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In Silico Exploring of the Antibiotic Adjuvant Potential of some Natural Ligands in Carbapenem-Resistance *Acinetobacter baumannii*

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ABSTRACT

Background: *A. baumannii* is a gram-negative pathogen that has become one of the most important challenges in the world due to its high antibiotic resistance, and today many efforts are being made to treat infections caused by it. In recent years, there have been many concerns about increasing resistance to the beta-lactam antibiotic, carbapenem. Because resistance to these antibiotics greatly narrows the treatment options for the infections. The main source of carbapenem resistance in *A. baumannii* is the production of class D carbapenemase enzymes.

Methods: In this study, 27 plant ligands that have been shown to have antibacterial effects against *A. baumannii* and other resistant bacteria were selected. The chemical structure of the ligands and the three-dimensional structure of carbapenemase OXA-58 were extracted. The requirements of oral consumption of ligands were examined and ligand and OXA-58 docking were performed. 9 ligands including baicalein, berberine, curcumin, ellagic acid, epicatechin, honokiol, magnolol, norwogonin, and thymol, which met the requirements of Rule 5 and had better binding affinity than 6-alpha-hydroxymethyl penicillanate were selected. Redocking with a focus on the active position was performed by AutoDock software.

Results: The amino acids involved in the hydrogen bonding of an antibiotic-representative ligand to the receptor were identified. Ligands that bind to at least one of these amino acids at the binding site by hydrogen bond were selected. Pharmacological and toxicity studies were performed and finally, the epicatechin ligand was introduced as the best ligand.

Conclusion: Plant ligands can be further investigated as promising antibiotic adjuvants and used in the future.

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Introduction

Acinetobacter baumannii, a gram-negative, pleomorphic, and opportunistic pathogen is the most clinically important member of the gram-negative *Acinetobacter* genus (1). This pathogenic bacterium is one of the common causes of nosocomial infections while having a high mortality risk of up to 26% for in-hospital patients and up to 43% for intensive care unit (ICU) patients among individuals with prolonged hospital stay (2). *A. baumannii* infections may occur in the blood, respiratory tract, genitourinary tract, soft tissue, pleural fluid, skin, urinary tract, CNS, and eyes (3). *A. baumannii* is a member of the global human health threat "ESKAPE" organisms. These microorganisms pose high mortality, morbidity, and therapeutic challenges due to their constantly increasing antimicrobial or antibiotic resistance (AMR) resistance especially in immunocompromised individuals (4).

Multidrug-resistant (MDR), extensive drug-resistant (XDR), and pan-drug-resistant (PDR) are common terminologies that have been used to describe the degree of antimicrobial resistance for *A. baumannii*. MDR microorganisms are resistant to at least three classes of antimicrobial agents (all penicillins, cephalosporins, fluoroquinolones, and aminoglycosides). Carbapenems and Polymyxins antibiotics are widely used treatment choices for MDR *A. baumannii* infections. Polymyxin usage should be limited due to its nephrotoxicity and neurotoxicity. *A. baumannii* is called XDR when shows additional resistance to carbapenems (5-7). Since the financial and clinical burdens of MDR infections have been challenging to patients and healthcare settings, WHO declared "combat drug resistance: no action today, no cure tomorrow" in 2011 (8).

Carbapenems are members of beta-lactam antibiotics with a unique structure containing carbapenem coupled to a β-lactam ring which protects against a spectrum of β-lactamases, consequently, carbapenems are considered a reliable antibiotic and the appearance of bacteria that are carbapenem-resistant has been a major concern (9). It has been observed that the most frequent and most concerning mechanism of carbapenem resistance in *A. baumannii* is the expression of beta-lactamases enzymes called carbapenemases. The four main classes of beta-lactamases (A, B, C, and D) are identified based on the amino acid sequence of these enzymes (10). Carbapenem-hydrolyzing class D-lactamases (CHDLs), also known as oxacillinases (OXA), are the major source of carbapenem-resistant *A. baumannii* outbreaks (11) 5 subtypes of this class of beta-lactamases in *A. baumannii* are: OXA-23, OXA-40/24, OXA-51, OXA-14, and OXA-58. OXA-58 is an extracellular enzyme that is secreted externally through the outer membrane vesicles, which are produced in the absence of carbapenem, but their production increases during carbapenem treatment (12).

