RESEARCH ARTICLE

In Silico profiling of the Angiotensin converting enzyme binding affinities, toxicity and Pharmacokinetics of compounds from the nuts of *Areca Catechu*, Linn. and its bioisosteres

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ABSTRACT

Background: Hypertension is a worldwide epidemic that has been recognized as the most leading global risk for mortality, with its prevalence associated with increased blood pressure, concomitant risks of cardiovascular and kidney diseases, and major commonality in individuals advanced in age. With the current treatment options for hypertension management, there is still a need to develop therapies that directly target receptors that aid in hypertension treatment.

Methodology: The study focused on the in-silico profiling of the reported compounds from Areca catechu L. (fam. Arecaceae) towards the n-domain and c-domain angiotensin converting enzyme (ACE) receptor models. Bioisosteric replacement was used to create bioisosteres investigated for similar binding affinity.

Results: Some A. catechu compounds exhibited favorable binding energies towards the n- and c-domain receptor models of ACE, binding in the same ACE ligand binding site as lisinopril, benazepril, and sampatrilat via similar interactions and amino acid residues. The majority of A. catechu compounds with favorable ACE binding energies belong to the phytochemical classes of flavonoids, polyphenols and phenolics, glycosides, and steroids. After in silico toxicity and pharmacokinetic profiling, the bioisosteres Leuco-DM02-39, Leuco-DM02-66, Leuco-DM05-60, Querc-DM09-63, and Querc-DM14-31 with binding energies higher than their parent compounds and comparable to lisinopril, benazepril, and sampatrilat were deemed the best.

Conclusion: A. catechu compounds have the potential to target ACE n-domain and c-domain receptor models. Three leucocyanidin and two quercetin bioisosteres exhibited favorable binding to the n-domain and c-domain ACE receptor models and could be further optimized to derive a promising antihypertensive agent through ACE inhibition.

Keywords: *Angiotensin Converting Enzyme (ACE) receptor models, Areca catechu, A. catechu Compounds, Bioisosteres, Binding Energy, Hypertension*

Introduction

Cardiovascular diseases are the leading cause of death for individuals between the age of 50 and 69, with high blood pressure holding the world record for highest number of deaths by risk factor. Hypertension is an important public-health challenge worldwide because of its high frequency and concomitant risks of cardiovascular and kidney diseases [1]. Hypertension has been reported to have no vivid symptoms, but sometimes it precipitates symptoms that include headache, dizziness, shortness of breath, nose bleeds, chest pain, and heart palpitations. Men are more prone to hypertension compared to their female counterparts and is also more common in people over the age of 65 than in younger individuals [2].

In the control of human blood pressure, angiotensin-converting enzyme (ACE) inhibitors are key players, which inhibit ACE from converting angiotensin I into angiotensin II [3]. ACE exists as two isoforms in mammals namely somatic ACE (sACE) and testis/testicular/germinal ACE (tACE) [4]. Each ACE isoform has one or two catalytic domains, which are both

zinc-metallopeptidase with the active motif HEMGH site of coordination between the zinc ion and 2 histidine residues. ACE is anchored to the membrane by the stalk, and upon its cleavage results in plasma ACE activity. sACE is composed of 1,306 amino acid residues, and with two catalytic domains (N- and C-domains) and a C-terminal transmembrane segment (stalk), whereas tACE possesses only one catalytic domain (C-domain) and has 665 amino acid residues. sACE is largely expressed in the retina and lung and also expressed in blood vessels, liver, intestine, adrenal gland, kidney, and uterus. tACE is manifested by post-meiotic male germ cells with high levels of expression in round and elongated spermatids [5]. Structurally, ACE contains two homologous domains, N-domain and C-domain, that have a similar sequence and three-dimensional (3D) structure, but are characterized by a different pharmacological profile, with distinctive specificities for substrates. Bradykinin is hydrolyzed by both domains of ACE. However, the hydrolysis of angiotensin I is entirely dependent on ACE C-domain and the hydrolysis of the anti-inflammatory peptide N-acetyl-SDKP is accomplished by ACE N-domain solely [6]. Though highly homologous in structure, the regulation of hematopoietic stem cell proliferation is associated with N-domain ACE, whereas C-domain ACE is primarily involved in the control of blood pressure [7]. This selectivity in angiotensin I conversion inhibition creates avenue for developing domain-specific drug molecules that could selectively bind to ACE C-domain. Hence, it is safe to assume that these selective ACE C-domain inhibitors would reduce angiotensin-II production while retaining normal bradykinin hydrolysis and reducing the side effects of currently marketed ACE inhibitors that are bradykinin mediated.

As about 25% of currently commercialized medications have been estimated to have been derived from plants used in traditional medicine, which indicates the importance of plants in drug development and as an aid in fighting diseases and chronic conditions, Areca catechu Linn. was utilized as the starting point for development of ACE inhibitor [8]. A. catechu Linn. (commonly known as Betel palm or Betel nut tree) is a species of palm which belongs to the Arecaceae family, with its nuts reported to contain polyphenols-mostly flavonoids and tannins, polysaccharides, fiber, fats, proteins, minerals, and alkaloids [9,10]. Inokuchi and team reported that Areca II-5-C, a fraction isolated from areca nuts, showed the most potent angiotensin-converting enzyme (ACE) inhibitory activity [11]. In addition, A. catechu nuts have also been reported to have antioxidant activity, antifungal activity, antimicrobial activity, antilipidemic activity, anthelmintic activity, larvicidal property, molluscicidal activity, and protection against ovariectomy-induced

osteoporosis, etc. [12–19]. With this information, the study geared its objective towards characterizing compounds reported in A. catechu and their bioisosteres for their ACE binding energies, the ACE C- and N-domains' amino acids residue they bound to, the types of receptor-ligand interaction displayed, as well as the structural similarities and differences among A. catechu compounds and their respective bioisosteres. Therefore, employing in silico molecular docking, toxicity studies, and pharmacokinetics profiling, this study used commercially marketed ACE inhibitors (benazepril, lisinopril, and sampatrilat) as its reference point for the investigation, comparison, and identification of the compounds and bioisosteres from A. catechu that possess comparable binding affinity in the ACE C-domain and N-domain active binding site.

Methodology

Literature Search

Using ScienceDirect and PubChem databases, a comprehensive literature search and review was conducted to identify all compounds that had ever been reported to be present in A. catechu. The resulting compounds were subjected to molecular docking, pharmacokinetic study, and toxicological profiling with the exclusion of fatty acids, minerals, amino acids, mycotoxins, trace elements, and carbohydrates and sugars.

Molecular Docking

The selection criteria for ACE C- and N-domain amino acids models entailed that models would have study reference (lisinopril and sampatrilat) bound within in it or bound with another compound (omapatrilat) related to the study reference (sampatrilat), and the model must have a resolution of 3.0 or below. For ACE C-Domain receptor models, 1O86, 6F9T, and 6H5W were bound with lisinopril, sampatrilat, and omapatrilat, and had resolutions of 2.00, 1.60, and 1.37, respectively [20–22]. For N-Domain ACE receptor models, 2C6N, 6H5X, and 6TT4 were bound with lisinopril, omapatrilat, and omapatrilat, and had resolutions of 3.00, 1.80, and 1.80, respectively [22–24]. These amino acid models also had to have an RMSD value of 2.00 or less after redocking with the ligands originally deposited inside them.

The crystalline structures of the amino acid models were downloaded in their PDB format from the Protein Data Bank and then converted to PDBQT format using the Autodock Tools v1.5.7. The 3D structures of the ligands were

downloaded from PubChem as SDF file format. The SDF format was converted to MOL2 format using Open Babel. Subsequently, the MOL2 format was converted into PDBQT format using Autodock Tools. MMFF94 energy optimization of the PDBQT format was performed using Open Babel. Grid Box optimization was performed, and co-crystallized ligands were redocked in the receptors using Autodock Tools v1.5.7 and Autodock Vina. Redocking was performed five times, with each resulting PDBQT file of the molecules having RMSD values less than 2 with respect to the crystallized ligand's original pose. Pymol and Discovery Studio Visualizer were used to visualize PDBQT files [25].

Bioisosteric Replacement

MolOpt was used to generate bioisosteres using a string of SMILES as input with a subsequent provision of a CSV output file containing the 2D bioisostere structures as well as the absorption, distribution, metabolism, excretion, and toxicity (ADMET) profile [26]. Stereoisomers were not recognized because the web server treated molecules in two-dimensional configurations. The ADMET profiles of all the bioisosteres generated by the web server were used to screen them prior to molecular docking. Bioisosteres were generated in their energy optimized 3D formats using Autodock Tools v1.5.6 and Open Babel.

Toxicity and Pharmacokinetic Screening

SWISS-ADME and pkCSM were used to analyze the pharmacokinetic properties (absorption, distribution, metabolism and excretion) of the bioisosteres, while pkCSM and Toxicity Estimation Software Tool (TEST) App were used for toxicity profiling. Inclusion criteria for bioisosteres were no toxicity (AMES toxicity/mutagenicity, hERG I and II inhibitor, and hepatotoxicity), good gastrointestinal absorption or bioavailability, and passing any of the drug-likeness rules. Cardiotoxicity of bioisosteres were investigated on eMolTox [27–32].

