Pak. j. sci. ind. res. Ser. A: phy. sci. 2023 **66A**(3) 266-280

Identification of Potential Antioxidant Compounds of *Artemisia annua* L. Using Computational Approaches

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(received July 7, 2021; revised September 30, 2021; accepted August 26, 2022)

Abstract. The global antioxidants market was valued at $2,923 million in 2015 and expected to reach $4,531 million by 2022. Globally people have become more concerned to use natural products over synthetic ones. That is why this research is planned to discover potential antioxidants from *Artemisia annua.* Thirty-seven bio-compounds representatives of all classes namely as α-terpinene, apigenin, arteannuin B, arteether, artemether, artemetin, artemisia ketone, artemisinic acid, artemisinin, artesunate, β-caryophyllene, β-selinene, camphor, casticin, chrysosplenol D, coumarin, cynaroside, deoxy-artemisinin, epifriedelanol, friedelin, germacrene D, isorhamnetin, kaempferol, limonene, luteolin, mearnsetin, myrtenol, quercetagetin, quercetin, quinic acid, retusin, rutin, scoparone, scopoletin, scopoline, stigmasterol and trans-pinocarveol were selected. Virtual screening of these ligands was carried out against drug targets that were catalase, superoxide dismutase 2 and glutathione peroxidase 1 by CB-dock. Quercetin, luteolin, apigenin, kaempferol and mearnsetin were shown as hit compounds. Further, refining by screening filters represented quercetin as a lead compound. Nebivolol was used as the standard for comparison. Quercetin is also far more active than the standard drug which is a novel finding. All the interaction visualization analysis studies were performed by PyMol molecular visualization tool and Ligplot^{+}. Finally, quercetin was identified as the most potent antioxidant which might be a drug candidate to treat oxidative stress and related chronic diseases in future.

Keywords: antioxidants, *Artemisia annua*, CB-dock, enzymatic antioxidants, nebivolol, quercetin

Introduction

Medicinal plants or herbal medicine is one of the major sources of medicine all over the World. Ayurvedic, Unani and Chinese traditional medicine are some examples of the oldest herbal medicine systems. Herbal medicines are used worldwide especially in south Asia, Africa, America, China, Australia and Japan are some countries since ancient times. Among the top twenty pharmaceutical dealers of the world, seven deals with plant compounds and their derivatives and earn 20 billion dollars annually. Approximately 0.4 million plant metabolites are reported worldwide but only 0.1 has been chemically isolated (Aslam and Ahmad, 2016). In Pakistan, only 600 angiosperm plants are reported out of 6000 for their medicinal usage (Adnan *et al.*, 2015). Scientifically proven herbal medicines use only purified and standardized efficient phytochemicals in a systematic way for the prevention and treatment of diseases (Firenzuoli and Gori, 2007). A decrease in efficacy and an increase in the side effects of synthetic drugs bring again natural medicines at top usage (Petrovska, 2012).

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Artemisia annua L. commonly known as scented worm wood (belongs to the family of *Asteraceae*) is a shrub indigenous to parts of Asia. Wild species are found in Europe, the United States and Argentina. Now, *A. annua* is cultivated throughout the world for *artemisinin* (Soni *et al*., 2022). Genus *Artemisia* has more than 400 species. This is the only species with an annual cycle so-called *Annua*. In China, *A. annua* had been used as a remedy for haemorrhoids, fever, malaria and as a food additive. Now, World Health Organization recommended *Artemisinin* combination therapies for malaria. *A. annua* has many different classes of compounds such as sesquiterpenes, monoterpenes, triterpenoids, coumarins, flavonoids, steroids, aliphatic and sweet hydrocarbons (Willcox, 2009). Flavonoids present in *A. annua* are highly antioxidant and are being assessed for cancer and parasitic diseases. Leaves are saturated with essential oil that shows antimicrobial and antifungal activity. Furthermore, the plant also shows cytotoxic, antioxidant and antipyretic properties (Skowyra *et al.*, 2014).

Virtual screening (VS) is low cost, effective and direct drug discovery approach as compared to experimental

approaches such as nuclear magnetic resonance spectroscopy and crystallography. Virtual Screening (VS) can be done by ligand based and structure based methods to find out lead compounds and molecular docking is one important tool of structure based methods. It predicts the interactions between small molecules called ligands and target proteins, also known as receptors (Pagadala *et al.*, 2017; Meng *et al.*, 2011).

Atomic or free radicles are those molecules that have single electrons in their outer orbits. Cigarette smoke and pollutants constantly produce free radicals in our environment. Cellular metabolisms like respiration and enzyme reactions also produce free radicals. Radon and cosmic radiations are also sources of free radicals. Excessive free radicals can cause damage to bio-molecules like DNA, proteins, lipids, glial cells and neurons. Oxidative stress results in cancer, diabetics, myocardial infarction, atherosclerosis, rheumatoid arthritis, cardiovascular diseases, re-oxygenation injury, stroke, persistent swelling, septic shock, ageing, hypertension, vasospasm and other regressive diseases in humans. Antioxidants are those compounds that remove, inhibit and scavenge reactive oxygen species. Catalase, glutathione peroxidase and superoxide dismutase are natural antioxidant enzymes, while non-enzymatic antioxidants are mostly polyphenols, carotenoids, lipoic acid and ascorbic acid which are derived from dietary sources. These non-enzymatic compounds provide defense against oxidative stress (Uttara *et al.*, 2009).