The challenge of antibiotic resistance has encouraged research at the chemistry–microbiology interface through the development of inhibitors of current resistance mechanisms. In the latter, the antibiotic is co-administered with an inhibitor molecule called an antibiotic adjuvant that has weak or no antimicrobial activity but enhances the activity of minimizing or blocking the resistance. The advantage of this strategy is the utility of available antibiotics with known properties even after resistance emerges (13). Clavulanic acid, sulbactam, and tazobactam are important adjuvants used along β-lactam antibiotics but do not inhibit class D carbapenemases (14).

Since the β-lactam/inhibitor combinations resistance has also been reported by emerging new inhibitors hydrolyzing enzymes, the growing efforts need to obstacle this kind of resistance through the modification of the inhibitors or finding novel inhibitors. To our knowledge, there is no report about in silico investigation of finding new class D carbapenemases OXA-58 inhibitors from natural origins. Therefore, in this study

suitability of some chemicals from plant origins as carbapenem antibiotic adjuvant were explored through the application of bioinformatic tools.

Materials and Methods

Selection of and preparation of structures

By studying relevant articles, plant compounds that showed anti-bacterial effects against *A. baumannii* were chosen, and the appropriate crystallographic structure of OXA-58 containing catalytic part where downloaded from PDB (https://www.rcsb.org/) (15); The collected structures were further observed by UCSF chimera (16).

Preparation for Docking

The 2D structure of ligands where obtained from PubChem and ChemDraw in SDF and Molfile format (17), furthermore, these structures were converted to SYBYL MOL2 using Open Babel(http://www.cheminfo.org/Chemistry/Cheminformatics/FormatConverter/index.html) (18) and turned into PDBQT by Racoon for further analysis (https://autodock.scripps.edu/resources/raccoon/) (19).

Blind Docking

Blind docking is the docking of a ligand to the whole surface of a protein without any prior information about the target binding sites (20). The blind docking was carried out by virtual screening method using PyRx (https://pyrx.sourceforge.io/) (21) and binding energy for the plant compounds and 6-alpha-methyl penicillate (as a carbapenem antibiotic representative) was calculated in both Autodock and Autodock Vina (22, 23).

Drug likeliness and Lipinski's rule

To evaluate the pharmacological and biological activity of the ligands, they were screened based on Lipinski's rule of five, and their properties were inspected using Molinspiration (https://www.molinspiration.com/) (24). The rule of five summarizes the following criteria:

a) Molecular weight must be less than or equal to 500 Dalton

b) The number of hydrogen bond acceptors (including all nitrogen and oxygen atoms) must be greater than or equal to 10.

c) the number of hydrogen bond donors (including all hydrogen-nitrogen and hydrogen-oxygen bonds) must be greater than or equal to 5.

d) Molecules should have an n-octanol-water partition coefficient less than or equal to five (logP $\leq$5).

Compounds that violated more than one of these criteria and or their sum of binding energy which was calculated by both docking software was greater than the calculated binding energy of antibiotic representative agent were eliminated (25).

Focused docking of selected ligands

To find the active site and involved amino acids of the receptor, docking between receptors dock and an antibiotic agent was necessary, to perform this action grid box of atoms was calculated using Autogrid, furthermore, this grid box was provided to AutoDock 4. 2. 6 so that further ligand docking is done appropriately. Subsequently, a rigid docking was performed by Autodock which uses the Lamarckian genetic algorithm (LGA) to optimize binding energy and to create a set of possible conformations, over 2,500,000 binding conformations were identified through 100 iterations, and the best binding conformations were evaluated based on the lowest binding energy and highest stabilizing interactions, both software's are available in MGLTools 1. 5. 7 (22).

Final selection of ligands

Amino acids involved in hydrogen bonds in receptors were recognized through docking between receptor and antibiotic representative agent, ligands that had hydrogen interaction with at least one of these amino acids were identified and chosen. Furthermore, these compounds were evaluated based on drug likeliness rules other than the rule of five through PreADMET (https://preadmet.qsarhub.com/druglikeness/) and SwissADME (http://www.swissadme.ch/) (26) packages and their pharmacokinetic properties were analyzed through SwissADME subsequently. To assess the cytotoxic characteristics of chosen ligands, ProTox-II (https://tox-new.charite.de/protox_II/) (27) was used.

Result

Preparation of enzyme and phytochemicals

By studying the articles, 27 plant ligands whose antibacterial effects against *A. baumannii* have been proven in the laboratory were selected. The name and characteristics of this plant are given in Table 1. Three-dimensional structure of OXA-58 enzyme with identification code 4Y0U was selected (Figure 1).

