Molecular Docking Studies of *Antirrhinum majus*-derived Compounds for CNS Stimulant Activity: Insights into Ligand-Receptor Interactions and Potential Therapeutic Candidates

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***Abstract- Antirrhinum majus*, also known as snapdragon, is a medicinal plant that has a long history of traditional use for an array of therapeutic purposes. Molecular docking studies have gained significance as an indispensable tool for predicting the binding interactions between compounds and target proteins. This research employs molecular docking studies to explore the CNS stimulant activity of compounds derived from *Antirrhinum majus*. The investigation focuses on their interactions with the DAT receptor (PDB code: 4M48), which is known to play a significant role in CNS stimulant activity. The main objective was to investigate the binding affinities of the phytoconstituents and identify the particular amino acid residues involved in the biochemical interactions at the binding site. The top 5 hits exhibited binding affinities from -10.9 to -8.8 kcal/mol. Notably, the designed compounds demonstrated favorable biochemical interactions with the target protein, establishing strong hydrogen bonding with Arg476, Asp475, and Ala117, along with pi bonding with Tyr124 and Phe325. These findings provide critical insights into the potential of these inhibitors as promising candidates for modulating neuronal activities in the context of CNS stimulant activity.**

Keywords: **Molecular docking, *Antirrhinum majus*, CNS stimulant activity, ligand-receptor interactions, neurological disorders.**

1. INTRODUCTION

Antirrhinum majus, commonly known as snapdragon, is a medicinal plant that has been traditionally used for various therapeutic purposes (Kumar et al., 2022). It contains a diverse array of phytochemicals, including alkaloids, flavonoids, and terpenes, which are known to possess potential pharmacological activities (Jang et al, 2020; Saqallah et al., 2022). The pursuit of CNS stimulants holds significant importance in addressing neurological disorders, and molecular docking simulations have emerged as a crucial tool for predicting compound interactions with target proteins, facilitating the discovery of potential drug candidates (Parvez et al, 2018; Tsolaki et al, 2023). In recent years, scientific interest has grown regarding the potential CNS stimulant effects of *Antirrhinum majus* (Kumar et al.,2022). Historically, this flowering plant has been utilized in traditional medicine to address various conditions, including anxiety, depression, and pain (Al-Snafi et al., 2015).

In this study, we directed our attention towards discovering plant-based inhibitors that target a DAT receptor known to be involved in CNS stimulant activity (Bu et al., 2021; Vaughan et al., 2013). The main goal of the study was to explore the interactions between plant-based inhibitors derived from *Antirrhinum majus* with DAT receptor, with a focus on key amino acid residues, to assess their potential as modulators of CNS stimulant activity for therapeutic intervention in neurological disorders.

2. METHODOLOGY

2.1 Ligand and target data collection

The IMPPAT database, developed by researchers at the Institute of Mathematical Sciences in Chennai, India, is a comprehensive repository of Indian medicinal plants, phytochemicals, and therapeutic uses (Mohanraj et al., 2018). For this study 25 phytoconstituents derived from *Antirrhinum majus* were collected from the IMPPAT database (table 1). Before conducting molecular docking, the geometry of the compounds was optimized and energy minimized using open Babel (O'Boyle et al., 2011). Subsequently, the optimized structures were converted to pdbqt format to prepare them for the docking simulations.

The Protein Data Bank, offers a vast collection of 3D structural data for proteins, nucleic acids, and other biological molecules. For this study, the three-dimensional structure of the *Drosophila melanogaster* dDAT receptor (PDB ID:

4M48) was downloaded from PDB (Penmatsa et al., 2013). This DAT structure serves as a well-established model for the dopamine transporter (DAT) and shares approximately 50% sequence similarity with mammalian DAT. Researchers commonly employ this dDAT model to investigate the mechanism of action of various compounds. The protein structure was prepared by removing solvents and hetero atoms and energy minimization using chimera dock prep module.

Table 1: Dataset of phytoconstituents of *A. majus*.

