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Identification of the Role of Ajoene from *Aegle marmelos* Correa for its Anti-Diabetic Action: Role of NRF2/KEAP-1 Signaling

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Abstract

Diabetes mellitus is a chronic metabolic disorder that affects millions of people worldwide. The management of diabetes is a significant for new and effective therapeutic agents. Ajoene, a compound found in garlic, has been shown to have anti-diabetic properties. The Nrf2/Keap-1 signaling pathway has been identified as a potential target for the treatment of diabetes. The aim of this study is to investigate the role of ajoene from Aegle Marmelos Correa in the activation of the Nrf2/Keap-1 signaling pathway and its potential as an anti-diabetic agent. In silico analysis was performed using molecular docking and molecular dynamics simulations to investigate the interaction between ajoene and the Nrf2/Keap-1 signaling pathway. The molecular docking studies revealed that ajoene binds to the Nrf2/Keap-1 complex with high affinity, indicating a potential interaction between ajoene and Cul3 was establishment of a single hydrogen bond involving ILE258. This interaction contributed to the formation of a binding pocket encompassing key residues such as LEU-253, ILE-258, VAL-260, LEU-266, LEU-292. These findings suggest that ajoene may activate the Nrf2/Keap-1 signaling pathway, leading to the upregulation of antioxidant genes and the inhibition of oxidative stress, which are known to contribute to the development of diabetes. The results of this study suggest that ajoene from Aegle Marmelos Correa may have potential as anti-diabetic agent through its activation of the Nrf2/Keap-1 signaling pathway. The specific interaction between ajoene and Cul3, characterized by the establishment of a single hydrogen bond involving ILE258, contributes to the formation of a binding pocket encompassing key residues.

Keywords: *Ajoene, Diabetes Mellitus, Health and Well-being, Nrf2/Keap-1 Signaling, Oxidative Stress, Public Health.*

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Introduction

Diabetes mellitus is one of the most common metabolic disorders characterized by an elevation in blood glucose levels and poses one of the significant health risks globally. Explorative searches for its effective treatments have been directed toward natural products, among them Aegle marmelos Correa, which reportedly bears the potential for anti-diabetic action. Ajoene, among the bioactive principles, has been gaining interest lately for its therapeutic potential against diabetes [1].

Aegle marmelos is a medicinal plant with a wide array of pharmacological activities, such as anti-diabetic, anti-inflammatory, and antioxidant effects [2]. Garlic and Aegle marmelos seem to have promising anti-diabetic activities through studies related to their sulfur compound, Ajoene [3]. In this regard, molecular interactions of Ajoene within biological systems would somehow be important for explaining the therapeutic mechanism accruing to it.

In silico analysis is thus a computational way that can be used to ab initio predict interactions between the bioactive compounds and target proteins, revolutionizing drug discovery and development. Through molecular interaction simulation, tentative targets of drugs or mechanisms of action could be identified, or therapeutic outcomes maximized. There is an in-silico analysis in relation to Ajoene from Aegle marmelos that would provide a very strong platform for the evaluation at a molecular level of its anti-diabetic properties [4].

The Nrf2/Keap-1 pathway is considered the major regulator of cellular resistance to oxidative stress and inflammation. Nrf2 is a transcription factor that plays a master role in the activation of antioxidant response elements, resulting in enhanced cellular antioxidant defenses [5]. Keap-1 is a regulator of Nrf2 activity by holding it in the cytoplasm under normal conditions. On activation, Nrf2 translocates to the nucleus, resulting in the transcription of genes related to antioxidant and detoxification pathways. The present study on Ajoene from Aegle marmelos Correa explores with bioinformatics studies its possible role in regulating the Nrf2/Keap-1 signaling pathway for anti-diabetic action. This paper will, therefore, explain the molecular interactions between Ajoene and key proteins involved in the Nrf2/Keap-1 pathway underlying anti-diabetic actions of Ajoene and explore the possibility of developing novel therapeutic strategies against diabetes [6].

Diabetes Mellitus is the disturbance in metabolic activity that results in high levels of blood sugar due to the inability of the human body either to produce or adequately use insulin, a hormone that assimilates blood sugar [7]. It can cause cardiovascular complications, kidney failure, damage to the nerves, and blindness, thus creating a major health problem worldwide. Already in 2019, an estimated 463 million adults were living with diabetes, with numbers projected to rise to 700 million by 2045 [8]. The rising trends in diabetes across the world have led to a continuous search for effective treatments; in this respect, natural products like Aegle marmelos Correa have been tried out for their potential anti-diabetic activity, traditionally being used in various systems of medicine [9].

