≤ MW ≥50, logD (logP at physiological pH 7.4) should be −2 ≥ logD ≤5n. Compounds that satisfy the Golden Triangle rule may have a more favorable ADMET profile. Only phytochemicals that were able to satisfy at least one of the drug rules and predicted as a NOX-inhibitor were selected for docking stimulation.

The biological activities; such as, antioxidant, free radical scavenging and NOX inhibition by phytochemicals were carried out using the Predict Activity Spectra of Substances (PASS) (http://www.way2drug.com/passonline/predict.php).

Selection of receptor and preparation

Under this stage, a water-forming NOX from Lactobacillus sanfranciscensis, with 452 residues, and PDB ID: 2CDU was downloaded in PDB format from the protein databank (http://www.rcsb.org/), and its structure was cross-checked in the 3D structure for optimized conformation. The choice for the use of this NOX enzyme from Lactobacillus sanfranciscensis was born out of the literature reports; in that, it gives the best protein-ligand binding geometries that play a key role in generating ROS; particularly superoxide anion, which is the precursor of most other ROS, and contains diverse amino acids in its catalytic site.[25-28] The enzyme structure was prepared by deleting water molecules and adding polar hydrogen as well as various charges using AutoDock Tool: as performed by Morris et al.[29]

Molecular docking

The phytochemical that satisfied at least one of the drug rules and predicted as a NOX-inhibitor was selected for the molecular docking exercise, so as to evaluate their binding interaction into the active pocket of the NOX: as performed by Trott and Olson[30] and Laksono et al.[31] The enzyme's grid box coordinate used for the docking was 1.687x, 9.885y and 54.962z; as determined by Costa et al.[32] The docked conformations and the configuration of the binding pocket residue amino acids for the target proteins were analyzed using Protein-Plus online software at https://proteins.plus/

Results

Antioxidant phytochemical compounds

A total of 22 phytochemicals with antioxidant and free radical scavenging activities were obtained from the literature, and identified from the chemical database (PubChem database). The list of the phytochemicals; including, the reference inhibitor, their chemical details; i.e., Pubchem CID number, and Canonical SMILE are presented in Table 1.

Table 1 Chemical properties of phytochemical compounds with proven antioxidant activities and reference inhibitor (Apocynin) from chemical databank

S/No.	Phytochemicals	Classification	Canonical SMILES	CID
1	Allicin	Organosulfur	C=CCSS(=O)CC=C	65036
2	Apocynin	Reference inhibitor	C1C(C(OC2=C1C(=CC3=C2C(CC(=O)O3)C4=CC(=C(C=C4)O)O)O)C5=CC(=C(C(=C5)O)O)O)O	9804654
3	Caffeic acid	Phenolic acids	C1=CC(=C(C=C1C=CC(=O)O)O)O	689043
4	Catechin	Flavanols	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C=C3)O)O)O	9064
5	Chlorogenic acid	--	C1C(C(C(CC1(C(=O)O)O)OC(=O)C=CC2=CC(=C(C=C2)O)O)O)O	1794427
6	Crocin	Carotenoids	CC(=CC=CC=C(C)C=CC=C(C)C(=O)OC1C(C(C(O1)COC2C(C(C(O2)CO)O)O)O)O)O)C=CC=C(C)C(=O)OC3C(C(C(C(O3COC4C(C(C(C (O4)CO)O)O)O)O)O)O)O	5281233
7	Curcumin	--	COC1=C(C=CC(=C1)C=CC(=O)CC(=O)C=CC2=CC(=C(C=C2)O)OC)O	969516
8	Epicatechin	Isoflavonoids	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C=C3)O)O)O	72276
9	Epicatechin gallate	--	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C=C3)O)O)OC(=O)C4=CC(=C(C(=C4)O)O)O	107905
10	Eriodictyol	Flavanones	C1C(OC2=CC(=CC(=C2C1=O)O)O)C3=CC(=C(C=C3)O)O	440735
11	Ferulic Acid	--	COC1=C(C=CC(=C1)C=CC(=O)O)O	445858
12	Genistein	--	C1=CC(=CC=C1C2=COC3=CC(=CC(=C3C2=O)O)O)O	5280961
13	Hesperetin	--	COC1=C(C=C(C=C1)C2CC(=O)C3=C(C=C(C=C3O2)O)O)O	72281
14	Lutein	--	CC1=C(C(CC(C1)O)(C)C)C=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC2C(=CC(CC2(C)C)O)O)C)C)C	5281243
15	Luteolin	Flavones	C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O)O	5280445
16	Lycopene	--	CC(=CCCC(=CC=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC=C(C)CCC=C(C)C)C)C)C)C	446925
17	Morin	Flavonols	C1=CC(=C(C=C1O)O)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O	5281670
18	Naringenin	--	C1C(OC2=CC(=CC(=C2C1=O)O)O)C3=CC=C(C=C3)O	932
19	Phloretin	Flavonoids	C1=CC(=CC=C1CCC(=O)C2=C(C=C(C=C2O)O)O)O	4788
20	Quercetin	--	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O	5280343
21	Resveratrol	--	C1=CC(=CC=C1C=CC2=CC(=CC(=C2)O)O)O	445154
22	Tetrahydroxystilbene glucoside	Stilbenes	C1=CC(=CC=C1C=CC2=C(C(=CC(=C2)O)O)O)OC3C(C(C(C(O3)CO)O)O)O)O	5321884
23	Zeaxanthin	--	CC1=C(C(CC(C1)O)(C)C)C=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC2=C(CC(CC2(C)C)O)O)C)C)C	5280899

Source of antioxidant phytochemicals: Zhang et al.[33]

SMILES=simplified molecular-input line-entry system, CID=chemical identification numbers

Drug likeness

The results of the drug-likeness analysis for the phytochemicals is presented in Table 2. The following were observed by the study; about three of the phytochemicals failed all the four drug rules test, one of the compounds passed only one out of the four drug rules test, five phytochemicals and the reference inhibitor (Apocynin) passed at least two or three out of the four drug rules test, while thirteen passed all four drug rules testing. The phytochemicals that passed all four drug rules testing were: catechin, chlorogenic acid, curcumin, epicatechin, eriodictyol, genistein, hesperetin, luteolin, morin, naringenin, phloretin, quercetin and resveratrol; whereas, lutein, lycopene and zeaxanthin all failed all the drug rules test.