Results

Literature Search

A total of 195 entries were gathered after literature review using ScienceDirect and PubChem databases only. These included polyphenols and phenolics, alkaloids, amino acids, anthraquinones, carbohydrates and sugars, carotenoids, cyclic peptides, fatty acids, flavonoids, glycosides, minerals, mycotoxins, other phytochemicals, steroids, stilbenoids,

tannins, trace elements, and triterpenes. The 195 entries were narrowed down to 105 compounds by removing amino acids, carbohydrates and sugars, fatty acids, minerals, mycotoxin, and trace elements. Since amino acids, sugars, and fatty acids were the simplest building components for proteins, carbohydrates, and lipids, respectively, they were eliminated from the study. Minerals and trace elements were excluded, because these elements were in their atomic or ionic states, and this study did not involve the modification of atomic structure. Mycotoxin were omitted as they were naturally occurring poisonous substances produced by various forms of mold (fungi). Entries that represented a group or class of phytochemical compounds rather than a single compound were also excluded. In addition, structures were excluded if their SMILES, Structure, or Identity could not be located on PubChem or generated with the TEST app. One of each duplicate entry was also eliminated. Thereafter, 105 compounds investigated in the study were composed of 24 polyphenols and phenolics, 13 alkaloids, two anthraquinones, one carotenoid, one cyclic peptide, 21 flavonoids, 10 glycosides, five other phytochemicals, six steroids, two stilbenoids, 13 tannins, and seven triterpenes (Supplemental Material 1).

Molecular Docking and Bioisosteric Replacement

Based on in silico results, lisinopril was used as the benchmark for identifying A. catechu compounds with binding energies of -7 and below because it has a binding energy in the range of -7, which was lower compared to -5 free binding energy of captopril. Additionally, lisinopril has been reported to have key features such as a long half-life, hydrophilic, and ability to not be broken down by the liver unlike captopril. Lisinopril is the commonly recommended first line ACE inhibitor for hypertension [33,34]. Therefore, the average of five trials of binding energies of the A. catechu compounds with binding energies of -7 or lower for each of the six receptor models were presented in Table 1. Of the 105 A. catechu compounds, 38 compounds were found to have binding energies of lines of -7 or lower across all six ACE receptor models. These 38 compounds included six polyphenols, two anthraquinones, 21 flavonoids, four glycosides, three steroids, one stilbenoid, and one tannin. Of the 38 A. catechu compounds, 18 compounds had at least in one receptor model a binding energy in the line of -9. The top 5 compounds in the 18 compounds had at least in two receptor models binding energies in the line of -9 across all receptor models. The next 5 compounds in the 18 compounds had only in one or two receptor models a binding energy in the line of -7. Therefore, these top ten A. catechu compounds (naringenin-7-O-

glucoside, isorhamnetin 3-O-(6"-O-α-L-rhamnopyransoyl) β-D-glucopyranoside, acatechu B, (-)-gallocatechin gallate, naringin, epigallocatechin gallate, jacareubin, rutin, leucocyanidin, and quercetin) based on binding energy were subjected to bioisosteric replacement for the generation of bioisosteres.

The top ten A. catechu compounds based on binding energy criteria of at least one binding energies in the line of -9 and only one or two binding energies in the line of -7 across all six receptor models were subjected to bioisosteric replacement using MolOpt website. Utilizing the data mining function of the MolOpt website, 14916 bioisosteres were generated from these ten A. catechu compounds. Upon generation of bioisosteres, the MolOpt website provides a csv file containing information about the bioisosteres, which include their SMILES, chemical structure and properties, pharmacokinetic properties, and several toxicity profiles. The inclusion criteria for the bioisosteres based on MolOpt data generated in a .csv file were synthetic accessibility lower than 6, high human intestinal absorption, no mutagenic potential, no carcinogenic potential, does not induce genotoxicity, does not cause drug induced liver injury, and not a hERG blocker [27–32].

Synthetic accessibility depicts the ease for which a drug-like molecule could be developed and validated and is scored between one (easy to make) and ten (very difficult to make), therefore, 6.0 is proposed as the threshold for distinguishing between easy- and difficult-to-synthesize drug-like molecules [27,28]. High human intestinal absorption is an essential factor during drug development especially for orally intended medicines as this affects the bioavailability of the developed drug molecule [29]. Mutagenic and genotoxic potential of compounds from A. catechu were investigated using the AMES test, which is one of the most used tests to assess the mutagenic potential of medicinal plants [30]. The ability of a drug-molecule to induce liver injury and its carcinogenic potential are critical issues identified by Food and Drug Administration (FDA) and International Council for Harmonization (ICH) guidelines as these issues impact prediction of human risk during drug development [31]. Drug-induced blockage of the human ether-a-go-go-related gene (hERG) continues to be a key hurdle to bringing safe medications to market because the inhibition of hERG results in sudden death attributed to side effect of non-antiarrhythmic drugs [32]. Based on these criteria, only 376 bioisosteres met the inclusion criteria and were subjected to molecular docking (Supplemental Material 2).

After the 376 compounds were subjected to molecular docking, benazepril and sampatrilat which had binding energies in the lines of -8 were utilized as points of reference to identify bioisosteres with binding energies of -8 and lower. Sampatrilat as a dual inhibitor of ACE and neutral endopeptidase, is more potent than Lisinopril [6]. Sampatrilat has been reported to show more selectivity towards the subsites of the C-Domain ACE model [21]. Additionally, the in silico study of Ningsih & Novianty, which investigated bioisosteres of arecoline (an A. catechu reported compound) utilized a benchmark binding energy of -8.1 kcal/mol for arecoline bioisosteres compared to the -5.8 kcal/mol free binding energy of arecoline [35]. Hence, the binding energies criteria for the bioisosteres of A. catechu compounds were set at -8, which is higher than the binding energies criteria for their parent compounds. Of the 376 bioisosteres, 31 bioisosteres were found to have binding energies of lines of -8 or lower across all six ACE receptor models. These 31 bioisosteres were identified to be from three A. catechu compounds – naringenin-7-O-glucoside (nine bioisosteres), leucocyanidin (ten bioisosteres) and quercetin (13 bioisosteres). The binding energies of the top-performing bioisosteres of naringenin-7-O-glucoside, leucocyanidin, and quercetin were presented in Table 2.

Observation of the docking of lisinopril, naringenin-7-O-glucoside, leucocyanidin and quercetin into the C-domain 1O86 ACE receptor model, and N-domain 2C6N ACE receptor model revealed that these compounds had similar forms of interactions in the binding sites (Figure 1). The common chemical interactions observed were hydrogen (conventional and carbon) bonds, electrostatic (salt bridge, attractive charges, cationic and anionic) interactions, hydrophobic (Pi-Sigma, Pi-Pi Stacked, Pi-Pi T-shaped, Pi-Alkyl and Alkyl) interaction, metal-acceptor interaction with the charged zinc ion, and unfavorable interactions.

Leuco-DM02-39, Leuco-DM02-66, Leuco-DM05-60, Querc-DM09-63 and Querc-DM14-31 all displayed similar binding-site interactions when docked into the C-domain 1O86 and N-domain 2C6N ACE receptor models (Figure 2). The most frequently observed chemical reactions were hydrogen (conventional, carbon and Pi-donor) bonds, electrostatic (salt bridge, attractive charges, cationic and anionic) interactions, hydrophobic (Pi-Sigma, Pi-Pi Stacked, Pi-Pi T-shaped, Pi-Alkyl and Alkyl) interaction, metal-acceptor interaction with the charged zinc ion, and unfavorable interactions.

Naringenin-7-O-glucoside is a flavone 7-O-beta-glucoside with IUPAC name of 5-hydroxy-2-(4-hydroxyphenyl)-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6- (hydroxymethyl)oxan-2-yl]oxy-2,3-dihydrochromen-4-one created when naringenin was

Table 1. *ACE Receptor Models Binding Energies of Captopril, Lisinopril and A. catechu Compounds.*

