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FULL PAPER

In silico studies for the antihypertensive effect of hibiscus acid and related compounds from *Hibiscus sabdariffa*

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The use of herbal medicines has been part of human culture for years. For example, hibiscus (*Hibiscus sabdariffa*) is known for its use in the management of hypertension. Pharmacological studies showed that hibiscus extract is indeed effective in controlling hypertension. This study investigates the effects of acidic constituents in hibiscus extract on several antihypertensive targets using *in silico* docking techniques. Based on the results, we predict that hibiscus acid may affect human angiotensin-converting enzyme (hACE), potentially responsible for the antihypertensive effect of hibiscus.

KEYWORDS

Antihypertensive; *in silico* docking techniques; hibiscus acid; *Hibiscus Sabdariffa*; angiotensin converting enzyme (ACE).

Introduction

For millennia, traditional medicines have utilized a wide range of medicinal plant species [1]. Modern medicine has evolved from folk medicine, and many of today's medicinal compounds are natural products or their derivatives derived from plants [2-3]. However, some medicinal plants have not yet been developed as an antihypertension drugs. In folk medicine, extracts of *Hibiscus sabdariffa* are commonly used for their hypotensive diuretic, and body temperature-lowering effects [4-5]. The biologically active compound hibiscus acid, (2S,3R)-3-hydroxy-5-oxo-2,3,4,5-tetrahydrofuran-2,3-dicarboxylic acid, has been isolated from *Hibiscus sabdariffa* plants. Due to the lack of commercial production of hibiscus acid from *Hibiscus sabdariffa*, little research exists on this compound. Particularly, scientific studies focused on its therapeutic activity and mode of action are scarce. However, its diastereomer, garcinia acid (extracted from *Garcinia cambogia*) is commercially available and well-studied. The most representative evidence on hibiscus acid extraction, properties, and chemical characteristics has been analyzed by Zheoat *et al.* (2019) and Portillo-Torres *et al.* (2019) [6-7]. Crystallographic analysis and X-ray spectroscopy confirmed that hibiscus acid is a five-membered lactone ring with four carbon atoms and one oxygen atom. C3 (sp2) has a double-bonded oxygen atom, C1 has an OH group and a COOH group, and C2 has a COOH group (Figure 1) [8]. In addition to garcinia acid and hibiscus acid, other related compounds extracted from *Hibiscus sabdariffa* are included in our study, as displayed in Figure 1.

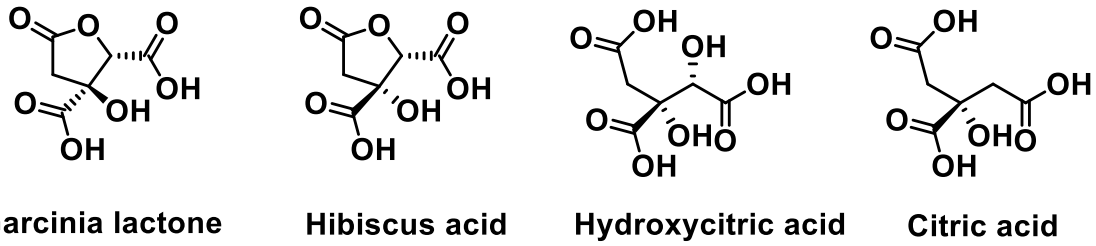

FIGURE 1 Structures of investigated compoundsRegarding its therapeutic and/or pharmacological properties, some studies suggest that hibiscus acid has an inhibitory effect against α-amylase and α-glucosidase; as well as vasorelaxant activity and a possible antihypertensive potential. In the first case, [9-10] isolated hibiscus acid and its 6-methyl ester from Hibiscus sabdariffa, which showed a high inhibitory activity against porcine pancreatic α-amylase. Therefore, hibiscus acid could be considered as an agent with a mechanism of action like that of some drugs used in type 2 diabetes, such as acarbose. Moreover, the antioxidant, anti-lipid peroxidative, hypoglycemic, and hepatoprotective effects in type 1 diabetic rats effects of Hibiscus trionum tea (HTT) was recently reported [11]. Furthermore, Hibiscus sabdariffa was shown to have arole on the inhibition of oral bacteria [12].

The vasorelaxant activity of hibiscus acid has been confirmed for the first time by [6]. As they conducted an *ex-vivo* study on Sprague-Dawley rat aorta to discover the direct vasorelaxant effect of the pure hibiscus acid derived from a methanolic crude extract of Hibiscus sabdariffa. The researchers suggested that hibiscus acid was more potent and effective as a vasorelaxant compound when compared to the crude extract of the plant (*Hibiscus sabdariffa*). In addition, they suggested that the vasorelaxant action of hibiscus acid could be attributed to the blockage of Ca^{2+} influx via Ca^{2+} channels. Moreover, in the same, they observed that the isomer of hibiscus acid, which is garcinia acid has a similar vasorelaxant activity.