Table 2 Predicted drug likeness for reference inhibitor (Apocynin) and phytochemicals candidate by drug rules predictor

S/No.	Phytochemicals	Classification	Lipinski Rule	Pfizer Rule	GSK Rule	Golden Triangle	Remark
1	Allicin	Organosulfur	Accepted	Accepted	Accepted	Rejected	Fair
2	Apocynin	Reference inhibitor	Accepted	Accepted	Rejected	Accepted	Fair
3	Caffeic acid	Phenolic acids	Accepted	Accepted	Accepted	Rejected	Fair
4	Catechin	Flavanols	Accepted	Accepted	Accepted	Accepted	Good
5	Chlorogenic acid	--	Accepted	Accepted	Accepted	Accepted	Good
6	Crocin	Carotenoids	Rejected	Accepted	Rejected	Rejected	Poor
7	Curcumin	--	Accepted	Accepted	Accepted	Accepted	Good
8	Epicatechin	Isoflavonoids	Accepted	Accepted	Accepted	Accepted	Good
9	Epicatechin gallate	--	Accepted	Accepted	Rejected	Accepted	Fair
10	Eriodictyol	Flavanones	Accepted	Accepted	Accepted	Accepted	Good
11	Ferulic acid	--	Accepted	Accepted	Accepted	Rejected	Fair
12	Genistein	--	Accepted	Accepted	Accepted	Accepted	Good
13	Hesperetin	--	Accepted	Accepted	Accepted	Accepted	Good
14	Lutein	--	Rejected	Rejected	Rejected	Rejected	Bad
15	Luteolin	Flavones	Accepted	Accepted	Accepted	Accepted	Good
16	Lycopene	--	Rejected	Rejected	Rejected	Rejected	Bad
17	Morin	Flavonols	Accepted	Accepted	Accepted	Accepted	Good
18	Naringenin	--	Accepted	Accepted	Accepted	Accepted	Good
19	Phloretin	Flavonoids	Accepted	Accepted	Accepted	Accepted	Good
20	Quercetin	--	Accepted	Accepted	Accepted	Accepted	Good
21	Resveratrol	--	Accepted	Accepted	Accepted	Accepted	Good
22	Tetrahydroxystilbene glucoside	Stilbenes	Accepted	Accepted	Rejected	Accepted	Fair
23	Zeaxanthin	--	Rejected	Rejected	Rejected	Rejected	Bad

Keynote:

Bad: signifies compound fail all of the four drug rule test

Good: signifies compound pass all of the four drug rule test

Fair: signifies compound pass at least two or three out of the four drug rule test

Poor: signifies compound pass only one out of the four drug rule test

Biological activities

About 19 phytochemicals that were predicted as NOX-inhibitors, antioxidant and free radical scavengers following the satisfaction of the drug rules test is shown in Table 3. All the 19 phytochemicals, including the reference inhibitor (Apocynin), were predicted to exhibit antioxidant activities; out of which 17 phytochemicals and the reference inhibitor displayed free radical scavenging activity. The probability for the phytochemicals to exert antioxidant activity is in the range of 0.318–0.872; whereas the probability of not being able to exhibit antioxidant is between 0.003–0.020, respectively, with quercetin having the highest probability (0.872) for exerting antioxidant effect. In contrast, allicin had the least; with a probability at 0.318; additionally, it was the highest for probability of not being able to exert antioxidant activity.

The results for the probability of free radicals scavenging effect of the phytochemicals is in the range of 0.375–0.939, with epicatechin gallate being the highest for free radical scavenging; with probability at 0.939. The compound, phloretin is less for free radical scavenging; concernig probability value (0.375), but highest (0.020) for not being able to scavenge free radicals. The results for the phytochemicals to act as inhibitors of NOX showed 9 out of the 19 phytochemicals were analyzed and had a reference inhibitor (Apocynin). The predicted probability

values for molecules to act as NOX–inhibitor showed 0.634 for the reference inhibitor, and for the phytochemicals this was in the ranges of 0.151–0.928. Quercetin had the highest probability value of 0.928; while curcumin is the least with 0.151 probability for NOX–inhibition.

Molecular docking

The illustration of the molecular docking is presented in Figure 1 and 2. Figure 1 presents molecular interactions of phytochemicals and the reference inhibitor, which interacted via a common amino acid of the enzyme. Figure 2 presents the interaction of the phytochemicals with enzyme via multiple amino acids. The reference inhibitor, Apocynin showed good binding energy, at –8.3 Kcal/mol, and interacts through hydrogen bond formation with Ser115A and Thr9A residues of amino acids. The phytochemicals also display good binding energies in the ranges from –6.1 to –8.7 Kcal/mol, and interacts with varied amino acids in the catalytic site of the enzyme. The residue amino acid (Ser115A) was found to have been involved in the interaction of the reference inhibitor as well as the phytochemicals: caffeic, eriodictyol, hesperetin, and morin with the enzyme through polar hydrogen bond formations: as shown in Figure 3. On the other hand, curcumin, epicatechin gallate, genistein, quercetin and resveratrol were found to have interacted with different catalytic site amino acids: Asp179B, Asp246B, Asp282A, Cys242B, Gly158B, Gly180B, Phe245B, Thr118B, Tyr296A, Val214B via hydrogen bond formation: as presented in Figure 4.

