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Original Article

POTENTIAL ACTIVITY OF KAEMPFEROL AS ANTI-PARKINSON'S; MOLECULAR DOCKING AND PHARMACOPHORE MODELLING STUDY

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ABSTRACT

Objective: This study examined molecular docking and pharmacophore modeling to evaluate the potential antiparkinson activity of Kaempferol on various types and classes of receptors.

Methods: The molecular docking was performed on various classes of receptors, namely transcription factor Nrf2, A2A Adenosine, and catechol-O-methyl transferase, using auto dock 4.0.1 software.

Results: Kaempferol exhibited potential effects on two of the three tests (A2A adenosine and COMT receptors) as indicated by the lowest free energy binding values (-5.42 kcal/mol,-7.16 kcal/mol, and-8.33 kcal/mol, respectively). Kaempferol also had lower inhibitory constant values on transcription factor Nrf2, A2A adenosine, and COMT receptors (106.06 µM, 5.63 µM, and 779.51 nM, respectively). Kaempferol and the natural ligand had similar functional groups according to the critical components of the interaction between amino acid residues. The pharmacophore modeling revealed that hydroxyl functional groups strongly interact with crucial amino acid residues of the receptors.

Conclusion: This study concludes that kaempferol is a potential antiparkinson agent against multiple receptors.

Keywords: Antiparkinson's, Kaempferol, Molecular docking, Pharmacophore modeling

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INTRODUCTION

Parkinson's disease is a neurological disorder affecting 2-3% of people under 65. In Europe, the estimated prevalence and incidence rates of Parkinson's disease range from 65 to 12,500 per 100,000 and 5 to 346 per 100,000 person-years, respectively. Male gender and age are factors that contribute to the disease. Some types of pesticides and rural living have been linked to an increased incidence of Parkinson's disease. It has been observed that certain chemicals, such as 1-methyl-4-phenyl tetrahydropyridine (MPTP) and annonacin, can lead to the death of nigrostriatal cells and a particular type of atypical parkinsonism [1]. Neuropathological indicators of Parkinson's disease include the presence of intracellular inclusions containing aggregates of alpha-synuclein and neuronal death in the substantia nigra, which leads to a deficiency of dopamine in the striatum. As the disease progresses, more cell types from the central and peripheral autonomic nervous systems become affected [2–4].

While bradykinesia and other key motor symptoms are necessary for a clinical diagnosis of Parkinson's disease, the condition is also accompanied by a range of non-motor symptoms contributing to overall disability. There are multiple pathways and mechanisms, including alpha-synuclein proteostasis, mitochondrial function, oxidative stress, calcium homeostasis, axonal transport, and neuroinflammation, involved in the underlying molecular etiology of the disease. Recent studies on diagnostic biomarkers have benefited from neuroimaging techniques such as positron emission tomography (PET), single photon emission computed tomography (SPECT), and advanced magnetic resonance imaging (MRI) as they enable early and differential diagnosis. Parkinson's disease is primarily pharmacologically treated by replacing striatal dopamine, non-dopaminergic methods to address motor and non-motor symptoms, and deep brain stimulation for those experiencing untreatable L-DOPA-related motor problems. Alpha-synuclein aggregation and cellular transit have been potential therapeutic targets in experimental therapies that restore striatal dopamine. It is challenging to identify the markers of prodromal stages of the disease, which would allow for the early implementation of disease-modifying treatments.

Currently, there are no disease-modifying treatments for Parkinson's disease, and treatment options are largely focused on managing symptoms and targeting the dopaminergic pathway. The most effective medication for motor symptoms is levodopa, the gold standard for treating Parkinson's. The remaining dopaminergic neurons in the substantia nigra convert levodopa to dopamine once it has crossed the blood-brain barrier. Levodopa is usually administered as tablets multiple times daily, but in advanced cases, it can also be given through duodenal infusion. The medicine significantly reduces the symptoms and supports a diagnosis of Parkinson's disease through a pharmacological test. Decarboxylase inhibitors, such as carbidopa or benserazide, can reduce levodopa's peripheral dopaminergic side effects, such as nausea and hypotension [5, 6]. Other side effects of levodopa include drowsiness, confusion, hallucinations, and impulse control disorders (ICDs), such as hypersexuality, compulsive shopping, gambling, and punding. However, developing motor problems, such as fluctuations, dyskinesia, dystonia, and wearing off, is a major limitation of levodopa [7].