Ligands	Phytochemical Group	C- Domain Models			N- Domain Models		
		1O86	6F9T	6H5W	2C6N	6H5X	6TT4
Captopril	Not Applicable	-5.36	-5.36	-5.8	-5.38	-5.74	-5.82
Lisinopril	Not Applicable	-7.02	-6.94	-7.14	-6.88	-7.26	-7.26
Naringenin-7-O-glucoside	Flavonoid	-8.8	-9.4	-8.3	-8.9	-9.46	-9.14
Isorhamnetin 3-O-(6"-O-α-L-rhamnopyransoyl) β-D-glucopyranoside	Glycoside	-8.9	-9	-8.12	-8.74	-9.5	-9
Acatechu B	Glycoside	-8.82	-8.06	-8.88	-8.88	-9.38	-9.06
(-)-Gallocatechin gallate	Flavonoid	-8.46	-7.84	-9.18	-8.52	-9.4	-8.64
Naringin	Polyphenol	-8.7	-9.5	-7.7	-9.26	-8.62	-8.86
Epigallocatechin gallate	Polyphenol	-8.74	-8.4	-8.24	-8.28	-9.6	-8.68
Jacareubin	Flavonoid	-8.3	-8.2	-8.88	-8.32	-9.14	-8.66
Rutin	Polyphenol	-7.92	-8.52	-8.58	-10.02	-8.96	-8.92
Leucocyanidin	Polyphenol	-8	-7.3	-9.2	-8.2	-8.7	-7.4
Quercetin	Flavonoid	-8.2	-7.3	-9.1	-8.4	-8.8	-7.7
Hyperoside [Quercetin-3-O-galactoside]	Flavonoid	-8.16	-7.52	-7.76	-7.98	-9.14	-8.1
Trans-piceid (a.k.a. Polydatin)	Stilbenoid	-8.02	-7.9	-8.7	-7.8	-9.04	-7.84
Ergosterol peroxide	Steroid	-8.32	-9.3	-7.56	-7.68	-8.22	-7.96
Leucopelargonidin	Flavonoid	-8.1	-7.2	-9.1	-7.9	-8.2	-7.46
(+/-)-Catechins	Flavonoid	-7.9	-7.2	-9	-7.8	-8.3	-7.62
L-epicatechins	Flavonoid	-7.9	-7.2	-9	-7.8	-8.2	-7.62
D-epicatechins	Flavonoid	-7.9	-7.1	-9	-7.82	-8.6	-7.48
Astragalin [Kaempferol-3-O-glucoside]	Flavonoid	-7.5	-7.3	-7.28	-7.96	-9.38	-7.96
Chrysoeriol	Flavonoid	-8.3	-7.4	-8.3	-8.32	-8.2	-8
4',5'-dihydroxy-3',5',7'-trimethoxyflavonone	Flavonoid	-8	-7.7	-8.36	-8.1	-8.22	-8
β-sitostenone	Steroid	-8.46	-8.84	-8.96	-7.3	-8.18	-8.34
Luteolin	Flavonoid	-8.38	-7.4	-8.3	-8.6	-8.5	-7.9
Naringenin	Flavonoid	-8.3	-7.6	-8	-8.6	-8.3	-7.8
Isorhamnetin	Flavonoid	-8.2	-7.4	-8.9	-8.2	-8.6	-7.74
5,7,4'-trihydroxy-3',5'-dimethoxy flavanone	Flavonoid	-8.16	-7.32	-8.1	-8.08	-8.32	-7.86
Liquiritigenin	Flavonoid	-8.4	-7.3	-8.4	-8	-8.2	-7.7
(s)-5-hydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-7-methoxychroman-4-one	Flavonoid	-8.1	-7.42	-7.66	-8.26	-8.3	-8.1
Kaempferol	Flavonoid	-8.2	-7.1	-8.8	-8.12	-8.3	-7.5
Guaiaverin	Glycoside	-8.06	-7.64	-7.58	-8.04	-8.9	-8.38
Ellagic acid	Tannin	-8.1	-7.4	-8.6	-7.7	-8.6	-7.88
(±)-5-hydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-7-methoxychroman-4-one	Flavonoid	-7.92	-7.6	-7.9	-8.16	-8.18	-8
Isohamnetin-3-O-galactoside	Flavonoid	-7.36	-7.42	-7.44	-8.14	-8.9	-8.06
Physcion (a.k.a. Physione)	Anthraquinone	-7.9	-7.1	-8.5	-7.6	-8.1	-7.7
Stigmasta-4-en-3-one	Steroid	-7.16	-8.22	-7.6	-8	-7.64	-7.78
Flavan-3,4-diol	Polyphenol	-7.3	-7.7	-8.2	-7.52	-7.4	-7.74
Hexahydroxyflavan	Polyphenol	-7.6	-7.36	-8.1	-7.14	-7.78	-7.7
Chrysophanol	Anthraquinone	-7.8	-7.6	-8.7	-7.2	-7.92	-7.48
Acatechu A	Glycoside	-7.9	-7.1	-7.68	-7.08	-8.06	-7.58

Table 2. *Average ACE Receptor Models Binding Energies of Benazepril, Sampatrilat and Bioisosteres across Six Receptor Models.*

Bioisosteres	C Domain Models			N Domain Models		
	1O86	6F9T	6H5W	2C6N	6H5X	6TT4
Benazepril	-8.22	-7.84	-8.42	-7.7	-8.26	-7.8
Sampatrilat	-8.1	-7.46	-7.74	-7.94	-8.72	-8.36
Narin-DM05-07	-10.3	-9.58	-9.58	-9.38	-10.34	-10.4
Narin-DM05-08	-10.12	-9.3	-9.58	-9.9	-10.4	-10.2
Narin-DM05-14	-9.9	-9.2	-9.6	-9.7	-10.18	-10.1
Narin-DM05-19	-9.92	-9.6	-9.66	-9.9	-9.8	-9.2
Narin-DM14-00	-9.44	-8.8	-9.1	-9.8	-9.98	-9.36
Narin-DM14-02	-9	-9.1	-8.82	-8.9	-9.38	-9.08
Narin-DM14-39	-8.86	-9.76	-8.5	-9.36	-9.52	-9.22
Narin-DM14-49	-8.98	-9.32	-8.5	-9.08	-9.46	-9.14
Narin-DM14-50	-9.4	-9.26	-9.28	-9.1	-9.44	-9.5
Leuco-DM01-50	-8.32	-8.5	-8.3	-8.38	-8.76	-8.16
Leuco-DM01-89	-8.38	-8.2	-8.56	-8.04	-9.14	-8.32
Leuco-DM02-39	-8.34	-8.3	-8.88	-8.02	-8.6	-8.34
Leuco-DM02-66	-8.3	-8.1	-9.1	-8.1	-8.34	-8.1
Leuco-DM03-50	-8.24	-8.32	-9.74	-8.38	-8.72	-8.46
Leuco-DM03-70	-8.32	-8.8	-8.5	-8.08	-9.2	-8.42
Leuco-DM05-53	-8.24	-8.06	-9.8	-8.48	-8.76	-8.38
Leuco-DM05-60	-8.52	-8.26	-9.2	-8.3	-8.36	-8.02
Leuco-DM05-63	-8.58	-8.3	-9.2	-8	-8.84	-8.3
Querc-DM00-35	-9.3	-8	-8.58	-8.3	-9.4	-8.3
Querc-DM00-73	-8.38	-8.2	-8.7	-8.1	-9.66	-8.4
Querc-DM05-91	-8.7	-8.04	-9.28	-8.12	-9.06	-8.48
Querc-DM09-63	-8.64	-8.7	-9.1	-8.22	-8.8	-8.8
Querc-DM12-20	-8.04	-8.36	-8.6	-8.1	-9.04	-8.12
Querc-DM12-35	-8.5	-8.5	-8.78	-8.5	-9.52	-8.6
Querc-DM13-20	-8.6	-8.3	-8.7	-8.52	-9.04	-8.8
Querc-DM13-35	-9.08	-8.26	-9.2	-8.9	-9.32	-9.1
Querc-DM13-52	-8.78	-8.04	-8.68	-8.2	-8.64	-8.3
Querc-DM14-31	-8.18	-8.2	-8.1	-8.12	-8.46	-8.02
Querc-DM14-35	-8.72	-8.5	-8.58	-8.78	-9.14	-8.08
Querc-DM15-20	-8.52	-8	-8.12	-8	-9.2	-8.14
Querc-DM15-73	-8.5	-8.3	-8.44	-8.5	-10	-8.66
Legends: *Narin - Naringenin-7-O-glucoside; Leuco - Leucocyanidin; Querc - Quercetin*						

substituted by a beta-D-glucopyranosyl moiety at position 7 via a glycosidic linkage [36]. Leucocyanidin is a leucoanthocyanidin with IUPAC name of 2-(3,4- dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,4,5,7-tetrol [37]. Quercetin is a polyphenolic flavonoid with IUPAC name of 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy chromen-4-one [38]. The absolute configuration for the docked bioisosteres were (2R,3S,4S) 2-[3-(trifluoromethyl)phenyl] chromane-3,4,5,7-tetrol for Leuco-DM02-39, (2'R,3'S,4'S) 3-(3,4,5,7-tetrahydroxychroman-2-yl) benzoic acid for Leuco-DM02-66, (2R,3S,4S) 2-[4-hydroxy-3-(trifluoromethyl)phenyl] chromane-

3,4,5,7-tetrol for Leuco-DM05-60, 3,5,7-trihydroxy-2-[3-hydroxy-5-(trifluoromethyl)phenyl] chromen-4-one for Querc-DM09-63, and 2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-7-(1-hydroxy-1-methyl-ethyl) chromen-4-one) for Querc-DM14-31 (Figure 3).