Table 3 Predicted antioxidant activities of the reference inhibitor (Apocynin) and phytochemical candidates

S/No.	Phytochemicals	Classification	Antioxidant	Free radical scavenger	NADPH oxidase inhibitor
1	Allicin	Organosulfur	0,318; 0,020	NA	NIN
2	Apocynin	Reference inhibitor	0,626; 0,004	0,742; 0,003	0,634; 0,003
3	Caffeic acid	Phenolic acids	0,603; 0,005	0,647; 0,005	0,171; 0,034
4	Catechin	Flavanols	0,810; 0,003	NA	NIN
5	Chlorogenic acid	II	0,785; 0,004	0,856; 0,002	NIN
6	Crocin	Carotenoids	0,513 0,006	0,663 0,004	NIN
7	Curcumin	II	0,610; 0,004	0,766; 0,003	0,151; 0,045
8	Epicatechin	Isoflavonoids	0,810; 0,003	0,842; 0,002	NIN
9	Epicatechin gallate	II	0,786; 0,004	0,939; 0,001	0,191; 0,027
10	Eriodictyol	Flavanones	0,817; 0,003	0,809; 0,003	0,292; 0,012
11	Ferulic acid	II	0,540; 0,005	0,731; 0,004	NIN
12	Genistein	II	0,765; 0,004	0,458; 0,013	0,470; 0,004
13	Hesperetin	II	0,746; 0,004	0,878; 0,002	0,337; 0,008
14	Luteolin	Flavones	0,775; 0,004	0,755; 0,003	NIN
15	Morin	Flavonols	0,850; 0,003	0,759; 0,003	0,890; 0,002
16	Naringenin	II	0,794; 0,003	0,769; 0,003	NIN
17	Phloretin	Flavonoids	0,434; 0,010	0,375; 0,020	NIN
18	Quercetin	II	0,872; 0,003	0,811; 0,003	0,928; 0,002
19	Resveratrol	II	0,546; 0,005	0,572; 0,007	0,296; 0,011
20	Tetrahydroxystilbene glucoside	Stilbenes	0,768; 0,004	0,914; 0,002	NIN

Keynote: Values in the green column indicate Pa (probability to be able to exert activity), while those in the red column indicate Pi (Probability of being unable to exert activity)

NADPH = Nicotinamide adenine dinucleotide phosphate (reduced), NA=no activity found, NIN=not an inhibitor of NADPH oxidase, and II sign indicates the same as the above

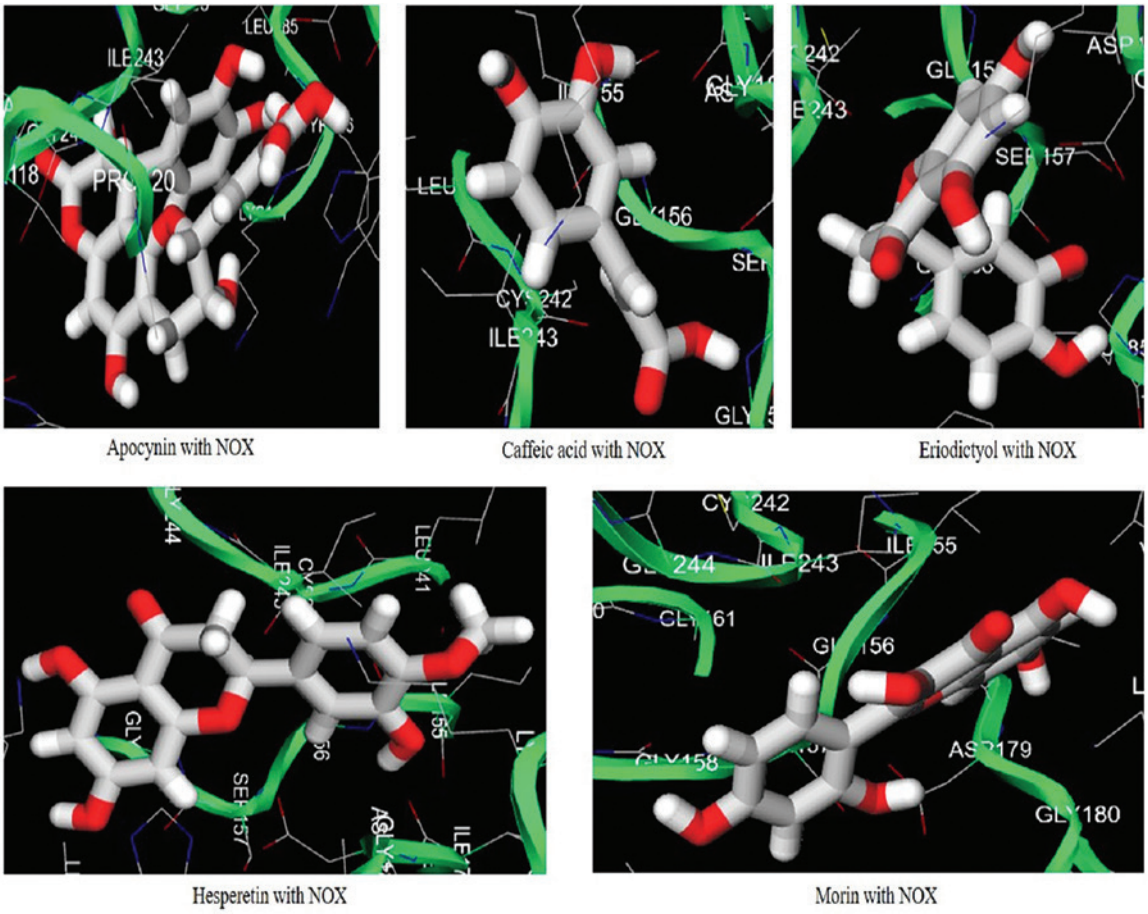

Figure 1 Illustrations of phytochemicals docked with NADPH oxidase (PDB: 2CDU) involving the same catalytic amino acids with the reference inhibitor (Apocynin)