Researchers have explored the use of a novel alternative for the treatment of Parkinson's disease that is derived from a secondary metabolite called Kaempferol to address the negative effects associated with standard levodopa therapy. Kaempferol is a common secondary metabolite found in plants with pharmacological properties such as antibacterial, antifungal, antiplasmodial cytogenetic, antiulcerogenic, antioxidant, antiviral, antiprotozoal, anti-colon cancer, and cytotoxic activity [8]. However, the literature on kaempferol's phytochemical makeup and biological effects is limited. Hence, it is necessary to examine kaempferol's bioactivity and pharmacological properties.

In this study, a molecular docking study was performed to assess the activity of kaempferol as an antiparkinsonian agent using structure-based drug design and pharmacophore modeling. This study explains the molecular basis of kaempferol's potential as a treatment for Parkinson's disease

MATERIALS AND METHODS

Identification of target receptors and the lead compounds

To identify the targets for this study, receptors commonly used to assess anti-parkinsonian effects, such as catechol-O-

methyltransferase inhibitors (COMT), A2A adenosine antagonists, and an important regulator of cellular protection against oxidative stress, were used. Parkinson's disease is a neurodegenerative condition strongly associated with oxidative stress. Therefore, the initial screening of lead compounds and receptor targets will be based on factors such as the method used to extract the receptor, the number of amino acids present, the origin of the organism, and the resolution of each receptor.

Validation using the molecular docking method

Several receptors from various classes modulated in Parkinson's disease were validated using the molecular docking approach. The molecular docking validation method was used on several types of receptors, including transcription factor Nrf2 (protein binding), A2A adenosine (signalling protein), and catechol-O-methyltransferase (transferase) [9, 10].

The first step in the process was to download the receptors in. pdb format from the Protein Data Bank database (https://www.rcsb.org/) [10, 11]. Each receptor was prepared using Discovery Studio Visualizer software by removing it from the complex lead compounds. To reduce variance in hydrogen bonding interactions, the water molecules at the receptor were also removed.

Autodock 4.0.1 software was used where kollman charges and compute gasteiger charges were added to the native ligand, and each receptor, polar hydrogen, was added to the protein molecule. Non-polar merged hydrogen was added to the ligand molecules to complete the process. The results were recorded in. pdb format, with "pdbq" referring to Protein Data Bank partial charge (q) and "t" standing for atom type (t). To generate the Grid Parameter File (.gpf) and Docking Parameter File (.dpf) with GA runs set to 100 and energy evaluation set to 2500000, the data for the receptor and ligand (.pdb) were combined. Finally, redocking was performed as a final step to examine the information gained from the molecular docking validation results using Command Prompt (CMD) tools.

Virtual screening on test compound

The potential of Kaempferol as a treatment for Parkinson's disease was evaluated using the structure-based drug design (SBDD). Multiple structures of receptors that have upregulated or down-regulated expression in Parkinson's disease were used as test targets, and the native ligands (3S)-1-(4-{[(2,3,5,6-tetramethyl phenyl)sulfonyl]amino}naphthalen-1-yl)pyrrolidine-3-carboxylic acid, oleic acid, and 8-hydroxy-6-(2-methylpyridin-3-yl)-3H-quinazoline-4-one were used as lead compounds for the test targets. The molecular test compound was modelled in ChemDraw 2D, and the energy minimization of the model was performed in ChemDraw 3D using MM2. The results were saved in. pdb format after the minimization step was completed. Using Autodock 4.0.1, Compute Gasteiger charges and non-polar merged hydrogen were added to structures and compounds. The test compound was then paired with each target receptor to create. gpf and. dpf files in the final steps [10].

Pharmacophore modelling

SBBD method was employed in the form of pharmacophore modelling on each complex of Kaempferol and receptors that had previously undergone molecular docking. Ligandscout 4.4 was used to load each complex into the structure-based perspective. The pharmacophore was generated, its 2D depiction was created to interpret and evaluate the results, and the interaction was analyzed by selecting the yellow box [11–13].