Primary ligand selection

The initial screening of the ligands was performed in terms of energy values for receptor-ligand binding and Rule Five. The results of these studies are presented in Tables 2 and 3, respectively. The results in Table 2 showed that the sum of the best binding energy reported for the antibiotic-representative (first row) was -10.48. Thus, ligands such as allyl methyl disulfide, allyl methyl trisulfide, carvacrol, diallyl disulfide, diallyl sulfide, diallyl tetrasulfide, diallyl trisulfide, eugenol, paeonol, trans-cinnamaldehyde, and α-elemene had more positive binding energies and were discarded. According to Table 3, the ligands baicalin, chebulinic acid, chebulagic acid, corilagin, epigallocatechin gallate, terchebulin, and theaflavin violated more than one rule five and were excluded. Finally, baicalein, berberine, curcumin, ellagic acid, epicatechin, honokiol, magnolol, norwogonin, and thymol ligands were selected for further studies.

Final selection of candidate ligand

The grid box was first determined with the specifications listed in Table 4. The distance between the points on the axis was 0.375 angstroms. The file related to the coordinates of the grid box was provided to the dock software. The accuracy of the box coordinates was determined from the RMSD less than 2 (1.25) in the docking of the antibiotic-representative with the receptor enzyme.

The binding energies, the number of hydrogen bonds, and the amino acids involved in the bonding between the selected ligands and the antibiotic-representative to the receptor were calculated by the relevant software (Table 5). The binding energies of all ligands were better than the binding energies of the antibiotic-representative ligand (-5.22). Ser221, Ala219, Tyr208, and Gln128 were the four amino acids involved in the hydrogen bonding of the receptor with the representative ligand. Berberine ligand was not hydrogen bonded despite binding to the receptor. The alginic acid ligand and epicatechin, similar to the antibiotic-representative ligand, formed 4 hydrogen bonds with the receptor. Three of These bonds were similar to the amino acids involved in the interaction of the antibiotic-representative ligand with the receptor. Due to the importance of the hydrogen bond, the berberine ligand was removed from further studies. Images of the binding of epicatechin ligand and 6-alpha-hydroxymethyl penicillanate with the receptor as an example, are shown in Figure 2.

Drug-likeness and pharmacokinetic properties of selected ligands

To further examine the suitability of ligands as drugs, several other important drug-likeness laws such as Lead-like law (28), CMC-like law (29), MDDR-like law (30), WDI-like law(31), Veber law (32), Eggan's law (33) and Muegge's law (34) were examined. The bioavailability of ligands was also extracted and the results are presented in Table 6. The three ligands baicalin, epicatechin, and norwogonin are within the permissible limits of all laws. The bioavailability score determines the oral absorption of drugs. Any drug molecule that achieves Rule Five with a score of 0.55 is considered sufficiently orally absorbable (35). Therefore, all of the ligands listed in Table 6 were orally absorbable. The pharmacokinetic properties of the selected ligands are presented in Table 7. Predictive results showed that all ligands were absorbed in the upper intestine and, two ligands honokiol and magnolol could pass across the blood-brain barrier (BBB). According to the summarized results in this table, epicatechin was the only compound that showed no inhibitory effect on members of the cytochrome p450 family and exhibited p-glycoprotein inhibitory properties. The skin permeation coefficient (Kp) is the measure of skin conductance for a specific compound, this coefficient has a direct linear correlation with molecular size and lipophilicity. The more negative log Kp the higher molecular conductance through the skin (36). As displayed in Table 7, honokiol and magnolol showed the highest skin permeability whereas curcumin showed the lowest permeability among ligands.

Relative cytotoxicity of selected ligands

Category I (LD50 ≤ 5 mg/kg) is the highest toxicity category. Category II (moderately toxic) includes chemicals with 5 < LD50 ≤ 50 mg/kg. Category III (slightly toxic) includes chemicals with 50 < LD50 ≤ 300 mg/kg. category IV includes chemicals that display adverse effects through oral use with 300< LD50 ≤ 2000 mg/kg. category V includes compounds that may display harmful effects with 2000< LD50 ≤ 5000 mg/kg and Safe chemicals (LD50 > 5000 mg/kg) are included in Category VI (37). As shown in Table 8, four ligands were in Category IV, three in Category V, and only epicatechin was in Category VI with LD50 of 10000. Other predictive results of this table are as follows. All chosen ligands had no cytotoxic effects in kidneys and none of them were tumor or cancer-inducing agents. Curcumin could have adverse immune responses among selected compounds. Ellagic acid and epicatechin were two ligands that didn't have any effect on the nuclear signaling pathways and stress response pathways.