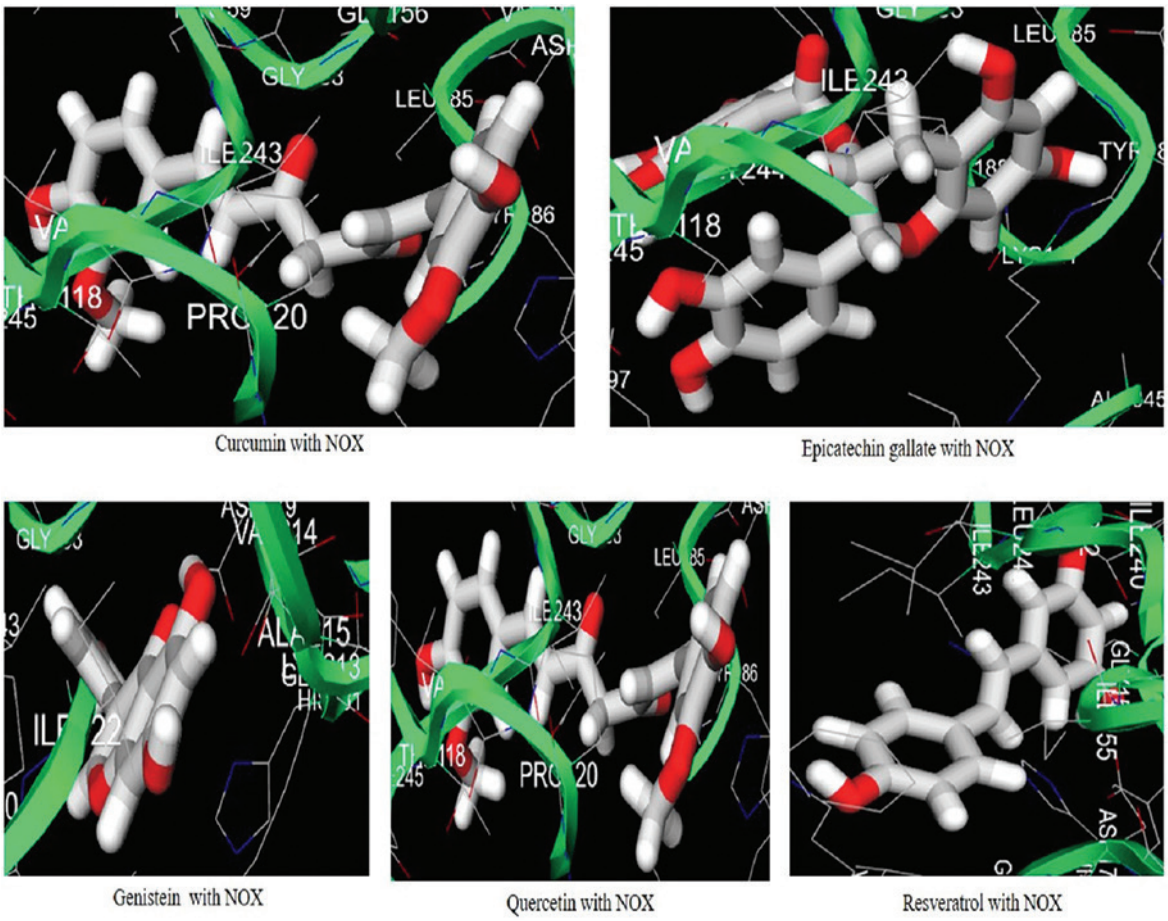

Figure 2 Illustrations of phytochemicals docked with NADPH oxidase (PDB: 2CDU) via different catalytic amino acids from the reference inhibitor (Apocynin)