Superoxide dismutase (SOD2), catalase (CAT) and glutathione peroxidase (GPX1) work as first line defense systems within the human body which are degraded by free radicals. Anti-oxidative compounds agonists and increase the activity of antioxidant enzymes and suppress or prevent the formation of free radicals or reactive species in cells. So, control the formation of free radicals and suppress their degrative effects, antioxidant compounds are a competent choice. Thus, this study aimed to find an efficient and cost-effective treatment for oxidative stress with lesser side effects as compared to other synthetic medicines. The objectives included identification of artemisinin, its derivatives and bio compounds from *A. Annua* as novel agonists of antioxidant enzymes. To study the interaction between SOD2, GPX1 and CAT as target protein and compounds from *A. annua* as ligands and to analyze the binding conformation between antioxidant enzymes and highly antioxidative compounds as standard antioxidant agents and to determine lead and hit compounds with antioxidant properties.

Materials and Methods

Selection of receptors. It is possible to prevent and cure chronic diseases related to oxidative stress with natural exogenous antioxidants which enrich the body system and first line defence enzymes. These enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) were selected as receptors in this research work. These human specific proteins have codes 2P4K, 2F8A and 1DGH are available in the protein data bank (PDB) (Abdulfatai *et al.*, 2017).

Refining of receptors. All extra water molecules, atoms, ions and residues were removed from receptors superoxide dismutase, SOD2 (2P4K), glutathione peroxidase, GPX1 (2F8A) and catalase, CAT(1DGH) by using pymol software (v1.7.4.5) (Yuan *et al.*, 2017).

Primary sequence retrieval. The primary sequence of target proteins (2P4K, 2F8A and 1DGH) was taken in FASTA format from protein sequence database UniProt under accession numbers P04179, P07203 and P04040 with residue length of 222, 203 and 527 amino acids respectively (Montella *et al.*, 2017).

Analysis of physico-chemical properties. Physico-chemical properties are vital in the determination of the functional role of protein. These properties of 2P4K, 2F8A and 1DGH were predicted by a computational tool ProtParam (Kaur *et al.*, 2020).

Functional domain identification of targeted proteins. Database Interpro was used to identify the domains and functional sites of 2P4K, 2F8A and 1DGH (Punta *et al.*, 2012).

3D structure predictions of proteins. 3D Structures of targeted proteins were downloaded from RCSB PDB in PDB format. The protein data bank is a database for the three dimensional structural data of large biological molecules, such as proteins and nucleic acids.

Preparation of ligands. Bioactive antioxidant compounds of *A. annua* were selected as ligands for the present study. The 3 D structures and information of selected ligands that are α-terpinene, apigenin, arteannuin B, arteether, artemether, artemetin, artemisia ketone, artemisinin, artemisinic acid, artesunate, β-caryophyllene, β-selinene, camphor, casticin, chrysosplenol D, coumarin, cynaroside, deoxy artemisinin, epifriedelanol, friedelin, germacrene D,

isorhamnetin, kaempferol, limonene, luteolin, mearnsetin, myrtenol, quercetagetin, quercetin, quinic acid, retusin, rutin, scoparone, scopoletin, scopolin, stigmasterol, transpinocarveol were downloaded from PubChem. This database is a public repository for information on chemical substances and their biological activities (Kim *et al.*, 2006). The compounds are shown in Table 1. Energy minimization of ligands was carried out by Chem pro software (chem 3D v 12.0.2) (Chaudhary and Mishra, 2016). This was a mandatory step in the preparation of ligands for docking because unstable ligands would show unreliable vina scores in docking results.

Molecular docking of the dataset with the target proteins. Molecular docking without having information of binding sites was performed by using a user friendly blind docking webserver called as CB Dock, which predicts and estimate a binding site for a given protein and calculate centres and sizes with a novel rotation cavity detection method and perform a docking with the popular docking program known as Auto dock Vina (Morris *et al*., 2009). Molecular dockings were performed by using SOD2, GPX1 and CAT as receptors and 37 selected compounds as ligands (Shivanika *et al.*, 2020). The interactive 3D structures were drawn by NGL viewer (Liu *et al.*, 2020).

Ligand-protein interaction. The docking analysis was performed by using Ligplot+(version v.1.4.5) and PyMol Edu (v1.7.4.5). Interactions of ligands and target proteins were predicted by using Ligplot plus (version v.1.4.5). The graphical system of Ligplot automatically generates multiple 2D diagrams of interactions from 3D coordinates. These 2D diagrams portray the hydrogen bond interaction pattern and hydrophobic contacts between the ligand and the main chain or side chain elements of the protein (Laskowski and Swindells, 2011). After the detailed analysis of protein and ligand interaction, docking score and ADMET studies, the most active agonist was identified as the lead compound.

Docking of standard anti-oxidative drug with the target proteins. The docking results of these 37 compounds were compared with 12 FDA approved and investigational drugs namely α-tocopherol, ascorbic acid, allopurinol, β-carotene, catechin, carvedilol, metformin, methionine, N-acetyl cysteine, nebivolol, resveratrol and serotonin. Their structures were downloaded from the PubChem database and minimized their energy by Chem 3D Pro (version 12.0) and saved in sdf format. The docking of these drugs as ligands against CAT, SOD2 and GPX1 as receptors was performed by CB dock. Among these 12 drugs, carvedilol was screened out due to its size, 18.6 KB, (because CB dock accepts files up to 15 KB). The remaining 11 drugs showed their 5 best poses with selected receptors. For the selection of the most efficient drug, physico-chemical parameters including molecular formula, molecular weight, absorption, water solubility, log P, H-bond donors and acceptors, bioavailability, polarizability, ADMET probability (must be less than 1) and side effects of these drugs were studied by using PubChem and Drug bank databases and pkCSM online tool. After docking and physico-chemical properties analyses, nebivolol was selected as the standard for comparison with lead compound.

