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Novel Insights in the Hypertension Treatment & Type 2 Diabetics Induced by Angiotensin Receptor Blockers: MD Simulation Studies & Molecular Docking of Some Promising Natural Therapies

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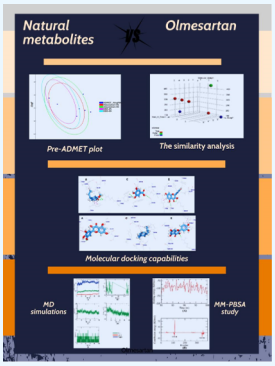
ABSTRACT: Angiotensin receptor blockers (ARBs) are commonly used to treat hypertension that target the hormonal system (renin-angiotensin system (RAS)), which regulates various physiological functions in the body. ARBs work by blocking the binding of angiotensin II to its receptor, thereby preventing a rise in blood pressure. These drugs not only normalize the overactivation of RAS but also provide protective effects against cardiovascular, renal, and type 2 diabetic patients. Inappropriate RAS activity has been linked to insulin resistance of type 2 diabetes. Olmesartan, as an ARB, was found to have a beneficial role in reducing postprandial glucose levels in type 2 diabetes. However, ARBs can cause side effects, prompting a search for new compounds that have fewer adverse effects. This study explores the potential of natural metabolites, specifically eugenol, gallic acid, myricetin, *p*-cymene, quercetin, and kaempferol, as ARB inhibitors compared to the current standard, olmesartan. Using in silico studies, the binding affinity of these natural substances to the ARB receptor was evaluated. The results showed that myricetin and kaempferol had affinities higher than those of olmesartan, suggesting that they could serve as promising ARB inhibitors for hypertension treatment. These natural compounds could provide an alternative approach to conventional antihypertensive drugs, which may have fewer side effects. However, more research is needed to validate the efficacy and safety of these natural compounds as antihypertensive drugs. Further in vitro and in vivo studies are needed to confirm their effectiveness and safety. This study provides a promising starting point for future investigations into the potential of natural metabolites as alternative treatments for hypertension. The findings also highlight the importance of exploring natural alternative treatments for hypertension and the protective effects of ARBs on early stage type-2 diabetics.



INTRODUCTION

Angiotensin receptor blockers (ARBs) are used to treat hypertension, congestive heart failure, and diabetic nephropathy. ARBs bind to and inhibit the angiotensin II type 1 receptor. There have been numerous articles on the association between insulin resistance, type 2 diabetes, and improper RAS activation.[1] Not only are systemic RAS components primarily derived from the kidney, liver, and lung related to these associations, but greater activation of local RAS in adipocytes and the pancreas may also contribute to impaired β-cell activity and insulin sensitivity.[2] Thus, type 2 diabetes with new onset may be uncommon when RAS inhibition occurs with ARBs or angiotensin converting enzyme inhibitors (ACEIs).[3,4] They are often prescribed as an alternate to ACEIs for patients who cannot tolerate ACEIs due to their adverse effect of inducing chronic, nonproductive cough. ARBs are well-tolerated with low adverse effects. While angioedema and cough may still arise with ARBs, their incidence is lower than that with ACE inhibitors because ARBs do not elevate bradykinin levels. However, ARBs can cause hypotension and/or renal failure in patients whose blood pressure (BP) or renal function is highly dependent on the renin−angiotensin−aldosterone system (RAAS). Patients with bilateral renal artery stenosis or heart failure patients with hypotension should not take ARBs.[5,6] Losartan, the first ARB to be marketed, has been relatively ineffective in controlling BP for 24-h/day. Olmesartan, a new ARB,[7] is a prodrug that is hastily and completely converted to its active metabolite, olmesartan, after oral administration.[8] Olmesartan with a half-life of 12−18 h[9] and was effective to control BP once per day with a safety profile like that of a placebo.[10] Both ACE inhibitors and ARBs lower hypertension

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Table 1. Tested Metabolites with Olmesartan and the Properties of the Molecules[a]

Compounds	A-Log p	M. wt.	Chemical Structure	HBA	HBD	Rotatable bonds	Rings	Aromatic rings	MFPSA	Minimum Distance	Is similar
Olmesartan	3.766	446.502	C24H26N6O3	7	3	8	4	4	0.278	0	Reference
Kaempferol	1.872	286.236	C15H10O6	6	4	1	3	2	0.415	1.37443	TRUE
Quercetin	1.63	302.236	C15H10O7	7	5	1	3	2	0.473	1.44971	TRUE
Myricetin	1.388	318.235	C15H10O8	8	6	1	3	2	0.526	1.5668	TRUE
Eugenol	2.579	164.201	C10H12O2	2	1	3	1	1	0.156	1.86929	TRUE
p-cymene	3.51	134.218	C10H14	0	0	1	1	1	0	2.16851	FALSE
Gallic acid	0.733	170.12	C7H6O5	5	4	1	1	1	0.605	2.10857	FALSE

[a]A-Log *p*: lipid−water partition coefficient, M.Wt.: molecular weight, HBA: hydrogen bond acceptor, HBD: hydrogen bond donor, MFPSA: molecular fractional polar surface area, Minimum Distance: the shortest distance between a tested compound and the reference one.

by acting on the RAS. ARBs act by blocking angiotensin II from binding to its receptor.[11] In contrast to ACE, ACE2 is changing angiotensin I and angiotensin II into other forms, which can be beneficial to the heart and lungs.[12,13] In terms of comparative studies, olmesartan has been shown to have a significant reduction in high-sensitive C-reactive protein after stenting compared to valsartan,[14] while telmisartan was more beneficial than olmesartan for improving glucose and lipid profiles.[15] However, olmesartan was found to have a more significant effect on decreasing serum IL-6 and hsCRP than telmisartan.[16]