Virtual screening is a valid method for discovery and development of drug, which is started by searching small molecules. The computational method (i.e. molecular docking) is an important powerful knowledge-based approach that is widely used to describe interaction between molecules and target protein [13]. This computational method can certainly be used to investigate the way by which the phytochemicals can interact with target receptors. *In silico* simulations can be used to propose protein ligand binding characteristics for molecular structures [14], e.g., known constituents of a plant material. Compounds that perform well in *in silico* predictions can be used as promising starting materials for experimental work. Activity predictions using virtual screening have intriguing success rates [15], and can be conducted with a wide variety of computational methods [16]. *In silico* studies can focus on the main constituents of herbal remedies [17]. Thus, the aim of this study was to explore hibiscus acid as natural product which can act as calcium channel blocker for development of a vasorelaxant drug.

Experimental

General

The structures of the test compounds were obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The structures of target proteins were obtained from protein data bank (https://www.rcsb.org/) using PDB IDs. All proteins were prepared as previously reported [18-19] by removing all water molecules and other small fragments, except ions. Hydrogens were added and charges were calculated using 'make macromolecule'

command in PyRx [20]. Small molecules and co-crystalized ligands were also prepared using 'make ligand' command in the same software after short energy minimization using Avogadro [21].

Docking was performed using AutoDock Vina [22] with a grid box centered on the co-crystallized ligand. The size of the grid box was adjusted to cover all amino acids around ligand and kept constant for each protein. An exhaustiveness value of 16 was used for all the runs. Validation was done by calculating root mean square value (RMSD) between co-crystalized ligand poses obtained from docking and crystal structure using DockRMSD server [23]. Images were prepared using PyMOL software (The PyMOL Molecular Graphics System, Version 1.2, Schrödinger, LLC).

Results and discussion

In this study, we want to investigate potential targets that could contribute to the known hypotensive effect of *Hibiscus sabdariffa* and its extracts. For the purpose of this study, we selected three of the main constituents of Hibiscus which include hibiscus acid, hydroxycitric acid, and citric acid, in addition to Garcinia acid lactone, from *Garcinia cambogia* (Figure 1). The selected compounds were docked against four important hypertension targets which include the human Angiotensin Converting Enzyme (hACE), Renin, human angiotensin II receptor as well as Human T-type voltage-gated calcium channel. The crystal structures of these target proteins along with known co-crystalized ligands were downloaded from the protein databank (https://www.rcsb.org/). The human Angiotensin Converting Enzyme (hACE) co-crystalized with lisinopril was downloaded using PDB ID: 1O86. Renin co-crystalized with Aliskiren was downloaded using PDB ID: 2V0Z. The human angiotensin receptor in complex with Olmesartan was obtained from protein data bank under PDB ID: 4ZUD. Finally, human low-voltage activated T-type calcium channel Cav3.3 in complex with mibefradil was downloaded using PDB ID: 7WLJ.

Docking validation for all the four targets was done through the redocking of the co-crystalized ligand, and then comparing the docked pose with the crystal structure pose using RMSD values calculated through the DockRMSD server [23]. The docking procedure is considered valid if the RMSD value is less than 2 Å which is seen in all the four targets. Docking poses of the docked and crystal poses for all the four targets are shown in Figure 2.

After we confirmed the validity of our docking procedures, our test compounds were docked in the active site of target proteins. Docking scores of test compounds along with co-crystalized ligands expressed as kcal/mol are indicated in Table 1. Docking scores reflect the affinity between the target protein and tested ligands. With smaller the number (or larger absolute value), the binder of the compound is performed well. We usually compare the docking score of the tested compound to those of the co-crystalized ligand which is usually a known inhibitor of this target.

These docking results show that among tested compounds, hibiscus acid showed better binding with all targets compared to the other three compounds which was not significantly different in terms of their docking scores. It worth to mention here that docking scores of all compounds are less than their corresponding co-crystalized ligands docking scores. This might be attributed to the smaller structures of the acids under investigation. In addition, hibiscus acid best docking score is seen with the human angiotensin converting enzyme target (hACE). It is worth mentioning that several previous studies have tried to investigate the hypotensive effect of *Hibiscus spp.* which was

attributed to calcium channel block, ACE inhibition and/or diuretic effect. [24]. In addition, a crude hydroalcoholic extract from *Hibiscus sabdariffa L. calyces* was able to inhibit Angiotensin I- Converting Enzyme (ACE) *in vitro* as reported by Jonadet *et al.* [25].

These studies agree well with our findings but never discussed the potential chemical entity responsible for this effect. Furthermore, the binding mode between hibiscus acid and ACE was not reported before which is demonstrated in Figure 3.

