BIOCOMPUTATIONAL AND PHARMACOLOGICAL ANALYSIS OF PHYTOCHEMICALS FROM ZINGIBER OFFICINALE (GINGER), ALLIUM SATIVUM (GARLIC), AND MURRAYAKOENIGII (CURRY LEAF) IN CONTRAST TO TYPE 2-DIABETES

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ABSTRACT

Objective: This study was aimed to analyze the inhibitory effect of the flavonoid class of phytochemicals present in ginger (Zingiber Officinale), garlic (Allium sativum), and curry leaf (Murraya koenigii) against some receptors of type-2 diabetes such as human aldose reductase receptor, mitogen synthase kinase receptor, as well as dipeptidyl peptidase receptor by implementing several in silico analysis techniques.

Methods: The 3D structures of the flavonoid class of phytochemicals of all the three plants were retrieved from the PubChem database in 3D SDF format and were converted to PDB format using PyMol software. These phytochemicals were subjected to in silico tools such as SwissADME, Pre-ADMET, and iMDS web server. The PDB-IDs of the targeted receptors human aldose reductase, dipeptidyl peptidase-IV, and mitogen synthase kinase were retrieved from Protein Data Bank in PDB format. All these receptors were then prepared for docking procedure using Autodock Tools. Now, both the prepared proteins and ligands were subjected to docking analysis using Pyree (AutodockVina).

Results: Naringenin and kaempferol showed excellent docking results with the aldose reductase receptor. On the other hand, rutin showed the best docking score with dipeptidyl peptidase receptor-IV, whereas, epigallocatechin showed the best docking results with mitogen synthase kinase receptor. The ADME analysis showed that resveratrol had the best gastrointestinal absorption as well as high blood-brain barrier permeability.

Conclusion: Overall, the molecular docking results when analyzed showed a good binding affinity with the targeted receptors of diabetes. The ADME analysis and molecular docking results of the phytochemicals concluded that these compounds can be used as a potential cure for treating diabetes.

Keywords: Diabetes, Phytochemicals, ADME, Molecular docking

INTRODUCTION

Ginger, scientific name Zingiber officinale, family Zingiberaceae, is a flowering plant. Its rhizome is commonly consumed as folk medicine or as a spice in food. Based on scientific evidence ginger can be used as an Antiplatelet, antimicrobial, anticancer agent [1]. The ginger extract can be used to prevent autoxidation of fat at an earlier stage i.e. as an antioxidant agent [2]. Garlic, scientific name Allium sativum, Family Amaryllidaceae is a bulbous type flowering plant. It is commonly used as a flavoring agent in food as well as in traditional medicine. Meta-analysis Animal studies and epidemiological studies have proved that garlic consumption can reduce the expression of cancer, for example, stomach, colon, esophagus, cervix, skin, lung, breast, and uterine cancers. Clinical data shows that along with cancer garlic is also effective as an antifungal, antimicrobial, antioxidant, antidiabetic, and antihypertensive drug [3, 4]. The curry tree, scientific name Murraya koenigii, family Rutaceae is a tropical and sub-tropical plant. Due to its characteristic aroma and chemical constituents, it is used as a flavoring agent in Indian dishes. The essential oil present in Murraya koenigii leaves shows mosquito-cidal. Antibacterial activity, Antifungal activity, and Antiprotozoal activity [5]. This study is mainly based on the phytochemical screening of the three above-mentioned plants. Flavonoids are a group of phytochemicals with different types of phenolic structures. Their antioxidant effects of flavonoids can cure various diseases like Alzheimer’s disease (AD), cancer, atherosclerosis, etc. A significant amount of flavonoid intake can maintain coronary heart disease [6]. Chemically flavonoids contain A, B, and C ring systems. B ring when linked in position 3 of the ring C are called isoflavones similarly when B ring is linked in position 4 are called neoflavonoids. When the B ring is linked in position 2 of the C ring, it can be further subdivided into several subgroups like flavones, flavonols, flavanones, flavonols, flavanones, catechins, and anthocyanins [7]. In this study, we have mainly selected the important flavonoid phytochemicals of the three plants i.e. Ginger, Garlic, and Curry leaves to carry out a comparative molecular docking analysis against some important receptors aldose reductase, dipeptidyl peptidase-IV, and mitogen synthase kinase. Moreover, this study also included pharmacological analysis of our selected phytochemicals to predict and analyze drug-likeness properties of the phytochemicals.