Ajoene is a bioactive compound containing sulfur, largely extracted from plants like Aegle marmelos and garlic, and recently seems to have great potential for therapeutic applications in the management of diabetes. In this respect, according to several studies, Ajoene can exert anti-diabetic potentials through mechanisms involving improvements in the homeostasis of glucose, antioxidant effects, and modulation of various biochemical parameters in diabetes [10]. Hence, it becomes very important to understand the molecular mechanisms underlying the antidiabetic effects of Ajoene for the design of appropriate

therapeutic strategies and the optimization of its clinical applications.

The Nrf2/Keap-1 pathway represents an essential regulator of cellular defense against the development of oxidative stress and inflammation, both tightly linked to pathogenesis of diabetes. Nrf2 is a pivotal transcription factor in the activation of the ARE-mediated gene expression, promoting genes involved in antioxidant and detoxification pathways [11]. In contrast, Keap-1 is the negative regulator of Nrf2, although under normal conditions, it generally sequesters Nrf2 in the cytoplasm. Recently available data show that dysfunction in the Nrf2/Keap-1 pathway is correlated to the initiation and development of diabetes [12]. Defective Nrf2 signaling was observed to be linked with high oxidative stress, inflammation, and disturbed glucose homeostasis driving the pathogenesis of diabetes. Therefore, targeting the Nrf2/Keap-1 pathway with bioactive compounds such as Ajoene from Aegle marmelos Correa may be an exciting approach for management of diabetes and its complications. Such computational approaches have established in silico analysis as one of the key tools in the discovery and development of drugs, where in-depth probes of molecular interactions of bioactive compounds with target proteins can be made. In this regard, in silico analysis of Ajoene from Aegle marmelos Correa may give insight into probable mechanisms by which Ajoene modulates the Nrf2/Keap-1 signalling pathway for anti-diabetic effects [13]. Mapping the binding interactions between Ajoene and key proteins involved in the Nrf2/Keap-1 pathway, like Nrf2 and Keap-1, will help in identifying potential binding sites, their binding affinities, and whether and how these interactions impact the overall signaling cascade of the pathway. The information that may be derived from such simulations helps in explaining the molecular mechanisms underlying the anti-diabetic action of Ajoene and might open new pharmaceutical strategies for the management of diabetes [14].

Diabetes mellitus remains one of the major challenges to global health, with the quest for therapeutic remedies directing researchers toward the investigation of a number of natural products, including Aegle marmelos Correa [15]. Ajoene is one such bioactive compound from the plant Aegle marmelos that seems to have promising anti-diabetic potential, documented through a host of studies. Thorough elucidation of molecular mechanisms of action, especially on the modulation of the Nrf2/Keap-1 signaling pathway of ajoene, confers anti-diabetic effects and can be helpful in developing new therapeutic strategies [16].

The study employed in silico analysis in an attempt to probe into the binding of Ajoene with key proteins in the Nrf2/Keap-1 pathway, trying to explore some probable modes of actions underlying the antidiabetic potential of Ajoene [17]. The molecular interactions explored herein are therefore envisioned to contribute towards developing innovative approaches for diabetes management and associated complications.

Materials and Methods

Preparation of Ligand

The Ajoene (CID: 5386591) [18] 3D structure was downloaded by PubChem database. SDF file format, converted into PDB file using online translator. Ligand format was changed by using Auto Dock tool for analysis.

Preparation of Receptors

The three-dimensional coordinates of NRF2-(Q16236) [19], KEAP1-(Q14145) [20], Cul3-(Q13618), Rbx1-(P62877), and Ubiquitin-(P62068) [21] were extracted from the Uniprot Protein Data Bank, while the 3D chemical structure of ajoene was sourced from the PubChem database. The ajoene structure was initially downloaded in SDF format and subsequently converted into PDB format using

online translators. The AutoDock tool was utilized to generate the receptor molecule, following which adjustments were made to the protein molecules, including the insertion of missing atoms and polar hydrogen. Finally, the file format was converted to PDBQT format to facilitate further analysis.

Active Site Identification

Using the CASTp server, the process of identifying binding sites was successfully carried out. This server has the capability to detect atoms outlining pockets and hidden crevices, determine the number and positions of these pockets and crevices, and assess the location and size of entrance openings.

