In vivo and *In silico* Analysis of Antihypertensive Activities of *Ficus religiosa* Fruit Extract

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(Received: August 14, 2023; Accepted: May 2, 2024; Published (web): June 13, 2024)

ABSTRACT: Hypertension is one of the major cardiovascular diseases leading to serious health consequences including cardiovascular events such as stroke and even death. These may also include significant damages to the body's most organs like heart, kidneys and brain. This deadly disease requires proper treatment drugs with lower cost and fewer adverse effects. In this study, *Ficus religiosa* fruit has been selected as a candidate plant and its fruit extract was used to perform different pharmacological tests in hypertensive rat model. It was observed that the extract decreased the heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) moderately in comparison to deoxycorticosterone acetate (DOCA) in ethanol-induced hypertensive rats. These effects were similar to that of atenolol, a standard antihypertensive drug. While testing liver function, it was seen that SGOT and SGPT levels were reduced significantly. The creatinine value was decreased to 0.43±0.2 U/L from 2.9±1.3 U/L (induced by DOCA) and 2.6±1.6 U/L (induced by ethanol). While observing cardiovascular parameters, the fruit extract given at dose of 400 mg/kg, lowered total Cholesterol (TC) to 97 mg/dl from 176 mg/dl, low-density lipoprotein (LDL) to 40 mg/dl from 75 mg/dl and Triglycerides (TG) to 61 mg/dl from 118 mg/dl. The increased values were induced by DOCA. In the same test, the high-density lipoprotein (HDL) was increased to 80 mg/dl from 44 mg/dl. These changes were comparable to those of the standard drug Atenolol. *F. religiosa* fruit contains different chemical constituents such as isofucosterol, leucopelargonidin, quercetin and beta sitosterol as reported earlier. Molecular docking studies with some of these constituents showed good binding affinity with the targeted protein (receptor) as compared to the standard drugs. Additionally, all the compounds have satisfied Lipinski's rules and other pharmacokinetic parameters. Moreover, they showed no adverse effects in aquatic and non-aquatic environments. We, thus, conclude that *F. religiosa* fruit extract could be taken for the discovery of a safe alternative for managing hypertension.

Key words: *Ficus religiosa*, Hypertension, Heart rate, Creatinine, Molecular docking, Lipinski's rule.

INTRODUCTION

Hypertension is the most frequent modifiable risk factor for cardiovascular diseases (CVD) and mortality.[1] The primary organs damaged by hypertension are the heart, brain, kidney and arteries. Uncontrolled hypertension hastens the degeneration of these organs, which ultimately leads to organ failure, functional impairment and mortality.[2] Untreated hypertension is a significant contributor to hemorrhagic stroke.[3] Sometimes, it is called a "silent killer" because there are almost no clear indicators seen before any medical emergency like heart attack, stroke or chronic renal disease.[4-6]

The elevated risk associated with high blood pressure (BP) may considerably be decreased by therapy with antihypertensive medications that

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Dhaka Univ. J. Pharm. Sci. **23**(1): 23-36, 2024 (June)
DOI: https://doi.org/10.3329/dujps.v23i1.74088

reduce not only BP but also related target organ damage. It is becoming more common, particularly, in low- and middle-income countries (LMICs).[7] According to estimates, 31.1 percent of people (1.39 billion) globally had hypertension in 2010. Adult hypertension prevalence was greater in LMICs (31.5 percent, 1.04 billion persons) than in high-income countries (28.5 percent, 349 million people).[8] Variations in the levels of hypertension risk factors such as excessive salt intake, low potassium intake, obesity, alcohol use, physical inactivity and poor nutrition may explain part of this variation in hypertension prevalence.[9] Although different FDA-approved antihypertensive drugs are available[10] such as atenolol, aliskiren, lisinopril and carazolol; yet many people die due to hypertension. These deaths are attributed to unwanted side effects of these drugs.

The preparation of *Ficus religiosa* bark has been reported to exhibit parasympathetic nervous system activation.[11] The tone and amplitude shrinkage in the rat and guinea pig ileums, as well as the rabbit uterus, were all reduced by the alcoholic extract of bark.[11] The acetylcholinesterase-inhibiting activity of the alcoholic bark extract demonstrated parasympathetic and antihypertensive properties.[12] This work investigated the therapeutic efficacy of the fruit extract of *F. religiosa* in rat model through testing levels of blood pressure, creatinine, cholesterol, etc.; as well as computational techniques such as molecular docking, ADMET properties and Lipinski's rules.

MATERIALS AND METHODS

Fruit collection and extract preparation. The fruits of *F. religiosa* were collected from the local market and then the specimen was certified by the experts of Bangladesh National herbarium at Mirpur and provided the accession number 66768 for the future references. The *F. religiosa* fruits was carefully cleaned and then powdered coarsely after shade drying. After that, the extraction of powdered fruits was done by using 70% ethanol for few days. The extract was filtered after every 3 days. The combined ethanol extract was evaporated under rotary to get the crude mass for subsequent use.

Experimental animal procurement, nursing and grouping. 105 healthy Wistar rats of either sex weighing between 150-200 gm were purchased from Department of Pharmacy, Jahangir Nagar University, Savar, Dhaka and each of them was nourished carefully by keeping them in the Institute of Food and Nutrition Science of the University of Dhaka in a well-controlled environment (relative humidity 55 ± 5%, 12 ± 1h light/dark cycle and temperature 25 ± 3°C) for 2 weeks. After that, considering equal body mass index, 21 groups were created where each group was constituted with 5 rats (n = 5) as shown in Table 1. All rats were provided with a standard food supplement and filtered water. The experimental procedures were carried out according to the Institutional Animals Ethics Committee (IAEC).

Deoxycorticosterone acetate (DOCA)-induced hypertension. Experimental hypertension in rats were induced by uninephrectomy and administration of deoxycorticosterone acetate (DOCA). According to Tiritilli and Ruff, after undergoing a unilateral nephrectomy; intraperitoneal administration of DOCA 300 mg/kg body weight were given for five days in a week for a total of six weeks. At the end of the sixth week following surgery, hemodynamic measurements were carried out and the animals were then euthanized to evaluate the safety characteristics.[13]

Ethanol-induced hypertension. Chan and Sutter described the ethanol feeding protocol in which rats were given 5% ethanol (v/v) in the drinking water during the first week, 10% over the following two weeks, and 20% throughout weeks 3 to 6. Average daily ethanol intake was between 10 and 11 g/kg.[14]

Different pharmacological tests were performed before and after administration of specified amount of either DOCA, Ethanol, standard drug atenolol or extract of *F. religiosa* at different doses according to the protocol shown in table 1.