RESULTS

Table 1: Validation using molecular docking method

PDB ID (Resolution)	Organism	Receptor (Classification)	Complexed ligand	Amino acid interaction	Free energy (ΔG Gibs)	Inhibition constant (CI)	RMSD
5CGJ (3.36 A)	*Mus Musculus*	Transcription Factor Nrf2 (Protein Binding)	(3S)-1-(4-{[(2,3,5,6-tetramethyl phenyl)sulfonyl] amino}naphthalen-1-yl) pyrrolidine-3-carboxylic acid	SER508, ARG483, PHE577, ALA556, ARG415, TYR334,	-10.18 kcal/mol	34.53 nM	0.99 A
5NM4 (1.70 A)	*Homo Sapiens, Escherichia coli*	A2A Adenosin (Signaling Protein)	Oleic Acid	ASN 358, GLU178, ALA72, ILE75, ILE379, MET186, PHE177, HIS355, LEU354,	-9.22 kcal/mol	174.23 nM	3.33 A
5P9V (1.04 A)	*Rattus norvegicus*	Catechol-O-methyl transferase (Transferase)	8-hydroxy-6-(2-methyl pyridine-3-yl)-3H-quinazoline-4-one	ASP141, HIS142, GLY66, MET89, ILE91	-11.73 kcal/mol	2.53 nM	1.24 A

Table 1 shows the validation outcome. From the root mean standard deviation (RMSD), where the population cluster is the main parameter, RMSD requires 2 Armstrong to measure the variation in the native ligands' position between docking and redocking [12]. The test results demonstrated that two of the three receptors met the requirements as seen from the RMSD values for the receptors of Transcription Factor Nrf2 (Protein Binding) and catechol-O-methyltransferase (Transferase) that were smaller than 2A 0.940 A and 1.24 A, respectively.

The RMSD value for the A2A adenosine (Signaling protein) receptor was higher than the required level (2.33 A). How these receptor clusters distributed the data from 100 different docking conformations, they are referred to as the best receptor clusters and the best molecular docking [14]. The clustering of docked conformations was dictated by the RMS tolerance specified by "rmstol" in the docking parameter file (dpf). More cluster indicates a greater probability that the preferred conformation will bind to the protein target [15]. The docking results showed the free energy binding values of the (3S)-1-(4-{[(2,3,5,6-tetramethyl phenyl)sulfonyl]amino}naphthalen-1-yl)pyrrolidine-3-carboxylic acid, Oleic Acid, and 8-hydroxy-6-(2-methylpyridin-3-yl)-3H-quinazoline-4-one are-10.18 kcal/mol,-9.22 kcal/mol, and-11.73 kcal/mol, respectively. They corresponded to an inhibitory constant of 34.53 nM, 174.23 nM, and 2.53 nM. The native ligand on the Transcription Factor Nrf2 receptor has hydrogen bonding interaction on amino acids SER508 and ARG483 and non-hydrogen bonding interaction on amino acids PHE577, ALA556, ARG415, and TYR334. As for the native ligand on the A2A Adenosine receptor, hydrogen binds the bonding interaction on amino acids ASN358 and GLU178, and non-hydrogen bonding interacts with amino acids ALA72, ILE75, ILE379, MET186, PHE177, HIS355, and LEU354.

Then the native ligand on the COMT receptor interacts with amino acids ASP141 and HIS142 and non-hydrogen bonding interactions with amino acids MET89 and ILE91. The results are generally consistent with earlier investigations. These data are used as a reference to evaluate the antiparkinson's activity of the Kaempferol tested using molecular docking screening [16].

Table 2: Virtual screening results

PDB ID	Amino acid residue		Free energy (ΔG Gibs)		Inhibition constant	
	Kaempferol	Native ligand	Kaempferol	Native ligand	Kaempferol	Native ligand
5CGJ	SER508, ARG483, SER555, GLY462, GLY364, ARG415, TYR225, ALA556,	SER508, ARG483, PHE577, ALA556, ARG415, TYR334,	-5.42 kcal/mol	-10.18 kcal/mol	106.06 µM	34.53 nM
5NM4	ASN358, GLU178, ILE75, TYR376, PHE177, ILE379, LEU354,	ASN 358, GLU178, ALA72, ILE75, ILE379, MET186, PHE177, HIS355, LEU354,	-7.16 kcal/mol	-9.22 kcal/mol	5.63 µM	174.23 nM
5P9V	HIS142, GLU90, VAL42, ASP141, ALA67, TYR68, MET40	ASP141, HIS142, GLY66, MET89, ILE91	-8.33 kcal/mol	-11.73 kcal/mol	779.51 nM	2.53 nM

Table 2 compares the virtual screening results of the test compound on each receptor to the native ligands of each receptor using the SBDD approach with GA runs set to 100 and medium energy set to 250,000. The data includes the residues of amino acids (a measure used to compare the activity of the test compound and the lead compounds based on the type of interaction and amino acids), the free energy binding (a parameter used to evaluate the strength of the interaction formed, with lower energy indicating a stronger bond and a spontaneous bond formed) [17], and values of the inhibition constants (a method for estimating pharmacological potency based on the inhibition constant, with lower values indicating higher biological activity).