Recent studies have explored the use of natural metabolites as potential inhibitors of the angiotensin II type-1 receptor, a key target in RAAS for the treatment of hypertension, type-2 diabetics, and other cardiovascular diseases. In one study, the effect of myricetin on prediabetes, as determined by *in vitro* and *in vivo* assays, was described. The research study established a theoretical foundation for the subsequent clinical investigation and development of myricetin as a potent immunomodulatory agent in prediabetes.[17] In addition, treatment with myricetin resulted in a substantial reduction in glomerulosclerosis as well as blood urea nitrogen, urinary volume, and protein excretion, all of which were markedly elevated in rodents with diabetes. A substantial increase in the decreased creatinine clearance was observed in diabetic rodents subsequent to treatment with myricetin. In diabetic rodents, altered renal activities were decreased; myricetin restored these activities, which were increased. In conclusion, myricetin restored renal activities in diabetic rodents and ameliorated altered renal functions.[18] The data obtained indicate that myricetin may have therapeutic potential in the treatment of diabetic nephropathy. A recent study suggests that treating diabetes and its consequences might be significantly improved by using the natural substance kaempferol.[19] In streptozotocin-induced diabetic rats, kaempferol boosted plasma insulin levels and decreased blood glucose levels,

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according to a study that used the insulin secretagogue glibenclamide as the control medication. Additionally, kaempferol enhances insulin-dependent glucose absorption.[20,21] Thus, the findings of this study revealed that these two compounds exhibited superior affinity toward the targeted site compared to olmesartan, a common ARB. Based on these results, natural metabolites such as myricetin and kaempferol may reduce the incidence of type 2 diabetes and show promise as supplements or alternatives to conventional ARBs in the treatment of hypertension, and this validates the findings of our study.

The use of molecular docking (MD) has been employed to screen numerous natural compounds, including polyphenols and flavonoids, for their potential to bind to the angiotensin receptor and inhibit its activity. Such studies have yielded promising results, with certain natural compounds exhibiting equivalent or even superior activity compared with conventional ARBs. Thus, molecular docking provides critical insights into the therapeutic potential of natural metabolites as angiotensin receptor inhibitors, paving the way for new and effective treatments for hypertension and related disorders. MD is a powerful computer technique that is frequently used in drug design and discovery. Recently, it has been increasingly applied to screen natural metabolites as potential inhibitors of vital drug targets.

The aim of this investigation was an assessment of the inhibitory potential of natural metabolites, namely, kaempferol, eugenol, gallic acid, myricetin, *p*-cymene, and quercetin, in comparison to the established standard, olmesartan. By utilizing MD simulation and molecular interactions in silico studies, it was possible to assess the binding affinity of these natural compounds toward the ARB receptor. In order to offer innovative perspectives on the management of hypertension and type 2 diabetes.

RESULTS AND DISCUSSION

Molecular Similarity. Table 1 shows the computational properties of the tested natural metabolites. The results of the similarity study provide a comprehensive comparison of several compounds to the reference compound, olmesartan, considering various molecular descriptors.

A-Log *p* (Partition Coefficient). Olmesartan has an A-Log *p* value of 3.766, indicating its partition coefficient between octanol and water. Compounds like kaempferol (1.872), quercetin (1.63), myricetin (1.388), and eugenol (2.579) have A-Log *p* values relatively close to olmesartan, suggesting similar lipophilicity.[22,23]

Molecular Weight (M. Wt.). Olmesartan has a molecular weight of 446.502 g/mol. Among the compared compounds, myricetin has the closest molecular weight (318.235 g/mol), followed by quercetin (302.236 g/mol) and kaempferol (286.236 g/mol). Eugenol (164.201 g/mol) has a lower molecular weight.[24]

Hydrogen Bond Acceptors (HBA) and Donors (HBD). Olmesartan has 7 hydrogen bond acceptors and 3 donors. Quercetin and myricetin both have similar numbers of hydrogen bond acceptors (7 and 8) and donors (5 and 6), respectively, indicating potential similar interaction patterns. Kaempferol also has 6 hydrogen bond acceptors and 4 donors. Eugenol has fewer HBAs and HBDs compared to those of olmesartan.

Rotatable Bonds, Rings, and Aromatic Rings. Olmesartan possesses 8 rotatable bonds and 4 rings, including 4 aromatic rings. Compounds, such as quercetin, myricetin, and kaempferol, have similar numbers of rotatable bonds and rings. Eugenol has fewer rotatable bonds, rings, and aromatic rings.

Molecular Polar Surface Area (MFPSA). Olmesartan has an MFPSA of 0.278. Compounds, such as quercetin, myricetin, and kaempferol, exhibit slightly higher MFPSA values, indicating comparable molecular polar surface areas. Eugenol, with an MFPSA of 0.156, has a lower value.

Minimum Distance and Similarity Classification. The minimum distance between olmesartan and each compound is calculated as a measure of similarity. Compounds with smaller distances are considered more similar. Based on this criterion, kaempferol, quercetin, and myricetin are classified as similar to olmesartan, while eugenol, *p*-cymene, and gallic acid are classified as dissimilar.

In conclusion, compounds, such as kaempferol, quercetin, and myricetin, exhibit structural and physicochemical similarities to olmesartan across multiple molecular descriptors, suggesting potential pharmacological relevance or therapeutic implications. On the other hand, compounds, such as eugenol, *p*-cymene, and gallic acid, demonstrate distinct characteristics compared to olmesartan, indicating their dissimilarity in terms of molecular properties. These findings can guide further research into the pharmacological activity and therapeutic potential of these compounds. The minimum distance property shows the distance between the reference molecule and the compared molecule in angstroms (Figure 1).