Toxicity and Pharmacokinetic Screening

Per the MolOpt generated data, most of the 14,916 generated bioisosteres caused myocardial damage including the top-performing bioisosteres of naringenin-7-O-glucoside,
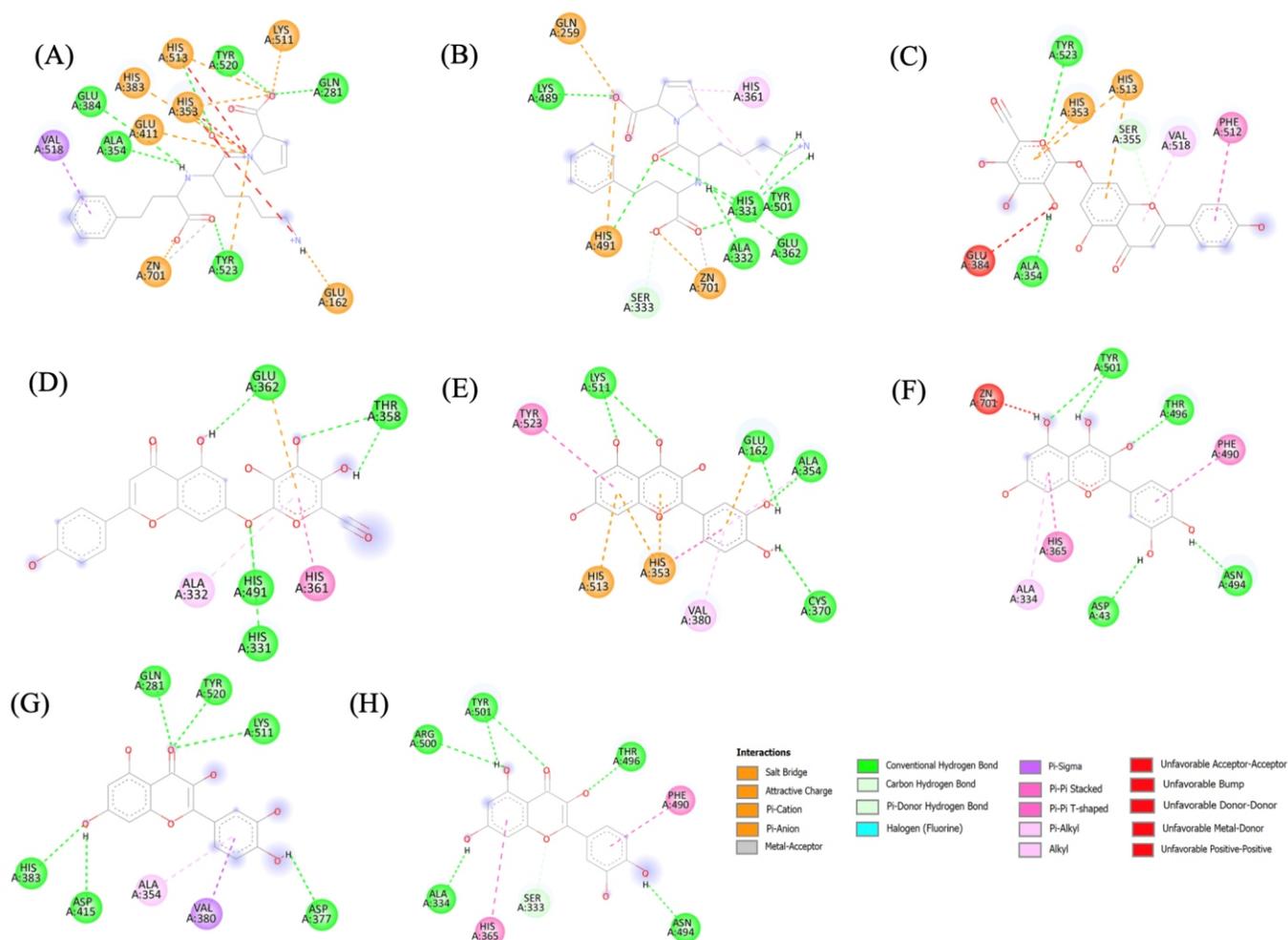
Figure 1. *Docking of molecules into ACE receptor models. (A) Molecular docking of lisinopril into ACE C-domain receptor model 1O86. (B) Molecular docking of lisinopril into ACE N-domain receptor model 2C6N. (C) Molecular docking of naringenin-7-O-glucoside into ACE C-domain receptor model 1O86. (D) Molecular docking of naringenin-7-O-glucoside into ACE N-domain receptor model 2C6N. (E) Molecular docking of leucocyanidin into ACE C-domain receptor model 1O86. (F) Molecular docking of leucocyanidin into ACE N-domain receptor model 2C6N. (G) Molecular docking of quercetin into ACE C-domain receptor model 1O86. (H) Molecular docking of quercetin into ACE N-domain receptor model 2C6N.*

leucocyanidin, and quercetin. Therefore, these bioisosteres were further investigated for cardiotoxicity using the eMolTox website (Table 3).

A total of 31 bioisosteres were identified to have binding energies of -8 and below, which was comparable to that of benazepril and sampatrilat. These bioisosteres per MolOpt data has the potential to cause myocardial damage and were therefore investigated for their ability to cause cardiotoxicity using the eMolTox website. Of these 31 bioisosteres, 15 were tagged with potential for cardiotoxicity by eMolTox – cardiotoxicity (Table 3). Based on toxicity and pharmacokinetics results generated by pkCSM, TEST App and Swiss ADME in addition to eMolTox results, only five bioisosteres met criteria of synthetic accessibility lower than 6, high human intestinal

absorption, good bioavailability score, non- mutagenic, non-hepatotoxic, and a non-hERG I and II blocker [27–32]

Discussion

A review of the scientific literature was conducted to identify all molecules reported to be present in A. catechu. The study was able to reduce the number of compounds from 195 to 105 by eliminating the following: amino acids, carbohydrates and sugars, fatty acids, minerals, mycotoxin, and trace elements; entries that represented a group or class of phytochemical compounds rather than a single compound; molecules whose SMILES, Structure, or Identity could not be located on PubChem or generated with the TEST app; and one of each duplicate entry. The binding
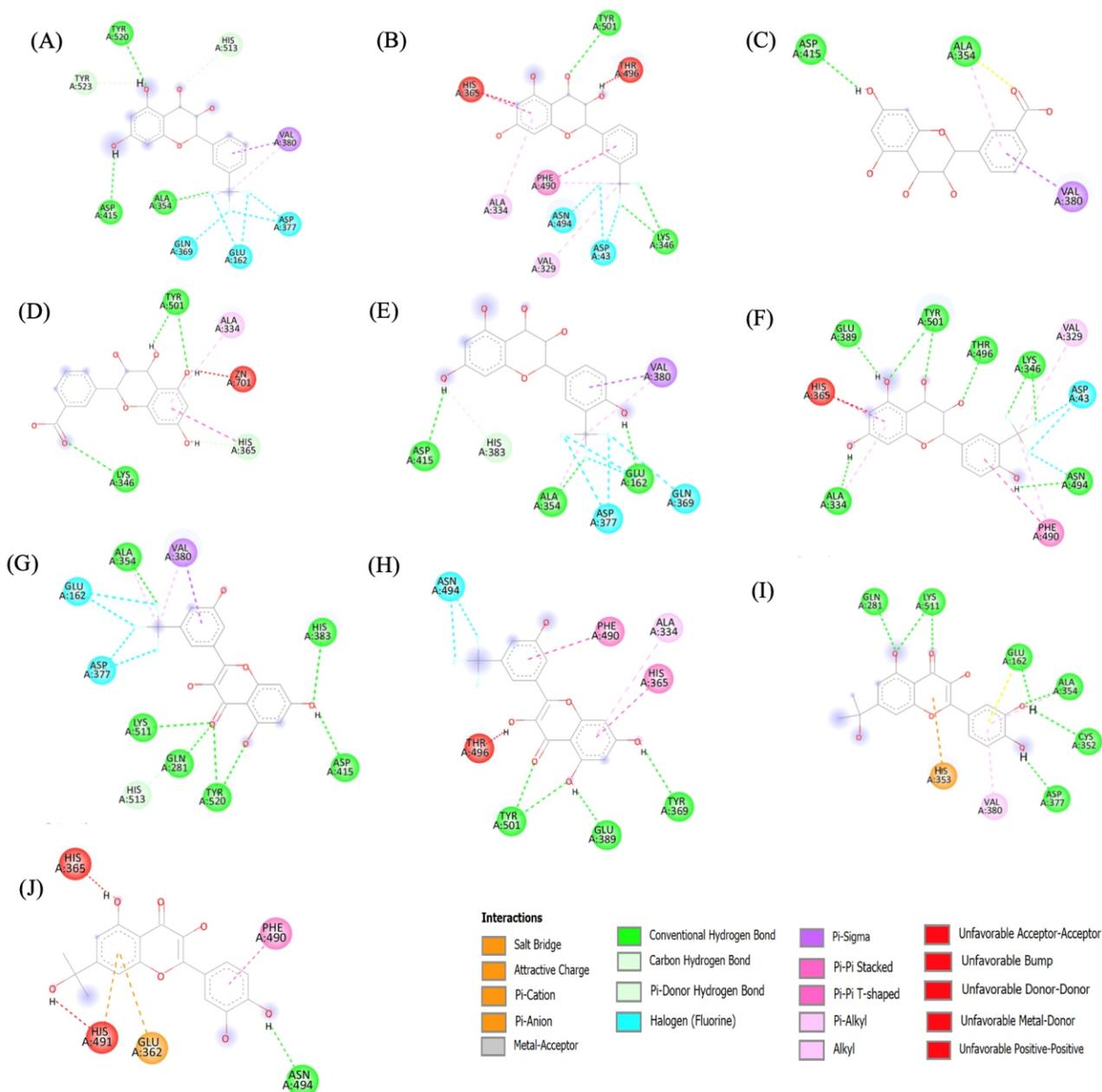
Figure 2. *Docking of molecules into ACE receptor models. (A) Molecular docking of Leuco-DM02-39 into ACE C-domain receptor model 1O86. (B) Molecular docking of Leuco-DM02-39 into ACE N-domain receptor model 2C6N. (C) Molecular docking of Leuco-DM02-66 into ACE C-domain receptor model 1O86. (D) Molecular docking of Leuco-DM02-66 into ACE N-domain receptor model 2C6N. (E) Molecular docking of Leuco-DM05-60 into ACE C-domain receptor model 1O86. (F) Molecular docking of Leuco-DM05-60 into ACE N-domain receptor model 2C6N. (G) Molecular docking of Querc-DM09-63 into ACE C-domain receptor model 1O86. (H) Molecular docking of Querc-DM09-63 into ACE N-domain receptor model 2C6N. (I) Molecular docking of Querc-DM14-31 into ACE C-domain receptor model 1O86. (J) Molecular docking of Querc-DM14-31 into ACE N-domain receptor model 2C6N.*

energies of these 105 compounds at the active binding site of ACE were then determined by docking them into the crystal structure of ACE.