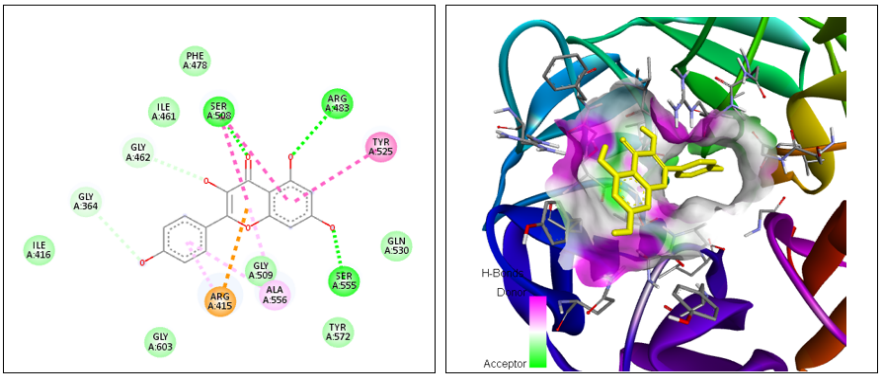

Fig. 1A: 2D and 3D visualization between kaempferol and transcription factor_Nrf2

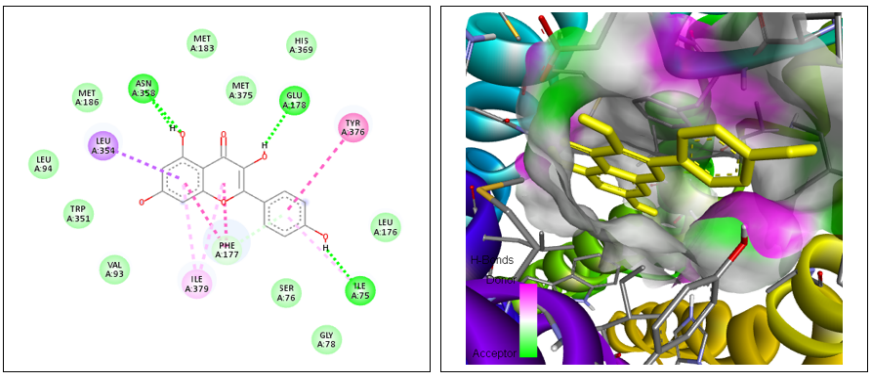

Fig. 1B: 2D and 3D visualization between kaempferol and A2A adenosine

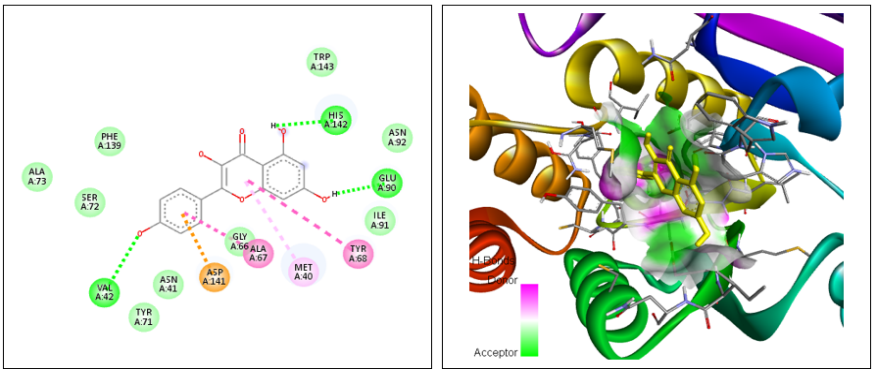

Fig. 1C: 2D and 3D visualization between kaempferol and COMT

Fig. 1A-C shows 3D visualization of kaempferol's binding site on the receptors, which can be used to determine if the drug has an either competitive or non-competitive inhibitory function. Kaempferol binding to receptors in all experiments had the same active site as the lead compounds. The interactions between the lead compounds and kaempferol's amino acid residues were similar. For example, there are four comparable interactions between the transcription factor receptor, and two of them involve hydrogen bonds at SER508 and ARG483. The A2A adenosine receptor also has six comparable interactions, three of which involve hydrogen bonds at ASN358, GLU178, and ILE75. The COMT receptor has two comparable interactions, one involving a hydrogen bond at HIS142. This essential amino acid can be used to show how similar the active lead compounds and the kaempferol compound are to each receptor [18].