Discussion

Antibiotic-resistant bacterial infections are deemed as one of the greatest threats to public health. New antibiotics and therapeutic strategies must be developed for an ever-growing number of infection cases. The variety in metabolic, genetic, and physiologic of antibiotic-resistant microbes has led researchers to look for new options one of them being plant-derived compounds as an antibiotic or supplementary agents. Plant derivative compounds such as polyphenols, alkaloids, and tannins show great potential to combat bacterial infection, whether as an antibiotic agent or in a synergic combination with other antibiotics. In this study, the focus was on *A. baumannii*, one of the major antibiotic-resistant bacteria, and OXA-58 enzyme which is involved in antibiotic resistance. It was tried to introduce appropriate adjuvant antibiotic candidates from important plant ligands. In this regard, several selective criteria were applied.

The number of hydrogen bonds between the ligand and the receptor was an important factor in the selection of ligands in this study (Table 5). Different types of protein-ligand interactions (for

Table 1. Names and characteristics of phyto-ligand used in this study.

Molecular Formula	Herbal Origin	PubChem ID	Name of the Ligand
Allyl methyl disulfide	*Allium sativum*	62434	C4H8S2
Allyl methyl trisulfide	*Allium sativum*	61926	C4H8S3
Baicalein	*Scutellaria baicalensis*	5281605	C15H10O5
Berberine	*Coptidis chinensis* Franch	2353	C20H18NO4+
Baicalin	*Scutellaria baicalensis*	64982	C21H18O11
Chebulagic acid	*Terminalia chebula*	250397	C41H30O27
Carvacrol	*Oreganum vulgare*	10364	C10H14O
Chebulinic acid	*Terminalia chebula*	72284	C41H32O27
Corilagin	*Terminalia chebula*	73568	C27H22O18
Curcumin	*Curcuma longa*	969516	C21H20O6
Diallyldisulfide	*Allium sativum*	16590	C6H10S2
Diallylsulfide	*Allium sativum*	11617	C6H10S
Diallyltetrasulfide	*Allium sativum*	75552	C6H10S4
Diallyltrisulfide	*Allium sativum*	16315	C6H10S3
Ellagic acid	*Rosa rugosa*	5281855	C14H6O8
Epicatechin	*Camellia sinensis*	72276	C15H14O6
Epigallocatechin gallate	*Camellia sinensis*	65064	C22H18O11
Eugenol	*Syzygium aromaticum*	3314	C10H12O2
Honokiol	*Magnolia dealbata*	72303	C18H18O2
Magnolol	*Magnolia dealbata*	72300	C18H18O2
Norwogonin	*Scutellaria baicalensis*	5281674	C15H10O5
Paeonol	*Paeonia suffruticosa Andr*	11092	C9H10O3
Terchebulin	*Terminalia chebula*	16175789	C48H28O30
Theaflavin	*Camellia sinensis*	135403798	C29H24O12
Thymol	*Thymus*	6989	C10H14O
Trans-cinnamaldehyde	*Cinnamomum zeylanicum*	637511	C9H8O
α-elemene	*Commiphora molmol*	80048	C15H24

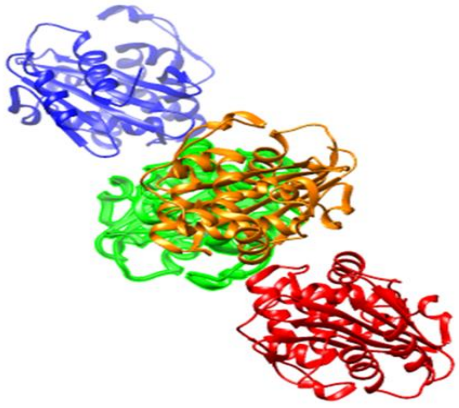

Figure 1. 3D structure of OXA-58.

Table 2. Energy values for receptor-ligand binding.

