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Drug-like Properties Analysis and In Silico Anti- Antibiotic Resistant *Klebsiella pneumoniae* Activity of Extracts of *Nigella sativa* and *Cassia angustifolia* in Comparison with Sulbactam- a Novel Anti-drug Resistance Drug

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Abstract

One big problem in developing the treatment of infectious diseases is increased number of multidrug resistant (MDR) bacteria that are responsible for serious health issues. Research has revealed that the medicinal herbs such as *Nigella sativa* and *Cassia angustifolia* have antimicrobial properties against the bacterial strains that have resistant against synthetic antibiotic compounds, which are commonly used for treatment. Using in silico methods, this study aimed to identify the effects of bioactive phytochemicals of *Nigella sativa* and *Cassia angustifolia* against antibiotic resistant bacteria, '*Klebsiella pneumoniae'*. Our study shows that the extracts of *Nigella sativa* and *Cassia angustifolia* have activity against the drug resistant enzyme beta lactamase. Their binding ability is comparable to the widely used drug 'sulbactam'. They also show good pharmacokinetic properties. This advocates their potential to be used as anti-drug resistance drugs. Further, in vitro analysis and animal trials are required to confirm the results of this study.

Keywords: Klebsiella pneumoniae

Introduction:

One big problem in developing the treatment of infectious diseases is increased number of multidrug resistant (MDR) bacteria that are responsible for serious health issues. To overcome this problem, it is important to know about the molecular mechanism that is responsible for resistance development in bacteria. According to biomedical, the increasing feature, that is common in bacteria, viruses, parasites, protozoa, and malignant tumor cells, is resistance against treatment. [2].

In the case of bacterial infections, common mechanisms that are involved in antibiotic resistance include the presence of drug-inactivating enzymes, modification of drug binding sites, changes to influx and efflux mechanisms, and alterations in enzyme pathways [3]. The β-lactams form a group of antibiotics that includes penicillin, cephalosporins, monobactams, and carbapenems which inactivate glycopeptide transpeptidases, thereby inhibiting bacterial cell wall synthesis. This leads to its main bactericidal properties via cell lysis [3, 4]. This interaction is particularly used for treating bacteria with multiple layers of peptidoglycan such as Staphylococcus aureus and other Gram-positive bacteria [4]. However, in recent years, it has been noticed that there is increase in resistance against staphylococci in hospital settings.

Resistance development can be seen in enterococci commonly causing hospital acquired infections. Enterococcus faecium and faecalis which are the part of the

commensal microbiota lining the intestinal mucosa, have clinical importance. Enterococci can spread antibiotic resistance properties through gene transfer from one to other susceptible bacteria. As a result, vancomycin-resistant enterococci (VRE) have increasingly become a serious problem in the clinical (particularly hospital) setting as this broad-spectrum antibiotic compound is commonly used as a reserve drug to treat intractable infections [6]. The decrease in treatment options of bacterial infections has become critical in treating patients that are in hospitals and, therefore, there is the need of new pharmacological therapeutical and preventive measures. we can handle this problem by exploring the therapeutical properties of medicinal plants.

Plants have often laid the foundation of pharmaceutical drug development and allowed breakthroughs in treating diseases on a greater and more efficient scale. Notably, 60% of currently available antimicrobial and antitumoral drugs are derived from plants [7). One of the oldest documented herbal medicinal plants is N. sativa that has been used for centuries in traditional Arabic medicine. This herbal plant is already well known for its safety and treatment. The chemical ingredients of NS are Thymoquinone, linoleic and oleic acid, trans-anethole, p-cymene, alpha pinene, limonene, and carvone. Extensive studies of NS have explored its pharmacological actions such as anti-cancer, immunomodulator, analgesic, antidiabetic, anti-microbial, anti-inflammatory, spasmolytic, bronchodilator, hepato-protective, renal protective, gastroprotective, and as an antioxidant [9].

Research has revealed that the medicinal herb N. sativa, that have antimicrobial properties, used against bacterial strains that have resistance against commonly used synthetic antibiotic compounds. Both Gram-positive and Gram-negative bacteria were susceptible to N. sativa at different concentrations. N. sativa plant is used for growth inhibition of Entero-pathogens such as Salmonella, H. pylori, and E. coli strains. The herbal plant also applies to B. cereus, S. aureus, and, strikingly, to MDR S. aureus strains including MRSA and even VRSA [10].