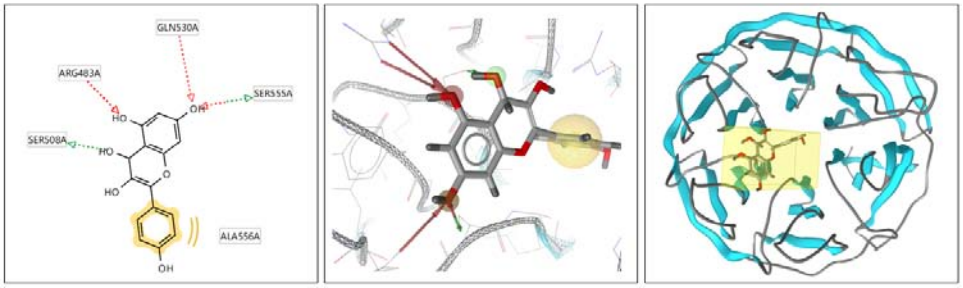

Fig. 2A: 2D visualization of pharmacophore modelling between kaempferol and transcription factor_Nrf2

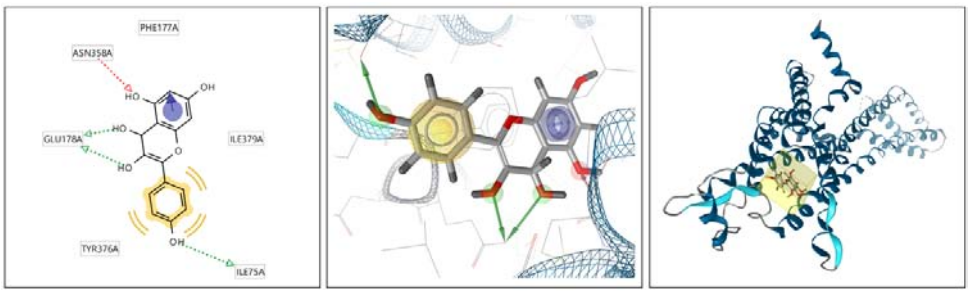

Fig. 2B: 2D visualization of pharmacophore modelling between kaempferol and A2A adenosine

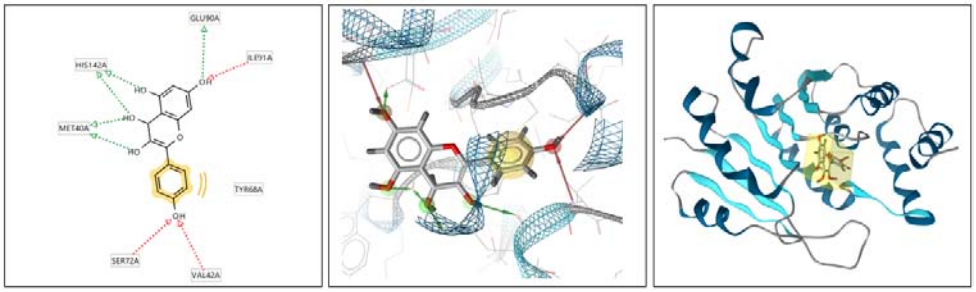

Fig. 2C: 2D Visualization of pharmacophore modelling between kaempferol and COMT

Fig. 2 shows the Kaempferol pharmacophore model on antiparkinson's receptors. Pharmacophore modelling was used to examine the functional groups interacting with the targets and potential structural changes that may be made to improve efficacy and address kaempferol's physicochemical limitations. Benzopiran moiety might be a potential component for modification in future drug development, for it has unintended interaction with amino acid residues. Hydroxyl groups are the main functional groups that bond with each receptor's key amino acid residues.

DISCUSSION

The data from the Protein Data Bank database infer that X-ray crystallography, NMR spectroscopy, and electron microscopy can be utilized to ascertain the structure of the receptors. Each strategy comes with its own set of benefits and drawbacks. The molecules' structures are shown in the X-ray diffraction pattern in X-ray crystallography, which details the conformation and separation of close-knit atoms for NMR spectroscopy. The final atomic model was created for each strategy based on the data. The X-ray technique was used to obtain the receptors used in this work. The receptors also made a proper model for research on antiparkinsons. The receptor with a resolution value of 3A was regarded as the most standard-compliant because it had a resolution value near 2 Armstrong [15]. The clones of the structure resemble the original receptor structure, as shown by the resolution value. The importance of the RMSD values was highlighted by the molecular docking method's validation results on antiparkinson's receptors. The RMSD value closer to or lesser than 2 Armstrong showed that variation in native ligand locations follows the docking and redocking [19].