Results and Discussion

Catalase has two functional domains that are catalase core domain and the catalase immune responsive domain starting from 28 and 437 amino acids and ending at 413 and 496 amino acids sequence respectively (Fig. 1). Superoxide dismutase 2 also has two functional domains which are Mn/Fe-SOD-C terminal domain with residue length 113-216 and Mn/Fe-SOD-N terminal domain with residue length 25-106 (Fig. 2). Glutathione peroxidase 1 belongs to the Glutathione peroxidase family having a functional domain GSH-peroxidase with 15-192 residue length (Fig. 3).

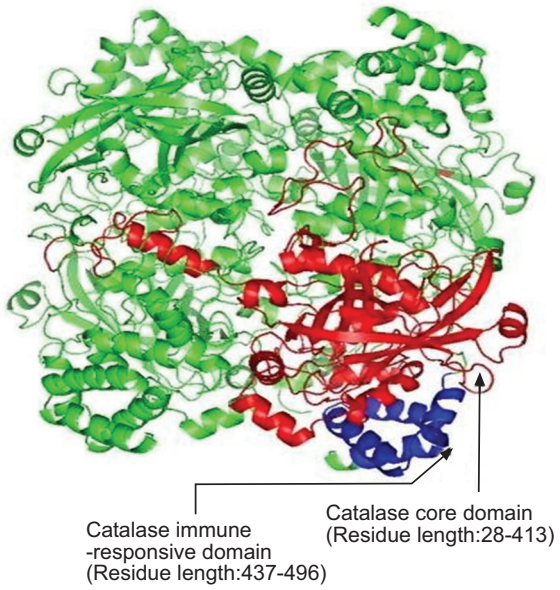
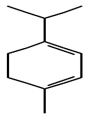
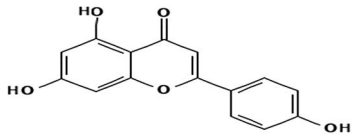
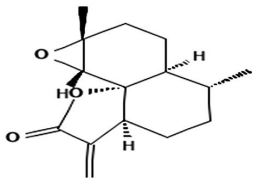
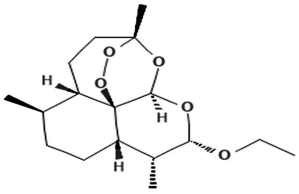
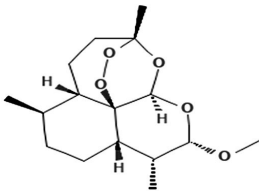
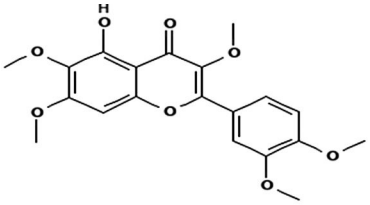
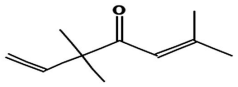
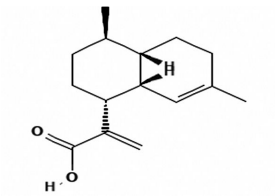


Fig. 1. Functional domains of CAT with residue lengths.

Table 1. Selected ligands with structural information

Name of ligand	Compound CID no	Molecular formula	Molecular weight	Structure
Alpha-Terpinene	7462	$C_{10}H_{16}$	136.23 g/mol	
Apigenin	5280443	$C_{15}H_{10}O_5$	270.24 g/mol	
Arteannuin B	6543478	$C_{15}H_{20}O_3$	248.32 g/mol	
Arteether	3000469	$C_{17}H_{28}O_5$	312.4 g/mol	
Artemether	68911	$C_{16}H_{26}O_5$	298.37 g/mol	
Artemetin	5320351	$C_{20}H_{20}O_8$	388.4 g/mol	
Artemisia ketone	68346	$C_{10}H_{16}O$	152.23 g/mol	
Artemisinic acid	10922465	$C_{15}H_{22}O_2$	234.33 g/mol	

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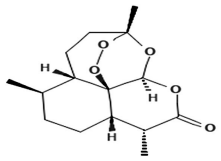
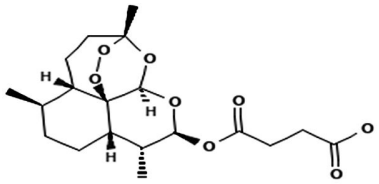
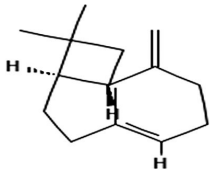
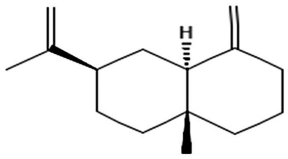
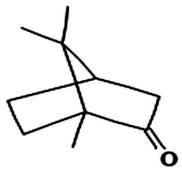
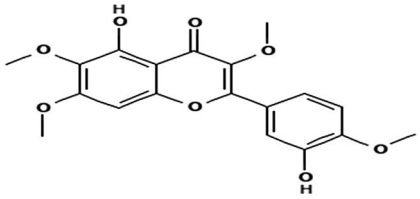
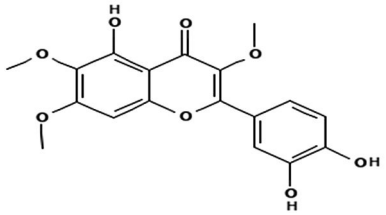
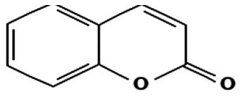
Name of ligand	Compound CID no	Molecular formula	Molecular weight	Structure
Artemisinin	68827	$C_{15}H_{22}O_5$	282.33 g/mol	
Artesunate	6917864	$C_{19}H_{28}O_8$	384.4 g/mol	
Beta- caryophyllene	5281515	$C_{15}H_{24}$	204.35 g/mol	
Beta-selinene	442393	$C_{15}H_{24}$	204.35 g/mol	
Camphor	2537	$C_{10}H_{16}O$	152.23 g/mol	
Casticin	5315263	$C_{19}H_{18}O_8$	374.3 g/mol	
Chrysosplenol D	5280699	$C_{18}H_{16}O_8$	360.3 g/mol	
Coumarin	323	$C_9H_6O_2$	146.14 g/mol	

