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Molecular Docking Studies of Some Novel Monoterpene's Derivation Against *Nitric Oxide Synthase* for Antidepressant Activity

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Abstract

Background: Major depressive disorder (MDD) is a significant and, in extreme instances, a fatal condition. Notwithstanding comprehensive study, understanding of the etiology, particular processes, and regulatory pathways of the disease remains insufficient. Prior research has demonstrated that monoterpene derivatives had significant antidepressant properties. Nonetheless, its mechanisms remain inadequately comprehended. The objective of our research is to elucidate the mechanisms of monoterpene derivatives in the treatment of depression. ***Purpose:*** The aim of current investigation is to reveal the mechanisms of monoterpene derivatives in treating depression. ***Methodology:*** Scientific validation of the current investigation was done by computational based molecular docking study of selected lead molecules against *NOS* enzyme. ***Result:*** The molecular docking results indicating binding energies of -5.2, -5.75, and -5.5 kcal/mol for α-pinene, limonene, and carveol, respectively. The IC50 values are 0.12, 0.10, and 0.11 for α-pinene, limonene, and carveol, respectively. ***Conclusion:*** The findings indicated that each selected lead chemical for additional investigation shown significant inhibitory activity against NOS, hence revealing its anti-depressant potential.

Keywords: Monoterpene, molecular docking, α-pinene, limonene and carveol.

INTRODUCTION

Depression manifests as low mood and reluctance to exercise, affecting an individual's thoughts, behaviours, emotions, and physical health. As many as 20% of individuals globally may be impacted by this chronic, recurrent, and devastating condition [1]. Depression is the leading cause of global disability, significantly contributes to suicide rates, and elevates the risk of cardiovascular disease and several neurological disorders, including dementia [2]. Despite substantial research on the neurobiology of depression and the mechanisms of antidepressant activity, the therapeutic management of the illness remains inadequate [3]. Depression, the most common affective disease, can vary from a moderate state that approaches normalcy to a severe (psychotic) condition characterized by hallucinations and delusions. Affective disorders are characterized as mood abnormalities rather than disruptions in thought or cognition. Furthermore, the ailment leads to an increase in the utilization of the healthcare system [4-5]. It appears that many individuals have a close friend, family member, or colleague who is experiencing depression. The World Health Organization forecasts that unipolar major depression will surpass ischemic heart disease as the second-leading cause of worldwide health problems by 2020 [6]. Consequently, depression becomes a substantial medical and societal concern. While the precise genes contributing to this risk remain unidentified, depression exhibits significant heritability, with 40–50% of the risk attributed to genetic factors. Numerous unresolved inquiries persist concerning the other 50–60% of non-genetic risk, potentially attributable to early childhood trauma, psychological stress, physical illness, or viral infection [7]. Most physicians agree that depression should be regarded as a syndrome rather than an illness. Consequently, the diverse array of symptoms that define depression and the inconsistent progression of the disorder, along with its varied responses to therapies, suggest that depression encompasses multiple disease states with different etiologies and maybe distinct pathophysiologies. Depression often coexists with several conditions, including anxiety disorders such as generalized anxiety disorder (GAD), post-traumatic stress disorder (PTSD), obsessive-compulsive disorder, pain disorder, and social phobia [8]. Essential oils (monoterpene derivatives) are traditionally utilized to

mitigate depressive symptoms, since their components can penetrate the blood-brain barrier and engage with biological receptors associated with depression, while demonstrating low toxicity and adverse effects. Furthermore, unlike traditional medications, they provide many methods of administration [9]. Nitric oxide is considered a crucial neurotransmitter involved in the development of certain neurological disorders, such as epilepsy, schizophrenia, substance dependency, anxiety, and serious depression [10].