Docking

The molecules obtained were subjected to docking using AutoDock Tools. Subsequently, the AutoGrid strategy was employed to generate three-dimensional grid boxes for evaluating binding energies at the macromolecule coordinates. Through AutoGrid, grid maps representing the entire ligand at the specific docking target site were created. The complete ligand was placed within the binding site, enclosed by cubic grids. Following this, the graphical user interface of AutoDock, version 4.2.6, provided by MGL Tools, was utilized to configure the types of AutoDock atoms. One of the most effective docking techniques available in AutoDock, the Lamarckian genetic algorithm, was utilized. The binding free energy and the optimal fit of a ligand conformation within the macromolecular structure were computed and evaluated using AutoDock. This approach can be valuable for understanding the binding characteristics and for developing more efficient drug candidates.

Results

Interaction Between CUL3 AND Ajoene

The results of our study revealed that ajoene demonstrated significant binding affinities towards Cul3, with a binding energy of -2.96 kcal/mol (Table 1). Furthermore, the interaction of superoxide dismutase involves the participation of ILE-258 in establishing a one hydrogen bond. The binding sites formed by Cul3 are LEU-253, ILE-258, VAL-260, LEU-266, LEU-292 by the CASTp docking website. And the binding pocket formed by Cul3 and ajoene such ASP-70,PHE-71,GLU-72,CYS-73,HIS-76,CYS-77,LEU-80,SER-81,GLN-84,SER-113,GLN-113,VAL-114,ALA-116,ALA-117,VAL-117,ALA-118,THR-119,GLU-120,ARG-120,ASP-121,THR-123,PRO-124,MET-124,ASP-127,ARG-128,ASN-138,TYR-140,ASN-141,LEU-144,TYR-160,MET-161,TYR-162,THR-163,GLY-164,LEU-184,ARG-184,LEU-185,ILE-186,ARG-187,CYS-187,GLN-188,LEU-188,MET-191,ILE-192,LEU-195,GLU-196,GLY-197,VAL-248,MET,249,HIS-250,CYS-251,LEU-252,ASP-253,LYS-254. (Figure 1)

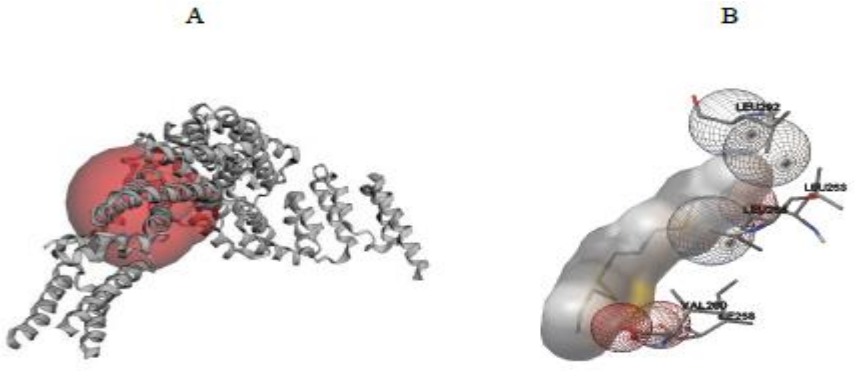

Figure 1. (A): Binding site of cul3. (B): Binding site of Cul3 with Ajoene

Interaction Between Keap1 and Ajoene

The results we obtained revealed strong binding affinities between ajoene and keap1, as evidenced by a binding energy of -5.6 Kcal/Mol (Table 1). The demonstrated a strong interaction by forming a one hydrogen bond network with specific amino acid residues, namely VAL-418. The binding sites formed by keap1 are GLY-367, GLY-417, VAL-418, GLY-464, VAL-465, GLY-511, VAL-512, GLY-605, VAL-606. Further analysis revealed that Keap1 had the highest affinity for ajoene, resulting in the formation of a distinct binding pocket. This binding pocket was characterised by the involvement of key residues such as TYR-334,SER-363,GLY-364,LEU-365,ALA-366,GLY-367,CYS-368,VAL-369,ARG-380,ASN-382,ASN-414,ARG-415,ILE-416,GLY-417,VAL-418,GLY-419,VAL-420,ILE-461,GLY-462,VAL-463,GLY-464,VAL-465,ALA-466,VAL-467,PHE-478,ARG-483,SER-508,GLY-509,ALA-510,GLY-511,VAL-512,CYS-513,VAL-514,TYR-525,GLN-530,SER-555,ALA-556,LEU-557,GLY-558,ILE-559,THR-560,VAL-561,SER-602,GLY-603,VAL-604,GLY-605,VAL-606,ALA-607 (see Figure 2).