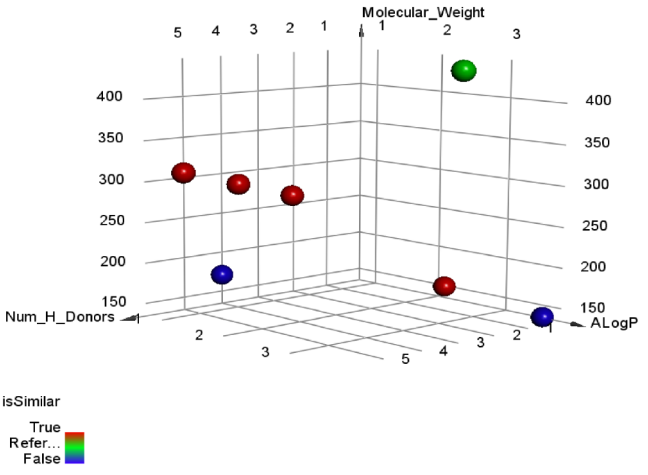

Figure 1. The similarity analysis between the tested molecules and olmesartan. Balls with green color indicated reference ligands (reference), balls with red color indicated similar ligands, and balls with blue color indicated not similar ligands.

Pre-ADMET and Pretoxicity Studies. Drug modeling and the development of novel compounds with medicinal value made it necessary to forecast the properties of drugs and their ADME data. Pre-ADMET data are shown in Table 2 with the ADMET properties that were predicted for the tested compounds. The level of BBB, the degree of solubility, the level of absorption, the degree of hepatotoxicity, the prediction of CYP 2D6 inhibition, and the prediction of PPB. According to the BBB level, most of the tested compounds have a low to very low chance of crossing the blood-brain barrier. Consequently, this outcome can be assessed favorably because

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Table 2. Predicted ADMET for the Tested Compounds

comp.	BBB level[a]	solubility level[b]	absorption level[c]	hepatotoxicity	CYP2D6 prediction[d]	PPB prediction[e]
eugenol	1	3	0	not true	not true	not true
gallic acid	3	4	0	true	not true	not true
kaempferol	3	3	0	true	not true	not Ttue
myricetin	4	3	3	not true	not true	not true
quercetin	4	3	1	true	not true	not true
***p*-cymene**	0	3	0	true	not true	true
olmesartan	4	2	2	true	not true	true

[a]The level of blood−brain barrier (BBB): zero indicates very high, one indicates high, two indicates medium, three indicates low, four indicates very low. [b]The level of blood−brain barrier (BBB): zero indicates very high, one indicates high, two indicates medium, three indicates low, four indicates very low. [c]Level of Absorption: zero indicates good, one indicates moderate, two indicates poor, three indicates very poor. [d]CYP2D6, cytochrome P2D6, is TRUE if indicates as an inhibitor, FALSE if indicates as noninhibitor. The cutoff Bayesian score of 0.161 was used to classify the compound if it is CYP2D6 inhibitor or not. [e]PPB, also known as plasma protein binding, is FALSE if it indicates less than 90%, and is TRUE if it indicates more than 90%. The cutoff Bayesian score of -2.209 was used to classify the compound if it is highly bounded (≥90% bound) to plasma proteins.

low brain bioavailability is more the norm than the exception for most medications since certain naturally occurring chemicals have the capacity to pass the blood-brain barrier and have negative effects. Furthermore, the integrity of the BBB may be impacted by exposure to certain chemicals. Because of these possible side effects, it is crucial to think through the implications of improving a drug's capacity to pass the blood-brain barrier, except for CNS disorders.[25] In terms of solubility, all compounds have good to optimal solubility except for olmesartan, which has a low solubility level. For absorption, all compounds have a good to moderate rating, except for myricetin and olmesartan, which showed poor to very poor absorptivity. Hepatotoxicity prediction indicates that gallic acid, kaempferol, quercetin, and olmesartan may have hepatotoxicity, while myricetin and eugenol have no hepatotoxicity. CYP2D6 inhibition prediction indicates that all compounds are noninhibitors of CYP2D6. PPB prediction shows that *p*-cymene and olmesartan have high PPB, while all other compounds have low PPB (Figure 2). Given that, a high degree of protein binding may cause a decrease in a drug's effectiveness and action.[26] The majority of the examined chemicals have a low PPB, which is regarded as a good indicative marker. It was observed that the most calculated values indicated that the majority of components demonstrated excellent drug-like features, making them a promising choice for fighting hypertension and other correlated illnesses.

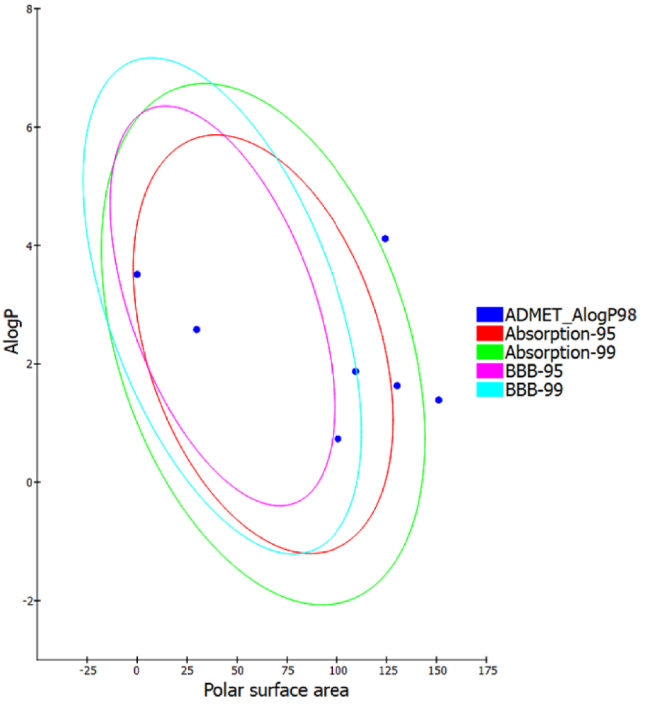

Figure 2. Pre-ADMET plot shows the relationship between ADMET descriptors.