Experimental work

Selection of lead molecules

Monoterpenes, composed of hydrocarbons, are the primary category of plant secondary metabolites and are commonly found in essential oils. Monoterpenes and their derivatives are crucial elements in the development and production of new physiologically active compounds. A literature survey reveals that α pinene has a wide range of pharmacological activities, including modulation of antibiotic resistance, anticoagulation, anticancer effects, antibacterial characteristics, antimalarial effects, antioxidant capabilities, anti-inflammatory actions, anti-Leishmania effects, and analgesic qualities. The primary effects of α- and β-pinene include cytogenetic, gastroprotective, anxiolytic, cytoprotective, anticonvulsant, and neuroprotective qualities, as well as effectiveness against H_2O_2-induced oxidative stress, pancreatitis, stress-induced hyperthermia, and pulpal pain [11]. Limonene is a monoterpene belonging to the Rutaceae family, exhibiting several biological activities including antioxidant, anti-inflammatory, anticancer, antinociceptive, and gastroprotective effects. There is increasing interest in examining the pharmacological effects of limonene on many chronic diseases due to its ability to alleviate oxidative stress, reduce inflammation, and regulate apoptotic cell death. Numerous studies illustrate the neuroprotective effects of limonene in neurodegenerative disorders, such as Alzheimer's disease, multiple sclerosis, epilepsy, anxiety, and stroke. The significant prevalence of limonene in nature, its safety profile, and diverse mechanisms of action render this monoterpene a promising candidate for development as a nutraceutical for preventive applications and as an alternative or adjunct to contemporary therapeutic agents in mitigating the onset and progression of neurodegenerative diseases [12]. Carveol is utilized in traditional Chinese medicine as an antispasmodic, carminative, and astringent, and has been assessed for its efficacy in treating indigestion and dyspepsia. Carveol has been shown to possess antioxidative, antihyperlipidemic, and anti-inflammatory properties, as well as to mitigate liver toxicity in a mouse model of carbon tetrachloride exposure [13].

Selection of Target protein

Nitric oxide is recognized for its substantial involvement in the pathophysiology of numerous bodily illnesses. Notwithstanding its brief half-life, nitric oxide is recognized for modulating different neurotransmitter systems in the body, suggesting its crucial involvement in the etiology of neurological illnesses. This "wonder" molecule frequently exhibits a "dual role" in many brain diseases. Evidence has demonstrated its significant significance in the etiology of severe depression. Nitric oxide regulates norepinephrine, serotonin, dopamine, and glutamate, the principal neurotransmitters implicated in the neurobiology of major depression. The nitric oxide modulatory effects of numerous new-generation antidepressants have been established. Nitric oxide is produced from l-arginine by the action of a NOS enzyme. NOS is a meticulously controlled enzyme including a heme domain that associates with the flavin mononucleotide (FMN)/flavin adenine dinucleotide (FAD) reductase enzyme, facilitating the transfer of electrons from nicotinamide adenine dinucleotide phosphate (NADPH) to the heme component. Three kinds of nitric oxide synthase (NOS) are recognized in mammals: nNOS (neuronal NOS; type I), iNOS (inducible NOS; type II), and eNOS (endothelial NOS; type III). All three isoforms of NOS are known to be expressed in various areas of the brain. Both nNOS and eNOS are constitutively expressed in the brain and are dependent on calcium/calmodulin. Nonetheless, research has demonstrated that these two isoforms of NOS may also be activated under specific stressful circumstances. Conversely, the expression of iNOS is meticulously regulated and is independent of calcium. nNOS is located in various regions of the brain linked to stress and depression, specifically the hippocampus, hypothalamus, dorsal raphe nucleus, and locus coeruleus. Numerous studies in the literature have revealed the critical function of nitric oxide in serious depression. These citations include:

> Plasma nitric oxide metabolites are significantly elevated in suicidal patients compared to nonsuicidal psychiatric patients and normal control subjects. Additionally, a clinical study indicates increased nitric oxide production in depressed patients, implying altered nitric oxide levels in individuals with major depression. Reducing the levels or inhibiting the generation of nitric oxide (by blocking NOS) in the brain can produce antidepressant-like effects, so suggesting the involvement of endogenous hippocampal nitric oxide in the pathophysiology of major depression [14].

Molecular docking studies

Ligand Preparation:

2D Structure of ligands like α-pinene, limonene and carveol were drawn using ChemSketch [15], the two-dimensional structures of the prepared ligands were converted into their 3-D structures optimized with 3D geometry. The optimized structures were saved in PDB format for AutoDock compatibility. The basic structures of the prepared ligands were given below:

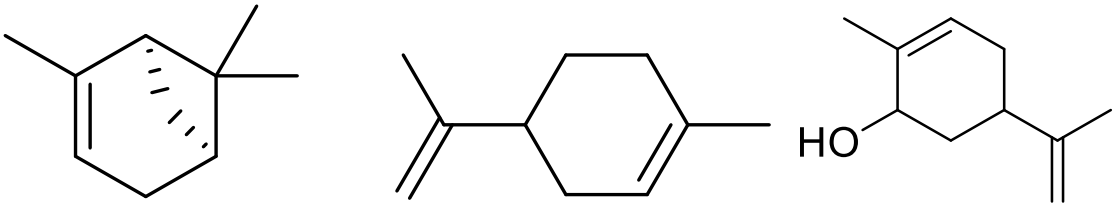


Figure 1: 2D structure of α-pinene, limonene and carveol

Preparation of the grid file

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimensions. The spacing between grid points can be adjusted with another thumbwheel, the value in the study taken is 0.436 Å and No. of points considered are 40, 40 and 40 points in the x, y, and z dimensions and 120.799, 248.904 and 358.058 as x, y, z centers [16-18].

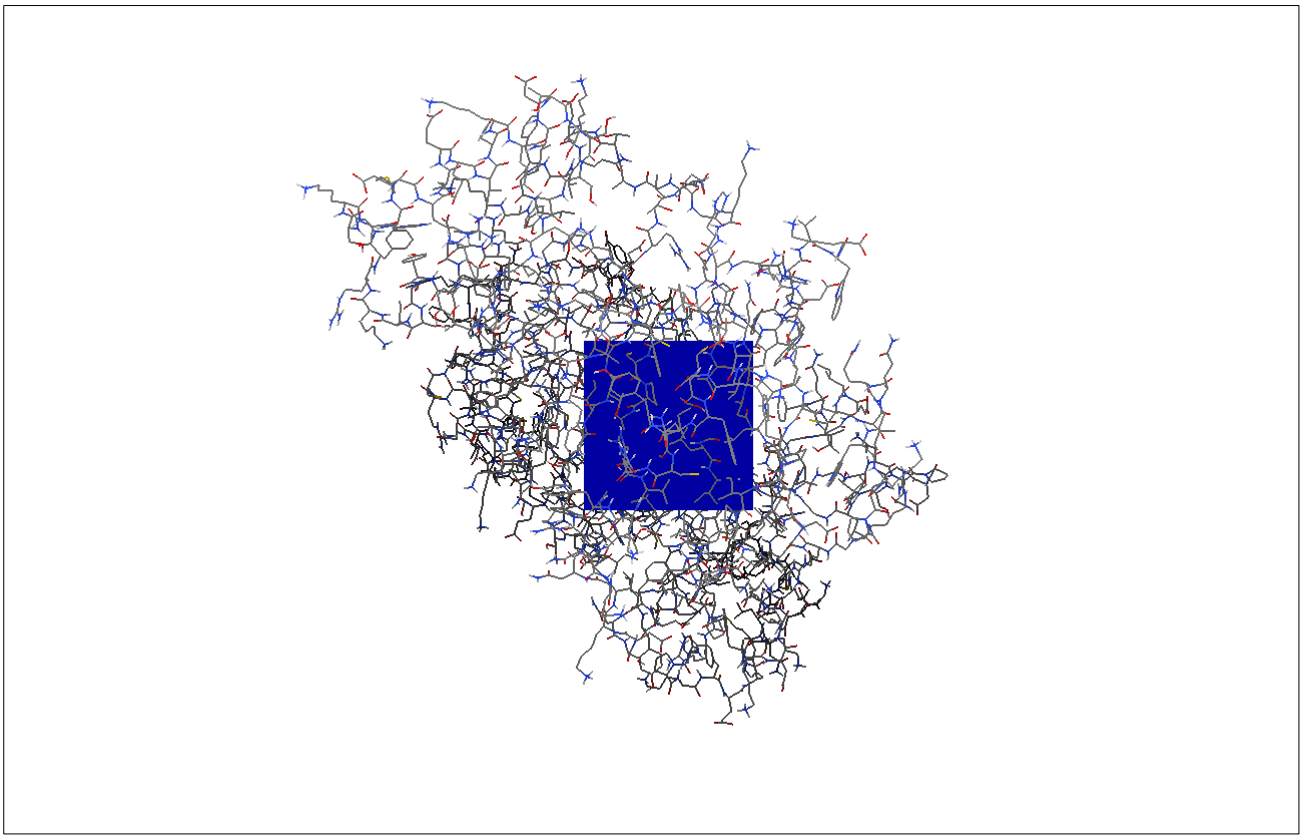


Figure 2: Grid box covering all active sites in NOS enzyme

Preparation of the docking file

All the calculations were carried out by using Autodock 4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus [19-21].

