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Original Article

Cardioprotective Potential of *Qusqualis indica* Leaves; An *in silico* Docking Study

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ABSTRACT

Background: Nowadays cardiac problems are the main cause of death. It includes coronary artery disease, angina pectoris (stable and unstable), congestive heart failure, etc. **Objectives:** The aim of this paper was to generate scientific data regarding *in silico* analysis of beta-sitosterol, coumaric acid, lupeol, qurecetin, and urosolic acid of Muscarinic (M_2) receptor. **Materials and Methods:** The leaf powder was treated with different reagents and prepared with different extracts. The phytochemical screening was carried out by treated the extracts with different reagents for the presence of various metabolites. RCSB protein data bank had used for docking studies. **Results:** The phytochemical screening clearly revealed the presence of various metabolites like flavonoids, alkaloids, saponins, etc. *In-silico* analysis of beta-sitosterol, coumaric acid, lupeol, qurecetin, and urosolic acid had very good interactions with cholinergic receptor (M_2). The obtained score is -7.96, -5.63, -6.7, -7.73, -5.82 for beta-sitosterol, coumaric acid, lupeol, quercetin, and urosolic acid, respectively, which lies in the standard scale. **Conclusion:** All these metabolites (compounds) are present in *Quisqualis indica* leaf extracts (aqueous and ethanolic) and found to be good cardio protective agents.

Keywords: *Quisqualis indica*, Cardio-protective, *in silico*.

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INTRODUCTION

Depending on a patient's physical makeup or a disease, the cardiac system may work differently from one patient to the next, and breathing may cause subtle variances. In extremely rare instances, the complete human structure may resemble a mirror image while still being normal or when a congenital heart abnormality is present. The body structures that typically exhibit lateralization are organised in isomeric way in various situations, which are more frequent than the mirror-imaged condition but still very uncommon.[1] The heart of a person is a crucial organ that continuously pumps blood in cycles throughout the body. The diaphragm, which is close to the thoracic cavity, supports the heart. It is located in the mediastinum, an area of the body where the sternum and spinal column meet anatomically (rib to the diaphragm, and between the lungs). The heart is always around two-thirds to the left of the body's midline. The pericardium encircles and shields the heart. It aids in keeping the heart confined to its location in the mediastinum and provides enough room for forceful, quick contraction. The epicardium (external layer), the myocardium (middle layer), and the endocardium are the three layers that make up the heart's wall (inner layer). Two inferior pumping chambers and two superior receiving chambers make up the heart (known as ventricles).[2]

Quisqualis indica Linn. (*Q. indica*) belongs to family-*Combretecea* can reach up to 9 meters. Locally known as 'Malti' It is cultivated all parts of India but also find in Africa and Indo-Malaysian region.[3,4] It generally requires an area with full sunlight and regular watering.[3] Leaf and root extracts/juices are traditionally used as anthelmintics, and leaves are used to ease flatulence. Externally, a leaf infusion is used to cure boils and ulcers. To expel worms, seeds are anthelimintic to youngsters.[4,5] It exhibits pharmacological activities such as excitotoxicity,[6,7] anti-inflammatory,[8,9] antipyretic,[10] immunomodulatory,[11,12]

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anti-staphylococcal activity,[13] acetyl cholinesterase inhibitor,[14,15] antioxidant activity,[16] and so on. This plant has a number of medicinally active phytochemical components that are responsible for a variety of pharmacological effects.

According to the literature review, there is no research-based data on *in silico* docking investigations on the leaves of *Q. indica*. As a result, the current research was carried out to evaluate the above-mentioned potential of leaves using WHO criteria and other official procedures. For the first time, a method for determining the quality of several metabolites in *Q. indica* has been devised that is relevant, responsive, and predictable. This research data may be used as a reference for new research, plant physiology, chemistry analysis, biochemistry, and the preparation of a plant monograph.

MATERIALS AND METHODS

Collection and authentication of leaf material

The leaves of *Q. indica* were collected from National Botanical Research Institute, Lucknow, India and authenticated by National Institute of Science Communication and Information Resources, Delhi, India (NISCAIR/RHMD/Consult/2015/2862/55-1).

Drugs and chemicals

Ethanol was purchased from Changshu Yangyuan Chemical, China, and the reagents had used in phytochemical screening, they were freshly prepared. All chemicals were analytical grade.