Table 1. Protocol of evaluation of antihypertensive activity of fruit extract of *Ficus religiosa*.

Group number	Group status	Treatment specimen	Volume of treatment specimen (mg/kg)	Group abbreviation
1	Negative control	Physiological saline	10 ml/kg	C
2	Disease control	Deoxycorticosterone acetate (DOCA)	300 mg/kg	D
3	Disease control	Ethanol (Et)	11 g/kg [5-20% (v/v)]	Et
4	DOCA + Atenolol	Atenolol (At)	300 mg/kg+0.8 mg/kg	D+At0.8
5	DOCA + Atenolol	Atenolol (At)	300 mg/kg+1.6 mg/kg	D+At1.6
6	DOCA + Atenolol	Atenolol (At)	300 mg/kg+2.4 mg/kg	D+At2.4
7	Et + Atenolol	Atenolol (At)	11 g/kg+0.8 mg/kg	Et+At0.8
8	Et + Atenolol	Atenolol (At)	11 g/kg+1.6 mg/kg	Et+At1.6
9	Et + Atenolol	Atenolol (At)	11 g/kg+2.4 mg/kg	Et+At2.4
10	DOCA + *Ficus religiosa*	*Ficus religiosa*	300 mg/kg+100 mg/kg	D+Fr100
11	DOCA + *Ficus religiosa*	*Ficus religiosa*	300 mg/kg+200 mg/kg	D+Fr200
12	DOCA + *Ficus religiosa*	*Ficus religiosa*	300 mg/kg+400 mg/kg	D+Fr400
13	Et + *Ficus religiosa*	*Ficus religiosa*	11 g/kg+100 mg/kg	Et+Fr100
14	Et + *Ficus religiosa*	*Ficus religiosa*	11 g/kg+200 mg/kg	Et+Fr200
15	Et + *Ficus religiosa*	*Ficus religiosa*	11 g/kg+400 mg/kg	Et+Fr400
16	Atenolol	Atenolol (At)	0.8 mg/kg	At0.8
17	Atenolol	Atenolol (At)	1.6 mg/kg	At1.6
18	Atenolol	Atenolol (At)	2.4 mg/kg	At2.4
19	*Ficus religiosa*	*Ficus religiosa*	100 mg/kg	Fr100
20	*Ficus religiosa*	*Ficus religiosa*	200 mg/kg	Fr200
21	*Ficus religiosa*	*Ficus religiosa*	400 mg/kg	Fr400

Optimization and ligand preparation. All the chemical structures were drawn by ChemDraw software 2012.[25] After that, the structures were optimized with the help of the avogadro Application for correcting the geometric configuration.[16] Finally, all the chemical structures were saved in protein data bank (PDB) file format for molecular docking analysis.

Protein preparation and molecular docking study. From the Research Collaboratory for Structural Bioinformatics (RSCB) Protein Data Bank, the four targeted proteins' crystal structures (ACE inhibitor PDB ID 2X97, ACE inhibitor PDB ID 2X8Y, Renin inhibitor PDB ID 2IKU, Beta 2 adrenoceptor PDB ID 5X7D) were downloaded as PDB file type. To obtain the protein, these were then evaluated and cleaned off all the access molecules such as water, ligand by Pymol version 2.

After that, the docking analysis was done for the drug-protein interaction by using the AutoDock Vina software package for the docking analysis. PyRx and AutoDock Vina Wizard were used to conduct docking experiments using the adjustable ligand and receptor grid box, respectively. At last, Discovery Studio was used to create the visual representation of the 2D interaction and ligand protein active amino acid residue.

Lipinski's rule, pharmacokinetics and drug likeness. The Lipinski's rule was first described by Christopher A Lipinski in 1997.[17] The pharmacokinetic features of pharmaceuticals are characterized by this rule which focuses on the importance of molecular characteristics. The Swiss ADME online free access database has been used to assess this Drug's likeness features. Molecular mass and the number of hydrogen bond acceptors, hydrogen bond donors, rotatable bonds, along with molar refractivity, bioavailability score, Lipinski's rule of five, topological polar surface area (TPSA) and solubility were estimated using this method.[18]

ADMET properties. The evaluation of pharmacokinetic qualities is vital for developing any new drug.[19] The PkCSM program package (http://biosig.unimelb.edu.au/pkcsm/) is an online application which helps in assessment putting physicochemical descriptors, predicting ADME parameters, pharmacokinetic properties, toxicity features of any chemical substances.[20] In this investigation, the water solubility Log S, Caco-2 permeability, P-glycoprotein substrate, permeability glycoprotein 1 (P-1) inhibitor, volume of distribution at steady-state (VDss) (human), CYP450 1A2 inhibitor, CYP450 2C9 substrate, total clearance (mL/min/kg), substrate have been listed. Besides, toxicity has also been analyzed in this study. In this case, the maximum tolerated dose (human) mg/kg/day, oral rat acute toxicity (LD50), oral rat chronic toxicity (mg/kg/day), hepatotoxicity, skin sensitization have been mentioned.

RESULTS AND DISCUSSION

Rats given *F. religiosa* 400 mg/kg, showed reduction in heart rate to 386 ± 4.7 beats/min from 454 ± 4.6 beats/min induced by ethanol (Figure 1a-c). It was observed that the extract of *F. religiosa* decreased the HR, SBP and DBP moderately in comparison to DOCA and ethanol induced hypertensive rats. These effects were comparable to that offered by atenolol.