Crystal structure

The crystal structure of the protein consisting of NOS enzyme is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (5uo1.pdb) registered in the Protein data bank was used [22-25].

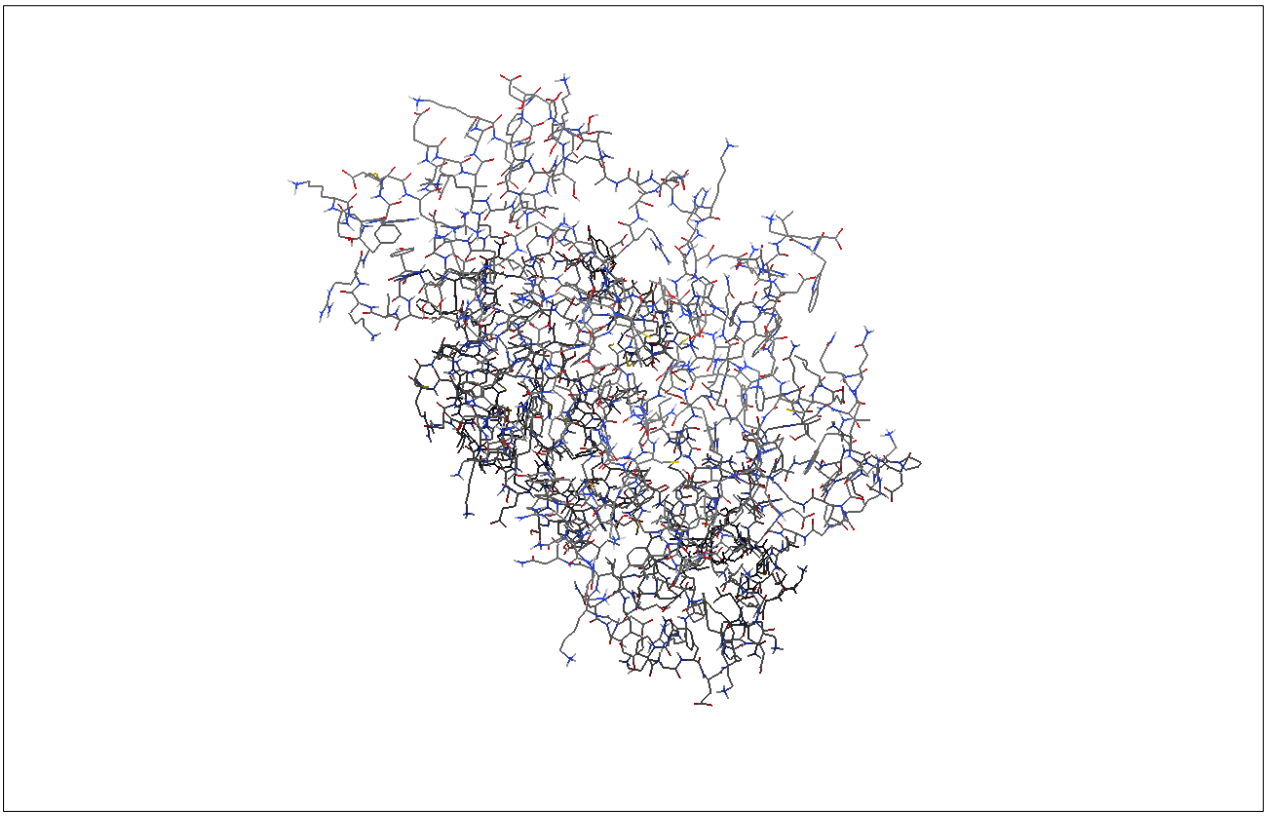


Figure 3: Crystal structure of NOS enzyme (PDB ID-5uo1)

Processing of Protein

The downloaded receptor protein is having two chains, i.e. chain A, and B. Out of these two chains, chain B was selected for experimental purpose and other chains were removed from it. The bound ions were separated from the macromolecular complex by using software Chimera [26-30].

Molecular Docking Simulation Studies

Docking of ligands like α-pinene, limonene and carveol against NOS enzyme was performed by Autodock. All the bonds of each ligand were kept flexible, while no residues in receptor were made flexible [31-32].