All three receptors: Transcription Factor Nrf2 (Protein Binding), A2A Adenosine (Signalling protein), and Catechol-O-methyltransferase (Transferase) had RMSD values of 3.36 A, 1.70 A, and 1.04 A, respectively showed free energy binding values of-10.18 kcal/mol,-9.22 kcal/mol, and-11.73 kcal/mol, respectively. They also show the constant inhibition value of 34.53 nM, 174.23 nM, and 2.53 nM, respectively. The receptors interacted with both hydrogen and non-hydrogen bonds on various amino acid residues, according to the values of energy binding (table 1). Results of molecular docking

screening show that these values met the requirements for evaluating kaempferol as an antiparkinson's [20]. According to the findings, two of the three tests (Kaempferol on A2A Adenosine and COMT) indicated a possible effect from the lowest free energy binding of-7.16 kcal/mol and-8.33 kcal/mol, respectively. A lower free energy binding corresponds to lower energy activation. Hence kaempferol compound and the receptors will interact and trigger a spontaneous reaction [16]. The kaempferol compound has a lower value of the inhibition constant determined by the constant inhibition results (106.06 µM, 5.63 nM, and 779.51nM on Transcription Factor Nrf2, A2A Adenosine, and COMT, respectively). Since the molecule has a significant inhibitory capacity at low dosages and shows the medicine in inhibiting receptors or enzymes, a relatively low value might carry significant power. The energy and inhibition constants indicate that the interaction between the compound's structures with the amino acids at its receptors affects the thectivity of the substance. Kaempferol molecule and the native ligands interact similarly through comparable amino acid residues. First, the Transcription Factor receptor has four similar interactions, two of which are hydrogen-bonding interactions at SER508 and ARG483. In addition, for A2A Adenosine receptor has six similar interactions, including three hydrogen-bonding interactions at ASN358, GLU178, and ILE75. Furthermore, COMT has two similar interactions, one of which is the hydrogen-bonding interaction at HIS142. (fig. 1.) This study highlights the kaempferol's hydrogen bonding interaction (table 2) following the awareness that this interaction is reversible and substantially more powerful than other types of interactions. The results of the molecular docking study showed that kaempferol had comparable interactions with the receptors as the lead compounds and demonstrated a potential effect on the receptors due to its low free energy binding and low inhibition constants. The pharmacophore model revealed that the benzopyran component of kaempferol might be a potential target for modification in future drug development. Overall, these findings suggest that kaempferol may have promise as a treatment for Parkinson's disease and warrant further investigation [21].

Overall, Kaempferol has shown potential as an antiparkinson's drug through its interaction with the receptors studied. Molecular docking results demonstrated that the compound had similar interactions with receptor amino acid residues as lead compounds. Pharmacophore modelling revealed that hydroxyl groups played a crucial role in these interactions. Further research and testing will be necessary to fully understand the potential of kaempferol as a treatment for Parkinson's disease.

The potential for kaempferol to be used in drug development is promising for its maximum efficacy, good physicochemical properties, and minimal side effects. Further research on kaempferol may reveal it to be a valuable candidate for drug development in the future.

CONCLUSION

There are several potential mechanisms of action for kaempferol's antiparkinson effects. One possibility is that it inhibits the transcription factor Nrf2, which regulates cellular defence against oxidative stress and is implicated in Parkinson's disease. Kaempferol may also function as an antagonist of A2A adenosine, a substance that regulates the central nervous system. Additionally, kaempferol can inhibit the enzyme COMT, which reduces dopamine levels. The combination of these actions may improve mobility in Parkinson's disease patients.

There are several possible mechanisms of action for kaempferol's antiparkinson effects. Firstly, Kaempferol inhibits the transcription factor Nrf2, produced by the NFE2L2 gene and plays a critical role in cellular defence against oxidative stress. Parkinson's disease has a strong link to oxidative stress. In addition, Kaempferol can act as an antagonist of A2A adenosine, a substance heavily involved in controlling the central nervous system. The striatum, a region in the brain, contains both adenosine A2A and dopaminergic D2 receptors, creating a milieu for antagonistic interaction between adenosine and dopamine. Experimental results show that simultaneous stimulation of dopaminergic D2 receptors and inhibition of adenosine A2A receptors can significantly improve mobility in Parkinson's patients. Kaempferol also can inhibit the COMT enzyme that reduces dopamine levels.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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