Name of the Ligand	The sum of the binding energies (Kcal/ mol)	The best binding energy in AutoDock Vina results (Kcal/ mol)	The best binding energy in AutoDock results (Kcal/ mol)	The sum of the binding energies (Kcal/ mol)
6-alpha-Hydroxymethylpenicillanate	-10.48	-3.38	-7.1	-10.48
Allyl methyl disulfide	-6.12	-3.02	-3.1	-6.12
Allyl methyl trisulfide	-6.26	-3.06	-3.2	-6.26
Baicalein	-15.63	-7.03	-8.6	-15.63
Berberine	-15	-6.1	-8.9	-15
Baicalin	-16.85	-6.15	-10.7	-16.85
Chebulagic acid	-19.34	-7.24	-12.1	-19.34
Carvacrol	-10.16	-4.56	-5.6	-10.16
Chebulinic acid	-14.74	-2.94	-11.8	-14.74
Corilagin	-18.43	-7.53	-10.9	-18.43
Curcumin	-12.35	-4.25	-8.1	-12.35
Diallyl disulfide	-6.71	-3.21	-3.5	-6.71
Diallyl sulfide	-5.95	-2.55	-3.4	-5.95
Diallyl tetrasulfide	-6.2	-2.9	-3.3	-6.2
Diallyl trisulfide	-6.09	-2.59	-3.5	-6.09
Ellagic acid	-15.32	-7.12	-8.2	-15.32
Epicatechin	-12.06	-3.86	-8.2	-12.06
Epigallocatechin gallate	-12.59	-3.39	-9.2	-12.59
Eugenol	-9.27	-5.7	-3.57	-9.27
Honokiol	-11.71	-4.01	-7.7	-11.71
Magnolol	-11.59	-3.79	-7.8	-11.59
Norwogonin	-15.16	-6.56	-8.6	-15.16
Paeonol	-9.86	-4.06	-5.8	-9.86
Terchebulin	-13.7	-2.7	-11	-13.7
Theaflavin	-18.74	-8.54	-10.2	-18.74
Thymol	-10.54	-4.64	-5.9	-10.54
Trans-cinnamaldehyde	-9.33	-4.13	-5.2	-9.33
α-elemene	-9.65	-3.35	-6.3	-9.65

*Ligands that were left out are shown in bold in the table

Table 3. Lipinski properties of diverse phytochemicals.

Name of phytochemical ligand	Molecular weight	Log P	H-bond donor	H-bond acceptor	Final result
Allyl methyl disulfide	120.24	1.98	0	0	Suitable

Allyl methyl trisulfide	152.31	2.48	0	0	Suitable
Baicalein	270.24	2.68	3	5	Suitable
Berberine	336.37	0.2	0	5	Suitable
Baicalin	446.36	0.55	6	11	Violated
Chebulagic acid	954.66	0.07	13	27	Violated
Carvacrol	150.22	3.81	1	1	Suitable
Chebulinic acid	956.68	0.4	13	27	Violated
Corilagin	634.46	0.31	11	18	Violated
Curcumin	368.38	2/30	2	6	Suitable
Diallyl disulfide	146.28	2.63	0	0	Suitable
Diallyl sulfide	114.21	2.13	0	0	Suitable
Diallyltetrasulfide	210.41	3.63	0	0	Suitable
Diallyl trisulfide	178.35	3.13	0	0	Suitable
Ellagic acid	302.19	0.94	4	8	Suitable
Epicatechin	290.27	1.37	5	6	Suitable
Epigallocatechin gallate	458.38	2.25	8	11	Violated
Eugenol	164.20	2.1	1	2	Suitable
Honokiol	266.34	5	2	2	Suitable
Magnolol	266.34	4.8	2	2	Suitable
Norwogonin	270.24	2.68	3	5	Suitable
Paeonol	166.18	1.81	1	3	Suitable
Terchebulin	1084.72	2.71	16	30	Violated
Theaflavin	546.5	2.35	9	12	Violated
Thymol	150.22	3.34	0	1	Suitable
Trans-cinnamaldehyde	132.16	2.48	0	1	Suitable
α-elemene	204.36	5.17	0	0	Suitable

*Violated ligands are shown in bold in the table.

Table 4. Grid size for the studied receptor (in Å).

Number of spots			Position from center		
X	**Y**	**Z**	**X**	**Y**	**Z**
60	70	60	-9.007	-0.826	67.809

Table 5. Selective plant compounds studied by molecular docking using AutoDock.