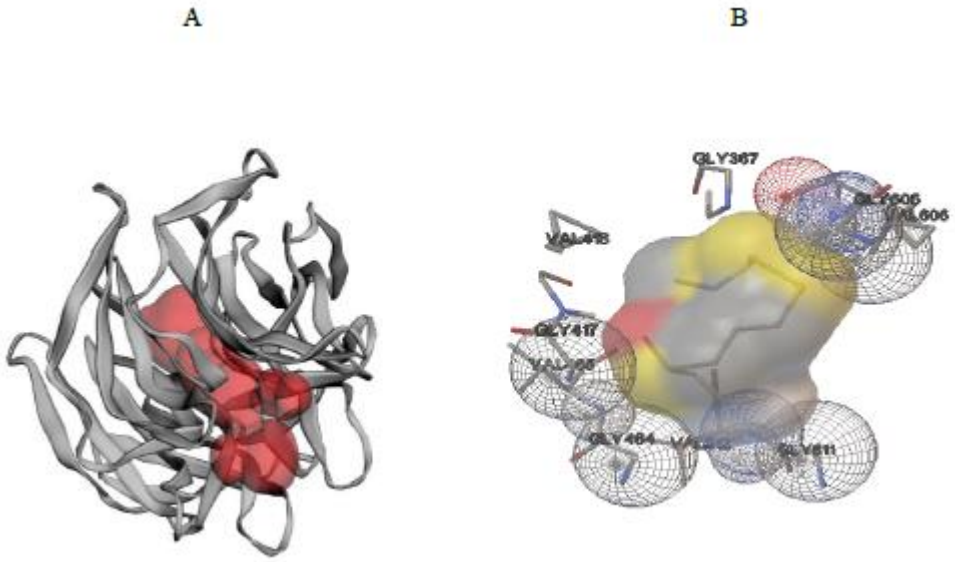

Figure 2. (A): Binding site of KEAP-1. (B): Binding site of KEAP-1 with Ajoene

Interaction Between NRF-2 and Ajoene

The results indicated that ajoene, the substance under investigation, had significant binding affinities for NRF2. The calculated binding energy of -5.35 Kcal/Mol, shown in Table 1, highlights the strength of this interaction. Further investigation uncovered a specific molecular mechanism involving the formation of a binding pocket. This pocket is critical for the interaction with key amino acids like LEU365, GLY417, GLY364, VAL465, VAL463, GLY462, ARG415, and GLY509. Further investigation revealed that NRF2 had the highest affinity for ajoene, resulting in the formation of a unique binding pocket. This binding pocket was characterised by the involvement of key residues such as ASP-77,ASP-79,THR-80,GLU-82,LEU-84,ARG-326,TYR-334,GLY-364,LEU-365,ALA-366,GLY-367,CYS-368,VAL-369,VAL-370,GLY-371,GLY-372,ARG-380,ASP-389,ASN-414,ARG-415,ILE-416,GLY-417,VAL-418,GLY-419,VAL-420,ILE-421,ASP-422,GLY-423,HIS-424,TYR-426 ,GLY-433,HIS-436, ARG-442,GLU-444,PRO-445,GLU-446, ARG-447,ILE-461,GLY-462,VAL-463,GLY-464,VAL-465,ALA-466,VAL-467,LEU-468,ASN-469,ARG-470,LEU-471,PHE-

478,ARG-483,TYR-491,PRO-492,GLU-493,ARG-494,GLY-509,ALA-510,GLY-511,VAL-512,CYS-513,VAL-514,LEU-515,HIS-516,ASN-517,CYS-518,TYR-520,ARG-536,ASP-538,ALA-556,LEU-557,GLY-558,ILE-559,THR-560,VAL-561,HIS-562,GLN-563,GLY-564,ARG-565,ASP-585,PRO-586,ASP-587,SER-602,GLY-603,VAL-604,GLY-605,VAL-606,ALA-607,VAL-608. (Figure 3)