Based on in silico results, the -7 binding energy of lisinopril, which was lower than the -5 binding energy of captopril, served as the standard by which A. catechu compounds with lower

binding energies were identified. Lisinopril was used as the benchmark as it is the commonly recommended first line ACE inhibitor for hypertension [33,34]. Of the 105 compounds found in A. catechu, 38 had binding energies of lines -7 or below for all six ACE receptor models. The top ten A. catechu compounds according to binding energies had binding energies in the -9 range in at least one receptor model, and binding
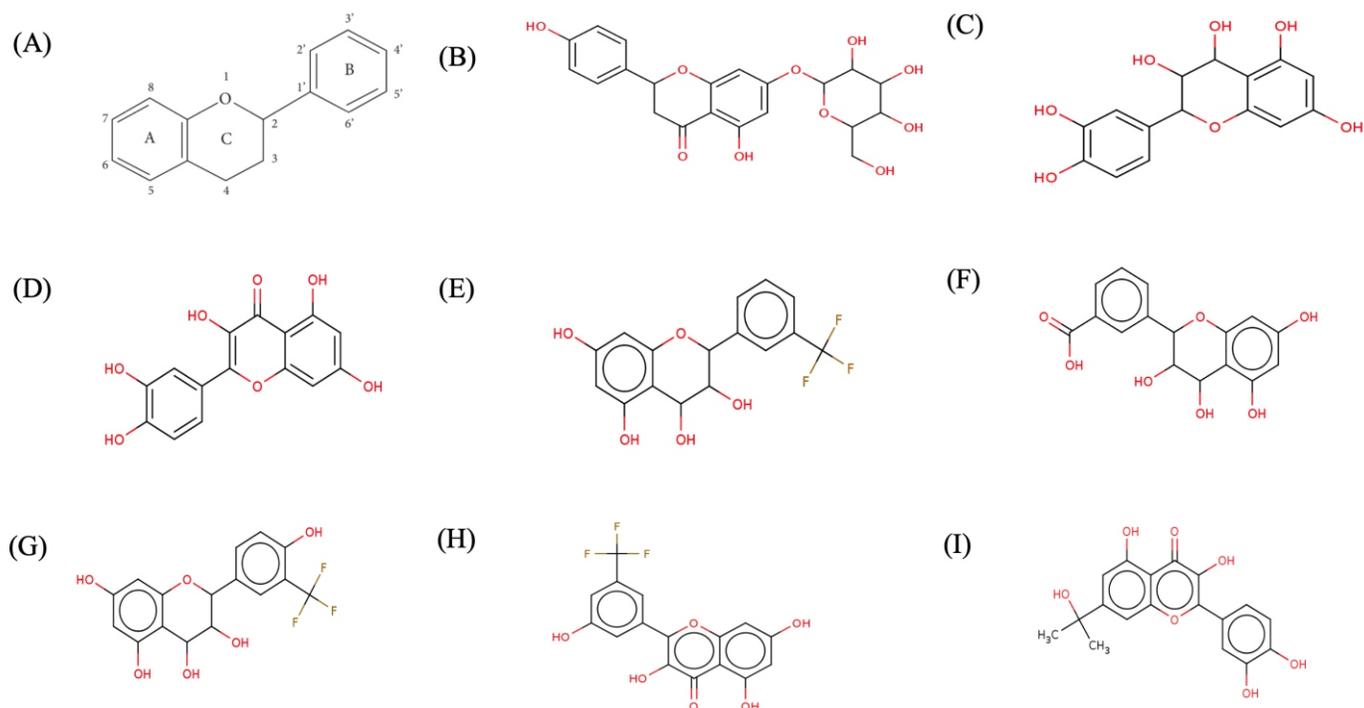
Figure 3. *Molecular structures of: (A) Unsubstituted flavonoid. (B) Naringenin-7-O-glucoside. (C) Leucocyanidin. (D) Quercetin. (E) Leuco-DM02-39 ((2R,3S,4S) 2-[3-(trifluoromethyl)phenyl] chromane-3,4,5,7-tetrol). (F) Leuco-DM02-66 ((2'R,3'S,4'S) 3-(3,4,5,7-tetrahydroxychroman-2-yl) benzoic acid). (G) Leuco-DM05-60 ((2R,3S,4S) 2-[4-hydroxy-3-(trifluoromethyl)phenyl] chromane-3,4,5,7-tetrol). (H) Querc-DM09-63 (3,5,7-trihydroxy-2-[3-hydroxy-5-(trifluoromethyl)phenyl] chromen-4-one). (I) Querc-DM14-31 (2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-7-(1-hydroxy-1-methyl-ethyl) chromen-4-one).*

energies in the -7 range in only one or two receptor models. These top ten A. catechu compounds were naringenin-7-O-glucoside, isorhamnetin 3-O-(6''-O-α-L-rhamnopyransoyl) β-D-glucopyranoside, acatechu B, (-)-gallocatechin gallate, naringin, epigallocatechin gallate, jacareubin, rutin, leucocyanidin, and quercetin, and they were subjected to bioisosteric replacement for the generation of bioisosteres.

Ten compounds from A. catechu were subjected to bioisosteric substitution using the data mining feature of the MolOpt website, yielding a total of 14916 bioisosteres. Bioisosteres were included in the study if they met the following criteria, as established by the MolOpt database: synthetic accessibility below 6, high human intestine absorption, no mutagenic or carcinogenic potential, does not cause genotoxicity, does not cause drug-induced liver damage, and is not a hERG blocker [27–32]. Consequently, only 376 bioisosteres met the criteria and were docked at the active site of ACE. Among the 376 compounds, 31 had binding energies of lines of -8 or below for all six ACE receptor models, which was comparable to benazepril and sampatrilat. Naringenin-7-O-glucoside (nine bioisosteres), leucocyanidin (ten bioisosteres), and quercetin (13 bioisosteres) are the three A. catechu compounds shown to be responsible for these 31 bioisosteres.

The docking of these compounds and bioisosteres into the ACE 1O86 model happened between the S1' and S2' subsites (Figure 1 and Figure 2). In the S1' subsite, the amino acid residues important for this binding pattern were GLU162 (conventional hydrogen bond, halogen, and anionic electrostatic interactions), ALA354 (conventional hydrogen bond, hydrophobic and halogen interactions), GLU369 (halogen interactions), ASP377 (conventional hydrogen bond and halogen interactions), and VAL380 (hydrophobic interactions). The fourth, fifth, and seventh positions of the chromene-3,4,5,7-tetrol and chromen-4-one rings were the key position for interactions in the ACE 1O86 models' S2' subsite. The hydroxyl and carbonyl groups at the fourth and fifth position had interactions with amino acid residues GLN281, LYS511, TYR520, and TYR523; and the hydroxyl group at the seventh position had interactions with amino acid residues HIS383 (conventional and carbon hydrogen bond) and ASP415 (conventional hydrogen bond).

In the docking of leucocyanidin in 2C6N, it had a binding energy of -8.2. Leuco-DM02-39, Leuco-DM02-66, and Leuco-DM05-60 had binding energies of -8.1, -8.1, and -8.3, respectively. Hence, Leuco-DM05-60 performed better than leucocyanidin, Leuco-DM02-39, and Leuco-DM02-66. This revealed that the presence of both hydroxyl and

Table 3. *Cardiotoxicity Results of Standards, Compounds and Bioisosteres.*

Standards, Compounds and Bioisosteres	Molecule Flagged Myocardial Damage by MolOpt	eMolTox Cardiotoxicity Results
Benazepril	N/A	6
Lisinopril	N/A	6
Sampatrilat	N/A	N/C
Naringenin-7-O-glucoside	N/A	N/C
Narin-DM05-07	YES	1, 3
Narin-DM05-08	YES	1
Narin-DM05-14	YES	1
Narin-DM05-19	YES	1
Narin-DM14-00	YES	1
Narin-DM14-02	YES	1
Narin-DM14-39	YES	1
Narin-DM14-49	YES	1, 3
Narin-DM14-50	YES	1, 3
Leucocyanidin	N/A	4, 5
Leuco-DM01-50	YES	4
Leuco-DM01-89	YES	N/C
Leuco-DM02-39	YES	N/C
Leuco-DM02-66	YES	N/C
Leuco-DM03-50	YES	4, 5
Leuco-DM03-70	YES	4
Leuco-DM05-53	YES	4, 5
Leuco-DM05-60	YES	N/C
Leuco-DM05-63	YES	N/C
Quercetin	N/A	2, 5
Querc-DM00-35	YES	N/C
Querc-DM00-73	YES	N/C
Querc-DM05-91	YES	A
Querc-DM09-63	YES	N/C
Querc-DM12-20	YES	N/C
Querc-DM12-35	YES	N/C
Querc-DM13-20	YES	N/C
Querc-DM13-35	YES	N/C
Querc-DM13-52	YES	N/C
Querc-DM14-31	YES	N/C
Querc-DM14-35	YES	N/C
Querc-DM15-20	YES	5
Querc-DM15-73	YES	N/C

Legends:
N/A - Not applicable; N/C - Not cardiotoxic; 1 - Antagonist of the peroxisome proliferator-activated receptor gamma (PPARg) signaling pathway (Kidney, heart, immune); 2 - Agonist of the peroxisome proliferator-activated receptor gamma (PPARg) signaling pathway (Kidney, heart, immune); 3 - Antagonist of the thyroid receptor (TR) signaling pathway (Endocrine & heart); 4 - Modulator of Dopamine D1 receptor (CNS, Kidney & Heart); 5 - Modulators of myocardial damage (Heart); 6 - Modulators of Angiotensin-Converting Enzyme (Heart, Kidney); A - Mitochondrial toxicity;

trifluoromethyl groups (in Leuco-DM05-60) at the third and fourth positions of phenyl group showed the lowest binding energy compared to one carboxyl group (in Leuco-DM02-66), one trifluoromethyl group (Leuco-DM02-39), or two hydroxyl groups (in leucocyanidin). In the docking of quercetin in 2C6N, it had a binding energy of -8.4. Querc-DM09-63 and Querc-DM14-31 had binding energies of -8.3 and -8.2, respectively. Quercetin performed better than Querc-DM09-63 and Querc-DM14-31.