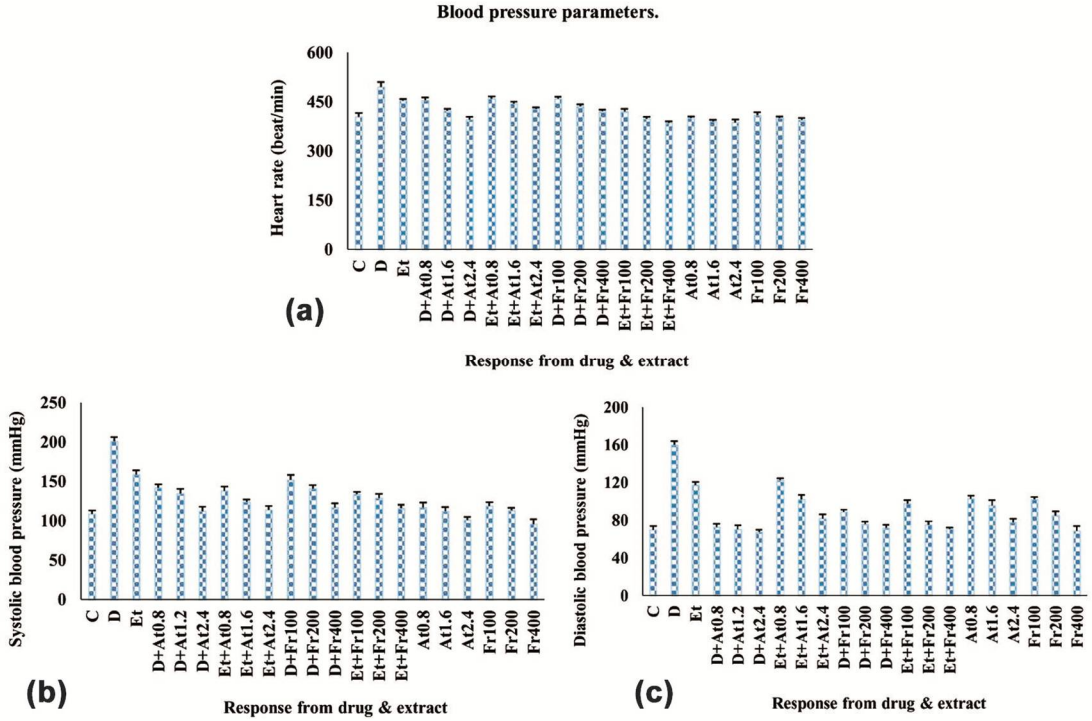

Figure 1. (**a**) Heart rate, (**b**) Systolic blood pressure and (**c**) Diastolic blood pressure in different rat groups.

It was seen that SGOT and SGPT levels were reduced significantly in comparison to DOCA and ethanol induced hypertensive rats as shown in figure 2a-b.

The creatinine value was decreased to 0.43 ± 0.2 U/L by *F. religiosa* from 2.9 ± 1.3 U/L induced by DOCA and 2.6 ± 1.6 U/L induced by ethanol. This result was more promising than standard drug atenolol.

The cardiovascular parameters also showed a similar pattern of decrease after administration of *F. religiosa* extract. TC was lowered to 97 mg/dl from 176 mg/dl by DOCA, HDL was increased to 80 mg/dl from 44 mg/dl by DOCA, LDL was decreased

to 40 mg/dl from 75 mg/dl by DOCA and TG was lowered to 61 mg/dl from 118 mg/dl by DOCA. These changes were near to changes caused by the standard drug atenolol.

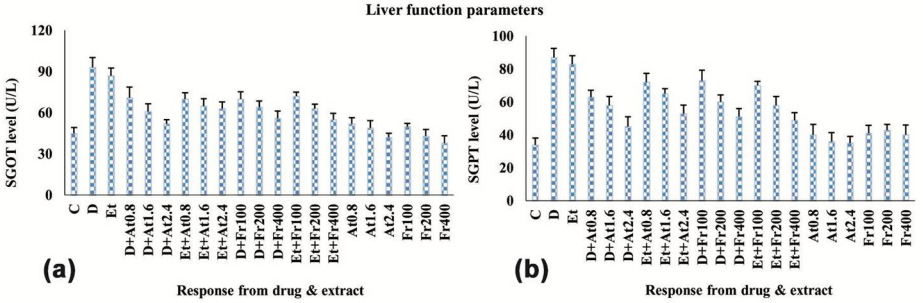

Figure 2. (**a**) SGOT and (**b**) SGPT levels in different rat groups.

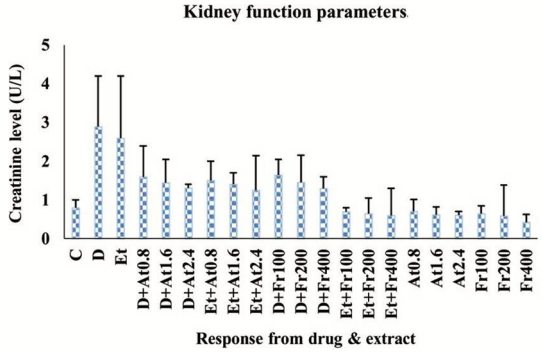

Figure 3. Creatinine levels in different groups of rats.

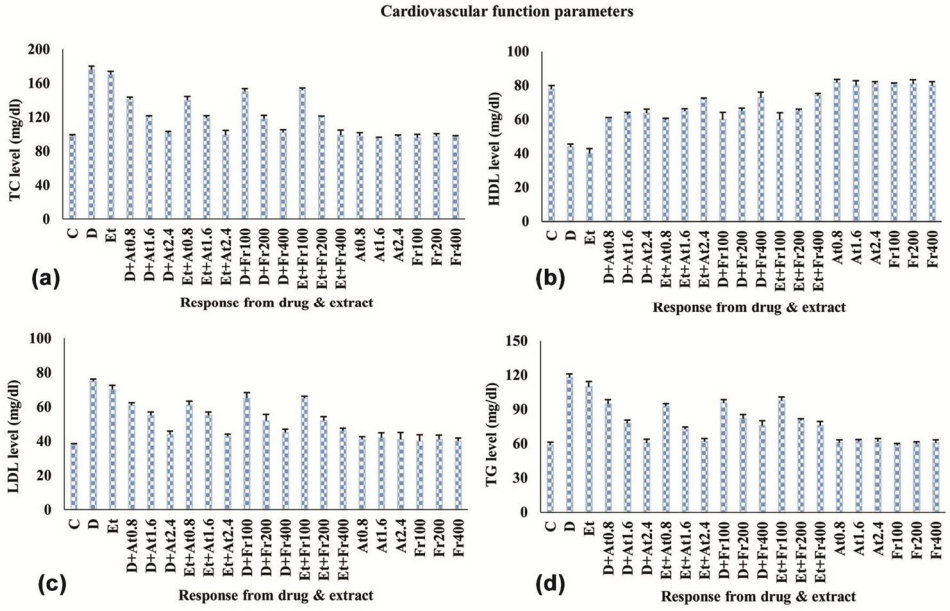

Figure 4. Cardiovascular system parameter (**a**) TC, (**b**) HDL, (**c**) LDL and (**d**) TG levels in different rat groups.