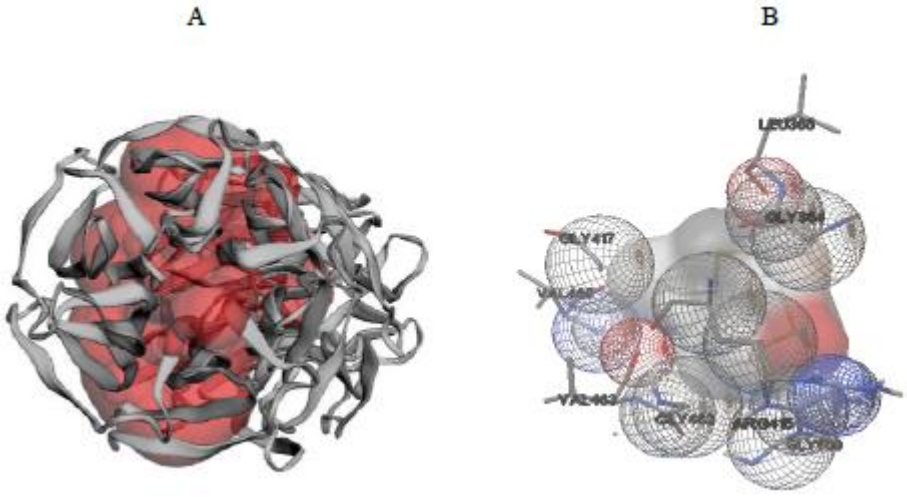

Figure 3. (A): Binding site of NRF2. (B): Binding site of NRF2 with Ajoene

Interaction Between RBX-1 and Ajoene

This study shows the interaction between ajoene and RBX1- A Chain revealed a binding energy of -4.94 kcal/mol, indicating a strong affinity between the two molecules. Additionally, one hydrogen bonds were identified between LYS-587, further highlighting the specific interactions at play. A comprehensive analysis of the amino acids involved in this interaction showcased a wide array of residues contributing to the binding process. These include LYS-587, LYS-586, VAL-475, LEU-582, GLN-538, TRP-581, ILE-537, THR-580 and a multitude of other amino acids, emphasizing the intricate nature of the molecular interaction between ajoene and RBX1- A Chain. Additional research revealed that RBX-1 had the highest affinity for ajoene, resulting in the formation of a distinct binding pocket. This binding pocket was defined by the participation of key residues such as LYS-26,ASN-28,ALA-29,VAL-30,LEU-32,ALA-34,TRP-35,ASP-36,ILE-37,VAL-38,VAL-39,ASN-41,CYS-42,ALA-43,ILE-49,MET-50,ASP-51,GLU-67,CYS-68,THR-69,VAL-70,TRP-72,VAL74,HIS-77,ALA-78,HIS-80,PHE-81,SER-85,LEU-96,GLU-102,PHE-103,GLN-104,LYS-105,TYR-106,PRO-363,ARG-424,ASP-427,SER-428,LYS-431,LYS-432,SER-433,SER-434,LYS-435,ASP-460,GLN-463,LYS-464,PHE-465,ALA-467,LYS-468,MET-469,ALA-471,LYS-472,ARG-473,VAL-475,HIS-476,GLN-477,ASN-478,GLU-499,TYR-500,SER-502,LYS-503,LEU-504,MET-507,SER-536,ILE-537,GLN-538,SER-542,GLY-543,SER-544,TRP-545,PRO-546,PHE-547,GLN-548,GLN-549,SER-550,CYS-551,THR-552,LYS-578,THR-580,TYR-583,GLN-584,LEU-585,SER-586,LYS-587,GLY-588,GLU-589,GLN-602,ALA-603,SER-604,THR-605,PHE-606,GLN-607,LYS-632,ILE-635,GLN-638,VAL-639,ILE-642,LYS-679,LEU-680,ARG-681,VAL-682,ASN-683,ASN-685,VAL-686,PRO-687,MET-688,LYS-689,GLU-691,GLN-692,GLN-694,GLU-695,GLN-696,THR-698,THR-699,ASN-702,ILE-703,GLU-705,ASP-706,ARG-707,LYS-

708,LEU-709,LEU-710,GLN-712,ALA-713,VAL-716,ARG-717,GLN-737,LEU-738,SER-740,ARG-741,PHE-742,LEU-756,LYS-759 and TYR-761. (Figure 4)