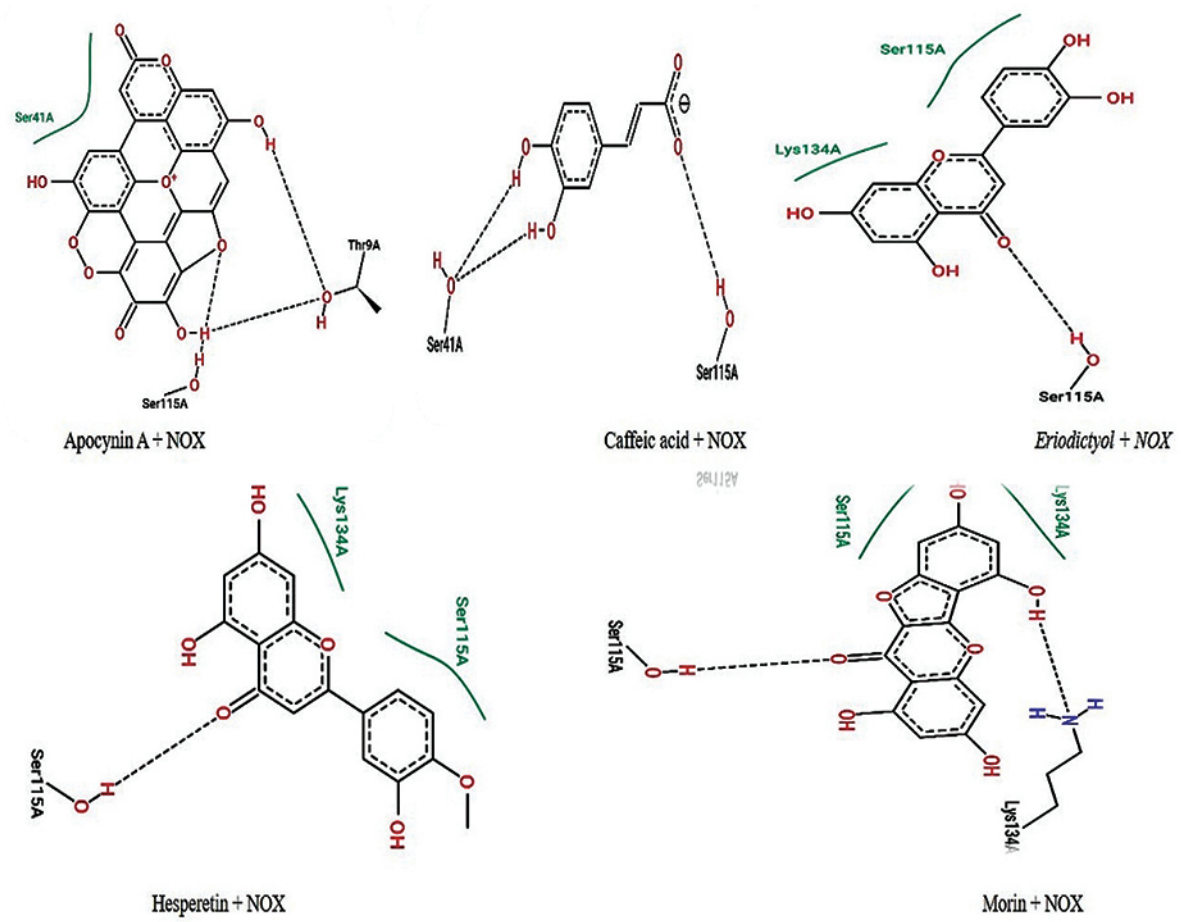

Figure 3 Illustrations of phytochemicals interaction with NADPH oxidase (PDB: 2CDU) involving the same catalytic amino acids with the reference inhibitor (Apocynin)

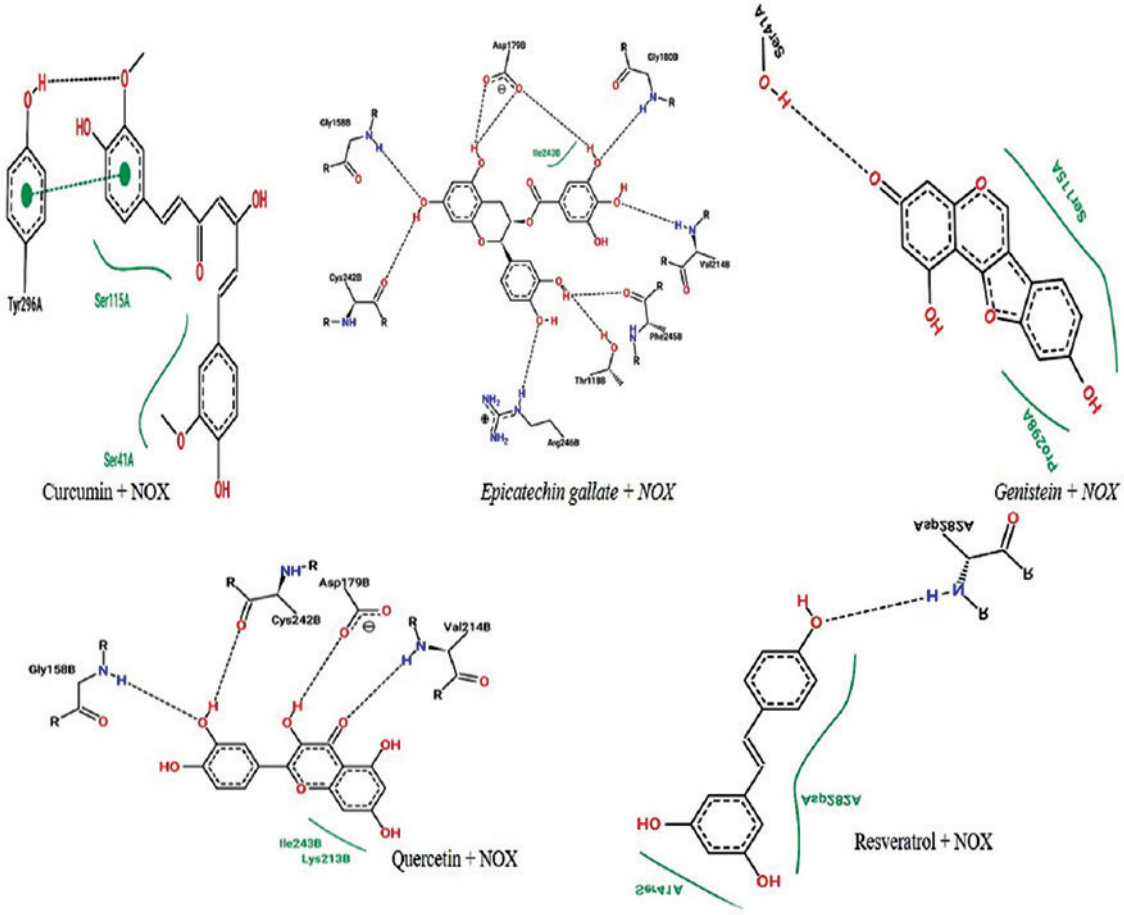

Figure 4 Illustrations of phytochemicals interaction with NADPH oxidase (PDB: 2CDU) via different catalytic amino acids from the reference inhibitor (Apocynin)

The results for the binding energies and the residue amino acids that were involved in the interactions between ligands and the receptor are presented in Table 4. The phytochemicals that display the lowest binding energies are epicatechin gallate and quercetin, with –8.7 and –8.1 kcal/mol, respectively, as against –8.3 kcal/mol by the reference inhibitor. These two phytochemicals were found to interact with residue amino acids' such as: Asp179B, Asp246B, Cys242B, Gly158B, Gly180B, Phe245B, Thr118B, and Val214B. The phytochemicals with the least binding energy is caffeic acid, with –6.1 kcal/mol and this interacted with Ser41A and Ser115A amino acids.