Name of the Ligand	H bond interaction residues	Number of H-Bonds	Binding energy (Kcal/ mol)
6-alpha-hydroxymethyl penicillanate	Ser221, Ala219, Tyr208, Gln128	4	-5.22
Baicalein	Ser221, Gln128, Lys220	3	-7.87

Berberine		0	-7.96
Curcumin	Gln128, Lys220, Lys264	3	-8.30
Ellagic acid	Ser221, Gln128, Lys220, Lys264	4	--8.54
Epicatechin	Ser221, Ala219, Tyr208, Lys220	4	-7.41
Honokiol	Ser221, Lys220	2	-7.16
Magnolol	Ser221, Lys220	2	-7.11
Norwogonin	Ser221, Lys220	2	-7.81
Thymol	Ser221, Lys220	2	-6.42

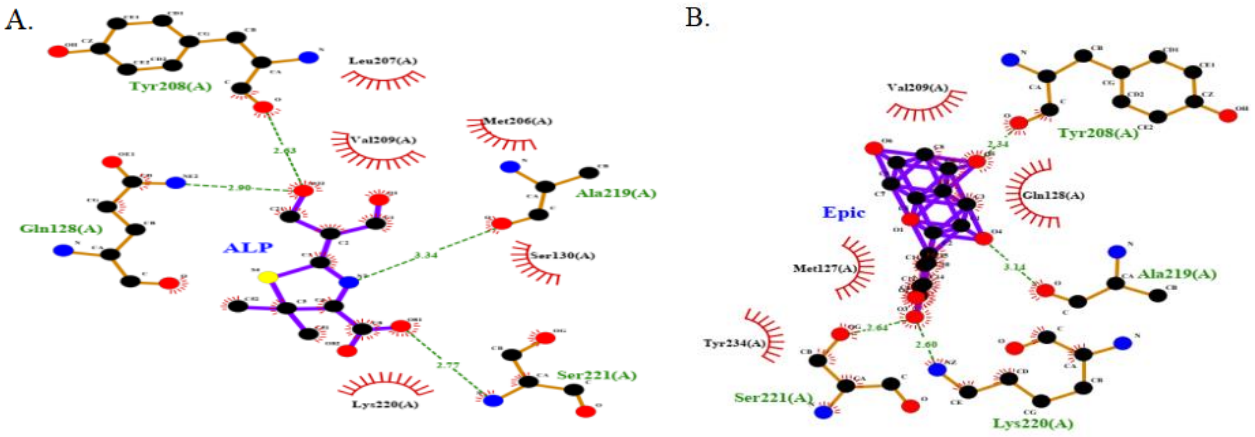

Figure 2. LIGPLOT image of the binding site of 6-alpha-hydroxymethyl penicillanate (A) and epicatechin (B) with the OXA-58.

Table 6. Drug-likeness prediction of selected ligands using PreADMET and SwissADME tool.

	PreADME							SwissADME
Herbal Ligand	**Bioavailability score**	**Muegge rule**	**Eggan rule**	**Veber rule**	**WDI-like law**	**MDDR-like rule**	**CMC-like rule**	**Lead-like rule**
Baicalein	0.55	Suitable	Suitable	Suitable	In cutoff 90%	Mid-structure	Qualified	Suitable if its binding affinity is greater than 0.1 µM

Curcumin	0.55	Suitable	Suitable	Suitable	Out of cutoff	90%	Mid-structure	Qualified	Violated	
Ellagic acid	0.55	Suitable	Violated	Violated	In cutoff	90%	Mid-structure	Qualified	Suitable if its binding affinity is greater than 0.1 µM	
Epicatechin	0.55	Suitable	Suitable	Suitable	In	90% cutoff	Mid-structure	Qualified	Suitable if its binding affinity is greater than 0.1 µM	
Honokiol	0.55	Suitable	Suitable	Suitable	In	90% cutoff	Mid-structure	Qualified		Violated
Magnolol	0.55	Suitable	Suitable	Suitable	In	90% cutoff	Mid-structure	Qualified		Violated
Norwogonin	0.55	Suitable	Suitable	Suitable	In	90% cutoff	Mid-structure	Qualified	Suitable if its binding affinity is greater than 0.1 µM	
Thymol	0.55	Violated	Suitable	Suitable	Out of cutoff	90%	Mid-structure	Not Qualified	Violated	

Table 7. Prediction of pharmacokinetics of phyto-ligands.

Herbal Ligand	**Skin permeation as (cm/s)**	**P-glycoprotein Inhibitor**	**CYP3A4 Inhibitor**	**CYP2D6 Inhibitor**	**CYP2C9 Inhibitor**	**CYP2C19 Inhibitor**	**CYP1A2 Inhibitor**	**Blood-Brain Barrier permeant**	**Human Intestinal Absorption**
Baicalein	-5.7	-	+	+	-	-	+	-	High
Curcumin	-8.23	-	+	-	+	-	+	-	High
Ellagic acid	-7.36	-	-	-	-	-	+	-	High
Epicatechin	-7.82	+	-	-	-	-	-	-	High
Honokiol	-4.39	-	+	+	+	+	+	+	High
Magnolol	-4.39	-	+	+	+	+	+	+	High
Norwogonin	-5.7	-	+	+	-	-	+	-	High
Thymol	-4.87	-	-	-	-	-	+	+	High

Table 8. Prediction of toxicity of phyto-ligands.