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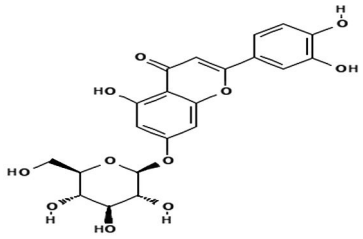
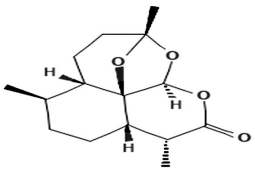
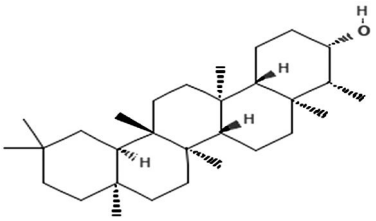
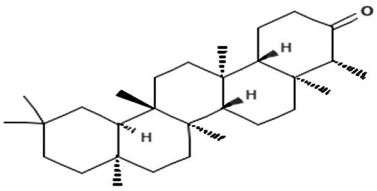
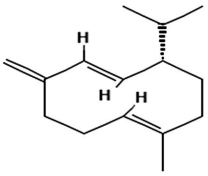
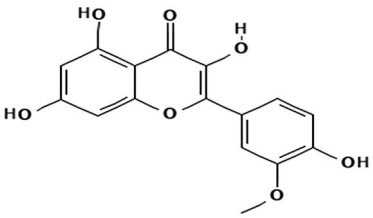
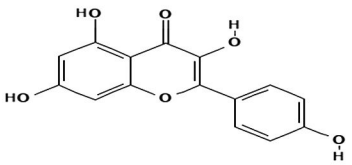
Name of ligand	Compound CID no	Molecular formula	Molecular weight	Structure
Cynaroside	5280637	$C_{21}H_{20}O_{11}$	448.4 g/mol	
Deoxyartemisinin	12814879	$C_{15}H_{22}O_4$	266.33 g/mol	
Epifriedelanol	119242	$C_{30}H_{52}O$	428.7 g/mol	
Friedelin	91472	$C_{30}H_{50}O$	426.7 g/mol	
Germacrene D	5317570	$C_{15}H_{24}$	204.35 g/mol	
Isorhamnetin	5281654	$C_{16}H_{12}O_7$	316.26 g/mol	
Kaempferol	5280863	$C_{15}H_{10}O_6$	286.24 g/mol	

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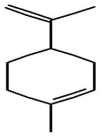
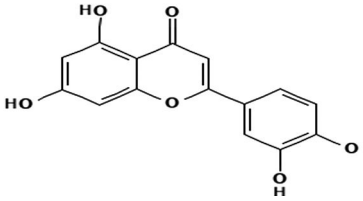
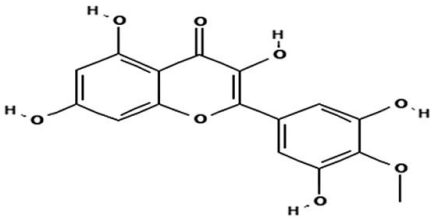
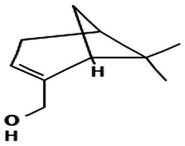
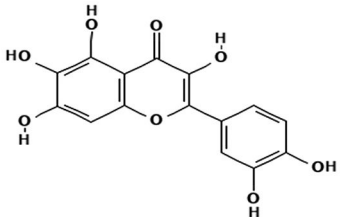
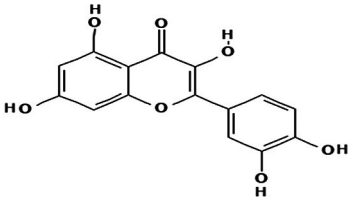
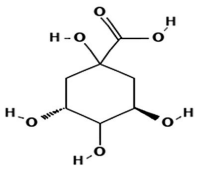
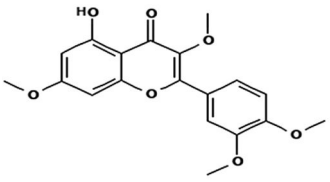
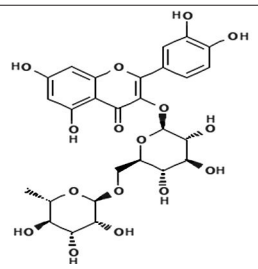
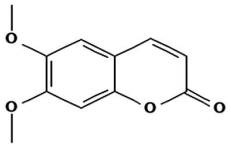
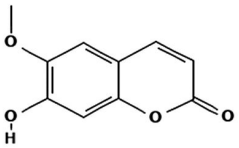
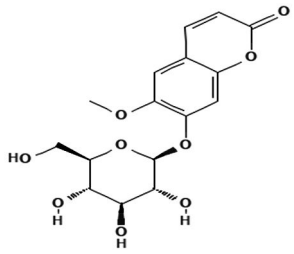
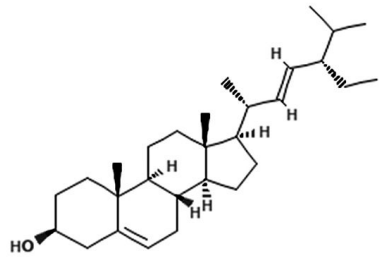
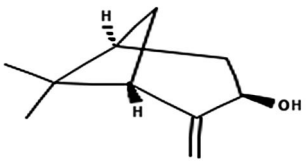
Name of ligand	Compound CID no	Molecular formula	Molecular weight	Structure
Limonene	22311	$C_{10}H_{16}$	136.23 g/mol	
Luteolin	5280445	$C_{15}H_{10}O_6$	286.24 g/mol	
Mearnsetin	10359384	$C_{16}H_{12}O_8$	332.26 g/mol	
Myrtenol	10582	$C_{10}H_{16}O$	152.23 g/mol	
Quercetagetin	5281680	$C_{15}H_{10}O_8$	318.23 g/mol	
Quercetin	5280343	$C_{15}H_{10}O_7$	302.23 g/mol	
Quinic acid	6508	$C_7H_{12}O_6$	192.17 g/mol	
Retusin	5352005	$C_{19}H_{18}O_7$	358.3 g/mol	