Table 4 Predicted residue amino acids and binding energy of NADPH oxidase enzyme with antioxidant phytochemicals

S/No.	Phytochemicals	Classification	Binding energies (kcal/mol)	Ligand–amino acid interaction
1	Apocynin	Reference inhibitor	–8.3	Ser115A, Thr9A
2	Caffeic acid	Phenolic acids	–6.1	Ser41A, Ser115A
3	Curcumin	---	–7.7	Tyr296A
4	Epicatechin gallate	Isoflavonoids	–8.7	Asp179B, Asp246B, Cys242B, Gly158B, Gly180B, Phe245B, Thr118B, Val214B
5	Eriodictyol	Flavanones	–7.4	Ser115A
6	Genistein	---	–7.7	Ser41A
7	Hesperetin	---	–7.7	Ser115A
8	Morin	Flavonols	–7.6	Lys134A, Ser115A
9	Quercetin	---	–8.1	Asp179B, Cys242B, Gly158B, Val214B
10	Resveratrol	---	–7.0	Asp282A

Keynote: the letter 'NA' signifies not available and the dotted (---) line means the same as the above

Discussion

According to the literature, NOX is an enzyme with the sole function of producing reactive oxygen species at physiological concentrations.[1] Additionally, its activity may be over–activated in some disease conditions; such as diabetes, cancer and COVID–19.[9,10] Several molecules have shown the ability to suppress the enzyme's over–activity, leading to the decrease in the excess ROS production; however, they are found to be unspecific in their inhibition.[19,20] Hence, the attempt to screen phytochemicals in this present study with the aim of finding the most specific and effective NOX–inhibitor. Of all the phytochemicals screened; caffeic, eriodictyol, hesperetin, and morin, are the only compounds that were predicted to have bonded to a common amino acid (Ser115), which was the same as the reference inhibitor (Apocynin) with ΔG –6.1 to –7.7, as against –8.3 kcal/mol displayed by Apocynin. On the other hand, phytochemicals; such as, epicatechin gallate and quercetin with ΔG –8.7 and –8.1kcal/mol did not bind to Ser115 but were predicted to have bonded to other amino acids in the catalytic site of the enzyme. By applying these tools, we are able to attest to the fact that NOX activity can be effectively inhibited by phytochemicals, and that novel agents that are more specific and effective in their inhibition can be ascertained.

Drug–likeness prediction has been a wonderful tool for early–stage discovery of drugs. For phytochemicals to be considered fit for drugs, they must not violate the drug rules filter. For instance; the Lipinski rule of five establishes that a drug may not have a MW greater than 500Da, a ratio for Log of the octanol and water partition coefficient (LogP) less than or equal to five, a hydrogen acceptor of less than or equal to ten, and a hydrogen donor less than or equal to five. According to Lipinski[34], a compound may demonstrate drug–likeness properties as long as it does not break two or more parameters. The phytochemicals that fail the Lipinski rule filter, as analyzed by the present study, are an indication that they might have broken at least two or more of the parameters. For the Pfizer rule, it is established that the logP value should be greater than 3 and the Topological Polar Surface Area (TPSA) is less than 75. Phytochemicals that were not accepted by the Pfizer rule in this study may either have their logP as less than three, have low TPSA; or both. Such compounds are likely

to be toxic. The GSk rule states that, for a compound to be considered accepted, its MW should be less than or equal to 400Da, and it should have a more favorable ADMET profile. The phytochemicals that were accepted by the GSK rule showed that they possess the required qualities. Those that have scaled the Golden triangle rule, showed that they have their an MW greater than or equal to 50, less than or equal to 200Da, a logP at physiological pH 7.4 between -2 to 5n, and a more favorable ADMET profile.[35]

NOX inhibition strategy is one of the most promising therapeutic target areas to minimize excess ROS produced in diseases associated with oxidative stress[12]. Phytochemicals seem to be the leading candidate, as they have the ability to inhibit NOX as part of their antioxidant activity. Identification of molecules that are more specific, selective, and effective NOX-inhibitors, with little or no influence on the physiological redox reaction, has been emphasized.[2] The analysis of the free radical scavenging ability of different phytochemicals in this study was a step targeted in the identification of their effectiveness. According to the literature, most phytochemicals that are capable of inhibiting NOX activity possess free radical scavenging properties.[36,37] Hence, the choice for selection of phytochemicals with antioxidant and free radical scavenging activities by this study. In addition, the ability of phytochemicals to act as NOX-inhibitors, as predicted by the bioactivity analysis, further aids in the selection of phytochemicals for docking stimulation.

The analysis of our results have predicted strong intermolecular interactions through hydrogen bonding between the phytochemicals (ligand) and the receptor (NOX), with binding energies from -6.1 to -8.7 kcal/mol. Other in silico studies, performed with this same NOX from Lactobacillus sanfranciscensis, have shown similar binding energies; ranging from -6.3 to-10.62 kcal/mol.[22,32,38] The binding of epicatechin gallate with amino acids: Asp179, Asp246, Cys242, Gly158, Gly180, Phe245, Thr118,Val214 and quercetin (-8.1 kcal/mol) with Asp179, Cys242, Gly158, Val214; other than the common Ser115 or Thr9 which was bonded by the reference inhibitor (Apocynin) as well as other phytochemicals like caffeic, eriodictyol, hesperetin, and morin, may be seen as a contradiction.