The seventh position of the chromen-4-one ring in naringenin-7-O-glucoside was the best possible site in the compound to make substitutions to generate bioisosteres. This was evident with the direct substitution at position seven of the chromen-4-one ring, or substitution at the adjoining oxygen atom between position seven of the chromen-4-one ring and position two of the 3,4,5-trihydroxy- 6-(hydroxymethyl) tetrahydropyran-2-yl ring. The second and fifth positions of the chromene-3,4,5,7-tetrol ring in leucocyanidin were the optimal possible site in the compound to make substitutions for generation of new bioisosteres that performed comparably well to leucocyanidin, benazepril and sampatrilat in the molecular docking. The second, third, fifth, and seventh positions of the chromen-4-one ring in quercetin were the optimal sites for the generation of new bioisosteres with binding energy results comparable to quercetin, benazepril, and sampatrilat.

The ACE inhibition capability of various flavonoids was determined by three structural characteristics (Fig. 3A): 1) the presence of a double bond between C2 and C3 in the C-ring that is necessary for the preservation of the flavonoid's planar structure; 2) the presence various hydroxyl (OH) groups at positions 3′ and 4′ in the B-ring, which is crucial for the formation of flavonoids' ACE inhibitory capability; and 3) the presence of a carbonyl (CO) group on C4 in the C-ring. Therefore, for ACE inhibition potential, the exact position and amount of hydroxyl and carbonyl functional groups as well double bond at C2 and C3 in the C-ring are crucial considerations [39–42]. In its structure, naringenin-7-O-glucoside has an OH group at position 4′ in the B-ring, the presence of a carbonyl (CO) group on C4 in the C-ring but lacked the double bond between C2 and C3 in the C-ring. Naringenin-7-O-glucoside has a total of five hydroxyl groups in its structure. The nine top-performing bioisosteres of naringenin-7-O-glucoside also had an OH group at position 4′ in the B-ring and a carbonyl (CO) group on C4 in the C-ring, but they lacked the double bond between C2 and C3 in the C-ring.

Leucocyanidin structure only showed the presence of OH groups at positions 3′ and 4′ in the B-ring as it lacked the

double bond between C2 and C3 in the C-ring and absence of a carbonyl (CO) group on C4 in the C-ring of leucocyanidin structure. However, it had a total of 6 hydroxyl groups in leucocyanidin, which were also considered crucial for the formation of flavonoids' ACE inhibitory capability. All ten top performing leucocyanidin bioisosteres lacked the double bond between C2 and C3 in the C-ring and the carbonyl (CO) group on C4 in the C-ring. Regarding the OH groups at positions 3′ and 4′ in the B-ring, some of the bioisosteres retained one or both hydroxyl groups, and others bioisosteres had no hydroxyl group, which was replaced by a trifluoromethyl group. The structure of quercetin revealed that it contained the double bond between C2 and C3 in the C-ring, the presence of OH groups at positions 3′ and 4′ in the B-ring, and the presence of a carbonyl (CO) group on C4 in the C-ring. All thirteen top performing quercetin bioisosteres retained the double bond between C2 and C3 in the C-ring and the carbonyl (CO) group on C4 in the C-ring. Regarding the OH groups at positions 3′ and 4′ in the B-ring, all the top-performing quercetin bioisosteres retained at least one of the hydroxyl groups, except for Querc 05-91, which lost both hydroxyl groups.

Binding energies of -8 or lower were found in 31 bioisosteres, placing them in the same ballpark as benazepril and sampatrilat. Based on the MolOpt findings, these bioisosteres were tested for cardiotoxicity on the eMolTox platform to determine if they could induce cardiac injury. Of these 31 bioisosteres, eMolTox - cardiotoxicity identified 15 as potentially harmful to the heart. Of the standards, lisinopril and benazepril were flagged as modulators of ACE, but sampatrilat was not flagged as cardiotoxic.

Naringenin-7-O-glucoside had been reported to have cardioprotective effects against doxorubicin induced cardiomyopathy [43–47]. It was not flagged by eMolTox - cardiotoxicity to have any potential for cardiotoxicity. However, all its top performing bioisosteres were flagged as having potential for cardiovascular toxicity specifically for either antagonist of the peroxisome proliferator-activated receptor gamma (PPARg) signaling pathway or antagonist of the thyroid receptor (TR) signaling pathway - endocrine and heart, or both.

Leucocyanidin had been reported to be present in guava (Psidium guajava L.), which was reported to possess antihypertensive properties, cardio-protective properties against myocardial ischemia-reperfusion injury, and cardio-inhibiting properties [48,49]. Nevertheless, leucocyanidin was flagged by eMolTox - cardiotoxicity as having cardiotoxic potential specifically for modulator of Dopamine D1 receptor and modulators of myocardial damage. Its bioisosteres Leuco-

DM03-50 and Leuco-DM05-53 were flagged to have cardiotoxic potential as modulator of Dopamine D1 receptor and modulators of myocardial damage. Bioisosteres Leuco-DM01-50 and Leuco-DM03-70 were flagged for cardiotoxic potential as modulator of Dopamine D1 receptor. The remaining six analogues Leuco-DM01-89, Leuco-DM02-39, Leuco-DM02-66, Leuco-DM05-60, and Leuco-DM05-63 were not flagged by eMolTox as cardiotoxic even though MolOpt flagged them as causing myocardial damage.

Although quercetin had been reported to have protective effects against doxorubicin induced cardiac damage, it was flagged by eMolTox - cardiotoxicity as having cardiotoxic potential specifically for agonist of the peroxisome proliferator-activated receptor gamma (PPARg) signaling pathway and modulators of myocardial damage [43–47]. Bioisostere Querc-DM05-91 was flagged to cause mitochondrial toxicity. Bioisostere Querc-DM15-20 was flagged for cardiotoxic potential as a modulator of myocardial damage as it was flagged by MolOpt. The remaining eleven bioisosteres Querc-DM00-35, Querc-DM00-73, Querc-DM09-63, Querc-DM12-20, Querc-DM12-35, Querc-DM13-20, Querc-DM13-35, Querc-DM13-52, Querc- DM14-31, Querc-DM14-35, and Querc-DM15-73 were not flagged by eMolTox as cardiotoxic even though MolOpt flagged them as causing myocardial damage.

Leucocyanidin and Quercetin were the A. catechu compounds, which provided the best bioisosteres that had binding energies comparable to benazepril and sampatrilat, as well as that met the toxicity and pharmacokinetic criteria of the study. Leucocyanidin had been reported to be isolated from the A. catechu seeds as well as a component of A. catechu seed polyphenol [50,51]. Quercetin had been isolated from the fruit of A. catechu, as well as from the seeds of A. catechu [52–54]. Therefore, only five bioisosteres met criteria of synthetic accessibility 6, high human intestinal absorption, good bioavailability score, non-mutagenic, non-hepatotoxic, and a non-hERG I and II blocker, as generated by pkCSM, TEST App, and Swiss ADME in addition to eMolTox results.

Conclusion

Using in silico methods, 105 compounds from A. catechu were characterized in terms of their ability to bind to the active site of the ACE receptor model. Based on the results of the molecular docking, 38 A. catechu compounds with average binding energies of -7 across six receptor models were identified, and these binding energies were comparable to those of lisinopril. After bioisosteric replacement, leucocyanidin and quercetin were the A. catechu compounds that produced the best analogues – Leuco-DM02-39, Leuco-DM02-66, Leuco-DM05-60, Querc-DM09-63, and Querc-DM14-31, which met the study's inclusion criteria. Therefore, the study showed the capacity of these flavonoid derived bioisosteres – Leuco-DM02-39, Leuco-DM02-66, Leuco-DM05-60, Querc-DM09-63, and Querc-DM14-31 – to become antihypertensive agents through ACE inhibition. The study finding support the motion of in silico screening methods and data as starting point for drug development, which could be adopted in personalized therapeutics. Nevertheless, due to its limitation regarding data translation to the human system, in silico data and modeling are not enough to account for the full potential of the bioisosteres of leucocyanidin and quercetin to become antihypertensive agents. Therefore, drug synthesis and analysis, preclinical studies comprising of in vitro and in vivo testing, and clinical trials are vital to establish their full potential as antihypertensive agents. Finally, it is suggested that future researchers use leucocyanidin and quercetin, as well as their bioisosteres Leuco-DM02-39, Leuco-DM02-66, Leuco-DM05-60, Querc-DM09-63, and Querc-DM14-31, as a starting point for the development of new antihypertensive agents via the ACE inhibition pathway by subjecting them to drug synthesis and analysis, preclinical in vitro and in vivo testing, and clinical trials.