MATERIALS AND METHODS

Ligand preparation

All the important flavonoid phytochemicals of the plants Murraya koenigii, Allium sativum, and Zingiber officinale were retrieved from the PubChem database in the form of 3D Standard Data Format (3D SDF) [8, 9]. PyMol was then used for converting the ligand from 3D SDF to Protein Data Bank (PDB) format [10].

Protein preparation

The targeted receptor molecules were selected and downloaded in PDB format from the protein data bank database [11]. The protein molecules were then loaded in AutoDock Tools software [12]. Firstly, the extraction of the co-crystallized ligand was done to validate the protein. Immediately, after this, protein preparation of the targeted proteins was started by removing water molecules, removing chains or heteroatoms not required, repairing missing atoms, the addition of hydrogen atoms, computing charges (Kollman charges) and finally converting it into pdbqt format. Finally, generation of the grid box was done keeping the co-crystallized ligand at the center of the grid. The dimension of the grid box was saved for docking using AutoDockVina as a config. txt file. The co-crystallized was then removed from the prepared protein pdbqt file.

ADMET and drug-likeness analysis

SwissADME and Pre-ADMET web servers were used to predict drug-likeness and ADMET properties of our selected phytochemicals [13]. Lipinski’s rule was used to virtually screen the best hit compounds.
from our selected list of phytochemicals. According to Lipinski’s rule of five, a compound, to qualify as a ligand, should have less than 500 Da molecular weight, high lipophilicity i.e. value of Log P less than five, hydrogen bond donors less than 5, and hydrogen bond acceptors less than 10. Compounds violating any two rules of Lipinski’s were eliminated for further screening. Other than Lipinski’s rule, physicochemical analysis, as well as Drug-likeness properties of all the ligand molecules, were also taken into consideration for the drug screening process.

**Boiled-egg**

For predicting blood-brain barrier permeability as well as gastrointestinal absorption of our selected phytochemicals, BOILED-EGG was used. According to BOILED-EGG plot analysis, compounds found in the yellow region were considered to be having higher blood-brain barrier permeability, whereas compounds found in the white region of the plot were considered to be having higher gastrointestinal absorption properties. The BOILED-EGG plot analysis was performed using the SwissADME webserver.

**Molecular docking analysis**

The molecular docking analysis was mainly performed to predict the interaction as well as inhibitory activity of our selected phytochemicals against our selected protein receptors. The docking study was carried out using PyRx (AutoDockVina) [14, 15]. The prepared ligands were docked with the prepared protein receptors. The results of docking were displayed in the terms of binding affinity along with good ADMET properties was chosen as the one that would bind with its target. The molecule with the best binding affinity along with good ADMET properties was chosen as the best hit compounds. The structural analysis of the compounds was done by using Discovery Studio Visualizer 2021 [16].