Predicted Toxicity Study. For the evaluated synthetic molecules, Discovery Studio 2019 software was used to produce toxicity predictions that were built by using the following validated and constructed models: Rat carcinogenicity as determined by the FDA,[27,28] rat carcinogenic potency as determined by the Toxcity Dose 50 (TD50),[29] rat maximum tolerated dose (MTD),[30,31] rat oral lethal dose 50 (LD50),[32] rat chronic LOAEL,[33,34] ocular irritation,[35] and skin.[36] Most of the synthetic compounds listed in Table 3 have low toxicity. Table 3 provides some in silico toxicity properties for a set of natural compounds. While these results are a useful starting point for evaluating the safety of the compounds, it is important to note that in silico methods do not provide a complete picture of a compound's toxicity, and further testing is needed to confirm these findings. Looking at the results in the table, it appears that most of the compounds are noncarcinogenic, except for gallic acid, which is listed as a multicarcinogen. This result evaluates the toxicity of gallic acid (by using software that can be used for prediction of toxicity in silico) but cannot be used to assess potential risks to humans, as developmental toxicity potential, which measures the extent to which substances can impede normal development and cause adverse effects in large numbers of animals, was not observed for gallic acid in the same results (Table 3). Furthermore, it has been demonstrated that gallic acid, the primary polyphenol, inhibits carcinogenesis in both in vitro and animal models of malignant cells. Gallic acid exerts its inhibitory influence on cancer cell proliferation through the regulation of genes responsible for cell cycle, metastasis, angiogenesis, and apoptosis. Based on many existing data from in vivo and in vitro investigations, this dietary polyphenol (gallic acid) exhibits potential as a chemopreventive agent for cancer.[37−39] The TD_{50} value listed for each compound is a measure of its carcinogenic potency in rats. The lower the TD_{50} value, the more potent the compound is as a carcinogen. The TD_{50} values listed in the table range from 9.95 to 141.57, with the lowest value belonging to olmesartan. It is important

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Table 3. *In silico* Toxicity Properties of the Tested Compounds[c]

comp.	FDA rodent carcinogenicity (male, mice)	carcinogenic potency TD 50 (rats)[a]	rat maximum tolerated dose (feed)[b]	developmental toxicity potential	rat oral LD50[b]	rat chronic LOAEL[b]	ocular irritancy (rats)	skin irritancy (rats)
eugenol	noncarcinogenic	141.57	0.169	no toxicity	1.423	0.0715	moderate	severe
gallic acid	multicarcinogenic	101.13	1.852	no toxicity	1.292	0.2914	no irritancy	medium
kaempferol	noncarcinogenic	54.54	1.036	no toxicity	0.955	0.1475	no irritancy	medium
myricetin	noncarcinogenic	24.93	2.437	no toxicity	0.846	0.2038	no irritancy	mild
quercetin	noncarcinogenic	48.79	1.594	no toxicity	1.191	0.2266	no irritancy	mild
***p*-cymene**	noncarcinogenic	29.27	0.062	no toxicity	1.496	0.1201	severe	none
olmesartan	noncarcinogenic	9.95	0.807	no toxicity	4.849	0.0872	no irritancy	mild

[a]Unit: mg/kg body weight/day. [b]Unit: g/kg body weight [c]FDA Rodent Carcinogenicity (Male, mice): this column indicates whether the compound is a carcinogen or a non-carcinogen in male mice, as determined by the FDA. Carcinogenic Potency TD50 (Rats): this column provides a measure of the carcinogenic potency of the compound in rats, as measured by the TD50 (tumorigenic dose 50%) value. Rat Maximum Tolerated Dose (Feed): this column gives the maximum tolerated dose of the compound in rats, as measured in feed. Developmental Toxicity Potential: this column indicates the potential of the compound to cause developmental toxicity. Rat Oral LD50: this column gives the oral LD50 (lethal dose 50%) of the compound in rats. Rat Chronic LOAEL: this column provides the chronic LOAEL (lowest observed adverse effect level) of the compound in rats. Ocular Irritancy (Rats): This column indicates the degree of ocular irritancy caused by the compound in rats. Skin Irritancy (Rats): this column indicates the degree of skin irritancy caused by the compound in rats.

to note that these values are specific to rats and may not be directly applicable to humans.[40,41] The maximum tolerated dose in the feed and oral LD_{50} values provide information about the acute toxicity of the compounds. The compounds listed in the table have relatively high maximum tolerated doses and LD_{50} values, suggesting that they are not likely to cause acute toxicity in rats at the doses tested. However, chronic toxicity may still be a concern, as indicated by the chronic LOAEL values provided in the table. Finally, the ocular and skin irritancy values provide information about the potential for these compounds to cause irritation or damage to the eyes and skin. The compounds listed in the table range from mild to severe in their irritancy potential, with *p*-cymene and eugenol causing severe skin and ocular irritancy, respectively. In rapid, the results revealed that natural substances are noncarcinogenic and have relatively low acute toxicity but may still have potential for chronic toxicity and skin/ocular irritancy. Further testing, including *in vivo* studies, would be needed to confirm these findings and fully evaluate the safety of these compounds.