Countinued

Name of ligand	Compound CID no	Molecular formula	Molecular weight	Structure
Rutin	5280805	$C_{27}H_{30}O_{16}$	610.5 g/mol	
Scoparone	8417	$C_{11}H_{10}O_4$	206.19 g/mol	
Scopoletin	5280460	$C_{10}H_8O$	192.17 g/mol	
Scopolin	439514	$C_{16}H_{18}O_9$	354.31 g/mol	
Stigmasterol	5280794	$C_{29}H_{48}O$	412.7 g/mol	
Trans- pinocarveol	1201530	$C_{10}H_{16}O$	152.23 g/mol	

Physiochemical characterization of SOD2, GPX1 and CAT was extracted from ProtParam. The computed parameters include the molecular weight, amino acid composition, theoretical pl, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY). The calculated pl greater than 7 represents the basic nature of the protein, while less than 7 shows the acidic nature of the protein. The extinction coefficient represents light absorption. Instability index if less than 40 shows the stability of the protein, while greater than 40 indicates the instability of protein (Morya *et al.*,

2012). The physico-chemical properties of superoxide dismutase 2, glutathione peroxidase 1 and catalase are shown in Table 2.

Physico-chemical and pharma-cokinetics properties of ligands. A drug like and non drug like compounds are separated by following certain parameters like Lipinski's rule of five and ADMET properties test (Kumar *et al.*, 2018). The original rules of five deal with four physico-chemical parameters (MWT≤500, log P≤5, H-bond donors≤5, H-bond acceptors≤10) which are associated with orally active compounds. The meaning of drug like is dependent on the mode of administration (Lipinski, 2004). A compound is considered to have drug likeness if it is complying with three or more of the RO5. If a compound violates more than two of these rules, it is assumed to be poorly absorbed (Daina *et al.*, 2017).

Physico-chemical and pharma-cokinetics properties determine the final destiny of compounds as drug or non-drug compounds. Physico-chemical properties or Lipinski's rule of five works as a primary filter and pharma-cokinetics studies as a secondary filter in the screening of potential compounds. Rutin and cynaroside did not obey Lipinski's rule of five, so they were knock out in primary screening while epifriedelanol, stigmasterol and friedelin did not comply with RO5 (All these three compounds have log P>5). Pharma-cokinetic studies of these compounds screened out α-terpinene, arteannuin B, artemether, artemisia ketone, β-caryophyllene, camphor and germacrene D (log BB > 0.3), epifriedelanol and friedelin (log BB >0.3 and log PS >-2), β-selinene, coumarin and stigmasterol (log PS> -2). Table 3 shows the applicability of Lipinski's rule of five on selected ligands.

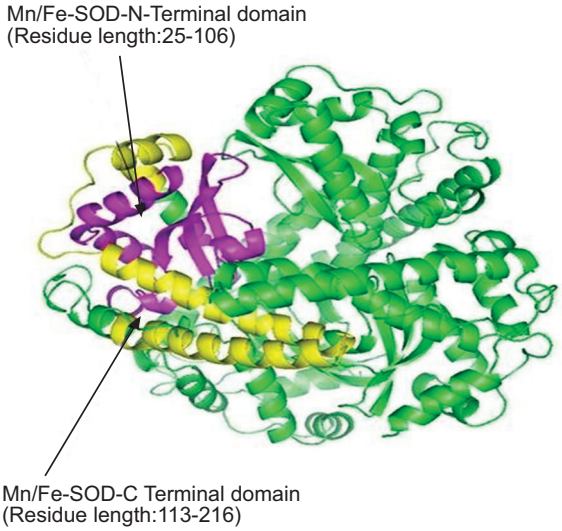


Fig. 2. Functional domains of SOD2 with residue lengths.

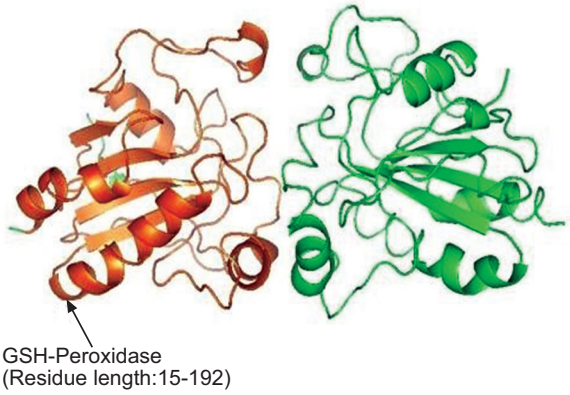


Fig. 3. Functional domains of GPX1 with residue lengths.