Herbal Ligand	Nuclear receptor signaling pathway	Stress response pathways	Cytotoxicity	Mutagenicity	Immunotoxicity	Carcinogenicity	Hepatotoxicity	Toxicity class	LD50% (mg/kg)
Baicalein	+	+	–	–	–	–	–	5	3919
Curcumin	+	–	–	–	+	–	–	4	2000
Ellagic acid	–	–	–	–	–	–	–	4	2991
Epicatechin	–	–	–	–	–	–	–	6	10000
Honokiol	+	+	–	–	–	–	–	4	1649
Magnolol	+	+	–	–	–	–	–	5	2200
Norwogonin	+	+	–	–	–	–	–	5	3919
Thymol	–	+	–	–	–	–	–	4	640

non-covalent bonds) include ionic interactions, hydrogen bonds, and van der Waals interactions (38). Hydrogen bonding is rightly called the "key to molecular detection." This interaction is weaker than the covalent bond and stronger than the van der Waals interaction. The permeability and flexibility of hydrogen bonds make them the most important physical interaction in biomolecular systems in an aqueous solution. Hydrogen bonding plays an important role in many chemical and biological processes including ligand binding and enzyme catalysis. In biological processes, both specificity and reversibility are important. Weaker interactions can be more easily created than stronger ones and broken (39). Drug-likeness and pharmacokinetic properties of compounds are an overall assessment of their potential to succeed in clinical trials. The investigation of these properties is essential for filtering ligands with unfavorable and poor development potential (40).

In this study, several drug-likeness rules were taken into consideration at the same time for choosing the best ligands (Table 6) and just three ligands were considered qualified. Among the pharmacokinetics properties, absorption in the upper intestine, ability to pass across the BBB, permeation from the skin, and inhibitory effect on cytochrome and p-glycoprotein were predicted for selected ligands (Table 7). The reasons for the importance of these factors are mentioned below. For a drug compound to reach the bloodstream when taken orally, it must first be absorbed from the gastrointestinal tract and transported to the liver via the hepatic vein. The drug and its metabolites are then distributed throughout the body by arterial circulation. Drugs are transported from a high-concentration area (such as digestive fluid) to a low-concentration area (such as blood) via simple diffusion. The diffusion rate is directly proportional to the gradient but also depends on the lipid solubility of the molecule, size, degree of

ionization, and surface area of the adsorbent. Fat-soluble drugs are released most rapidly due to the cell membrane mainly being made of phospholipids. Furthermore, Small molecules tend to penetrate membranes more rapidly than larger molecules (41). Passage of the ligands from the barrier is not a good feature in the case of candidates for drugs that are not therapeutic targets in the central nervous system. The pharmacological properties that are desirable to cross this barrier are high lipophilicity, small molecular size and weight, and low hydrogen bonding potential (42). Cytochrome (CYP) p450 family members are vital for the biosynthesis of cholesterol, steroids, prostacyclin, and thromboxane A2, these enzymes are also involved in detoxification and drug metabolism. There are more than 50 members of the cytochrome p450 family although CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5 metabolize 90% of drugs. Simultaneous consumption of multiple pharmaceutical compounds may show time-dependent deactivation or inhibition of the enzymes and result in unpleasant or cytotoxic reactions (43). P-glycoprotein plays a vital role in limiting cells drug absorption from bloodstream to brain parenchyma and from intestines lumen to epithelial cells involved in absorption, furthermore, p-glycoprotein is involved in urine and bile-related drug excretion. A relative amount of p-glycoprotein is not paramount in drug absorption unless the oral drug is used in small amounts or the solubility and diffraction rate of the drug is low. P-glycoprotein inhibitors exhibit vital roles in drug interference since simultaneous use of multiple drug compounds may lead to high plasma levels due to bile/urine-related excretion inhibition, the latter may cause adversary effects on drugs with limited therapeutic range (44). In case of therapeutic or cosmetic use, the subcutaneous injection has numerous advantages to oral intake or any other injection type. Furthermore, skin acts as a repository for injected compounds thus making it an apt source for long-term stable release sites. Subcutaneous injection also prevents systematic adverse effects. Nonetheless, there is a small number of drugs that can be delivered at a stable rate through subcutaneous injection (45).