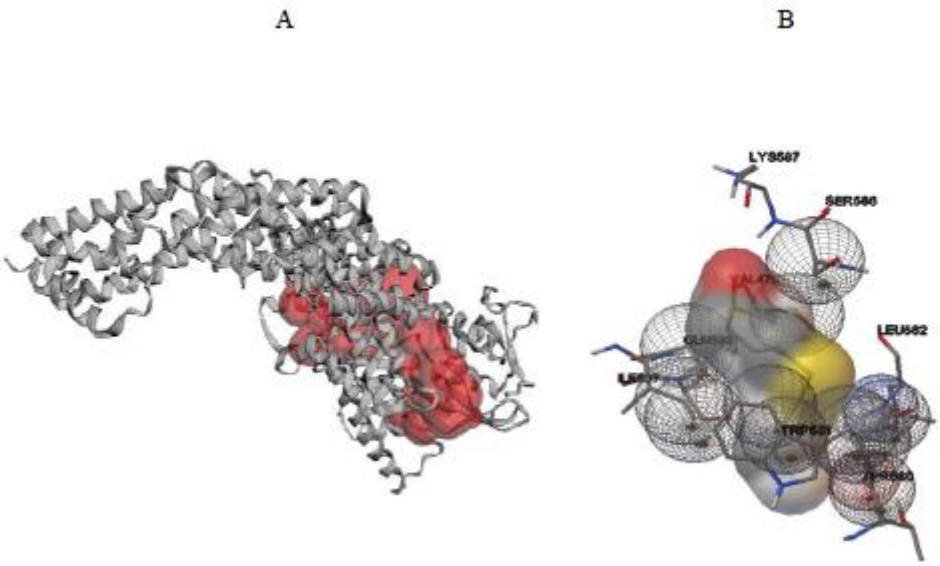

Figure 4. (A): Binding site of RBX1. (B): Binding site of RBX1 with Ajoene

Interaction Between Ubiquitin and Ajoene

The molecular docking analysis of the interaction between ajoene and ubiquitin revealed a binding energy of -4.31 kcal/mol, indicating a favorable affinity between the two molecules. Additionally, a hydrogen bond was identified with THR347, further emphasizing the specific interactions at play. The critical binding pocket involved in this interaction plays a crucial role in engaging key amino acids like LEU-346, GLU-341, GLU-342, GLY-345, THR-347, and GLU-355. Amino acids such as PHE-45, ALA-46, GLY-47, LYS-48, LEU-50, ARG-54, ASP-58, TYR-59, ASN-60, ASN-130, ALA-133, ASP-134, GLN-137, GLU-164, LEU-165, HIS-169, GLN-173, ASP-194, PHE-195 and LEU-196. This is the intricate nature of the binding process and the key amino acids involved in this molecular interaction. (Figure 5)