Table 2. Physio-chemical properties of (a) superoxide dismutase (SOD2), (b) glutathione peroxidase (GPX1) and (c) catalase (CAT)

(a) Superoxide dismutase (SOD2)								
MW	PI	NR	PR	Ext.Co1	Ext.Co2	Instability index	Aliphatic index	GRAVY
24750.14	8.35	20	22	48025	47900	40.26	84.41	-0.407
(b) Glutathione peroxidase (GPX1)								
MW	PI	NR	PR	Ext.Co1	Ext.Co2	Instability index	Aliphatic index	GRAVY
22088.17	6.15	21	20	17210	16960	47.96	86.11	-0.070
(c) Catalase (CAT)								
MW	PI	NR	PR	Ext.Co1	Ext.Co2	Instability index	Aliphatic index	GRAVY
59756.17	6.90	61	59	64540	64290	30.15	68.29	-0.586

Molecular docking. Molecular docking without having information of binding sites was performed by using a user friendly blind docking webserver called as CB Dock, which predicts and estimate a binding site for a given protein and calculate centres and sizes with a novel rotation cavity detection method and perform a docking with the popular docking program known as Auto dock Vina (20). Molecular dockings were performed by using SOD2, GPX1 and CAT as receptors and 37 selected compounds as ligands (21). After submitting input files (receptor file in PDB format and ligand file in SDF format), CB-Dock checked the input files and converted them to pdbqt formatted files using Open Babel and MGLTools. After that CB-Dock predicted cavities of the receptor and calculated the centres and sizes of the top N (n=5 by default) cavities. Each centre, size and pdbqt file were submitted to Auto Dock Vina for docking. The results were displayed after the computation of N rounds. The interactive 3D structures were drawn by NGL viewer (Liu *et al.*, 2020).

Among the 5 best confirmations, the best one was selected based on the highest affinity score of receptor ligand interaction. The best five compounds (Hit compounds based on primary and secondary filters, toxicity predicted values and binding score) were quercetin, luteolin, apigenin, kaempferol and mearnsetin (Binding scores with all three receptors shown in Table 4). The lead compound was found to be quercetin.

Nebivolol and antioxidant agent comparison. Nebivolol was used as a standard drug as is widely used in clinical practice for the treatment of hypertension and heart failure and proves itself as a highly selective beta-blocker with additional vasodilator properties (Briciu *et al.*, 2014). The standard drug and lead compounds were compared for their physicochemical and pharmacokinetic properties to assess their bioavailability, drug likeness, efficacy, and safety. Both compounds passed the drug likeness criteria (Lipinski's rule of five). However, quercetin has low molecular weight and log P value than nebivolol and showed 5 H-BD whereas nebivolol showed 3 H-BD. Molar refractivity of quercetin was also high than nebivolol (Table 5a).

Discovering protein ligand binding sites and conformations are particularly important in drug discovery. Therefore, a standard drug as a ligand was docked against selected receptors by CB-dock which predicted the cavities of the protein and calculated the centres and sizes of the top 5 cavities for all the three proteins separately. Results of docking of standard drug and lead compound against selected three receptors namely catalase (CAT), superoxide dismutase 2 (SOD2), and glutathione peroxidase 1 (GPX1) are shown in

Table 3. Applicability of Lipinski rule on ligands

Ligand	LogP Value	Molecular Weight	H-bond acceptor	H-bond donor
Alpha terpinene	3.3089	136.238 g/mol	0	0
Apigenin	2.5768	270.24 g/mol	5	3
Arteannuin B	2.4518	248.322 g/mol	3	0
Arteether	3.2309	312.406 g/mol	5	0
Artemether	2.8408	298.379 g/mol	5	0
Artemetin	3.2086	388.372 g/mol	8	1
Artemisia ketone	2.7339	152.237 g/mol	1	0
Artemisinic acid	3.6458	234.339 g/mol	1	1
Artemisinin	2.3949	282.336 g/mol	5	0
Artesunate	2.6024	384.425 g/mol	7	1
Beta caryophyllene	4.7252	204.357 g/mol	0	0
Beta selinene	4.7252	204.357 g/mol	0	0
Camphor	2.4017	152.237 g/mol	1	0
Casticin	2.9056	374.345 g/mol	8	2
Chrysosplenol D	2.6026	360.318 g/mol	8	3
Coumarin	1.793	146.145 g/mol	2	0
Cynaroside	-0.2445	448.38 g/mol	11	7
Deoxyartemisinin	2.4633	266.337 g/mol	4	0
Epifriedelanol	8.2488	428.745 g/mol	1	1
Friedelin	8.457	426.729 g/mol	1	0
Germacrene D	4.8913	204.357 g/mol	0	0
Isorhamnetin	2.291	316.265 g/mol	7	4
Kaempferol	2.2824	286.239 g/mol	6	4
Limonene	3.3089	136.238 g/mol	0	0
Luteolin	2.2824	286.239 g/mol	6	4
Mearnsetin	1.9966	332.264 g/mol	8	5
Myrtenol	1.9711	152.237 g/mol	1	1
Quercetagetin	1.6936	318.237 g/mol	8	6
Quercetin	1.988	302.238 g/mol	7	5
Quinic acid	-2.3214	192.167 g/mol	5	5
Retusin	3.2	358.346 g/mol	7	1
Rutin	-1.6871	610.521 g/mol	16	10
Scparone	1.8102	206.197 g/mol	4	0
Scopoletin	1.5072	192.17 g/mol	4	1
Scopolin	-1.0197	354.311 g/mol	9	4
Stigmasterol	7.8008	412.702 g/mol	1	1
Transpinocarveol	1.9695	152.237 g/mol	1	1

Table 4. Hit compounds with binding scores

Name of potential compound	Binding score with CAT	Binding score with SOD2	Binding score with GPX1
Quercetin	-10	-8.4	-6.5
Luteolin	-9.8	-8	-6.4
Apigenin	-9.5	-7.8	-6
Kaempferol	-9.5	-8.2	-5.9
Mearnsetin	-9.3	-8.6	-6.4

(Table 5b). The highest binding score was found to be -10 against CAT receptor which was shown by quercetin which was higher than Nebivolol that showed -9.4 against the same protein. Among the top 5 cavities (n=5 by default), the first one for both ligands is displayed in Figs. 4 and 5. Minimized energy pose of quercetin and CAT showed the best and strong cavity interaction with the involvement of three chains of protein as compared to nebivolol which had weak interaction at top of protein with the involvement of two chains only. All the interaction visualization analysis studies were performed by PyMol molecular visualization tool and Ligplot^{+} (V.1.4.5). Best docking scores of reference drug and lead compound were analyzed by Ligplot^{+} (V.1.4.5), (Fig 4 and 5, Table 6).