**Table 1: Physiochemical analysis**

| Ligand       | Molecular formula | Molecular weight (g/mol) | Monoisotropic mass (g/mol) | Heavy atom count | Tropological polar surface area |
|--------------|-------------------|--------------------------|---------------------------|------------------|---------------------------------|
| Naringenin   | C15H12O5          | 272.25                   | 27.0068473                | 20               | 86.99                           |
| Catechin     | C15H16O7          | 308.28                   | 308.0896029               | 22               | 111                             |
| Epigallocatechin | C15H14O7    | 306.27                   | 306.0793528               | 22               | 131                             |
| Epicatechin  | C15H14O6          | 290.27                   | 290.079038                | 12               | 110.38                          |
| Resveratrol  | C14H12O3          | 228.24                   | 228.078644                | 17               | 60.7                            |
| Quercetin    | C15H10O7          | 302.04                   | 302.042653                | 16               | 131.36                          |
| Apigenin     | C15H10O5          | 270.24                   | 270.052823                | 20               | 90.9                            |
| Quercetin    | C21H20O11         | 448.4                    | 448.100561                | 32               | 186                             |
| Rutin        | C27H30O16         | 610.5                    | 610.1533849               | 43               | 266                             |
| Kaempferol   | C15H10O6          | 286.24                   | 286.047738                | 22               | 111.13                          |
| Morin        | C15H11O7          | 302.23                   | 302.0426527               | 21               | 127                             |
| Myricetin    | C15H10O8          | 318.23                   | 318.0375673               | 23               | 148                             |

**Table 2: Lipinski’s analysis**

| Ligand       | Molecular formula | H-Bond donor | H-Bond acceptor | ClogP | Molar refractivity |
|--------------|-------------------|--------------|-----------------|-------|-------------------|
| Naringenin   | C15H12O5          | 3            | 5               | 2.16  | 71.57             |
| Catechin     | C15H16O7          | 6            | 7               | 1.51  | 77.38             |
| Epigallocatechin | C15H14O7   | 6            | 7               | 1.16  | 76.36             |
| Epicatechin  | C15H14O6          | 5            | 6               | 1.51  | 74.33             |
| Resveratrol  | C14H12O3          | 3            | 3               | 2.83  | 67.88             |
| Quercetin    | C15H10O7          | 5            | 7               | 1.49  | 78.03             |
| Apigenin     | C15H10O5          | 3            | 5               | 2.34  | 73.99             |
| Quercetin    | C21H20O11         | 11           | 11              | 0.58  | 1.09              |
| Rutin        | C27H30O16         | 10           | 16              | -1.26 | 141.38            |
| Kaempferol   | C15H10O6          | 4            | 6               | 1.84  | 76.01             |
| Morin        | C15H10O7          | 5            | 7               | 1.49  | 78.03             |
| Myricetin    | C15H10O8          | 6            | 8               | 1.14  | 80.06             |

**BOILED-egg analysis**

The BOILED-Egg analysis showed that resveratrol was the only compound showing both high blood barrier permeability property as well as good gastrointestinal absorption properties. The other compounds showing high gastrointestinal absorption other than resveratrol were apigenin, naringenin, kaempferol, morin, quercetin, epicatechin, epigallocatechin, catechin. The...
least gastrointestinal absorption ability was shown by morin, quercetin, and rutin.

**Molecular docking analysis**

Most of the phytochemicals showed good docking results for our three targeted receptors human aldose reductase, glycogen synthase kinase, and dipeptidyl peptidase-IV. For the aldose reductase receptor, the highest dock scores of about-10 and-9.9 with naringenin and kaempferol respectively. For the dipeptidyl peptidase-IV receptor, the highest dock score of about-9.7 was observed with rutin. Lastly, with mitogen synthase kinase receptor, the highest dock score of about-9.0 was observed with epigallocatechin. Thus, the compounds that showed the best dock score indicate good binding affinity with their respective receptor.