Docking Studies. To determine the molecular docking capabilities of the test compounds (eugenol, gallic acid, myricetin, *p*-cymene, quercetin, and kaempferol) with respect to the target enzyme (Angiotensin II receptor), molecular docking was implemented. Table 4 presents the docking scores obtained by the compounds in relation to their respective targets. Every compound successfully docked with the Angiotensin II receptor. 3D interaction diagrams indicate that distinct residues of the target proteins are involved in a variety of chemical bonds, including hydrophobic and hydrogen bonds, Pi-Pi interactions, Pi-Alkyl interactions, and van der Waals forces (Figures 3 and 4). In particular, the energy binding of ethanol against the Angiotensin II receptor was found to be −5.50 kcal/mol.

Table 4. Show DG, RMSD, Interactions in kcal/mol of the Tested Metabolites, Contrary to the Target Site (Angiotensin II Receptor)

targets screened	tested compounds	RMSD value (Å)	score of docking (affinity)	interactions	
				H.B.	pi interaction
Angiotensin II receptor	eugenol	1.11	−5.50	2	4
	gallic acid	1.03	−4.32	3	-
	kaempferol	1.07	−5.70	3	3
	myricetin	1.35	−5.80	1	4
	p-cymene	0.94	−5.05	-	4
	quercetin	1.34	−5.51	2	5
	olmesartan (reference)	1.02	−5.97	1	9

Eugenol formed four Pi-Alkyl and Pi-Pi interactions with Val108, Tyr292, Ile288, and Trp84, additionally interacting with Arg67 by two hydrogen bonds with distances of 2.57 and 2.89 Å (Figure 3). Moreover, the binding manner of gallic acid revealed an energy binding of −4.32 kcal/mol, contrary to Angiotensin II receptor, which interacts with Tyr35, Arg2.62, and Cys180 by 3 HB with a distance of 2.98, 2.62, 2.26, and 2.55 Å (Figure 3). The binding mode of kaempferol showed an energy binding of −8.45 kcal/mol against Angiotensin II receptor. Kaempferol formed 3 Pi-Alkyl and Pi-Pi interactions with Val108, Tyr92, & Ile288, and also interact with Tyr87 and Arg167 by 3 HB with a reserve of 2.45, 1.99, and 2.41 Å (Figure 3).

The binding manner of myricetin showed an energy binding of −5.80 kcal/mol against the Angiotensin II receptor. Myricetin formed four Pi-Alkyl and Pi-Pi interactions with Val108, Ile288, and Tyr87, additionally interacting with Cys180 by a single bond of hydrogen with a distance of 2.33 Å (Figure 4). Moreover, the binding method of *p*-cymene showed an energy binding of −5.05 kcal/mol against Angiotensin II receptor, which interacts with Val108, Ile288, and Tyr87 by four Pi-Alkyl interactions (Table 4). The binding mode of kaempferol exhibited an energy binding of −5.51 kcal/mol. against Angiotensin II receptor. Quercetin formed five Pi-Alkyl and Pi-Pi interactions with Val108, Tyr92, Tyr87, and Ile288, additionally interacted with Tyr87 and Arg167 by two bonds of hydrogen with a reserve of 2.16 and 2.34 Å (Figure 4). Docking of all natural substances with the Angiotensin II receptor reveals two additional hydrogen bonds of varied length at the binding site in addition to multiple hydrophobic interactions with amino acid residues, as seen in 3D figures. They have the highest score against the Angiotensin II receptor of any investigated ligand due to two more hydrogen bonds with residues Arg67, Tyr87, and Cys180, among other considerations. Moreover, hydrogen

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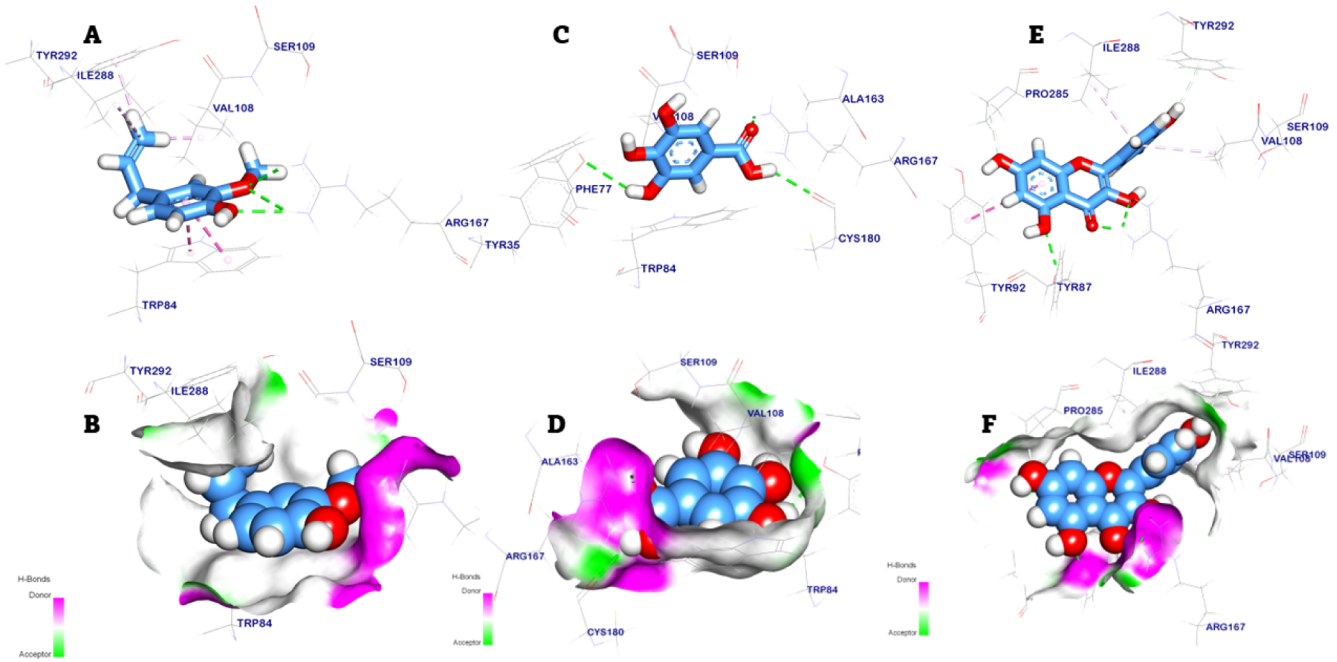

Figure 3. Eugenol (A), gallic (C) acid, and kaempferol (E) and their 3D ball-and-stick models (B, D, and F) are *docked in* Angiotensin II receptor. Hydrogen bonds (green) and pi-interactions are denoted by purple lines with surface mapping showing eugenol, gallic acid, and kaempferol inhabiting the active pocket of the Angiotensin II receptor.