ADMET properties comparison. Pharmaco-kinetics properties included absorption, distribution, metabolism, excretion and toxicity (ADMET) that play a critical role in the screening of compounds as drug candidates. ADMET properties were compared by using Byju's greater than calculator learning app. Pharma-cokinetic properties of reference drugs and lead compounds are listed in Table 7. The water solubility of the standard drug is less than the lead compound. Caco-2 permeability predicts the absorption of orally administered drugs. Predicted values of this earlier mention model were within the safe range for both compounds but quercetin showed less value than nebivolol. Nebivolol falls in the 'Yes' category for P-gp substrate and P-gp I/II inhibitors while quercetin stands in the 'No' category for all these three models. This means nebivolol as P-gp substrate shows low oral absorption and as P-gp I/II inhibitor, reduce the pumping out of xenobiotics and toxins activity of P-gp from cell and may have high absorption (Table 7).

Nebivolol showed itself as a substrate of CYP2D6 & CYP3A4 isoforms whereas quercetin is not predicted as a substrate of these isoforms. Nebivolol was predicted as an inhibitor of CYP3A4 which is the main isoform for drug metabolism while quercetin was found inhibiting CYP1A2 isoform (Table 8). Fu value of quercetin was found to be more than nebivolol which shows quercetin is more effective than reference drug in case of unbounded friction present in plasma. BBB permeability <-1 means no harm to the brain. CNS permeability <-3 is considered

Table 5a. The physico-chemical properties of nebivolol and quercetin based on Lipinski rule of five

Name of compound	Log P value	Molecular weight	H-bond donor	H-bond acceptor	Molar refractivity
Nebivolol	2.44	405.4	3	7	71A^{o2}
Quercetin	1.988	302.238	5	7	127A^{o2}

Table 5b. The predicted docking scores nebivolol and quercetin

Name of ligand	Binding score with CAT	Binding score with SOD2	Binding score with GPX1
Nebivolol	-9.4	-8.2	-6.5
Quercetin	-10	-8.4	-6.5

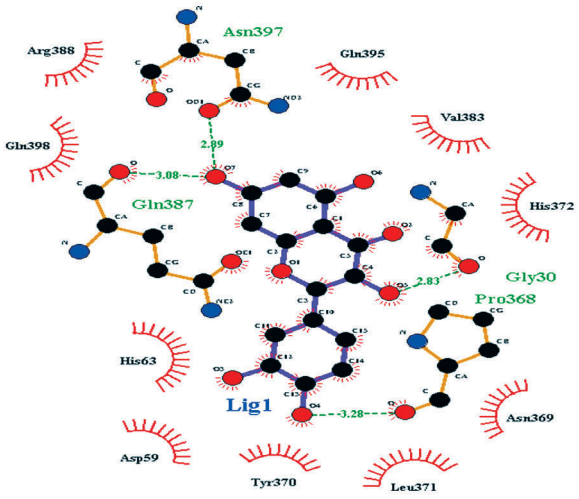


Fig. 4. Hydrogen bonds and interactions of quercetin (ligand) with CAT (receptor).

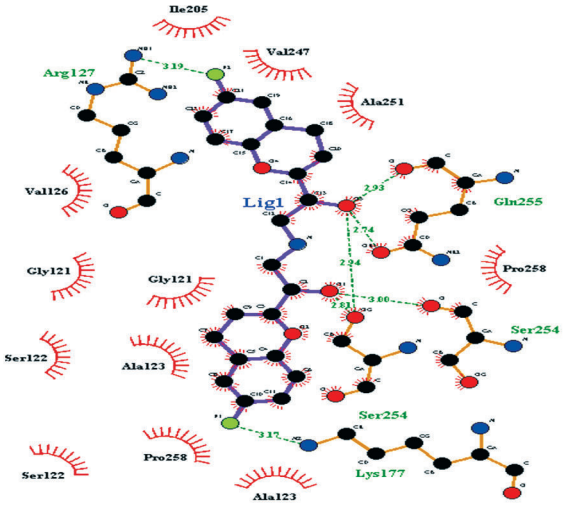


Fig. 5. Hydrogen bonds and interactions of nebivolol (ligand) with CAT (receptor)

safe (Table 9). The predicted value of drug clearance as total clearance of quercetin was high as compared to nebivolol. Total clearance is related to bioavailability and is important for determining dosing rates. Both compounds stand in the 'No' category for the Renal OCT2 substrate model, which means that they do not interfere in the normal functioning of organic cation transporter 2 who plays role in renal clearance of drugs (Table 10).