IMPPAT ID	Compound Name	Smiles
IMPHY004055	Choline	C[N+](C)(C)CCO
IMPHY006273	4-Methyl-2,6-naphthyridine	Cc1cncc2c1cncc2
IMPHY010914	2-Aminobutyric acid	CCC(C(=O)O)N
IMPHY012545	Antirrhinoside	CC12C3C(OC=CC3(C(C1O2)O)O)OC4C(C(C(C(O4)CO)O)O)O
IMPHY003138	Keracyanin	CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=CC4=C(C=C(C=C4[O+]=C3C5=CC(=C(C=C5)O)O)O)O)O)O)O)O)O)O.[Cl-]
IMPHY003437	Pelargonidin	C1=CC(=CC=C1C2=[O+]C3=CC(=CC(=C3C=C2O)O)O)O
IMPHY004388	Kaempferol	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O
IMPHY004619	Quercetin	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O
IMPHY004660	Luteolin	C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O)O
IMPHY004661	Apigenin	C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O
IMPHY004681	Aureusidin	C1=CC(=C(C=C1C=C2C(=O)C3=C(C=C(C=C3O2)O)O)O)O
IMPHY008945	Cyanidin	C1=CC(=C(C=C1C2=[O+]C3=CC(=CC(=C3C=C2O)O)O)O)O
IMPHY014890	Cyanidin 3-glucoside	C1=CC(=C(C=C1C2=[O+]C3=CC(=CC(=C3C=C2OC4C(C(C(C(O4)CO)O)O)O)O)O)O)O)O.[Cl-]
IMPHY014836	beta-Sitosterol	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C
IMPHY014990	Linoleic acid	CCCCCC=CCC=CCCCCCCCC(=O)O
IMPHY001699	4-Hydroxybenzoyl glucose	C1=CC(=CC=C1C(=O)OC2C(C(C(C(O2)CO)O)O)O)O

IMPHY002580	Chrysoeriol glucuronide	COC1=C(C=CC(=C1)C2=CC(=O)C3=C(C=C(C=C3O2)OC4C(C(C(C(O4)C(=O)O)O)O)O)O)O
IMPHY002996	Bracteatin 6-glucoside	C1=C(C=C(C(=C1O)O)O)C=C2C(=O)C3=C(C=C(C=C3O2)OC4C(C(C(C(O4)CO)O)O)O)O
IMPHY003010	Aureusin	OCC1O[C@@H](Oc2cc3OC(=Cc4ccc(c(c4)O)O)C(=O)c3c(c2)O)C([C@H]([C@@H]1O)O)O
IMPHY003173	Chalconaringenin 4'-glucoside	OC[C@H]1O[C@@H](Oc2cc(O)c(c(c2)O)C(=O)/C=C/c2ccc(cc2)O)[C@@H]([C@H]([C@@H]1O)O)O
IMPHY004753	Apigenin 7,4'-diglucuronide	OC(=O)C1O[C@@H](Oc2ccc(cc2)c2cc(=O)c3c(o2)cc(cc3O)O[C@@H]2O[C@@H](C(=O)O)[C@H](C(C2O)O)O)C(C([C@@H]1O)O)O
IMPHY006273	4-Methyl-2,6-naphthyridine	Cc1cncc2c1cncc2
IMPHY007296	3-Methyldotriacontane	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC(CC)C
IMPHY012872	Luteolin-7-glucuronide	OC(=O)[C@H]1OC(Oc2cc(O)c3c(c2)oc(cc3=O)c2ccc(c(c2)O)O)[C@@H]([C@H]([C@@H]1O)O)O
IMPHY014824	Astragalin	OC[C@H]1O[C@@H](Oc2c(oc3c(c2=O)c(O)cc(c3)O)c2ccc(cc2)O)[C@@H]([C@H]([C@@H]1O)O)O

2.2 Molecular docking study

PyRx, an open-source software package, revolutionizes virtual screening and structure-based drug design (Dallakyan et al., 2015). Written in Python, PyRx seamlessly integrates with AutoDock Vina, AutoDockTools, and Open Babel, providing a wide range of functionalities. This study utilizes PyRx autodock vina module to efficiently screen *A. majus* phytocompound libraries for potential drug dDAT target to discover potential hits. The 25X25X25 size grid was generated around X:39.01, Y: -1.8, Z:54.65 coordinates. To validate the docking protocol, we performed redocking of the co-crystallized drug, nortriptyline, back into the binding site. The predicted pose obtained from the redocking was then compared with the docked pose of nortriptyline from the initial docking study. After molecular docking the poses were ranked based on the vina binding energy. The top five hits were selected and visulized using The Free Maestro visualizer.