Chemical structure of tested ligand present in *F. religiosa* fruit.[21] The fruit portion of *F. religiosa* contains many chemical compounds (Figure 5). The chemical structures of those compounds have

been drawn by the ChemDraw application. The three standard FDA approved antihypertensive drugs are also displayed including lisinopril (PubChem CID 5362119), aliskiren (PubChem CID 5493444) and carazolol (PubChem CID 71739). Literature study shows that one of the important usages of *F. religiosa* is its use in the treatment of hypertension.[22] The *F. religiosa* contains a number of chemical compounds in their roots, seeds and bark. In this investigation, we have used the fruits of *F. religiosa*. The most abundant chemical found in fruits of *F. religiosa* was reported as kaempferol, quercetin and myricetin.[11] Besides, the chemical analysis revealed that *F. religiosa* flavonoids, with quercetin and myricetin being the most prevalent.[23]

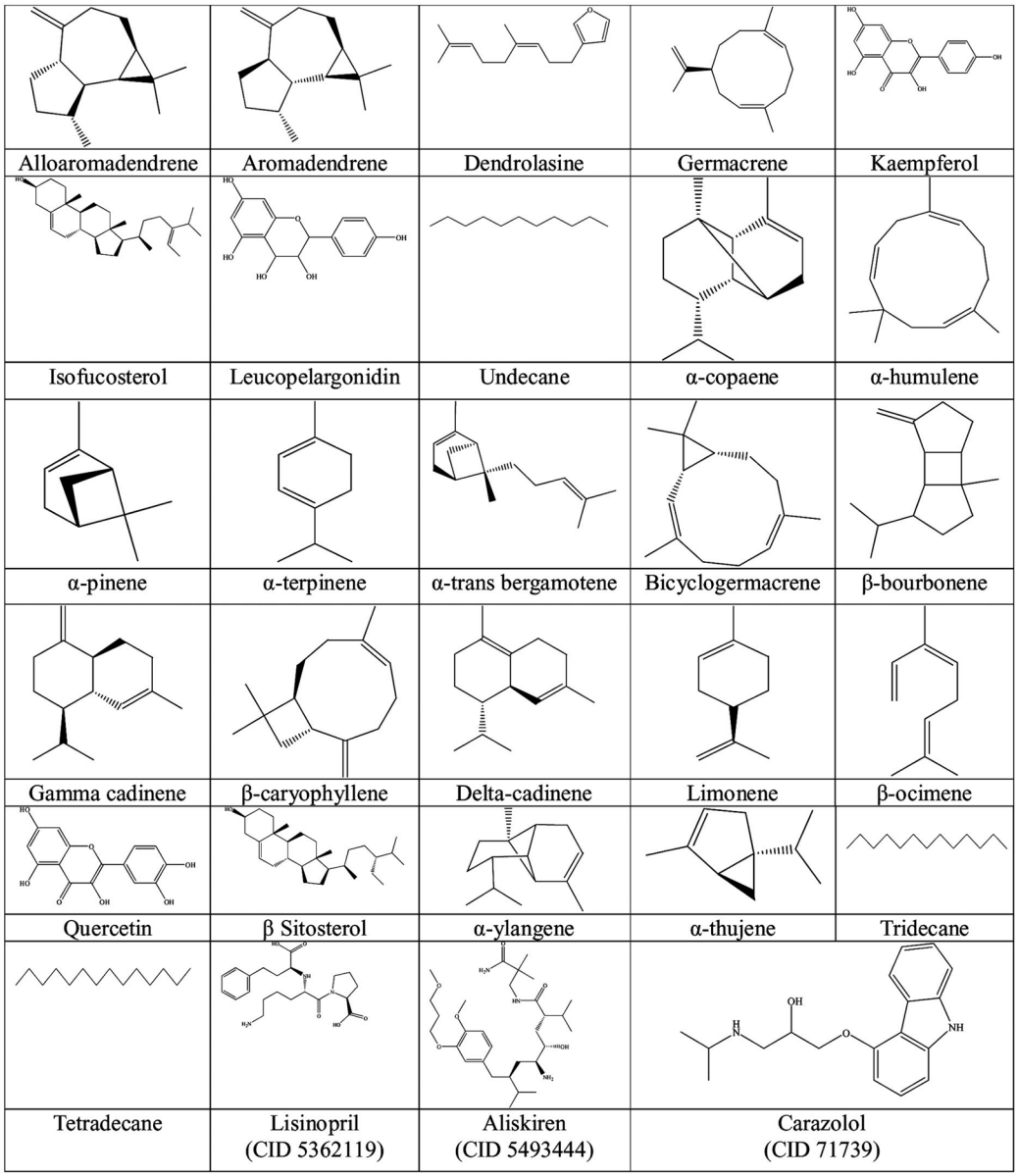

Figure 5. Chemical structures of phytoconstituents found in fruits of *F. religiosa*.

Lipinski's rule, pharmacokinetics and drug likeness. To analyze the Lipinski's rule, pharmacokinetics and drug likeness, several parameters have been measured (Table 2). Among

them, some notable parameters are - the number of rotating bonds (NRB), the number of hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD), octanol/water coefficient ($LogP_{o/w}$), gastrointestinal absorption parameter (GIA) and topological polar surface area (TPSA) and the molecular weights (MW). These are listed to describe drug substance physicochemical properties intended for oral use. The mentioned data showed that the number of rotating bonds were 0 to 11, the number of hydrogen acceptors were 0 to 7 and the hydrogen donor atoms were from 0 to 5. In most cases, the $LogP_{o/w}$ scores were below 5, with some compounds above 7.

Table 2. Data of Lipinski's rule, pharmacokinetics and drug likeness.