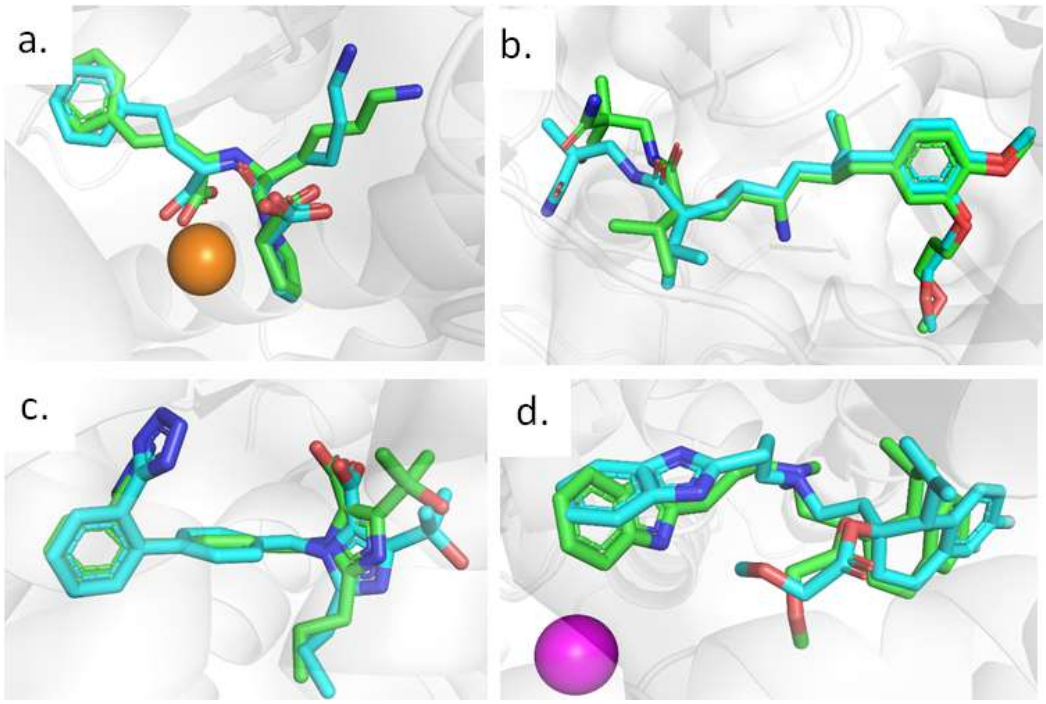

FIGURE 2 Validation of docking procedure. Green structure represents the crystal structure pose while blue represents docked poses. Orange sphere represents Zinc ions and magenta sphere is calcium ion. a) human angiotensin converting enzyme (hACE, 1o86). b) renin enzyme (2v0z). c) human angiotensin II receptor (4zud). d) voltage-dependent calcium channels (7wlj)

TABLE 1 Docking scores of test compounds and co-crystalized ligands

	Binding Affinity (kcal/mol)			
	hACE	**Renin**	**Angiotensin II receptor**	**T-type calcium channel**
	1O86	2V0Z	4ZUD	7WLJ
Garcinia acid lactone	-5.8	-5.3	-4.9	-5.1
Hibiscus acid	-6.3	-5.8	-5.5	-5.5
Hydroxycitric acid	-5.6	-5.4	-4.9	-5.0
Citric acid	-5.7	-5.0	-5.1	-5.0
Co-crystalized ligand	-8.2	-9.0	-8.8	-9.3
Co-crystalized ligand name	Lisinopril	Aliskiren	Olmesartan	Mibefradil

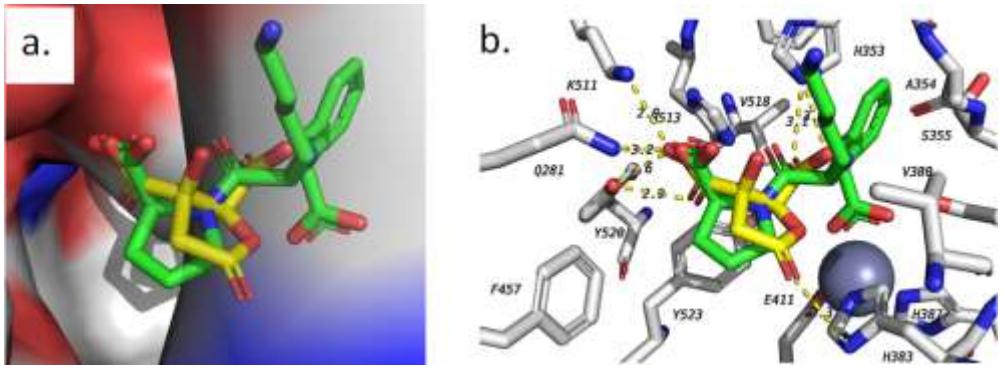

FIGURE 3 Docking pose of Hibiscus acid (yellow) in the active site of hACE I (PDB ID 1O86) overlapped with co-crystalized ligand (green). a. surface representation showing similar binding mood and b. Interactions with active site residues

Conclusion

In this study, we investigated potential effect of hibiscus acid and other related constituents in hibiscus extract against four different antihypertensive targets including hACE, Renin, angiotensin II receptor and T-type calcium channel. Potential interactions were investigated using molecular docking.

According to the results obtained, we propose that the antihypertensive effect of hibiscus extract might be attributed to the effect hibiscus acid on human angiotensin converting enzyme (hACE). These results require further follow-up experimental studies to confirm this finding.

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Authors' Contributions

Ali Ali and Ahmed Zheoat organized the study and wrote the manuscript. All authors collected, prepared and analysed the samples and extracted data.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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