### Table 3: Drug-likeness analysis

| Ligands    | Blood-brain barrier | GI absorption | Permeability glycoprotein substrate | LogS (scale insoluble<-10<poorly<-6<moderately<-4<soluble<-2<very<0<highly) [Water solubility] |
|------------|---------------------|---------------|------------------------------------|-----------------------------------------------------------------------------------------------|
| Naringenin | No                  | High          | Yes                                | -3.49                                                                                          |
| Catechin   | No                  | High          | No                                 | -2.02                                                                                          |
| Epigallocatechin | No    | High          | No                                 | -2.08                                                                                          |
| Epicatechin| No                  | High          | Yes                                | -2.22                                                                                          |
| Resveratrol| Yes                 | High          | No                                 | -3.62                                                                                          |
| Quercetin  | No                  | High          | No                                 | -3.16                                                                                          |
| Apigenin   | No                  | High          | No                                 | -3.94                                                                                          |
| Quercetin  | No                  | Low           | No                                 | -3.33                                                                                          |
| Rutin      | No                  | Low           | Yes                                | -3.3                                                                                           |
| Kaempferol | No                  | High          | No                                 | -3.31                                                                                          |
| Morin      | No                  | High          | Yes                                | -3.16                                                                                          |
| Myricetin  | No                  | Low           | No                                 | -3.01                                                                                          |

### Table 4: Molecular docking results with aldose reductase receptor

| Ligand     | PDB-ID | dock score |
|------------|--------|------------|
| Naringenin | 1US0   | -10        |
| Catechin   | 1US0   | -9.2       |
| Epigallocatechin | 1US0 | -9.6       |
| Epicatechin| 1US0   | -9.4       |
| Resveratrol| 1US0   | -8.8       |
| Quercetin  | 1US0   | -9.7       |
| Apigenin   | 1US0   | -9.7       |
| Quercetin  | 1US0   | -8.3       |
| Rutin      | 1US0   | -10        |
| Kaempferol | 1US0   | -9.9       |
| Morin      | 1US0   | -9.7       |
| Myricetin  | 1US0   | -8.7       |

### Table 5: Molecular docking results with mitogen synthase kinase receptor

| Ligand     | Pdbid | Dock score |
|------------|-------|------------|
| Naringenin | 3F7Z  | -6.2       |
| Catechin   | 3F7Z  | -7.7       |
| Epigallocatechin | 3F7Z | -9        |
| Epicatechin| 3F7Z  | -7.8       |
| Resveratrol| 3F7Z  | -7.3       |
| Quercetin  | 3F7Z  | -8.0       |
| Apigenin   | 3F7Z  | -8.0       |
| Quercetin  | 3F7Z  | -8.7       |
| Rutin      | 3F7Z  | -8.1       |
| Kaempferol | 3F7Z  | -7.5       |
| Morin      | 3F7Z  | -7.8       |
| Myricetin  | 3F7Z  | -8.0       |

Fig. 1: The left-sided diagram shows the 2D amino acid interactions of Naringenin with human aldose reductase receptors. The right-sided diagram shows the binding analysis of Naringenin (light blue) at the active site of the co-crystallized/native ligand (deep green) of the receptor human aldose reductase. In the right-sided diagram, protein is represented in light violet color, whereas, the amino acid residues are represented in deep blue color.

Table 5: Molecular docking results with mitogen synthase kinase receptor
Fig. 2: The left-sided diagram shows the 2D amino acid interactions of epigallocatechin with mitogen synthase kinase receptor. The right-sided diagram shows the binding analysis of Epigallocatechin (light blue) at the active site of the co-crystallized/native ligand (deep green) of the receptor mitogen synthase kinase. In the right-sided diagram, protein is represented in light violet color, whereas, the amino acid residues are represented in deep blue color

Table 6: Molecular docking results with dipeptidyl peptidase-IV receptor

| Ligand     | Pdbid | Dock score |
|------------|-------|------------|
| Narigenin  | 3F8S  | -7.1       |
| Catechin   | 3F8S  | -7.7       |
| Epigallocatechin | 3F8S | -8.1   |
| Epicatechin| 3F8S  | -7.5       |
| Resveratrol| 3F8S  | -6.9       |
| Quercetin  | 3F8S  | -7.8       |
| Apigenin   | 3F8S  | -7.8       |
| Quercitrin | 3F8S  | -8.8       |
| Rutin      | 3F8S  | -9.7       |
| Kaempferol | 3F8S  | -7.7       |
| Morin      | 3F8S  | -7.7       |
| Myricetin  | 3F8S  | -8         |