Extraction procedure

Leaves were dried on cool place and powdered with the help of electric grinder. soxhlation method was followed in which powder was defatted using 250 mL of petroleum ether for more than 6 hr, after that the powders were dried and extracted with different solvents. The obtained final fractions were concentrated under vacuum in a rotary evaporator at 40°C and stored at 4°C for further use.[17]

Phytochemical screening

Different metabolites like carbohydrates, alkaloids, flavonoids, tannins, proteins, terpenoids etc present in different leaf extracts of *Q. indica*.

In silico docking studies

Molecular docking of beta-sitosterol, coumaric acid, lupeol, quercetin and urosolic acid to muscarinic (M_2) receptors: To predict the contact of the drug molecule with receptor, the molecular docking simulation was carried out.[18] It involved the following steps.

Selection and preparation of protein

The RCSB protein data library was used to download a muscarinic (M_2) receptor coupled to ligand (PDB: 5ZHP).

Preparation of ligands

The ligand Tiotropium was prepared for molecular docking simulation by using the Auto Dock software to determine the number of rotatable, non-rotatable, and un rotatable bonds present in the ligand.

Grid box formation

The grid parameter points of the grid box required to perform the molecular docking simulation of ligand molecules were enumerated using the receptor binding site, which was found using the protein visualisation software PyMol. For all docking runs, these grid parameters were used. To ensure that all extended conformations of the ligand fit within the grid box, it was put in the middle of the ligand by covering all binding residues involved in the binding of the ligand.

Grid map preparation

The map files for different atom types in ligands and receptor viz. A, C, F, HD, N, OA, and SA were prepared by running Auto grid utility of the Auto dock suite.

Docking parameters

Lamarckian Genetic Algorithm (LGA) is the primary conformational search approach used in Auto Dock for molecular docking simulation. Docking parameter file for each ligand was prepared by using 150 Genetic Algorithm (GA) runs, 250000 maximum numbers of evaluations, 27000 maximum number of generations, and 0.02% rate of gene mutation.

Docking method validation

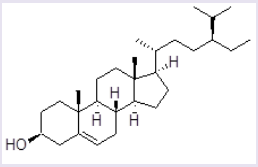
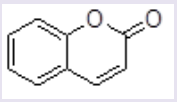
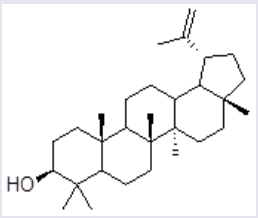
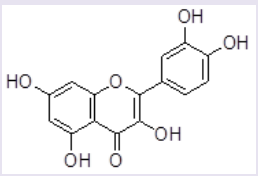
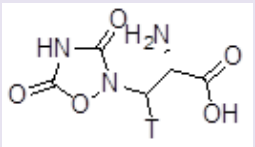
The position and orientation of the ligand obtained after the molecular docking study represent the potential binding modes of the inhibitors. The various docking parameters considered in the docking methods were validated by redocking individually crystallized ligands beta-sitosterol, coumaric acid, lupeol, qurecetin and urosolic acid over the muscarinic acetylcholine

Table 1: Phytochemical analysis of different extracts of *Q. indica*.

Phytoconstituents	AEQI	EEQI
Alkaloids	Present	Present
Glycosides	Present	Present
Tannins	Present	Present
Flavonoids	Present	Present
Fats and oil	Present	Present
Carbohydrates	Present	Present
Reducing sugar	Present	Present
Proteins	Present	Present
Saponin	Absent	Absent
Terpenoids	Present	Present

AEQI- Aqueous extract of *Q.indica*, EEQI- Ethanolic extract of *Q.indica*

Table 2: Docking results of certain drugs with M_2 Receptor (3UON).

Sl. No.	Name of drugs	Structure	Binding energy (Kcal/mol)
1	Beta-sitosterol		-7.96
2	Coumaric acid		-5.63
3	Lupeol		-6.7
4	Quercetin		-7.73
5	Urosolic acid		-5.92

receptor (M_2). The molecular docking simulation technique is validated by using different parameters.

RESULTS

Phytochemical testing of different extracts shows the presence of alkaloids, glycosides, sterols, carbohydrates, tannins, terpenoids, saponins and flavonoids as shown in Table 1 respectively. From Table 2, the maximum binding energy was obtained -7.96 Kcal/mol in case of beta-sitosterol.

An appropriate grid box was prepared by covering all residues involved in the active binding of the ligand 9EC to the M_2 muscarinic acetylcholine receptor. The coordinates used for the preparation of the grid box are tabulated in Table 3. Three-dimensional grid box covering and the active ligand binding sites present in the receptor molecule as well as all residues involved in the binding of the ligand show in Figure 1. Molecular Docking results of ligands with M_2 muscarinic acetylcholine receptor are shown in Table 4.