Authors Contribution

KCE did study designing and experimentation and wrote the manuscript. JVT and JBB provided guidance and supervision in the design of the study and writing of the manuscript. All authors read and approved the final manuscript.

Conflict of Interests

The authors declare that they have no conflicts of interest related to the subject matter discussed in this article. No funding was received for conducting this study nor for the preparation of this manuscript. However,

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Supplemental Materials

List of the 105 A. catechu Compounds that were subjected to molecular docking.

Smiles of 376 Bioisosteres that met inclusion criteria

References

1. WHO. (2022) DOH, WHO, RTSL, and WHF commit to strengthen collaboration for cardiovascular diseases prevention. https://www.who.int/philippines/news/detail/30-09-2022-doh-who-rtsl-whf-commit-strengthen-collaboration-cardiovascular-diseases-prevention

2. Vulupala, H. R., Sajja, Y., Bagul, P. K., Bandla, R., Nagarapu, L., & Benerjee, S. K. (2018). Potent ACE inhibitors from 5-hydroxy indanone derivatives. Bioorganic Chemistry, 77, 660–665. https://doi.org/10.1016/J.BIOORG.2018.02.022

3. Charlton, M., & Thompson, J. (2018). Drugs acting on the heart: antihypertensive drugs. Anaesthesia & Intensive Care Medicine, 19(7), 365–369. https://doi.org/10.1016/J.MPAIC.2018.04.005

4. Sturrock, E. D., Anthony, C. S., & Danilov, S. M. (2013). Peptidyl-Dipeptidase A/Angiotensin I-Converting Enzyme. Handbook of Proteolytic Enzymes, 1, 480–494. https://doi.org/10.1016/B978-0-12-382219-2.00098-3

5. Wong, M. K. S. (2016). Angiotensin Converting Enzymes. Handbook of Hormones: Comparative Endocrinology for Basic and Clinical Research, 263-e29D-4. https://doi.org/10.1016/B978-0-12-801028-0.00254-3

6. Caballero, J. (2020). Considerations for Docking of Selective Angiotensin-Converting Enzyme Inhibitors. Molecules 2020, Vol. 25, Page 295, 25(2), 295. https://doi.org/10.3390/MOLECULES25020295

7. Polakovičová, M., & Jampílek, J. (2019). Advances in Structural Biology of ACE and Development of Domain Selective ACE-inhibitors. Medicinal Chemistry, 15(6), 574–587. https://doi.org/10.2174/157340641566619051408 1132

8. Liperoti, R., Vetrano, D. L., Bernabei, R., & Onder, G. (2017). Herbal Medications in Cardiovascular Medicine. Journal of the American College of Cardiology, 69(9), 1188–1199. https://doi.org/10.1016/J.JACC.2016.11.078

9. Jaiswal, P. , Kumar, P. , Singh, V. , & Singh, D. (2011). Areca catechu L.: A Valuable Herbal Medicine Against Different Health Problems. Research Journal of Medicinal Plant, 5(2), 145–152. https://scialert.net/abstract/?doi=rjmp.2011.145.152

10. Bhat, S. K. , Ashwin, D. , & Sarpangala, M. (2017). Analgesic, Anti-inflammatory and Wound Healing Properties of Arecanut, A. catechu L.: A Review. International Journal of Ayurveda and Pharma Research, 4(12). https://ijapr.in/index.php/ijapr/article/view/518

11. Inokuchi, J. ichi, Okabe, H., Yamauchi, T., Nagamatsu, A., Nonaka, G. ichiro, & Nishioka, I. (1986). Antihypertensive substance in seeds of Areca catechu L. Life Sciences, 38(15), 1375–1382. https://doi.org/10.1016/0024-3205(86)90470-4

12. Kumar, M., Moon, U. R., & Mitra, A. (2012). Rapid separation of carotenes and evaluation of their in vitro antioxidant properties from ripened fruit waste of Areca catechu – A plantation crop of agro-industrial importance. Industrial Crops and Products, 40(1), 204–209. https://doi.org/10.1016/J.INDCROP.2012.03.014

13. Yenjit, P., Issarakraisila, M., Intana, W., & Chantrapromma, K. (2010). Fungicidal activity of compounds extracted from the pericarp of Areca catechu against Colletotrichum gloeosporioides in vitro and in mango fruit. Postharvest Biology and Technology, 55(2), 129–132. https://doi.org/10.1016/J.POSTHARVBIO.2009.09.003

14. Nelson Anthikat, R. R. (2009). Study on the Areca Nut for its Antimicrobial Properties. J Young Pharm, 1(1). https://doi.org/10.4103/0975-1483.51874

15. Keshava Bhat, S., Sarpangala, M., & Ashwin, D. (2017). Antilipidemic activity of arecanut, Areca catechu L.: A valuable herbal medicine. ~ 35 ~ International Journal of Herbal Medicine, 5(1).

16. Yamson, E. C., Gabriel, §, Tubalinal, A. S. P., Viloria, V. V, & Mingala, C. N. (2019). Electronic) A periodical of the Network for the Veterinarians of. JOURNAL OF ADVANCED VETERINARY AND ANIMAL RESEARCH, 6(1), 44–49. https://doi.org/10.5455/javar.2019.e310

17. Kiuchi, F., Miyashita, N., Tsuda, Y., Yoshimura, H., & Kondo, K. (1987). Studies on crude drugs effective on visceral larva migrans. I. Identification of larvicidal principles in betel nuts. Chemical & Pharmaceutical Bulletin, 35(7), 2880–2886. https://doi.org/10.1248/CPB.35.2880

18. Jaiswal, P., & Singh, D. K. (2008). Molluscicidal activity of Carica papaya and Areca catechu against the freshwater snail Lymnaea acuminata. Veterinary Parasitology, 152(3–4), 264–270. https://doi.org/10.1016/J.VETPAR.2007.12.033

19. Li, S., Chen, R., Luo, K., Guo, Y., Xiao, M., & Du, G. (2017). Areca nut extract protects against ovariectomy-induced osteoporosis in mice. Experimental and Therapeutic Medicine, 13(6), 2893–2899. https://doi.org/10.3892/ETM.2017.4362/HTML

20. Natesh, R., Schwager, S. L. U., Sturrock, E. D., & Acharya, K. R. (2003). Crystal structure of the human angiotensin-converting enzyme–lisinopril complex. Nature 2003 421:6922, 421(6922), 551–554. https://doi.org/10.1038/nature01370

21. Cozier, G. E., Schwager, S. L., Sharma, R. K., Chibale, K., Sturrock, E. D., & Acharya, K. R. (2018). Crystal structures of sampatrilat and sampatrilat-Asp in complex with human ACE – a molecular basis for domain selectivity. The FEBS Journal, 285(8), 1477–1490. https://doi.org/10.1111/FEBS.14421

22. Cozier, G. E., Arendse, L. B., Schwager, S. L., Sturrock, E. D., & Acharya, K. R. (2018). Molecular Basis for Multiple Omapatrilat Binding Sites within the ACE C-Domain: Implications for Drug Design. Journal of Medicinal Chemistry, 61(22), 10141–10154. https://doi.org/10.1021/ACS.JMEDCHEM.8B01309/SUPPL_FILE/JM8B01309_SI_001.CSV

23. Corradi, H. R., Schwager, S. L. U., Nchinda, A. T., Sturrock, E. D., & Acharya, K. R. (2006). Crystal Structure of the N Domain of Human Somatic Angiotensin I-converting Enzyme Provides a Structural Basis for Domain-specific Inhibitor Design. Journal of Molecular Biology, 357(3), 964–974. https://doi.org/10.1016/J.JMB.2006.01.048

24. Cozier, G. E., Lubbe, L., Sturrock, E. D., & Acharya, K. R. (2020). ACE-domain selectivity extends beyond direct interacting residues at the active site. The Biochemical Journal, 477(7), 1241–1259. https://doi.org/10.1042/BCJ20200060

25. Forli, S., Huey, R., Pique, M. E., Sanner, M. F., Goodsell, D. S., & Olson, A. J. (2016). Computational protein–ligand docking and virtual drug screening with the AutoDock suite. Nature Protocols 2016 11:5, 11(5), 905–919. https://doi.org/10.1038/nprot.2016.051

26. Shan, J., & Ji, C. (2020). MolOpt: A Web Server for Drug Design using Bioisosteric Transformation. Current Computer-Aided Drug Design, 16(4), 460–466. https://doi.org/10.2174/1573409915666190704093400

27. Ertl, P., & Schuffenhauer, A. (2009). Estimation of synthetic accessibility score of drug-like molecules based on molecular complexity and fragment contributions. Journal of Cheminformatics, 1(1), 1–11. https://doi.org/10.1186/1758-2946-1-8/TABLES/1

28. Voršilák, M., Kolář, M., Čmelo, I., & Svozil, D. (2020). SYBA: Bayesian estimation of synthetic accessibility of organic compounds. Journal of Cheminformatics, 12(1), 1–13. https://doi.org/10.1186/S13321-020-00439-2/FIGURES/11