Toxicity prediction is an important step in the drug discovery process due to the identification of the compounds with the higher potential of being safe and effective in humans (46). Predictions of the toxicity potential of selected phyto-ligands are presented in Table 8. It has been estimated that a 10% improvement in anticipation of cytotoxic effects before the costly clinical experiment can lead to over 100 million dollars in financial conservation, furthermore, there have been and there are numerous attempts in the early evaluation of compound safety in drug development programs (47). Immunotoxic effects of drugs may lead to downregulation or stimulation of the immune system, hypersensitivity, autoimmune responses, infectious side effects, and virus-related malignancies. Excessive allergic responses are the most common form of immunotoxic effects of drug compounds, whereas, systemic or organ-specific autoimmune reactions are rare. An overview of the immunotoxic effects of drug-related compounds suggests that this phenomenon is an important contributor to major side effects or even death (48). Mutagenicity is another major phenomenon that needs to be avoided in drug development. It is a wide term used in the description of chemical or therapeutic compounds that are used to induce genetic mutation (49). Plant derivatives and products are widely used in clinical settings for supplementary purposes and disease treatment, it has been estimated that over 80% of the populace in developing countries use ancient therapeutic herbals as their first line of choice, nonetheless, long-term use of herbal products is not deemed safe (50). According to the National Toxicology Program (Tox21), chemical compounds may potentially lead to disorders in the homeostasis of the human body and thus lead to adverse effects on health. Nuclear signaling

pathways and stress response pathways are two branches of signaling pathways in the body and play vital roles, studies suggest that many toxic compounds show cytotoxicity in lower concentrations than the necessary concentration needed for interaction with their receptor, which may lead to cell apoptosis before any ligand-receptor interaction takes place (51).

Overall docking and ligand structure analysis suggested that epicatechin can show inhibitory effects on the OXA-58 enzyme. furthermore, our result suggests that it is unlikely that epicatechin shows toxic effects in the body. *Clonorchis sinensis* also known as tea plant, tea shrub, and tea tree, is cultivated in tropical and subtropical regions in countries such as China, India, Sri Lanka, and Japan and some countries of southern America and Africa. Green tea due to no fermentation compounds such as polyphenols is reserved and the most desirable traits of green tea are due to poly phenol compounds which are mainly catechins that make up between 25% to 35% of dried green leaves weight. Catechins that exist in green tea are namely catechin, epicatechin, gallocatechin, epicatechin-3-gallate, epigallo-catechin, and epigallocatechin-3-gallate (52). Molecular dynamic stimulation has been used in the study of multiple catechins and bilateral phospholipid layers and it has been shown that in general, molecules without gallate groups such as catechin and epicatechin, are more apt in penetration of bilateral layers (53). In recent times, scientists have been analyzing the possibility of using green tea and catechins as antimicrobial agents and have shown the potential use of these compounds in various infections, in the following record, some of the researches related to *A. baumannii* are included. It has been shown that catechin can result in quinolones-induced redox imbalance as well as a significant reduction of glutathione in *A. baumannii* which leads to antibiotic-induced oxidative stress (54). It has been demonstrated that epigallocatechin-3-gallate can show bactericidal effects in clinical strains of *A. baumannii* (55). The synergetic effect of curcumin and epigallocatechin-3-gallate has been studied in an in vitro environment against multi-drug resistant *A. baumannii* in which the results show significant antibacterial effects (56). In a study antibiotic effect of pinus pinaster aqueous bark extract with its basic components including caffeic acid, catechin, epicatechin, gallic acid, and vanillin was tested against *A. buamanni* has been suggested (57). A combination of theaflavin-epicatechin has been also used against *A. baumannii*-infected larvae in which it has been demonstrated that polyphenol compound coupling produces better bactericidal effects (58). Epigallocatechin 3-gallate synergism with antibiotics in *A. baumannii* has been examined and the results suggested combination therapy may be an alternative therapeutic approach (59). To our knowledge, there has been no study on the direct effect of catechin compounds on carbapenemase enzymes.

Conclusion

Overall docking and ligand structure analysis suggested that epicatechin can show inhibitory effects on the OXA-58 enzyme. furthermore, our result suggests that it is unlikely that epicatechin shows toxic effects in the body.

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Ethics approval and consent to participate

This study did not require an ethics license.

Conflict of interest

Elaheh Zadeh Hosseingholi, Ghader Molavi, Mohammad Sadra Mohammadi have no conflict of interest regarding the present manuscript. There has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors.

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