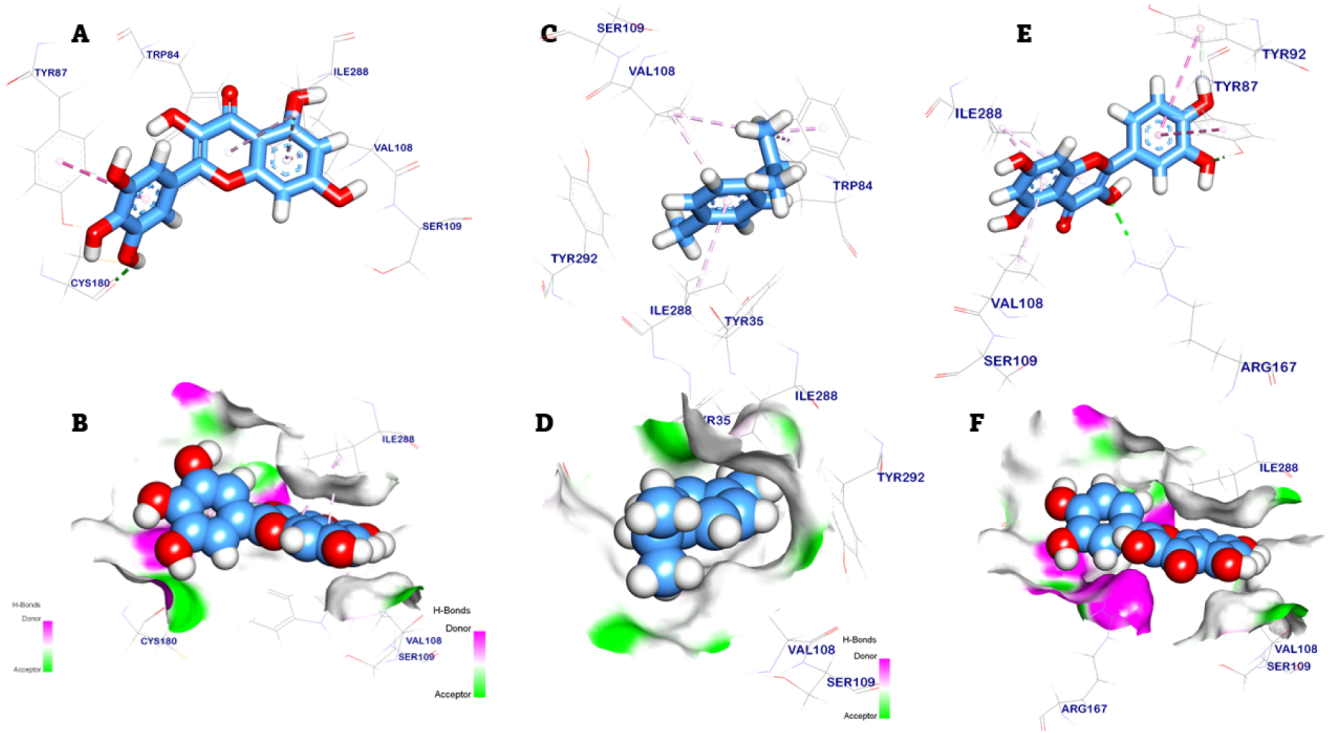

Figure 4. Myricetin (A), *p*-cymene (C), and quercetin *(E)* with their 3D ball-and-stick models (B, D, and F) are docked in Angiotensin II receptor. Bonds of hydrogen are represented with a green color, the pi-interactions are with purple lines with surface mapping showing the three compounds of myricetin, *p*-cymene, and quercetin, which inhabit the active concise of Angiotensin II receptor.

bonds predominated, indicating that the compounds interacted strongly with the receptor.[42,43] In particular, this is the first time that the docking interaction between these naturally occurring substances and the Angiotensin II receptor to accomplish the aforementioned biological activity as an antihypertensive has been identified.

Molecular Dynamic Simulation and MMPBSA. The study investigated the degree of stability and the changes that happened in the conformation of the protein−ligand complex through various computational methods. The RMSD analysis showed that the protein, ligand, and complex exhibited a lower RMSD, indicating their stability. It was observed that some