In case of validation, the following parameters were used to validate the molecular docking process for docking of muscarinic acetylcholine receptors with different legends.

Binding energy

Molecular docking should fall in the normal range of -5 to -15 kcal/mol. The molecular docking validated as the binding energy of the ligands beta-sitosterol, coumaric acid, lupeol, qurecetin and urosolic acid with the M_2 muscarinic acetylcholine receptor was found to be -7.96, -5.63, -6.7, -7.73 and-5.82 kcal/mol respectively, which lies in the predefined range of -5 to -15 kcal/mol.

Overlay methods

The overlaid conformation of the docked ligand with reference to the bioactive crystal structure of the ligand was obtained from RSCB protein data bank.

Chemical resemblance

The molecular docking method is validated when the docked ligand should have the same interactions with the residues of the macromolecule as those present in the downloaded crystallized macromolecule. The interactions present in the crystal structure and the docked structure are shown in Figure 2.

Table 3: **Coordinates of the grid box for the muscarinic acetylcholine receptor (M_2).**

Proteins	x-D	y-D	z-D	Spacing (Å)	x center	y center	z center
5ZHP	40	40	40	0.375	8.15	0.745	-3.75

Table 4: **Molecular Docking results of ligands with M_2 muscarinic acetylcholine receptor.**

Proteins	Interacting residues	Binding energy (kcal/mol)
5ZHP	Beta-sitosterol	-7.96
	Coumaric acid	-5.63
	Lupeol	-6.7
	Qurecetin	-7.73
	Ursolic acid	-5.82

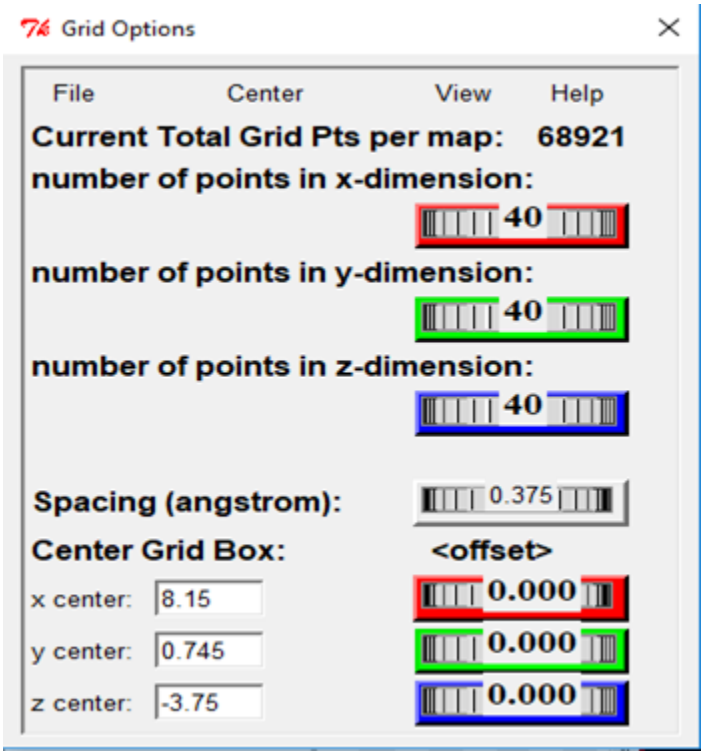


Figure 1: Three dimensional grid box covering the active ligand binding sites present in the receptor molecule as well as all residues involved in the binding of the ligand.

DISCUSSION

Cardiovascular diseases are the main and most important cause of death. According to Nature Reviews, Cardiology, Cardiovascular diseases are also the leading cause of death in mostly countries like China etc.[18] There are lots of types of diseases and they are all dangerous for heart in which coronary artery diseases, and also atherosclerotic heart disease, is the condition in which the accumulation of plague within the walls of coronary arteries. Coronary artery disease is the leading cause of death worldwide. While the symptoms and signs of coronary artery disease are