Name of active principles	NRB	HBA	HBD	TPSA, Å²	Consensus $LogP_{o/w}$	Log Kp (skin permeation), cm/s	Lipinski's rule		M.W.	G.I. absorption
							Result	Violation		
Alloaromadendrene	00	00	00	0.00	4.34	-4.20	Yes	01	204.35	Low
Aromadendrene	00	00	00	0.00	4.34	-4.20	Yes	01	204.35	Low
Dendrolasine	06	01	00	13.14	4.32	-4.03	Yes	00	218.33	High
Germacrene	01	00	00	0.00	4.57	-3.38	Yes	01	204.35	Low
Isofucosterol	05	01	01	20.23	7.08	-2.53	Yes	01	412.69	Low
Kaempferol	01	06	04	111.13	1.58	-6.70	Yes	00	286.24	High
Leucopelargonidin	01	06	05	110.38	0.61	-7.60	Yes	00	290.27	High
Undecane	08	00	00	0.00	4.56	-3.31	Yes	01	156.31	Low
α-copaene	01	00	00	0.00	4.30	-4.37	Yes	01	204.35	Low
α-humulene	00	00	00	0.00	3.74	-4.29	Yes	00	176.30	Low
α-pinene	00	00	00	0.00	3.44	-3.95	Yes	01	136.23	Low
α-terpinene	01	00	00	0.00	3.30	-4.11	Yes	00	136.23	Low
α-trans-bergamotene	03	00	00	0.00	4.02	-2.97	Yes	01	204.35	Low
Bicyclogermacrene	00	00	00	0.00	4.14	-4.61	Yes	01	204.35	Low
β-bourbonene	01	00	00	0.00	4.40	-4.20	Yes	01	204.35	Low
Gamma cadinene	01	00	00	0.00	4.18	-4.49	Yes	01	204.35	Low
β-caryophyllene	00	00	00	0.00	4.24	-4.44	Yes	01	204.35	Low
Delta-cadinene	01	00	00	0.00	4.14	-4.85	Yes	01	204.35	Low
Limonene	01	00	00	0.00	3.35	-3.89	Yes	00	136.23	Low
β-ocimene	03	00	00	0.00	3.42	-4.11	Yes	00	136.23	Low
Quercetin	01	07	05	131.36	1.23	-7.05	Yes	00	302.24	High
β sitosterol	06	01	01	20.23	7.19	-2.20	Yes	01	414.71	Low
Tetradecane	11	00	00	0.00	5.68	-2.40	Yes	01	198.39	Low
α-thujene	01	00	00	0.00	3.15	-5.11	Yes	01	136.23	Low
Tridecane	10	00	00	0.00	5.31	-2.70	Yes	01	184.36	Low
α-ylangene	01	00	00	0.00	4.30	-4.37	Yes	01	204.35	Low
Aliskiren	20	07	04	146.13	3.58	-7.15	Yes	01	551.76	Low
Carazolol	06	03	03	57.28	2.90	-5.57	Yes	00	298.38	High
Lisinopril	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

NBR=Number of rotating bonds, HBA= Hydrogen bond acceptor, HBD=Hydrogen donor atoms, $LogP_{o/w}$=Octanol/water coefficient, GIA=Gastrointestinal absorption parameter, TPSA=Topological polar surface area (TPSA) and MW=Molecular weights (MW).

Another critical parameter is GI absorption. Among the reported compounds, it is clear that most of the ligands have shown lower GI absorption and their molecular weight level ranged from 136.23 to

414.71. In the overall investigation, it has been seen that all the compounds had satisfied the Lipinski's rule. So, it can be said that they are eligible for oral medications.

Molecular docking. Molecular docking studies have been performed to assess the binding affinity with protein as shown in table 3.[24] It is considered that the docking score higher than -6.0kcal/mol is active in the physiological system and can bind with the target receptor.[25] In this research, four targeted proteins (ACE inhibitor PDB ID 2X97, ACE inhibitor PDB ID 2X8Y, renin inhibitor PDB ID 2IKU and beta 2 adrenoceptor PDB ID 5X7D) have been taken and analyzed for their binding affinity with the selected ligands. At the same time, three FDA approved antihypertensive drugs (lisinopril, aliskiren and carazolol) are also studied to compare with newly screened ligands from the *F. religiosa* fruit fraction. The studies revealed that the

Table 3. Binding affinity of selected drugs with targeted proteins.

Drug molecules name	ACE inhibitor PDB ID 2X97	ACE inhibitor PDB ID 2X8Y	Renin inhibitor PDB ID 2IKU	Beta 2 adrenoceptor PDB ID 5X7D
	Binding affinity (kcal/mol)	Binding affinity (kcal/mol)	Binding affinity (kcal/mol)	Binding affinity (kcal/mol)
Alloaromadendrene	-7.1	-7.2	-7.6	-8.3
Aromadendrene	-7.0	-6.9	-7.7	-8.0
Dendrolasine	-6.0	-6.0	-7.0	-7.5
Germacrene	-7.2	-7.4	-7.5	-8.0
Isofucosterol	-9.5	-10.0	-9.5	-9.6
Kaempferol	-8.7	-8.2	-7.1	-8.1
Leucopelargonidin	-8.1	-8.6	-7.5	-8.7
Undecane	-5.1	-4.9	-5.4	--5.8
α-copaene	-7.2	-7.4	-7.2	-7.8
α-humulene	-6.4	-6.2	-7.1	-7.6
α-pinene	-5.5	-5.3	-5.8	-6.5
α-terpinene	-5.4	-5.6	-6.1	-7.0
α-trans bergamotene	-6.6	-6.8	-6.7	-8.2
Bicyclogermacrene	-7.2	-6.6	-7.5	-7.9
β-bourbonene	-6.7	-6.7	-8.0	-7.6
Gamma cadinene	-7.0	-7.0	-7.6	-7.7
β-caryophyllene	-7.3	-7.2	-7.5	-8.0
Delta-cadinene	-6.8	-6.9	-7.6	-7.7
Limonene	-5.7	-5.4	-6.2	-6.6
β-ocimene	-5.1	-5.2	-5.7	-6.6
Quercetin	-9.0	-8.4	-8.3	-8.3
β sitosterol	-8.1	-8.3	-9.0	-10.0
Tetradecane	-4.4	-5.0	-5.4	-5.7
α-thujene	-5.3	-5.6	-5.8	-6.7
Tridecane	-5.4	-5.1	-5.6	-5.6
α-ylangene	-7.2	-7.2	-7.1	-8.1
Lisinopril	-7.5	-7.8	N/A	N/A
Aliskiren	N/A	N/A	-7.5	N/A
Carazolol	N/A	N/A	N/A	-8.2

binding energy of ligands α-pinene, α-terpinene, limonene, β-ocimene, tetradecane, α-thujene and Tridecane are much lower than standards in every case. But the remaining ligands crossed the standard binding energy against four targeted proteins. In ACE inhibitor (PDB ID 2X97), the maximum binding energy has been obtained at -9.5 kcal/mol in Isofucosterol, and ACE inhibitor (PDB ID 2X8Y) provided the maximum binding energy of -10.0kcal/mol in identical ligand which is higher than the standard FDA approved lisinopril. After that, two more targeted proteins were taken and performed docking analysis. In renin inhibitor (PDB ID 2IKU), the maximum docking score has been reported as -9.5 kcal/mol in lsofucosterol, where the standard aliskiren shown the value to be -7.5 kcal/mol. The last one, beta 2 adrenoceptor (PDB ID 5X7D) showed maximum docking score of -10.5 kcal/mol in β-sitosterol, which is also more significant than the standard drug carazolol's docking score. Finally, it could be concluded that the reported ligands could prove to be a promising lead for the treatment of hypertension.