The drought resistant herb is Senna Makki (*Cassia angustifolia) which* is native of Saudi Arabia and is now grown worldwide. Research has been completed to find out the chemical composition of Senna, various compounds that are found in it are Sitosterol, Sennosides A, B,C, anthraquinone, Cathartic acid, Rhamnetin, gluco-sennin, , chrysophenic acid, nigrin, kaemphrin, rhein, flavonoids, emodin and salicylic acid [11,12,13]. Several medicinal effects (like purgative, antibiotic, anti-malarial, anti-cancer, antipyretic, antioxidant, anti-inflammatory etc.) of Senna are described and studied through its chemical composition. [13]. Research also shows that Cassia angustifolia may have antibacterial principles that could be useful in microbial diseases especially against *Klebsiella pneumoniae* [14].

The efficient methods that are used for screening of bioactive compounds are silico molecular docking and drug-like properties analysis that used a pool of phytochemicals [15]. Docking can energize the interactions between a ligand and protein, calculate their binding energies and predict the possibility of whether a compound may bind to a pharmacological target, such as an enzyme. Drug-like properties analysis screens the phytochemicals with desired pharmacokinetic properties, including the absorption, distribution, metabolism, excretion and toxicity [16]. Docking has been widely used to identify bioactive compounds for further in vitro and in vivo studies. Using in silico methods, this study focused to identify the effects of bioactive phytochemicals of *Nigella sativa* and *Cassia angustifolia* that can inhibit antibiotic resistant bacteria, '*Klebsiella pneumoniae'*.

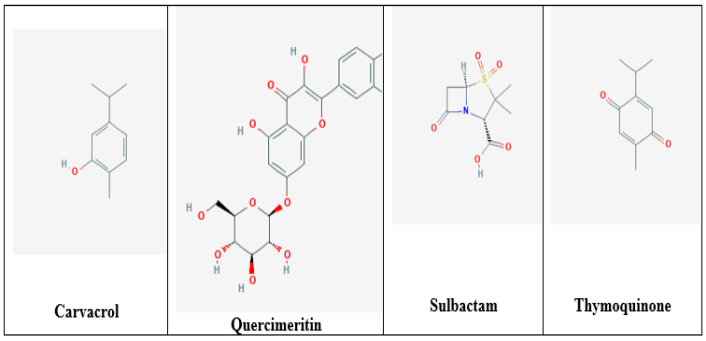
Methodology

In silico Molecular Docking and Drug Like Properties Analysis

The three-dimensional structure of Beta- Lactamase was obtained from Protein Data Bank under PDB ID 1HTZ [17]. Crystallographic properties of the compound are shown in Table 1.

Enzyme	PDB Code	Classification	Organism	Expression System	Resolution	Method	Total Structure Weight	Chain
TEM 52 Beta Lactamase	1HTZ	Hydrolase	*Klebsiella pneumoniae*	*Escherichia coli*	2.40 Å	X-Ray Diffraction	173.65 kDa	A

The three-dimensional structures of the phytochemicals of Sulbactam (drug used as a reference in this study), *Nigella sativa*, and *Cassia angustifolia* were obtained from PubChem [20] in SDF format and were converted to PDB format using Open Babel GUI [21] and were used as Ligands. The 2-dimensional structures of the compounds are shown in Table 2.



Ligands were prepared using ADT [19]. Molecular Docking was performed using Patch Dock [22] which is an online tool for molecular docking. Blind docking was performed to identify the active site and later on all the ligands were docked with active site using specific docking.

Drug like properties analysis of the compounds was done using online tool SWISS ADME [23]. For this purpose, Canonical SMILES of the ligands were retrieved from PubChem and used in Swiss ADME for analysis.