Fig. 3: The left-sided diagram shows the 2D amino acid interactions of Rutin with the dipeptidyl peptidase-IV receptor. The right-sided diagram shows the binding analysis of Rutin (light blue) at the active site of the co-crystallized/native ligand (deep green) of the receptor dipeptidyl peptidase-IV. In the right-sided diagram, protein is represented in light violet color, whereas, the amino acid residues are represented in deep blue color

Fig. 4: B-factor or mobility (The main-chain deformability is a measure of the capability of a given molecule to deform at each of its residues)
Fig. 5: Eigenvalues (The eigenvalue associated to each normal mode represents the motion stiffness. Its value is directly related to the energy required to deform the structure. The lower the eigenvalue, the easier the deformation)

Fig. 6: Variance (individual (red) and cumulative (green) variances)

Fig. 7: Covariance map (correlated (red), uncorrelated (white) or anti-correlated (blue) motions of coupled residues)

Fig. 8: Elastic network (Each dot denotes one spring within the respective atoms pair. The dots are colored based on the stiffness where the dark grey dots indicate the stiffer springs and vice versa)
insulin receptor substrate (IRS) -1, a key molecule participating in phosphorylation of the specific serine residues is responsible for the inactivation mechanism. In addition, it has been reported that insulin receptor substrate (IRS)-1, a key molecule participating in insulin-signaling cascades can also be phosphorylated by GSK-3. In skeletal muscle, insulin regulation is correlated by phosphorylating glycogen synthase kinase. Narin genin and kaempferol showed excellent inhibitory activity towards dipeptidyl peptidase-IV, aldose reductase, and mitogen synthase receptor. The most important diabetes type 2 receptors namely dipeptidyl peptidase-IV, aldose reductase as well as nitric oxide synthase receptor. The most important phytochemicals i.e. the flavonoid class of phytochemicals of these three plants was chosen as ligands for this study. Flavonoids are a group of phytochemicals with different types of phenolic structures. Their antioxidant effects of flavonoids can cure various diseases like Alzheimer's disease (AD), cancer, atherosclerosis, etc. A significant amount of flavonoid intake can maintain coronary heart disease [6]. These phytochemicals were subjected to various in silico techniques such as molecular docking and ADME-based pharmacological tools analysis. Through this study, we were finally able to screen and find our hit compounds for each receptor, which showed a good binding affinity with our selected type two diabetes receptors. In short, we can conclude by saying that our hit phytochemicals, can be considered as lead candidates in binding with our targeted receptors, and thus can help to treat type 2 diabetes. Results obtained from this research study will serve as an insight for future preclinical as well as in vivo studies.

CONCLUSION

Zingiber officinalis (Ginger), Allium sativum (Garlic) as well as Murraya koenigii (Curry leaf) have been previously used as a cure to various diseases. The flavonoids phytochemicals obtained from these three plants have overall good showed a binding affinity with the receptors dipeptidyl peptidase-IV, aldose reductase, and nitric oxide synthase kinase. Naringenin and kaempferol showed excellent docking results with the aldose reductase receptor. On the other hand, rutin showed the best docking score with dipeptidyl peptidase receptor-IV, whereas, epigallocatechin showed the best docking results with nitric oxide synthase kinase receptor. The ADMET analysis showed that resveratrol had the best gastrointestinal absorption as well as high blood-brain barrier permeability. Hence, we can conclude by saying that these phytochemicals can provide a cure to diabetic disorders. To find the effectiveness as well as to propose the exact mechanism, in vitro studies can be encouraged on these phytochemicals to understand the exact mechanism and potential cure for diabetes.

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AUTHORS CONTRIBUTIONS
All authors have contributed equally.

CONFLICTS OF INTERESTS
All authors have none to declare.

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