Ligand-protein interaction and molecular docking poses. The protein-ligand interaction has been graphically designed based on higher binding affinity. The Pymol application and Biovia Discovery Studio have created this ligand-protein interaction and molecular docking poses. Ligand-protein interaction and molecular docking poses are mainly performed to represent how a drug molecule binds with targeted protein and how many active amino acid residues are present during molecular docking investigation. The figures (from figure 6 to figure 11) below display the ligand-protein interaction and molecular docking pose in the binding pockets of the target molecules.

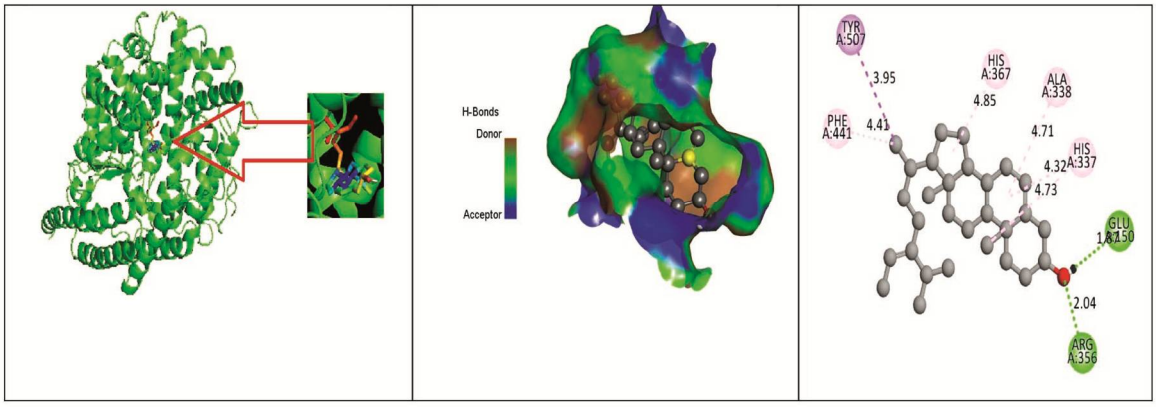

Figure 6. Molecular docking poses of lsofucosterol with ACE inhibitor (PDB ID 2X97).

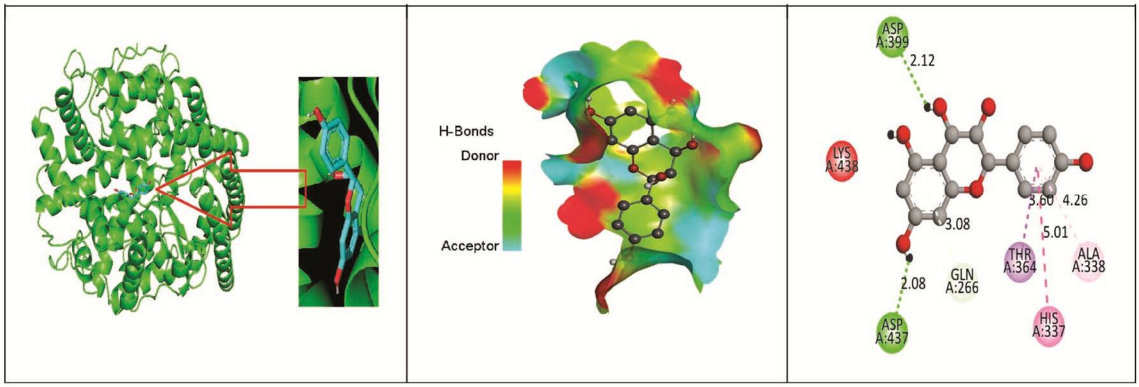

Figure 7. Molecular docking poses of leucopelargonidin with ACE inhibitor (PDB ID 2X8Y).

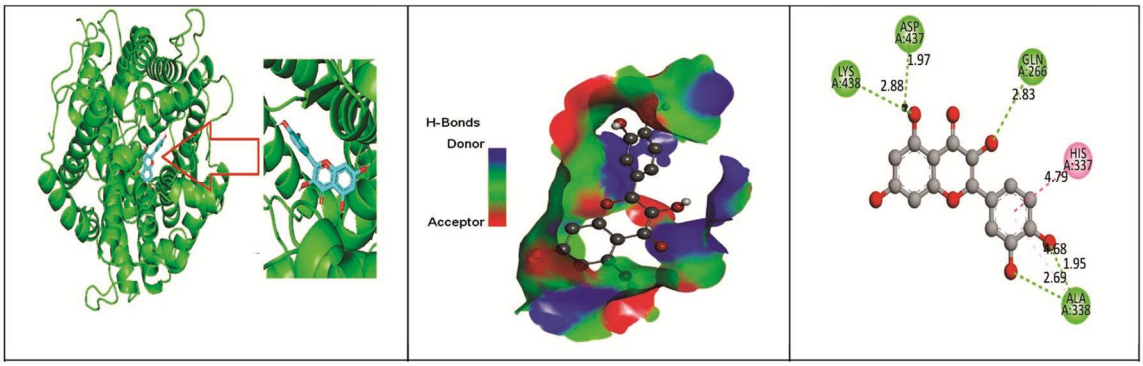

Figure 8. Molecular docking poses of quercetin with ACE inhibitor (PDB ID 2X97).