Results

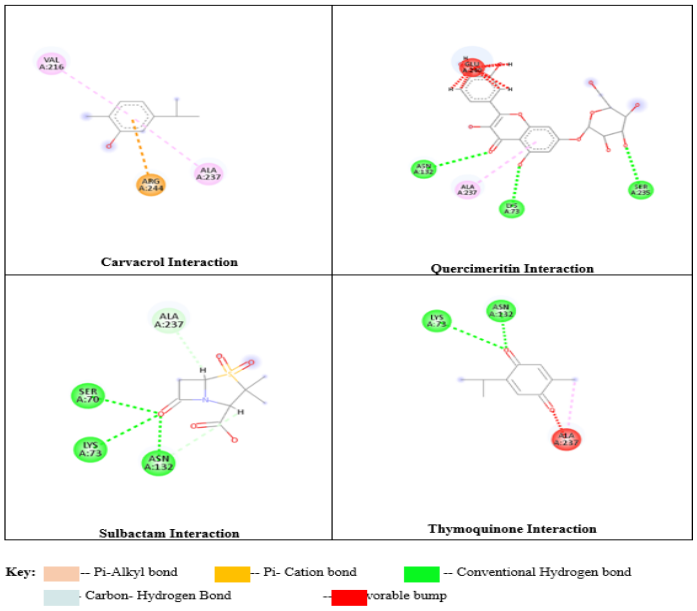
	Sulbactam	Quercimeritin	Carvacrol	Thymoquinone
Docking Score	2960	4742	2788	2762
Atomic Contact Score	-14.94	-85.25	-73.14	-21.70
Global Energy	-15.64	-22.69	-20.56	-16.73
Molecular Weight	233.24	464.38	150.22	164.20
Log P	-0.35	-0.37	2.82	1.85
Solubility (Ali)	57.8	0.02	0.03	0.462
GI Absorption	High	Low	High	High
BBB Permeability	No	No	Yes	Yes
Skin Permeation	-8.44	-8.88	-4.74	-5.74
Drug Likeness (Ghose,Veber)	Yes Yes	No (1 viol) No (1 viol)	No (1 viol) Yes	Yes Yes
BRENK	0	1 (catechol)	0	0
PAINS	0	1 (catechol)	0	0
Leadlikeness	No	No	No	No
Bioavailability Score	0.56	0.17	0.55	0.55
Synthetic Accessibility	3.84	5.31	1.00	2.83
CYP1A2	NI	NI	I	NI
CYP2C9	NI	NI	NI	NI
CYP2C19	NI	NI	NI	NI
CYP2D6	NI	NI	NI	NI
CYP3A4	NI	NI	NI	NI

The results of Molecular Docking and Drug like properties analysis are shown in Table 3.

Table 3. Results of Molecular Docking Analysis and Drug-like Properties Analysis

Ligand: I- Inhibitor, NI- Not an Inhibitor, Viol- Violation, CYP- Cytochrome P

Table 4 shows the interactions of the ligands with the Receptor in 2D diagrams. All the compounds showed interaction with similar amino acids at the same site.



Discussion

The in silico ADMET results demonstrated that these extracts were non-toxic, non-carcinogenic, absorb in the human intestine, have Caco-2 permeability, do not inhibit CYP enzymes except Carvacrol which is an inhibitor of CYP 1 A2, are non-inhibitors for RCT which suggested their significant pharmacokinetic properties. Quercimeritin shows low GI absorption that decreases its drug-likeness. It might be improved by processing the drug.

First three parameters in the Table 3 show the results of in- silico molecular docking. Docking score is Geometric shape complementarity score [see 22 for details]. Sulbactam, Carvacrol, and Thymoquinone show a comparable docking score of 2960, 2788, and 2762, respectively. Quercimeritin has a score of 4762 which is almost double to the other tested compounds. This score is based on the geometric orientation of the ligands with the receptor molecule in the space. Table 4 shows the amino acids involved in the interaction. All four drugs show interaction to similar amino acids. Possible Hydrogen bonds, Covalent bonds, Polar bonds, and Intermolecular interactions are color coded. Quercimeritin shows unfavorable bumping due to its large size which might be a false positive result that can be ruled out using in vitro studies.

Atomic Contact Energy is the measure of the binding affinity of the ligand. Highest contact energies are shown by Quercimeritin and Carvacrol. Global Energy refers to the energy of Receptor-Ligand Complex.[22] A negative global energy shows a stable ligand-receptor complex. All the four compounds under study bind and make stable complexes with beta-lactamase with the global energies of -15.64, -22.69, -20.56, and -16.73 for Sulbactam, Quercimeritin, Carvacrol, and Thymoquinone.

Our study is consistent with the previous studies. Previous research is also indicative that Cassia angustifolia may have antibacterial principles that could be useful in microbial diseases especially against *Klebsiella pneumoniae* [14]. Other studies revealed that the medicinal herb N. sativa exhibited antimicrobial properties against bacterial strains that were shown to be resistant against commonly used synthetic antibiotic compounds. [10]
Though in-silico studies provide a good foundation for drug discovery and drug interactions, yet they are not a substitute to experimental analysis and clinical studies. This is the major limitation of our study. We indicate in vitro analysis and experimental trials for the efficacy of *Nigella sativa* and *Cassia angustifolia* against drug resistant bacteria.

Conclusion

Our study shows that the extracts of *Nigella sativa* and *Cassia angustifolia* have activity against the drug resistant enzyme beta lactamase. Their binding ability is comparable to the widely used drug 'sulbactam'. They also show good pharmacokinetic properties. This advocates their potential to be used as anti-drug resistance drugs. Further, in vitro analysis and animal trials are required to confirm the results of this study.

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Conflict Of Interest

The authors declare no conflict of interest.

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