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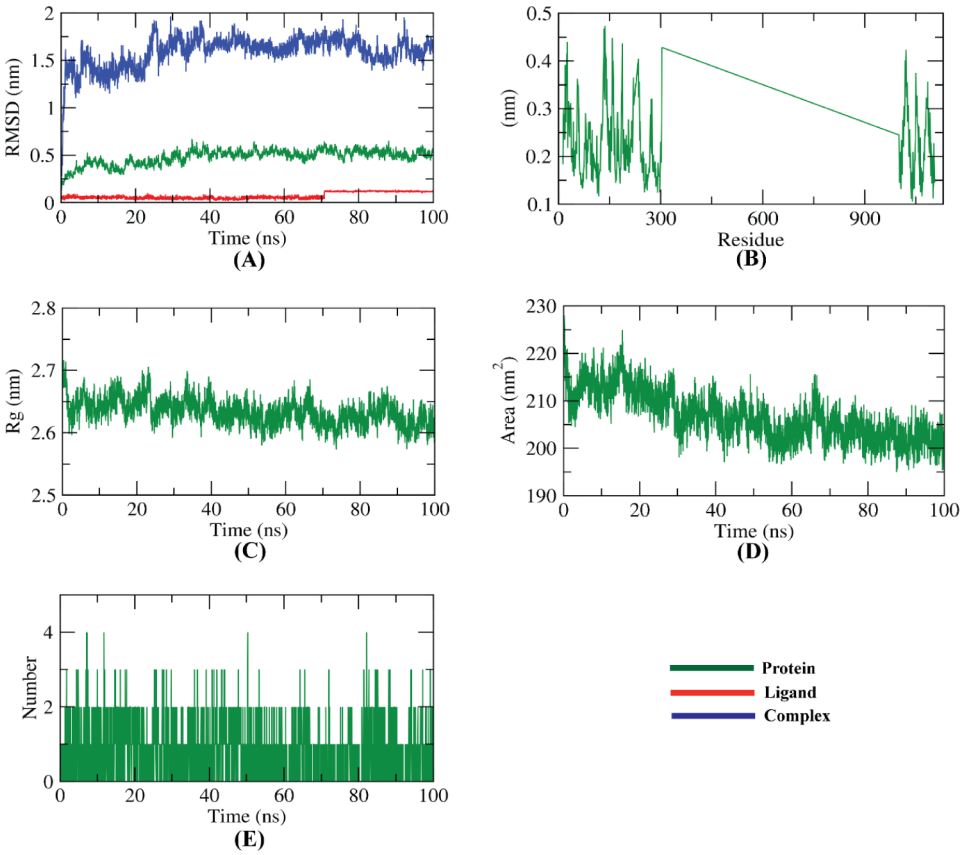

Figure 5. MD simulations of Angiotensin II receptor-Kaempferol complex: (A) RMSD, (B) RMSF, (C) Rg, (D) SASA, and (E) H-bond analysis.

minor fluctuations from 0 to 10 ns and 25−30 ns matched with amino acids between 300 and 900. Each residue flexibility was evaluated in terms of RMSF to have a better kind of protein regions that fluctuated during the imitation. Thus, the RMSF analysis showed that the binding of ligands did not allow the protein to be flexible in any residue areas, except the area between 300 and 900 amino acids showed some flexibility and minor fluctuations. The radius of gyration (Rg) analysis showed that the compound was compact, and its Rg was slightly lower than the starting period, indicating low movements and conformational changes. The SASA analysis showed that the protein presented a diminution in the surface area, indicating that it underwent fitting conformational changes during the interaction. Since hydrogen bonding between protein−ligand complexes is required for structural stability.[44,45] The study revealed that up to three hydrogen bonds are formed between the protein and the ligand, which are essential to stabilize the structure (Figure 5). In general, the examination of the MD simulation trajectory demonstrated that the presence of lead phytochemicals promoted the construction of a stable and energetically advantageous complex. The ligand-protein binding contribution of amino acids was demonstrated by the MM-PBSA analysis; therefore, the MM-PBSA further supports the docking results.

The Molecular Mechanics Poisson−Boltzmann Surface Area (MMPBSA). The MM/PBSA method was utilized to calculate the binding free energy of the protein−ligand complex with a 100 ps interval from MD trajectories of the last 20 ns of the invention track. The MmPbSaStat.py script was used to determine the average free binding energy and its standard deviation SD/error SE from the output files obtained from g_mmpbsa. The protein−ligand complex showed a binding free energy of −94 kJ/mol. Furthermore, the involvement of each residue in the protein to the required free energy was identified, allowing us to determine the residues that contribute favorably to the ligand-protein interaction.[46] The per-residue contribution energy was intended to be determined by disintegrating the total binding free energy of the system. This analysis revealed that CYS-18 and ILE-266 residues were crucial in binding with the ligand, as they donated >−5 kJ/mol binding energy and are considered flashpoint residues (Figure 6).

CONCLUSIONS

In conclusion, angiotensin receptor blockers (ARBs) are a widely used class of drugs that are well-tolerated and have a low occurrence of adverse reactions. They are commonly prescribed to treat elevated BP, congestive heart failure (CHF), and nephropathy due to DM, and are often used as an alternative to ACE inhibitors for patients who cannot tolerate them due to side effects. Olmesartan is a new ARB and was effective to control BP once per day, with a safety profile like that of a placebo. Recent studies have explored the potential of natural metabolites as inhibitors of the angiotensin receptor using molecular docking analysis, and promising results have been obtained for compounds such as myricetin and kaempferol. These natural metabolites hold promise as potential substitutes for conventional ARBs in the treatment of hypertension. Myricetin, kaempferol, and quercetin have structural and physicochemical similarities to olmesartan, suggesting pharmacological or medicinal applications. Eugenol, *p*-cymene, and gallic acid have more molecular features than

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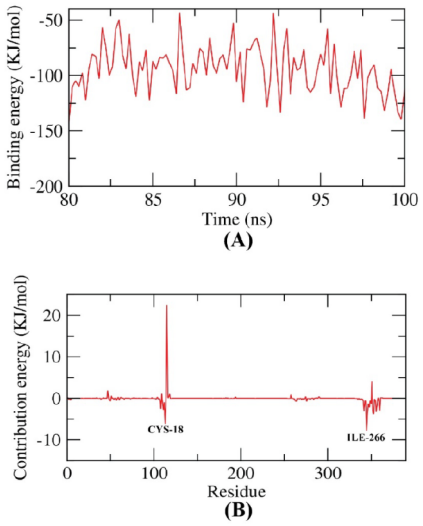