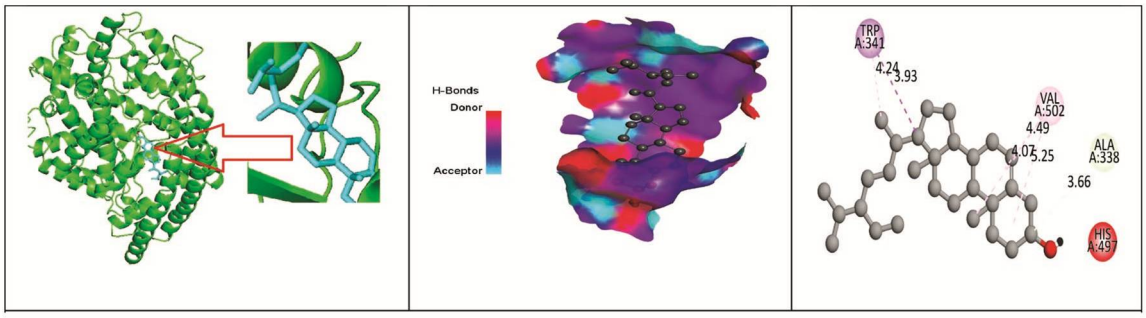

Figure 9. Molecular docking poses of beta sitosterol with ACE inhibitor (PDB ID 2X8Y).

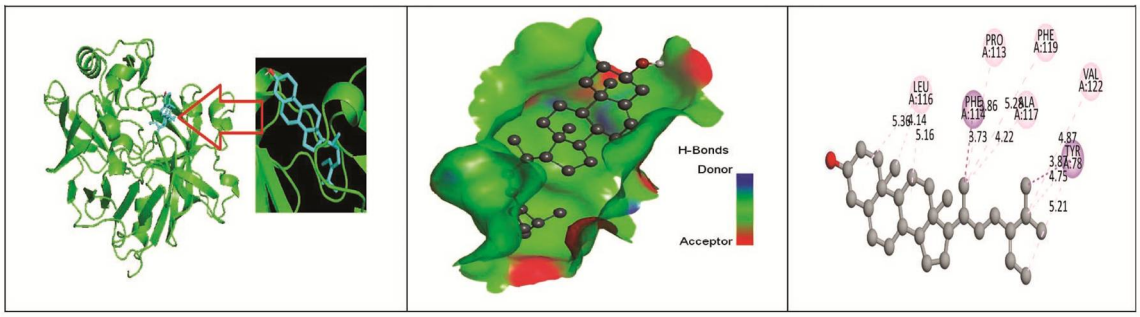

Figure 10. Molecular docking poses of Isofucosterol with Renin inhibitor (PDB ID2IKU).

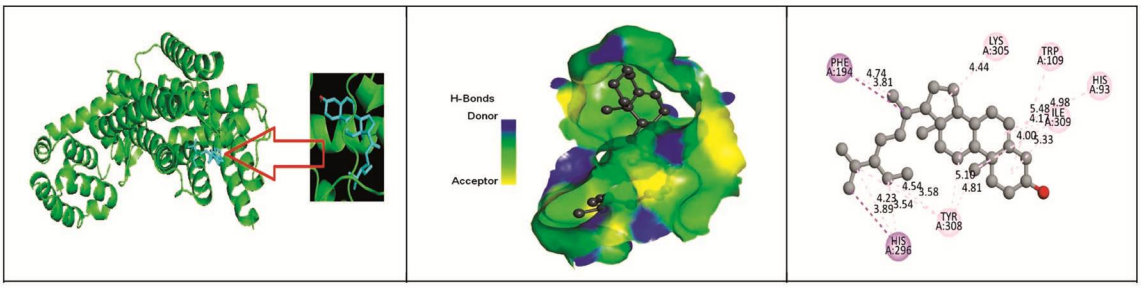

Figure 11. Molecular docking poses of beta sitosterol with beta 2 adrenoceptor (PDB ID 5X7D).

ADMET studies. The absorption, distribution, metabolism, excretion and toxicity (ADMET) prediction studies further help to understand the strategies that determine how drugs molecules remain in the physiological system from their administration until their excretion.[26] Forecasting ADMET characteristic is significant in pharmacology, toxicology and pharmacokinetics particularly for the

potential discovery of new drug candidates.[27] The parameters of pharmacokinetic (PK) investigations of the compounds' were done for ADMET prooerties using the PkCSM online tools and the results have been shown in table 4. The absorption of drugs depends on different factors including membrane permeability [indicated by colon cancer cell line (Caco-2)], P-glycoprotein substrate and P-1 glycoprotein inhibitor. It has been noticed that the range of Caco-2 permeability is better than the standard FDA approved drugs. Only β-sitosterol can actively inhibit the P-1 glycoprotein inhibitor, whereas the kaempferol, leucopelargonidin, α-humulene, limonene and quercetin can be actively substrated by the P-glycoprotein substrate. The ranges of water solubility (Log S) for slightly soluble drugs is considered to be -4 to -6 and for high solubility compounds to be -2 to -4.[28] It is seen that most of the mentioned compounds showed lower

Table 4. ADME properties of ligands present in fruits of *Ficus religiosa*.

Sl.No.	Absorption				Distribution	Metabolism		Excretion
	Water solubility, Log S	Caco-2 permeability	P-glycoprotein substrate	P-1 glycoprotein inhibitor	VDss (human)	CYP450 1A2 inhibitor	CYP450 2C9 substrate	Total clearance (ml/min/kg)
Alloaromadendrene	-5.764	1.395	No	No	0.753	No	No	0.926
Aromadendrene	-5.764	1.395	No	No	0.753	No	No	0.926
Dendrolasine	-5.732	1.56	No	No	0.607	Yes	No	1.549
Germacrene	-5.682	1.436	No	No	0.544	No	No	1.42
Isofucosterol	-6.888	1.202	No	No	0.053	No	No	0.54
Kaempferol	-3.25	1.031	Yes	No	0.007	Yes	No	0.655
Leucopelargonidin	-3.33	-0.459	Yes	No	0.244	No	No	0.222
Undecane	-6.156	1.379	No	No	0.537	No	No	1.654
α-copaene	-5.698	1.399	No	No	0.843	Yes	No	0.95
α-humulene	-4.324	1.408	Yes	No	0.441	No	No	0.197
α-pinene	-3.733	1.38	No	No	0.667	No	No	0.043
α-terpinene	-3.911	1.41	No	No	0.397	No	No	0.223
α-trans bergamotene	-5.934	1.401	No	No	0.848	No	No	1.176
Bicyclogermacrene	-5.352	1.414	No	No	0.653	Yes	No	1.09
β-bourbonene	-6.019	1.40	No	No	0.597	Yes	No	0.967
Gamma cadinene	-6.228	1.427	No	No	0.67	No	No	1.188
β-caryophyllene	-0.471	1.366	No	No	0.106	Yes	No	0.711
Delta-cadinene	-6.025	1.414	No	No	0.698	No	No	1.182
Limonene	-3.568	1.403	Yes	No	0.396	No	No	0.213
β-ocimene	-4.481	1.401	No	No	0.339	No	No	0.441
Quercetin	-3.372	0.587	Yes	No	0.239	Yes	No	0.578
β Sitosterol	-6.773	1.201	No	Yes	0.193	No	No	0.628
Tetradecane	-7.528	1.376	No	No	0.646	No	No	1.774
α-thujene	-4.294	1.386	No	No	0.575	No	No	0.077
Tridecane	-7.131	1.377	No	No	0.618	No	No	1.734
α-ylangene	-5.698	1.399	No	No	0.843	Yes	No	0.95
Lisinopril	-2.743	0.107	No	No	-1.224	No	No	0.476
Aliskiren	-4.342	0.12	Yes	Yes	-0.333	No	No	0.785
Carazolol	-3.546	1.159	Yes	No	1.452	Yes	No	0.948