3. RESULTS & DISCUSSION

3.1 Interaction of phytoconstituents with target DAT

A molecular docking study of the A. majus phytoconstituents was conducted on the dDAT receptor to identify potential hits and important interactions responsible for the biochemical interaction at the binding site. Molecular docking of the co-crystalised nortriptyline to its binding site generated pose with same orientation and geometry with the binding energy -10.6 kcal/mol (Figure 1, Figure 2 a). The stability analysis revealed that two compounds beta-setosterol and Luteolin-7-glucuronide exhibited higher or same stability with dDAT as the the reference molecule Nortriptyline (table 2). Overall, top five compounds showing the highest binding energy with the target were selected for visual analysis. The binding affinities for the top 5 compounds ranges from − 10.9 to − 8.8 kcal/mol. The results of our molecular docking simulation study shed light on the critical role of specific amino acid residues in the drosophila dopamine transporter (DAT) for CNS stimulant activity.

Among the phytoconstituents, beta-Sitosterol displayed the highest binding energy of -10.9 kcal/mol, indicating a strong interaction with the target protein (table 2). This interaction was predominantly mediated by hydrogen bonding with Arg476, an essential residue in the substrate recognition site of DAT (figure 2 b).

Luteolin-7-glucuronide, with a binding energy of -10.6 kcal/mol, formed strong hydrogen bonds with Asp475 and pi bond with Tyr124. Tyr124 is a conserved residue known to be crucial for the high-affinity binding of substrates to DAT, while Asp475 plays a significant role in coordinating the binding of dopamine and other ligands (figure 2 c).

Chrysoeriol glucuronide exhibited a binding energy of -10.3 kcal/mol, forming hydrogen bonds with Ala117, Asp475 and pi bond with Tyr124. Ala117 has been implicated in substrate recognition and binding within DAT. Additionally,

the interactions with Tyr124 and Asp475 further emphasize the potential of Chrysoeriol glucuronide as a modulator of dopamine reuptake and neurotransmitter regulation (figure 2 d).
Bracteatin 6-glucoside, with a binding energy of -10.1 kcal/mol, demonstrated strong pi bonding interactions with Tyr124. This observation highlights the significance of Tyr124 as a key residue in DAT-ligand interactions and suggests that Bracteatin 6-glucoside might affect dopamine reuptake via competitive bidding (Figure 2 e).
Apigenin1 displayed a binding energy of -8.8 kcal/mol, forming hydrogen bonds with Asp46, Val113, and pi bonding with Phe325. The interactions with Asp46 and Val113 indicate potential involvement in substrate binding and conformational changes required for dopamine translocation. Pi bonding with Phe325, which is in proximity to the substrate-binding site, further strengthens the potential of Apigenin1 as a modulator of dopamine transport (figure 2 f).
These findings underscore the importance of specific amino acid residues within the dopamine transporter for the binding and potential modulation of CNS stimulant activity by the designed inhibitors derived from Antirrhinum majus compounds. By interacting with key residues involved in substrate recognition and dopamine binding, these inhibitors have the potential to alter dopamine neurotransmission, thereby presenting opportunities for the development of novel therapeutic agents targeting CNS disorders.
The results obtained from this molecular docking simulation study serve as a foundation for further experimental investigations, such as *in vitro* and *in vivo* studies, to validate the inhibitory activity of the designed compounds on dopamine transporter and their potential as CNS stimulants. Furthermore, a deeper understanding of the specific interactions with DAT may facilitate the rational design of more potent and selective compounds with enhanced CNS stimulant effects while minimizing unwanted side effects (figure 1 and 2).

Table 2: Phytoconstituents of the plant and their binding energy and interacting residues with the target receptor.