Toxicity is the most important parameter of pharmacokinetic (ADMET) properties which consists of 9 models. Maximum tolerated dose helps to set maximum recommended tolerated dose, which was found to be negative value for nebivolol (log mg/Kg/day as-0.098) and log mg/Kg/day=0.499 for quercetin indicating that quercetin is ahead in safety than reference drug. From (Table 11), it is evident that nebivolol showed itself as h ERG II inhibitor. Mostly h ERG I/II inhibitors are withdrawn from the pharmaceutical market. The model named oral rat acute toxicity (LD50) expressed as mol/Kg is the amount of drug that can cause the death of 50% of rats (test animals). LD 50 value of nebivolol was slightly higher than quercetin. Oral rat chronic toxicity (LOAEL) determines the lowest dose of a drug which can produce adverse effects over long duration usage (chronic use) of the drug. LOAEL predicted value of nebivolol is less than quercetin which shows its potency to be more toxic than bio-compound. Hepatotoxicity simply indicates the injury to the liver which shows result in two categories yes/no. Nebivolol predicted result shows it as hepatotoxic whereas quercetin is not a hepatotoxic compound. Both compounds do not cause any allergic reactions. *T.*

Table 6. Comparison of nebivolol and quercetin for hydrogen bonds and hydrophobic interactions

Ligand name	No. of H-bonds	H-bonding		Hydrophobic
		Amino acid	Distance	
Nebivolol	6	N:Arg127:F1	3.19	Gly121
		O:Gln255:O5	2.93	Ala123
		O2:Gln255:O5	2.74	Val126
		O:Ser254:O1	3.00	Pro258
		O:Ser254:O5	2.94	Ala251
		N:Lys177:F2	3.17	Val247
Quercetin	4	OD1:Asn:O7	2.89	Arg388
		O:Gln:O7	3.08	Gln398
		O:Gly:O5	2.83	Gln395
		O:Pro:O4	3.28	Val383
				His372
				His63
				Asn369
				Asp59
				Tyr370
				Leu371

Table 7. ADMET properties of standard drug and lead compound

Model name	Predicted values of nebivolol	Predicted values of quercetin
Water solubility	-3.123	-2.925
$CaCO_2$ permeability	1.15	-0.229
Intestinal absorption (human)	90.554	77.207
Skin Permeability	-2.879	-2.737
P-glycoprotein substrate	Yes	No
P-glycoprotein I inhibitor	Yes	No
P-glycoprotein II inhibitor	Yes	No

Table 8. Metabolic properties of standard drug and lead compound

Model name	Predicted values of nebivolol	Predicted values of quercetin
CYP2D6 substrate	Yes	No
CYP3A4 substrate	Yes	No
CYP1A2 inhibitor	No	Yes
CYP2C19 inhibitor	No	No
CYP2C9 inhibitor	No	No
CYP2D6 inhibitor	No	No
CYP3A4 inhibitor	Yes	No

Table 9. Distribution properties of standard drug and lead compound

Model name	Predicted values of nebivolol	Predicted values of quercetin
VDss (human)	0.993	1.559
Fraction unbound (human)	0.283	0.206
BBB permeability	-0.888	-1.098
CNS permeability	-3.083	-3.065

Table 10. Excretion properties of standard drug and lead compound

Model name	Predicted values of nebivolol	Predicted values of quercetin
Total clearance	0.89	0.407
Renal OCT2 substrate	No	No

pyriformis toxicity is expressed as the negative logarithm of the concentration required to inhibit 50% growth (p IGC50). *T. pyriformis* toxicity predicted value of nebivolol is higher than quercetin which is again going in favour of quercetin. Minnow toxicity is the lethal concentration values (LC50 expressed as log LC50 in m M) of a compound that is necessary to cause the death of 50% minnows (small bait fishes). Nebivolol predicted value is 1.416 mM, whereas 3.721 mM is the predicted value of quercetin. Less quantity of reference drug than quercetin is enough to cause the death of 50% minnows, which again shows the standard drug's toxicity and highlights the efficacy and safety of the lead compound. All the 9 models of toxicity show quercetin as a safe compound than nebivolol (Table 11).

Table 11. Toxicity values of standard drug and lead compound

Model name	Predicted values	
	nebivolol	quercetin
Max.tolerated dose (human)	-0.098	0.499
hERG I inhibitor	No	No
hERG II inhibitor	Yes	No
Oral rat acute toxicity (LD50)	2.566	2.471
Oral rat chronic toxicity (LOAEL)	1.608	2.612
Hepatotoxicity	Yes	No
Skin sensitisation	No	No
T. pyriformis toxicity	0.365	0.288
Minnow toxicity	1.419	3.721

Quercetin is one of the important bioflavonoids present in more than twenty plants material and is known for its anti-inflammatory, anti-hypertensive, vasodilator effects, antiobesity, anti-hypercholesterolemic and anti-atherosclerotic activities (David *et al.*, 2016). It also has displayed the ability to prevent the oxidation of low density lipoproteins (LDL) by scavenging free radicals and chelating transition metal ions. As a result, quercetin may aid in the prevention of certain diseases, such as cancer, atherosclerosis and chronic inflammation (Dagher *et al*., 2021).

Conclusion

The motive of the present research work was to discover potential antioxidants from *A. annua* and its derivatives. Thirty seven phyto-compounds (which represents almost all classes of natural antioxidant compounds) were selected from literature and databases. Drug targets were three endogenous antioxidant enzymes that serve as first line defense within the human body, namely catalase, superoxide dismutase 2 and glutathione peroxidase 1. Molecular docking was performed by CB-dock an online tool and the five best scoring phyto-compounds namely quercetin, luteolin, apigenin, kaempferol and mearnsetin were identified as hit compounds. Drug likeliness of compounds was studied and reported by using primary and secondary filters (Lipinski rule of 5 as primary and pharma-cokinetics properties as a secondary filter). Quercetin belongs to class polyphenol was found to be a lead compound. Virtual screening results, physio-chemical properties and pharma-cokinetics properties of this compound were compared with an FDA approved drug namely nebivolol. Quercetin was found to be capable of binding protein targets (CAT, SOD2 and GPX1) more efficiently and showed less toxicity than standard drug nebivolol.

Acknowledgement

The authors are grateful to the Capital University of Science and Technology Islamabad for providing the platform to conduct this research.

Conflict of Interest. The author declare that they have no conflict of interest.

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