Figure 6. MM-PBSA study of the Angiotensin II receptor-Kaempferol complex: (A) binding energy and (B) contribution energy.

olmesartan. Furthermore, the shorter distances make compounds more comparable in similarity analysis, which can lead to more pharmacological and medical studies. Kaempferol, quercetin, and myricetin are similar to olmesartan, but eugenol, *p*-cymene, and gallic acid are dissimilar. The use of molecular docking is a powerful computational technique that provides critical insights into the therapeutic potential of natural metabolites as angiotensin receptor inhibitors, paving the way for the development of novel and effective natural-specific drugs as an alternative treatment for hypertension and related disorders such as type-2 diabetics. Myricetin and kaempferol have a higher affinity for the targeted site than olmesartan, a popular ARB. These findings show that natural metabolites such as myricetin and kaempferol may replace or enhance conventional ARBs for hypertension and type 2 diabetes. The current molecular docking investigation revealed that the phytochemicals exhibit notable binding capability at the protein−protein interaction sites and the active site of the ARB receptor. Additionally, the molecular dynamics (MD) simulation and MMPBSA calculations showed that the protein and ligand established a maximum of three hydrogen bonds, which are critical for the ligand complex's energy and structural stability. Moreover, the lead phytochemicals demonstrated that CYS-18 and ILE-266 residues were critical for ligand binding, as they contributed substantially to the complex's binding free energy and donated >−5 kJ/mol of binding energy, making them flashpoint residues. To sum up, as is generally accepted in documented pharmacological or clinical trials, the question of whether the actions of angiotensin receptor blockers will give more benefit for type 2 diabetes and more vascular profit than each type separately. This study could be the start of that, as more information about some naturally occurring ARBs that were the subject of this investigation is probably going to come to light. As adequately demonstrated by molecular docking studies and MD simulations, these blockers, particularly myricetin and kaempferol, exhibited properties that usually surpassed those of olmesartan as an ARB.

EXPERIMENTAL PROCEDURES

Materials and Methods. *Method of Molecular Similarity.* The molecular similarity calculation is a computer technique that evaluates the structural and physicochemical characteristics of two ligands, such as molecular weight, Log*p*, and distances between descriptors, to determine how similar the two molecules are. Using Discovery Studio software, the molecular similarity of six ligands with anticancer action against angiotensin receptor blockers was examined in this work using olmesartan as a reference. Rotatable bonds, cyclic rings, aromatic rings, hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), partition coefficient (ALog p), molecular weight, and molecular fractional polar surface area (MFPSA) were the molecular features investigated (Table 1).

Methodology of In-Silico Pre-ADMET and Pretoxicity Studies. The ADMET and toxicity studies were done using Biovia Discovery Studio software, which imported the compounds' structures in SDF format and calculated their properties using several models. In silico models predicted ADMET properties and toxicity, and evaluated based on established guidelines. Toxicity was predicted using the TOPKAT model, including FDA rodent carcinogenicity, carcinogenic potency in rats, maximum tolerated dose in rats, developmental toxicity potential, oral Lethal Dose 50 (LD50) in rats, chronic Lowest Observed Adverse Effect Level (LOAEL) in rats, ocular irritation in rats, and skin irritation in rats. These properties can be used to evaluate the safety of the compounds for human use and guide further research into their potential uses and toxicity. Compounds with favorable ADMET and toxicity profiles were selected and summarized in Table 2. Biovia Discovery Studio gave valuable insights into the safety and efficacy of the compounds, which can aid in further investigations.

Method of Docking Study. The MOE 19.0901 software was used to test ten natural metabolites against Angiotensin II receptor. The cocrystallized ligand within the crystal protein (PDB code: 4ZUD) that was acquired from the RCSB was utilized to create the binding sites.[47] In order to construct the targeted proteins, water molecules were eliminated, a quick preparation was done, missing amino acids were added, unfilled valence atoms were corrected, and CHARMM force fields were used to reduce the energy of the protein peptides. The protein's essential amino acids were chosen and ready for screening. Using Chem-Bio Draw Ultra17.0, the natural metabolites' 2D structures were created and saved in SDF file format. Then, using the MOE 19.0901 software, we studied the stored files. The ligands were protonated, and the energy was lowered using the MMFF94 force field with 0.1 RMSD kcal/mol. For molecular docking, the minimized structures were kept in storage. Using docking algorithms, the targeted pocket stayed rigid, while the ligands were given flexibility for easier molecular binding. During the refining, each molecule received permission to interact with protein in 20 ways. Discovery Studio 2019 Client was used to generate the 3D orientation and record the docking scores (affinity interaction energy) of the best-fitted poses with the Angiotensin II receptor active site.[48]

Method of Molecular Dynamic Simulation and MMPBSA. By the numerical solution of a system's equations of motion, molecular dynamics (MD) simulation is a computational technique used to study the motions of molecules and atoms.[49] The MD simulations are carried out using the commonly utilized Gromacs program. Chimaera was used to produce the protein−ligand combination, and Gromacs was used to create the topology and parameter files. NVT and NPT ensembles before a 100 ns production run were used to equilibrate the

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system after it had been solvated with water molecules. The binding free energy between the protein and ligand was calculated by the Molecular Mechanics Poisson−Boltzmann Surface Area (MMPBSA) method.[50] Gromacs tools were used to analyze the trajectory files in order to calculate the radius of gyration, hydrogen bonds, RMSD, RMSF, and SASA.[51] To understand the dynamic behavior of the protein−ligand complex and the impact of ligand binding on protein structure and stability, these were computed in 100 ns using Gromacs. The noted data can help with drug design and discovery as it provide a light on the stability and interactions of the protein−ligand complex.

■ ASSOCIATED CONTENT

Data Availability Statement

All data is contained in the published article.

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Notes

The authors declare no competing financial interest.

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