The amino acids; Asp179, Gly158, Gly180 and Val214 involved in the bonding formation of epicatechin gallate and quercetin with the NOX enzyme from Lactobacillus sanfranciscensis has been predicted by other similar studies. For instance, an in silico study by da Silva-Pantoja et al.[28], predicted that quercetin interacted with these amino acids; Asp 179, Gly158, Gly 180, and Val 214 with binding energy -8.3kcal/mol. In another similar study by da-Costa et al.[32], two caffeine analogies: ZINC08706191 (-7.8 kcal/mol) and ZINC08992920 (-7.5 kcal/mol), and a reference inhibitor (dextromethorphan -9.8 kcal/mol) were found to have interacted with Asp179 and Val214. In the same vein, catechins; including epicatechin gallate, was reported to have formed a hydrogen bond with Asp179 and/or Val214, much the same as NOX with PDB ID:2CDU,[36]

According to a literature review, different molecular structures seem to interact differently with the amino acid residue of an enzyme. For instance, in the Herrera-Calderon et al.[22] in silico study, different amino acids shown to have been involved in the interactions between three compounds of different chemical structures (α bisabolol; 5-methyl-1,2,3,4-tetrathiane and 4H-1,2,3-trithiine) with binding energies (-10.62, -9.33 and -9.05 kcal/mol, respectively). The α bisabolol interacted with Lys187 and Tyr188, 5-methyl-1,2,3,4-tetrathiane interacted with Cys133, Gly244, while 4H-1,2,3-trithiine was reported to have interacted with Cys133 only. A study by Ormachea and Ferretti[25] has also supported this claim; wherein, cinnamaldehyde phenylhydrazone was predicted to have interacted with amino acids; Lys187. Try188, Try288, Ala295, Try296, Pro298, Ser326, Leu346, Ala349 of NOX from Lactobacillus sanfranciscensis. with a binding energy of -7.15 kcal/mol. On this note, it could be deduced that

NOX from Lactobacillus sanfranciscensis is rich with different amino acids within its catalytic binding site, which are actively involved in the interaction with different molecules.

In line with the above findings, it is tempting to say that epicatechin gallate, with the lowest and best ΔG −8.7Kcal/mol, may have displayed the most energetically and strongest interaction with the NOX enzyme among other phytochemicals studied. Its wider interactions with the different amino acids; particularly Asp179, Gly158, Gly180 and Val214 among others, may be due to its structure. This is because it fits appropriately into the entire pocket of the active site of the enzyme. A previous study, by one of the authors, has pointed out that the compound's structure plays a major role in fitting into the active site of an enzyme[39] This justify its claim to have displayed the most energetically and strongest interaction with the enzyme. Farouk et al.[38] stated that the lower the ΔG the more significant the interaction between the receptor and the ligands; thus, the possibility of interrupting the enzyme leads to its retardation.[39] Hence, to the best of our knowledge, this is the first time epicatechin gallate and NOX is shown to have interacted via in silico, and this has call for further work to ascertain the claimed.

Conclusion

This study concluded that careful and diligence in silico studies has led to the prediction of phytochemicals with NOX-inhibitory effects. The lowest ΔG and the bindings of multiple amino acids by epicatechin gallate may be an indication of it being potentiality a specific and effective NOX-inhibitor. Hence, the need for further study to probe the enzyme's binding subunit for epicatechin gallate, and to corroborate the results of this study.

Acknowledgement

The authors acknowledge Mr. Theophilus T. Dawus for his technical assistance during the in-silico analysis.

Conflict of interest

The authors declare no competing interests.

References

1. Magnani F, Mattevi A. Structure and mechanisms of ROS generation by NADPH oxidases. Curr Opin Struct Biol 2019; 59:91–7.
2. Altenhofer S, Radermacher KA, Kleikers PWM, Wingler K, Schmidt HHHW. Evolution of NADPH oxidase inhibitors: selectivity and mechanisms for target engagement. Antioxid Redox Signal 2015;23:406–27.
3. Coso S, Harrison I, Harrison CB, Vinh A, Sobey CG, Drummond GR, et al. NADPH oxidases as regulators of tumor angiogenesis: current and emerging concepts. Antioxid Redox Signal 2012;16:1229–47.
4. Gaoand L, Mann GE. Vascular NAD(P)H oxidase activation in diabetes: a double-edged sword in redox signaling. Cardiovascular Res 2009;82:9–20.
5. Bedard K and Krause KH. The NOX family of ROS generating NADPH oxidases: physiology and pathophysiology. Physiological Rev 2007;87:245–313.
6. Komatsu D, Kato M, Nakayama J, Miyagawa S, Kamata T. NADPH oxidase 1 plays a critical mediating role in oncogenic Ras-induced vascular endothelial growth factor expression. Oncogene 2008;27:4724–32.
7. Jiang F, Zhang Y, Dusting GJ. NADPH oxidase mediated redox signaling: roles in cellular stress response, stress tolerance, and tissue repair. Pharmacological Rev 2011;63:218–42.
8. Sareila O, Kelkka T, Pizzolla A, Hultqvist M, Holmdahl R. NOX2 complex-derived ROS as immune regulators. Antioxid Redox Signal 2011;15:2197–208.
9. Kusaka I, Kusaka G, Zhou C, Ishikawa M, Nanda A, Granger DN, et al. Role of AT1 receptors and NAD(P)H oxidase in diabetes-aggravated ischemic brain injury. Am J Physiol Heart Circ Physiol 2004;286:H2442–51.
10. Santos AF, Povoa P, Paixao P, Mendonça A and Taborda-Barata L. Changes in Glycolytic Pathway in SARS-COV 2 Infection and Their Importance in Understanding the Severity of COVID-19. Front. Chem 2021;9:685196.
11. Baillet A, Hograindleur MA, El Benna J, Grichine A, Berthier S, Morel F, et al. Unexpected function of the phagocyte

NADPH oxidase in supporting hyperglycolysis in stimulated Neutrophils: Key Role of 6phosphofructo-2-kinase. FASEB J 2017;31:663–73.