water solubility ranging -4 to -6. The VDss (human) range was 0.007 to 0.848, where the higher distribution value was 0.848 and the lower distribution value was 0.007 in the ligand kaempferol. Few drugs can inhibit the CYP450 1A2 inhibitor enzyme in the metabolism portion, the total clearance (mL/min/kg) rate was excellent 1.774 ml/min/kg in tetradecane. This indicated them to be an interesting molecule suitable for further research activities.

Aquatic and non-aquatic toxicity. During the manufacturing process, drug molecules may mix into the aquatic and non-aquatic environments and produce significant impacts such as destroying the ecosystem. So, aquatic and non-aquatic toxicity has been studied to ensure drug molecules' environmental and non-environmental impact. So, total of seven aquatic and non-aquatic toxicity parameters including AMES toxicity, maximum tolerated dose (human), oral rat acute toxicity (LD50), oral rat chronic toxicity, hepatotoxicity and skin sensitization have been measured and the obtained results have been shown in table 5. In AMES toxicity, it has been

Table 5. Aquatic and non-aquatic toxicity of compounds present in fruits of *Ficus religiosa*.

Sl.No.	AMES toxicity	Max. tolerated dose (human) mg/kg/day	Oral rat acute toxicity (LD50) (mol/kg)	Oral rat chronic toxicity (mg/kg/day)	Hepatotoxicity	Skin sensitization
Alloaromadendrene	No	-0.007	1.549	1.397	No	No
Aromadendrene	No	-0.007	1.549	1.397	No	No
Dendrolasine	No	0.686	2.096	1.233	No	Yes
Germacrene	No	0.539	1.747	1.363	No	Yes
Isofucosterol	No	-0.509	2.333	2.42	No	No
Kaempferol	No	0.91	2.301	2.699	No	No
Leucopelargonidin	Yes	0.664	2.223	2.434	No	No
Undecane	No	0.389	1.597	2.698	No	Yes
α-copaene	No	-0.029	1.674	1.437	No	No
α-humulene	No	0.638	1.829	1.372	No	Yes
α-pinene	No	0.48	1.77	2.262	No	No
α-terpinene	No	0.695	1.734	2.418	No	No
α-trans bergamotene	No	0.188	1.641	1.357	No	Yes
Bicyclogermacrene	No	0.331	1.654	1.378	No	Yes
β-bourbonene	No	-0.475	1.753	1.455	No	No
Gamma cadinene	No	0.048	1.54	1.473	No	Yes
β-caryophyllene	No	0.427	1.643	1.425	No	Yes
Delta-cadinene	No	0.12	1.58	1.4	No	Yes
Limonene	No	0.77	1.88	2.368	No	Yes
β-ocimene	No	0.574	1.614	2.419	No	No
Quercetin	No	0.499	2.471	2.612	No	No
β Sitosterol	No	-0.431	2.332	0.845	No	No
Tetradecane	No	0.221	1.527	1.377	No	Yes
α-thujene	No	0.353	1.589	2.243	No	No
Tridecane	No	0.269	1.542	1.413	No	Yes
α-ylangene	No	-0.029	1.674	1.437	No	No
Lisinopril	No	0.505	2.223	2.227	Yes	No
Aliskiren	No	-0.111	2.052	2.511	Yes	No
Carazolol	Yes	0.021	2.257	0.684	Yes	No

noted that only leucopelargonidin can produce toxicity but the remaining drugs are free from AMES toxicity. The maximum tolerated dose has been found to be 0.91 mg/kg/day, whereas the lowest maximum tolerated dose was -0.007 mg/kg/day. The oral rat acute toxicity ranges have been found to be 1.527 mol/kg to 2.471 mol/kg, which indicated if the quantity of drugs administrated higher than 2.471 mol/kg, it might produce toxicity. The range of oral rat chronic toxicity has been found to be 0.845 mg/kg/day to 2.699 mg/kg/day.

CONCLUSION

In this investigation, it has been found that the extract of fruits of *F. religiosa* showed remarkable antihypertensive activity and at the same time it exerted no harmful effect on liver, kidney and cardiovascular system. On the other hand, the *in silico* study revealed that among the phytoconstituents of fruits of *F. religiosa*, isofucosterol, β sitosterol have shown promising affinities and efficiency in comparison to FDA approved drugs such as lisinopril, aliskiren and carazolol. It can be suggested that extract of fruits of *F. religosa* may offer a promising lead for the management of hypertension.

ACKNOWLEDGMENTS

We express our gratitude to the authority of the Department of Pharmaceutical Chemistry for using the computers of the Molecular Pharmacology and Herbal Drug Research Laboratory established under the HEQEP Project.

Competing interests. The authors declare that they have no competing interests.

Funding. This work has been funded by a grant from the Ministry of Science and Technology, Government of the Peoples Republic of Bangladesh. Serial: 523; Ref.No.:39.00.0000.009.14.019.21, Date: 15-12-2021.

Authors' contributions. MSA has originated the concept. NJS, KS and SA worked in the laboratory and wrote the original draft. JAC, AAC and SK critically reviewed the whole activities. MSA supervised the overall work.

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