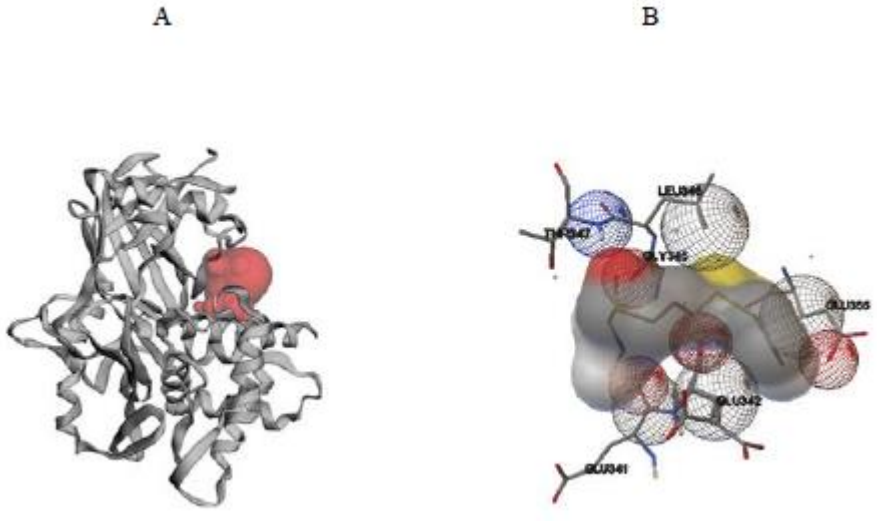

Figure 5. Binding site of Ubiquitin. (B): Binding site of Ubiquitin with Ajoene

TARGET MOLECULES	COMPOUND	BINDING ENERGY	AMINO ACID INTERACTED	BOND FORMED
Cul3	Ajoene	-2.96	LEU-253, ILE-258, VAL-260, LEU-266, LEU-292	1H- ILE258
KEAP1	Ajoene	-5.6	GLY-367,GLY-417,VAL-418,GLY-464,VAL-465,GLY-511,VAL-512,GLY-605,VAL-606	1H- VAL418
NRF2	Ajoene	-5.35	LEU365, GLY417, GLY364, VAL465, VAL463, GLY462, ARG415, GLY509	No HB
RBX1	Ajoene	-4.79	LYS-587, LYS-586, VAL-475, LEU-582, GLN-538, TRP-581, ILE-537, THR-580	1H- LYS-587,
Ubiquitin	Ajoene	-4.31	LEU-346,GLU-341,GLU-342,GLY-345,THR-347,GLU-355	1H- THR347

Table 1. The Target Molecule and Compound Binding Energies and Amino Acid Interaction

Discussion

To discuss the in-silico analysis of Ajoene from Aegle marmelos Correa for its anti-diabetic action focusing on the Nrf2/Keap-1 signaling pathway, we delve into the molecular mechanisms underlying the potential therapeutic effects of this bioactive compound. Ajoene, a sulfur-containing compound found in Aegle marmelos, has shown promise in managing diabetes, making it a subject of interest for in silico studies aiming to elucidate its anti-diabetic properties [23]. Molecular docking is a computational technique used to predict the binding modes and affinities of small molecules, like Ajoene, with target proteins, such as those involved in the Nrf2/Keap-1 signaling pathway. By conducting molecular docking studies, researchers can simulate the interactions between Ajoene and key proteins like Nrf2 and Keap-1, providing insights into the potential mechanisms through which Ajoene exerts its anti-diabetic effects. The Nrf2/Keap-1 pathway is crucial in cellular defense against oxidative stress and inflammation, both of which play significant roles in the pathogenesis of diabetes. Nrf2 activation leads to the transcription of antioxidant response elements, enhancing cellular antioxidant defences [24].

Keap-1 regulates Nrf2 activity by sequestering it in the cytoplasm, controlling its translocation to the nucleus upon activation. Understanding how Ajoene interacts with this pathway can shed light on its ability to modulate oxidative stress and inflammation in the context of diabetes. *In silico* studies aim to identify specific targets within the Nrf2/Keap-1 pathway that are modulated by Ajoene. By analysing the binding affinities and interactions between Ajoene and these targets, researchers can elucidate the molecular

mechanisms through which Ajoene influences the activity of Nrf2 and Keap-1. This information is crucial for understanding how Ajoene may enhance antioxidant defences and mitigate oxidative damage associated with diabetes. The findings from in silico analysis can have significant therapeutic implications by providing a molecular basis for the anti-diabetic effects of Ajoene. Insights into how Ajoene interacts with the Nrf2/Keap-1 pathway can guide the development of novel therapeutic strategies for diabetes management.

By targeting specific components of this signaling pathway, Ajoene may offer a promising avenue for combating the oxidative stress and inflammation characteristic of diabetes [22]. The in-silico analysis of Ajoene from Aegle marmelos Correa targeting the Nrf2/Keap-1 signaling pathway represents a valuable approach to understanding the molecular mechanisms underlying its anti-diabetic properties. By unravelling the intricate interactions between Ajoene and key proteins in this pathway, researchers can pave the way for the development of innovative treatments for diabetes that leverage the therapeutic potential of natural compounds like Ajoene.

Conclusion

In silico analysis of ajoene from Aegle marmelos Correa reveals its potential as an antidiabetic agent through the modulation of the Nrf2/Keap-1 signalling pathway, which is crucial for oxidative stress regulation. The findings suggest that ajoene activates this pathway, leading to an increase in antioxidant enzymes and a subsequent reduction in oxidative stress in diabetes. The specific interaction between ajoene and Cul3, characterized by the establishment of a single hydrogen bond involving ILE258, contributes to the formation of a binding pocket encompassing key residues. These results highlight the potential of ajoene as a promising candidate for diabetes management and underscore the value of in silico analysis in drug discovery. Further experimental validations are needed to explore the application of ajoene in diabetes management.

Conflict of Interest

The author hereby declares that there is no conflict of interest.

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