12. Delgado-Roche L, Mesta F. Oxidative stress as key player in severe acute respiratory syndrome coronavirus (SARS-CoV) infection. Arch Med Res 2020;51:384-7.
13. Das A, Durrant D, Koka S, Salloum FN, Xi L, Kukreja RC. Mammalian target of rapamycin (mTOR) inhibition with rapamycin improves cardiac function in type 2 diabetic mice: potential role of attenuated oxidative stress and altered contractile protein expression. J Biol Chem 2014;289:4145–60.
14. Davignon J, Jacob RF, Mason RP. The antioxidant effects of statins. Coron Artery Dis 200;15:51–8.
15. Esteghamati A, Eskandari D, Mirmiranpour H, Noshad S, Mousavizadeh M, Hedayati M, et al. Effects of metformin on markers of oxidative stress and antioxidant reserve in patients with newly diagnosed type 2 diabetes: a randomized clinical trial. Clin Nutr 2013;32:179–85.
16. Chen ZH, Qin WS, Zeng CH, Zheng CX, Hong YM, Lu YZ, et al. Triptolide reduces proteinuria in experimental membranous nephropathy and protects against C5b-9-induced podocyte injury in vitro. Kidney Int 2010;77:74–88.
17. Taye A, Morawietz H. Spironolactone inhibits NADPH oxidase-induced oxidative stress and enhances eNOS in human endothelial cells. Iran J Pharm Res 2011;10:329–37.
18. Wang W, Wu QH, Sui Y, Wang Y, Qiu X. Rutin protects endothelial dysfunction by disturbing NOX4 and ROS-sensitive NLRP3 inflammasome. Biomed Pharmacother 2017;86:32–50.
19. Altenhofer S, Radermacher KA, Kleikers PWM, Wingler K, and Schmidt HHW. Evolution of NADPH oxidase inhibitors: selectivity and mechanisms for target engagement. Antioxid Redox Signal 2015;23:406-27.
20. Augsburger F, Filippova A, Rasti D, Seredenina T, Lam M, Maghzal G, et al. Pharmacological characterization of the seven human NOX isoforms and their inhibitors. Redox Biol 2019;26:101272.
21. Mazumdar S, Marar T, Devarajan S, Patki J. Functional relevance of Gedunin as a bona fide ligand of NADPH oxidase 5 and ROS scavenger: an in silico and in vitro assessment in a hyperglycemic RBC model. Biochem Biophys Rep 202;26: 100904.
22. Herrera-Calderon O, Chacaltana-Ramos LJ, Huayanca-Gutiérrez IC, Algarni MA, Alqarni M, Batiha GES. Chemical Constituents, In vitro antioxidant activity and in silico study on NADPH oxidase of Allium sativum L. (Garlic) essential oil. Antioxidants 2021;10:1-16.
23. Dos Santos WH, Yoguim MI, Dare RG, da Silva-Filho LC, Lautenschlager SOS Ximene VF. Development of a caffeic acid–phthalimide hybrid compound for NADPH oxidase inhibition. Royal Soc Chem 2021;11:17880-90.
24. Laksono AB, Kusumawati R, Suselo YH, Indarto D. In silico development of new candidate of NADPH oxidase inhibitor for hypertension treatment. Earth Environ Sci 2021;819:012071.
25. Ormachea C and Ferretti CA. In Silico evaluation of antioxidant properties of cinnamaldehyde phenylhydrazone. Chem Proc 2021;3:1-6.
26. Bedard K and Krause KH. The NOX Family of ROS-Generating NADPH Oxidases: Physiology and Pathophysiology. Physiol Rev 2007;87:245–313.
27. Rahmani H, Ghavamipour F, Sajedi RH. Bioluminescence detection of superoxide anion using aequorin. Anal Chem 2019;91:12768–74.
28. da Silva Pantoja LVP, Trindade SSA, da Silva Carneiro A, Silva JPB, da Paixão TP, Romeiro CFR, et al. Computational study of the main flavonoids from Chrysobalanus icaco L. against NADPH-oxidase and in vitro Antioxidant Activity. Res Soc Development 2022;11:1-18.
29. Morris GM, Goodsell D S, Halliday RS, Huey R, Hart WE, Belew RK, et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem 1998;819:1639–62.
30. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multi-threading. J Comput Chem 2010;31: 455–61.
31. Laksono AB, Kusumawati R, Suselo YH, and Indarto D. In silico development of new candidate of NADPH oxidase inhibitor for hypertension treatment. Earth Environ Sci 2021:819.
32. da Costa JS, da Ramos RS, da Costa KS L, do Brasil DSB, de Paula da Silva CHT, Ferreira EFB, et al. An in silico study of the antioxidant ability for two caffeine analogs using molecular docking and quantum chemical methods. Molecules 2018;23:2801.
33. Zhang YJ, Gan RY, Li S, Zhou Y, Li AN, Xu DP, et al. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. Molecules 2015;20:21138-56.

34. Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discov Today Techno 2004;1:337–41.
35. Xiong G, wu Z, Yi J, Fu L, Yang Z, Cao T, et al. ADMETlab 2.0: an intergrated online platform for accurate and comprehensive prediction of ADMET properties. Nucleic Acids Res 2021;49: W5-14.
36. Kritsi E, Tsiaka T, Ioannou AG, Mantanika V, Strati IF, Panderi I, et al. In vitro and in silico studies to assess edible flowers' antioxidant activities. Appl Sci 2022;12:7331.
37. Ejembi SA, Johnson TO, Dabak JD, Akinsanmi AO, Oche JI, Francis T. Analysis of the oxidative stress inhibition potentials of Artemisia annua and its bioactive compounds through in vitro and in silico studies. J Pharm Bioresources 2021;18:245-59.
38. Farouk A, Mohsen M, Ali H, Shaaban H, Albaridi N. Antioxidant activity and molecular docking study of volatile constituents from different aromatic lamiaceous plants cultivated in Madinah Monawara, Saudi Arabia. Molecules 2021;26:41-5.
39. Mhya DH, Nuhu AA, Mankilik MM. In-silico discovery of antidiabetic drug potential of Balanites aegyptiaca leaf's phenolic compounds. Natr Resour Human Health 2021;1:90-6.