Phytoconstituent	Binding energy (kcal/mol)	Interacting Residues	
		Hydrogen bond	Pi bond
Beta-Sitosterol	-10.9	Arg476	-
Luteolin-7-glucuronide	-10.6	Asp475	Tyr124
Chrysoeriol glucuronide	-10.3	Ala117, Asp475	Tyr124
Bracteatin 6-glucoside	-10.1	-	Tyr124
Apigenin1	-8.8	Asp46, Val113	Phe325

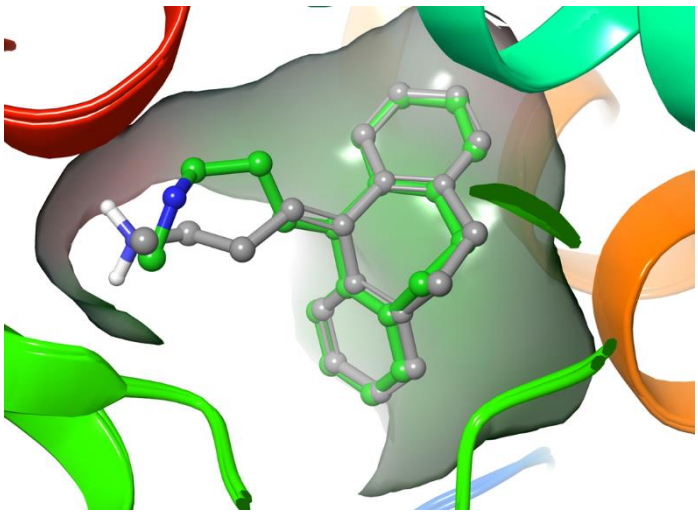
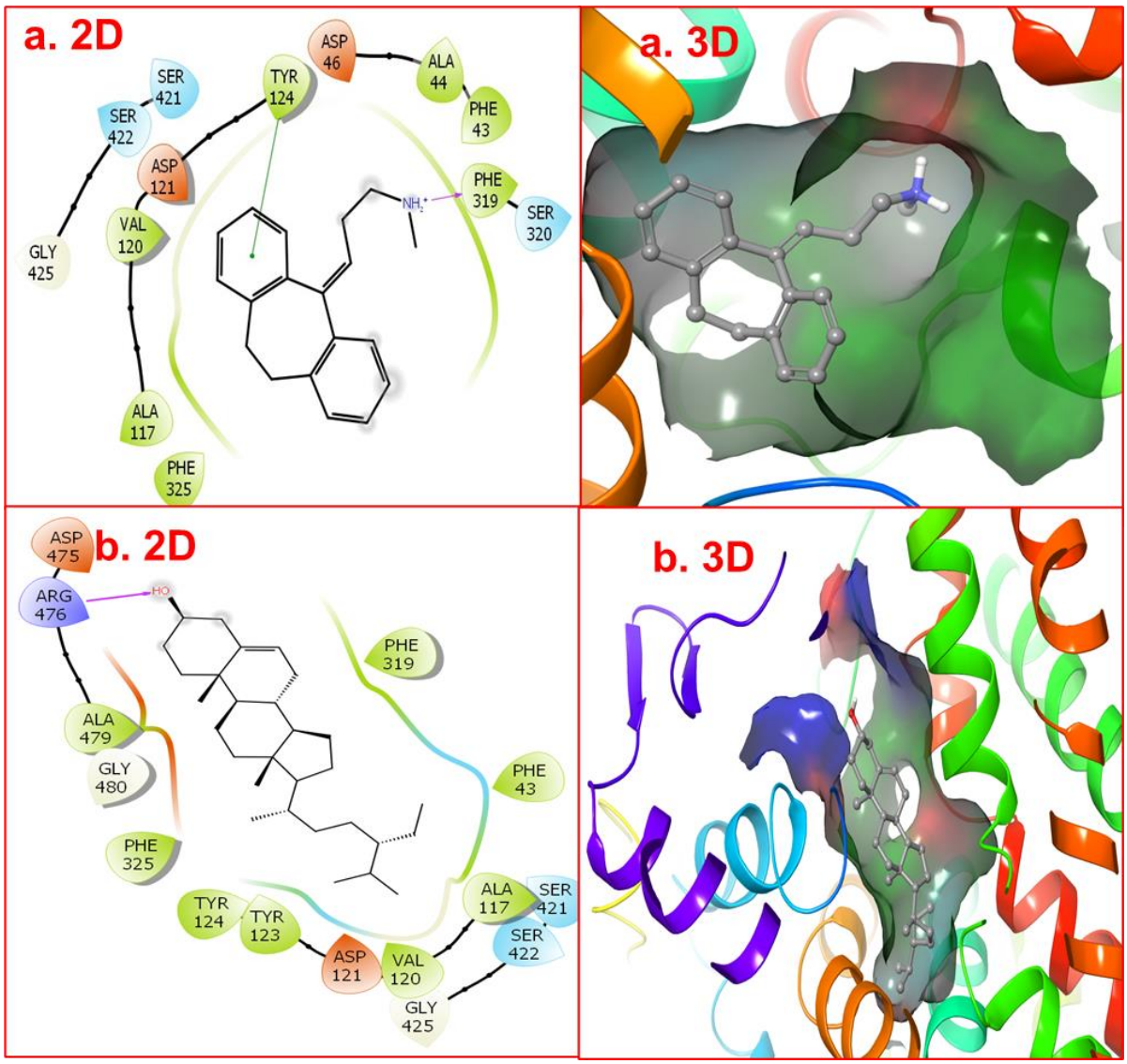
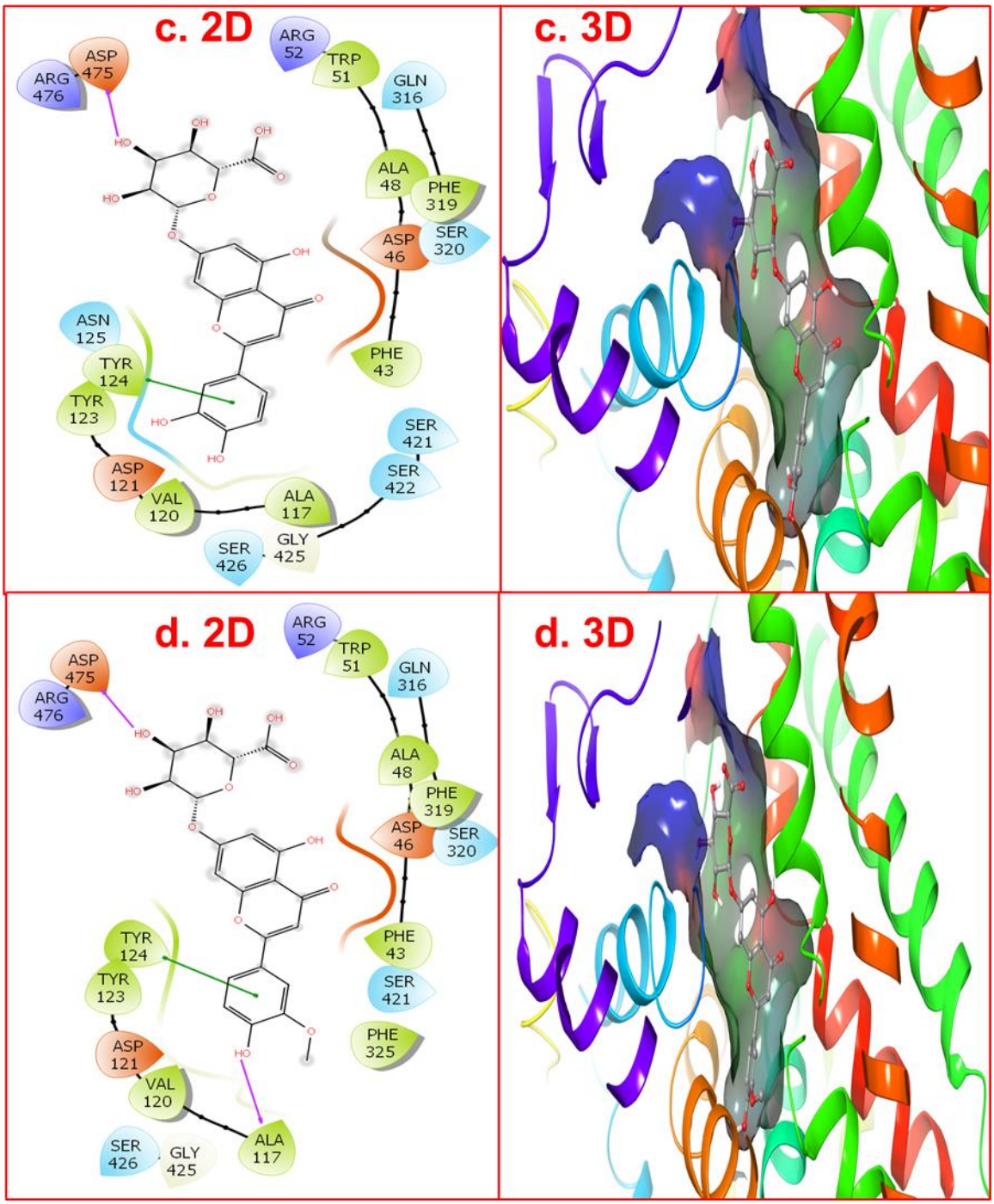