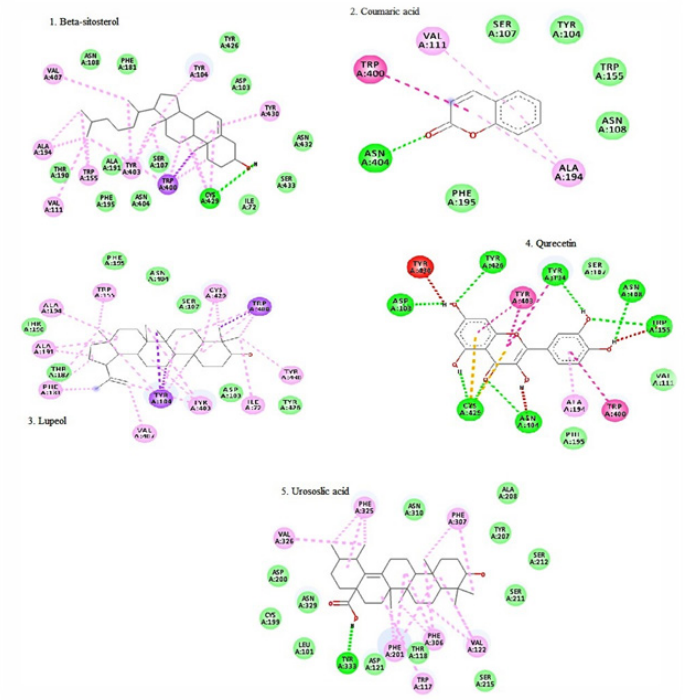


Figure 2: Binding mode and chemical interactions of the bound ligands (1) Beta-sitosterol, (2) Coumaric acid, (3) Lupeol, (4) Qurecetin, and (5) Ursolic acid within the active ligand binding site of M2 muscarinic acetylcholine receptor.

noted in the advanced state of disease, most individuals with coronary artery disease show no evidence of disease for decades as the disease progresses before the first onset of symptoms, often a "sudden" heart attack, finally arises.[19,20] Cholinergic receptors are classified into muscaranic and nicotinic, in which muscaranic is again classified into M_1, M_2, M_3, M_4, and M_5. The M_2 receptor has been selected for docking studies because the main location of M_2 is heart. Beta-sitosterol, coumaric acid, lupeol, qurecetin and urosolic acid have been selected for docking studies because they all have a protective mechanism of heart.[21] The 5ZHP protein complex of RCSB protein data bank consists of four identical chains of amino acids and bound ligand 9EC [(1R,2R,4S,5S,7s)-7-({[4-fluoro-2-(thiophen-2-yl) phenyl]carbamoyl}oxy)-9,9-dimethyl-3-oxa-9 azatricyclo[3.3.1.0~2,4~]nonan-9-ium]. After processing the receptor molecule it was saved in ".pdbqt" format by using AutoDock software. Beta-sitosterol is widespread used as a lipid lowering agent andimproves the condition of prostate gland. Beta-sitosterol may reduce the risk of Cardiovascular Disease (CVD) by lowering LDL cholesterol in the blood.[22] It is clearly proved that beta-sitosterol isis a good cardioprotective agent. Phytosterols like beta-sitosterol are homologues to cholesterol found in various fruits and vegetables and present in the daily

diet as similar to cholesterol (200-400 mg/kg).[23] Beta- sitosterol has a lipid lowering quality because of competitive inhibition of cholesterol absorption and a gene implicated in the metabolism of cholesterol.[24] Coumaric acid is a well-known phenolic compound that is also present in various fruits and vegetables and reported to have anti-inflammatory activity. p-coumaric acid can convert to phenolic acids such as chlorogenic acid, rosmarinic acid, flavonoids, and other secondary metabolites and possesses various effects including antioxidant, anti-angiogenic, anti-UV damage, and antiplatelet properties.[25,26]

Lupeol has also reported various pharmacological properties like antioxidant, anti-inflammatory, anti-hyperglycemic, anti-dyslipidemic, and anti-mutagenic effects.[27] Quercetin exhibits significant heart-related benefits such as inhibition of LDL oxidation, endothelium-independent vasodilator effects etc. Urosolic acid also has the good score and it exerts these effects in various tissues and organs. Mechanisms include suppressing nuclear factor-kappa B signaling in cancer cells, improving insulin signaling in adipose tissue, reducing the expression of markers of cardiac damage in the heart.[28]

CONCLUSION

Looking to future public health approaches, all these compounds are targeted for cardiovascular disease prevention. This paper has clearly emphasized the *Q. indica* has good cardioprotective potential, but detailed research is needed for prospects.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ABBREVIATIONS

***Q. indica*:** *Quisqualis indica*; **M_2:** Muscaranic receptor; **AEQI:** Aqueous extract of *Q. indica*; **EEQI:** Ethanolic extract of *Q. indica*.

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