29. Dahlgren, D., & Lennernäs, H. (2019). Intestinal Permeability and Drug Absorption: Predictive Experimental, Computational and In Vivo Approaches. Pharmaceutics 2019, Vol. 11, Page 411, 11(8), 411. https://doi.org/10.3390/PHARMACEUTICS11080411

30. Dantas, F. G. da S., Castilho, P. F. de, Almeida-Apolonio, A. A. de, Araújo, R. P. de, & Oliveira, K. M. P. de. (2020). Mutagenic potential of medicinal plants evaluated by the Ames Salmonella/microsome assay: A systematic review. Mutation Research/Reviews in Mutation Research, 786, 108338. https://doi.org/10.1016/J.MRREV.2020.108338

31. Avila, A. M., Bebenek, I., Bonzo, J. A., Bourcier, T., Davis Bruno, K. L., Carlson, D. B., Dubinion, J., Elayan, I., Harrouk, W., Lee, S. L., Mendrick, D. L., Merrill, J. C., Peretz, J., Place, E., Saulnier, M., Wange, R. L., Yao, J., Zhao, D., & Brown, P. C. (2020). An FDA/CDER perspective on nonclinical testing strategies: Classical toxicology approaches and new approach methodologies (NAMs). Regulatory Toxicology and Pharmacology, 114, 104662. https://doi.org/10.1016/J.YRTPH.2020.104662

32. Kalyaanamoorthy, S., & Barakat, K. H. (2018). Development of Safe Drugs: The hERG Challenge. Medicinal Research Reviews, 38(2), 525–555. https://doi.org/10.1002/MED.21445

33. Whelton, P. K., Carey, R. M., Aronow, W. S., Casey, D. E., Collins, K. J., Dennison Himmelfarb, C., DePalma, S. M., Gidding, S., Jamerson, K. A., Jones, D. W., MacLaughlin, E. J., Muntner, P., Ovbiagele, B., Smith, S. C., Spencer, C. C., Stafford, R. S., Taler, S. J., Thomas, R. J., Williams, K. A., … Wright, J. T. (2018). 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Journal of the American College of Cardiology, 71(19), e127–e248. https://doi.org/10.1016/J.JACC.2017.11.006

34. Regulski, Mil. osz, Regulska, K., Stanisz, B., Murias, M., Gieremek, P., Wzgarda, A., & Niz.nik, Bartl. omiej. (2014). Chemistry and Pharmacology of Angiotensin-Converting Enzyme Inhibitors. Current Pharmaceutical Design, 21(13), 1764–1775. https://doi.org/10.2174/1381612820666141112160013

35. Ningsih, U. P. (2021). STUDI IN SILICO SENYAWA ARECOLINE (Areca cathecu L.) SEBAGAI KANDIDAT OBAT ANTIDEPRESAN. https://repository.unri.ac.id/xmlui/handle/123456789/10284

36. National Center for Biotechnology Information. (2022). Prunin | C21H22O10 | CID 92794 - PubChem. https://pubchem.ncbi.nlm.nih.gov/compound/92794

37. National Center for Biotechnology Information. (2022). Leucocyanidin | C15H14O7 | CID 71629 - PubChem. https://pubchem.ncbi.nlm.nih.gov/compound/71629

38. National Center for Biotechnology Information. (2022). Quercetin | C15H10O7 | CID 5280343 - PubChem. https://pubchem.ncbi.nlm.nih.gov/compound/5280343

39. Hussain, F., Jahan, N., Rahman, K. ur, Sultana, B., & Jamil, S. (2018). Identification of Hypotensive Biofunctional Compounds of Coriandrum sativum and Evaluation of Their Angiotensin-Converting Enzyme (ACE) Inhibition Potential. Oxidative Medicine and Cellular Longevity, 2018. https://doi.org/10.1155/2018/4643736

40. Xu, Y. C., Leung, S. W. S., Yeung, D. K. Y., Hu, L. H., Chen, G. H., Che, C. M., & Man, R. Y. K. (2007). Structure–activity relationships of flavonoids for vascular relaxation in porcine coronary artery. Phytochemistry, 68(8), 1179–1188. https://doi.org/10.1016/J.PHYTOCHEM.2007.02.013

41. Navarro-Núñez, L., Castillo, J., Lozano, M. L., Martínez, C., Benavente-García, O., Vicente, V., & Rivera, J. (2009). Thromboxane A2 Receptor Antagonism by Flavonoids: Structure–Activity Relationships. Journal of Agricultural and Food Chemistry, 57(4), 1589–1594. https://doi.org/10.1021/JF803041K

42. Guerrero, L., Castillo, J., Quiñones, M., Garcia-Vallvé, S., Arola, L., Pujadas, G., & Muguerza, B. (2012). Inhibition of Angiotensin-Converting Enzyme Activity by Flavonoids: Structure-Activity Relationship Studies. PLOS ONE, 7(11), e49493. https://doi.org/10.1371/JOURNAL.PONE.0049493

43. Abushouk, A. I., Ismail, A., Salem, A. M. A., Afifi, A. M., & Abdel-Daim, M. M. (2017). Cardioprotective mechanisms of phytochemicals against doxorubicin-induced cardiotoxicity. Biomedicine & Pharmacotherapy, 90, 935–946. https://doi.org/10.1016/J.BIOPHA.2017.04.033

44. Carresi, C., Gliozzi, M., Giancotta, C., Scarcella, A., Scarano, F., Bosco, F., Mollace, R., Tavernese, A., Vitale, C., & Musolino, V. (2016). Studies on the protective role of Bergamot polyphenols in doxorubicin-induced cardiotoxicity. PharmaNutrition, 4, S19–S26. https://doi.org/10.1016/J.PHANU.2015.11.005

45. Ojha, S., Al Taee, H., Goyal, S., Mahajan, U. B., Patil, C. R., Arya, D. S., & Rajesh, M. (2016). Cardioprotective Potentials of Plant-Derived Small Molecules against Doxorubicin Associated Cardiotoxicity. Oxidative Medicine and Cellular Longevity, 2016. https://doi.org/10.1155/2016/5724973

46. Razavi-Azarkhiavi, K., Iranshahy, M., Sahebkar, A., Shirani, K., & Karimi, G. (2016). The Protective Role of Phenolic Compounds Against Doxorubicin-induced Cardiotoxicity: A Comprehensive Review. Nutrition and Cancer, 68(6), 892–917. https://doi.org/10.1080/01635581.2016.1187280

47. Yarmohammadi, F., Rezaee, R., & Karimi, G. (2021). Natural compounds against doxorubicin-induced cardiotoxicity: A review on the involvement of Nrf2/ARE signaling pathway. Phytotherapy Research, 35(3), 1163–1175.

https://doi.org/10.1002/PTR.6882

48. Dakappa, S., Adhikari, R., Sanjay, S. T., & Sajjekhan, S. (2013). A Review on the Medicinal Plant Psidium Guajava Linn. (Myrtaceae). Journal of Drug Delivery and Therapeutics, 3(2), 162–168. https://doi.org/10.22270/JDDT.V3I2.404

49. Kumari, N., Gautam, S., & Ashutosh, C. (2013). Psidium guajava A Fruit or Medicine – An Overview. Online Available at www.Thepharmajournal.Com THE PHARMA INNOVATION-JOURNAL, 2(7725). www.thepharmajournal.com

50. Gupta, A. K., Tulsyan, S., Thakur, N., Sharma, V., Sinha, D. N., & Mehrotra, R. (2020). Chemistry, metabolism and pharmacology of carcinogenic alkaloids present in areca nut and factors affecting their concentration. Regulatory Toxicology and Pharmacology, 110, 104548. https://doi.org/10.1016/J.YRTPH.2019.104548

51. Meng, K., Mei, F., Zhu, L., Xiang, Q., Quan, Z., Pan, F., Xia, G., Shen, X., Yun, Y., Zhang, C., Zhong, Q., & Chen, H. (2021). Arecanut (Areca catechu L.) seed polyphenol improves osteoporosis via gut-serotonin mediated Wnt/β-catenin pathway in ovariectomized rats. Journal of Functional Foods, 84, 104598. https://doi.org/10.1016/J.JFF.2021.104598

52. Cao, M., Yuan, H., Daniyal, M., Yu, H., Xie, Q., Liu, Y., Li, B., Jian, Y., Peng, C., Tan, D., Peng, Y., Choudhary, M. I., Rahman, A. ur, & Wang, W. (2019). Two new alkaloids isolated from traditional Chinese medicine Binglang the fruit of Areca catechu. Fitoterapia, 138, 104276. https://doi.org/10.1016/J.FITOTE.2019.104276

53. Yang, W. Q., Wang, H. C., Wang, W. J., Wang, Y., Zhang, X. Q., & Ye, W. C. (2012). [Chemical constituents from the fruits of Areca catechu]. Zhong Yao Cai = Zhongyaocai = Journal of Chinese Medicinal Materials, 35(3), 400–403. https://europepmc.org/article/med/22876678

54. Wang, R., Pan, F., He, R., Kuang, F., Wang, L., & Lin, X. (2021). Arecanut (Areca catechu L.) seed extracts extracted by conventional and eco-friendly solvents: Relation between phytochemical compositions and biological activities by multivariate analysis. Journal of Applied Research on Medicinal and Aromatic Plants, 25, 100336. https://doi.org/10.1016/J.JARMAP.2021.100336