Figure 1: Superimposed poses of co-crystalized (green) with the docked pose (grey) of Nortriptyline.


a. 2D
ASP 46
ALA 44
PHE 43
TYR 124
SER 421
SER 422
ASP 121
PHE 319
SER 320
VAL 120
GLY 425
ALA 117
PHE 325
a. 3D
b. 2D
ASP 475
ARG 476
HO
PHE 319
ALA 479
GLY 480
PHE 43
PHE 325
TYR 124
TYR 123
ASP 121
VAL 120
ALA 117
SER 421
SER 422
GLY 425
b. 3D


c. 2D
ARG 52
TRP 51
GLN 316
ASP 475
ARG 476
ALA 48
PHE 319
ASP 46
SER 320
ASN 125
TYR 124
PHE 43
TYR 123
SER 421
SER 422
ASP 121
VAL 120
ALA 117
SER 426
GLY 425
c. 3D
d. 2D
ARG 52
TRP 51
GLN 316
ASP 475
ARG 476
ALA 48
PHE 319
ASP 46
SER 320
TYR 124
PHE 43
TYR 123
SER 421
PHE 325
ASP 121
VAL 120
SER 426
GLY 425
ALA 117
d. 3D

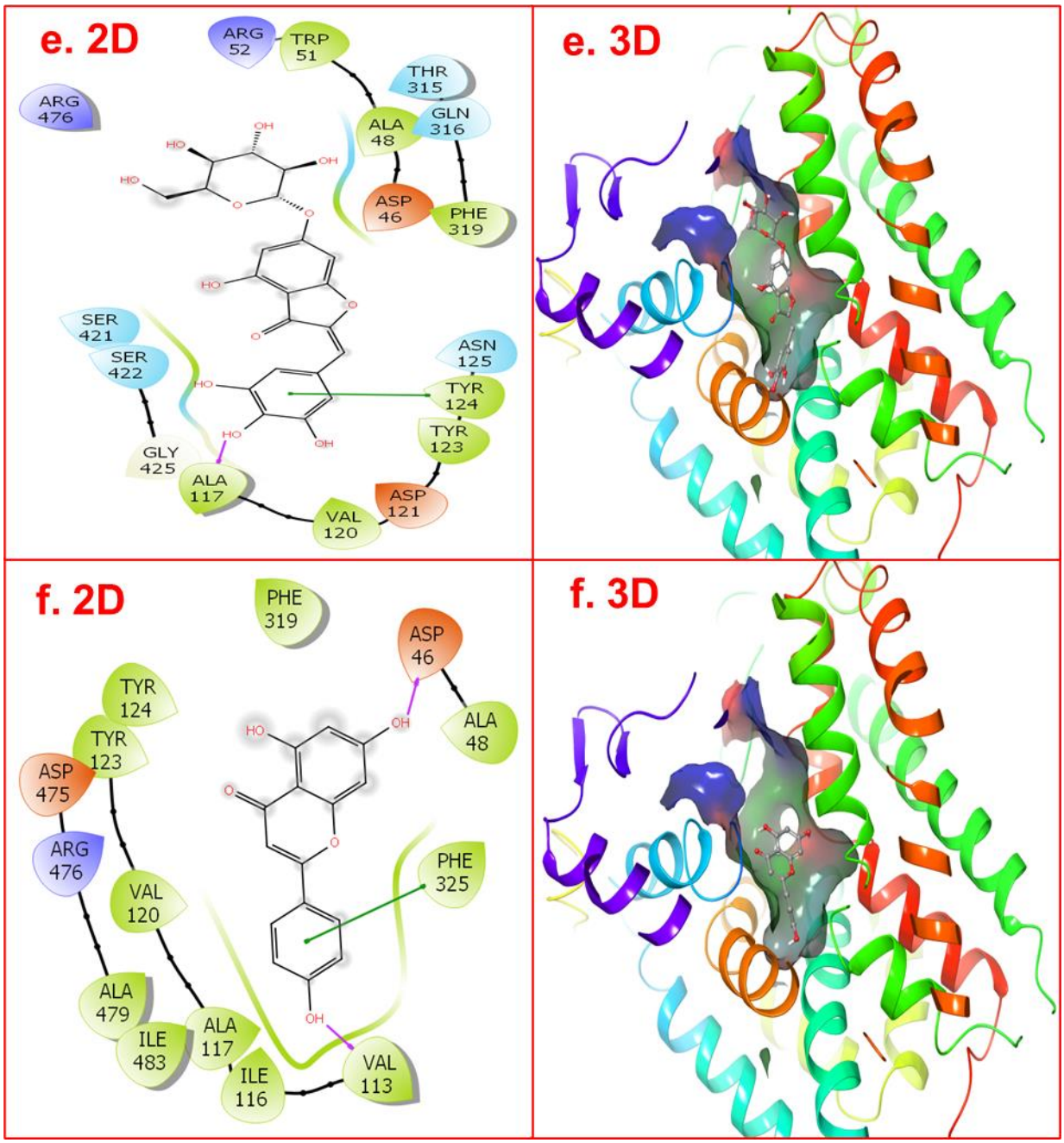


Figure 2: Interaction of different phytoconstituents present in the plant and interaction with residues.

4. CONCLUSION

The relationship between medicinal plants and humanity has been a subject of extensive exploration, particularly in the field of medicine. Recent research has increasingly focused on understanding the scientific basis behind the significant benefits offered by medicinal plants. This attention has led to the investigation of various phytochemical constituents and their ethnobotanical importance. In order to harness the potential advantages of medicinal plants with scientific validity, researchers are now emphasizing the documentation of the biological activities of phytochemical constituents. Advanced techniques, such as molecular docking are being employed to evaluate and identify promising compounds. This approach holds the potential to pave the way for the development of natural products that can offer substantial health benefits to human population.

In conclusion, this study delved into the molecular docking simulation of 25 phytoconstituents derived from *Antirrhinum majus* to explore their potential as CNS stimulants by interacting with the dDAT receptor. The results provided valuable insights into the critical role of specific amino acid residues within DAT for modulating CNS stimulant activity. These findings emphasize the relevance of specific amino acid residues within DAT for the interactions and potential modulation of CNS stimulant activity by the Antirrhinum majus-derived inhibitors. While the study provides essential insights, further experimental investigations, such as *in vitro and in vivo* studies, are essential to validate the inhibitory activity of the hits on DAT and their actual CNS stimulant effects. A more comprehensive

understanding of the specific interactions with DAT will aid in the rational design of potent and selective compounds with enhanced CNS stimulant effects and reduced side effects.

In conclusion, this research serves as a foundation for advancing the understanding of the potential CNS stimulant properties of Antirrhinum majus compounds and offers promising leads for drug development targeting CNS-related conditions. By elucidating the molecular mechanisms underlying the interactions between these inhibitors and DAT, this study contributes to the expanding knowledge in neuroscience and provides potential avenues for the development of novel therapeutic interventions in the field of CNS disorders.

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Conflict of interest

There is no conflict of interest between authors.

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