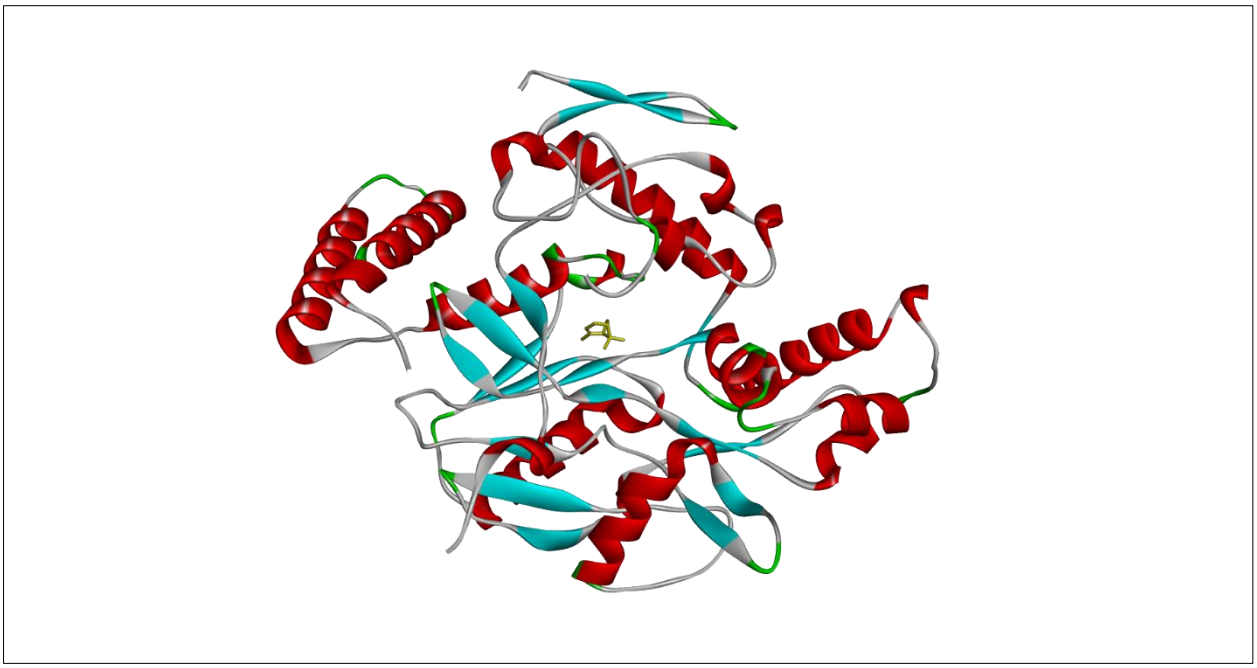


Figure 4: Binding mode of α-pinene within the active site of NOS enzyme

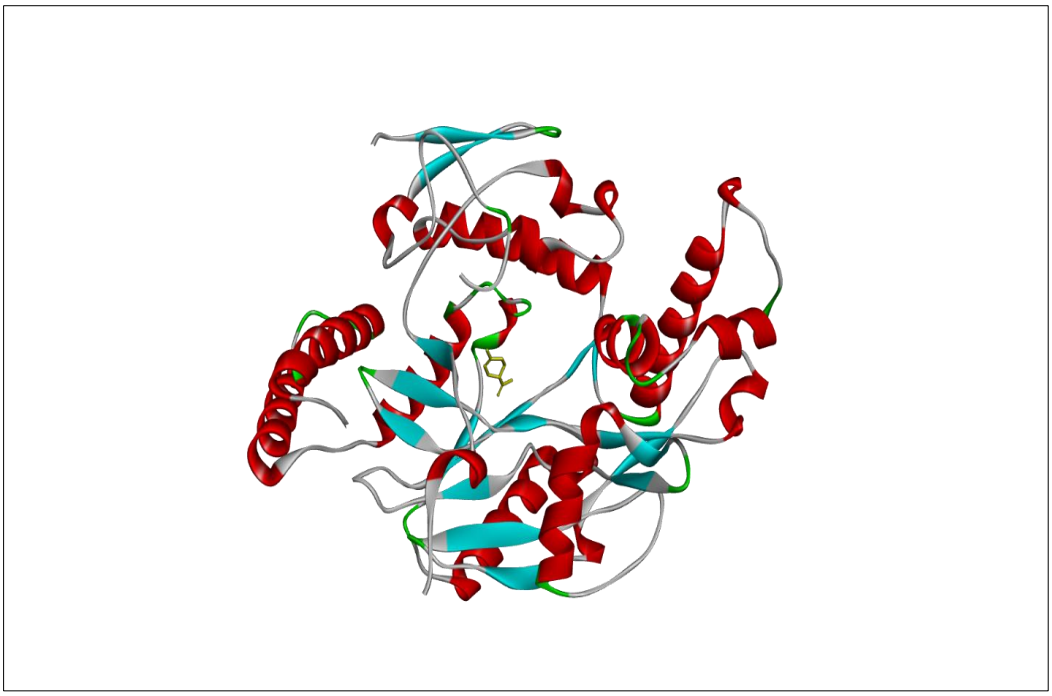


Figure 5: Binding mode of limonene within the active site of NOS enzyme

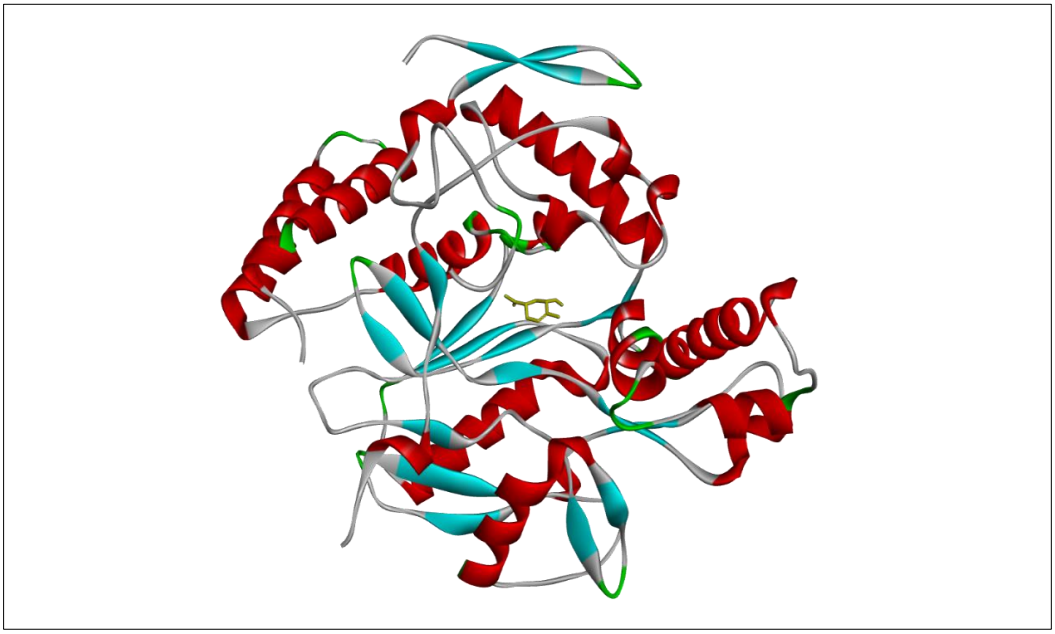


Figure 6: Binding mode of carveol within the active site of NOS enzyme

Toxicity & ADME-T Studies

The ligand molecules viz. α-pinene, limonene and carveol were studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME- T properties [33].

RESULT AND DISCUSSION

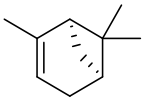
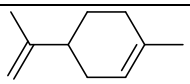
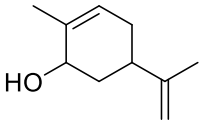
A clinical investigation has shown elevated nitric oxide production in depressed patients, indicating that nitric oxide levels are modified in persons with significant depression. Reducing nitric oxide levels or inhibiting its synthesis (by blocking NOS) in the brain can produce antidepressant-like effects, so suggesting the involvement of endogenous hippocampal nitric oxide in the pathophysiology of severe depression. Consequently, NOS inhibitors may emerge as the preferred pharmacological agents for addressing stress and associated diseases. The molecular docking results are presented in Table 1, indicating binding energies of -5.2, -5.75, and -5.5 kcal/mol for α-pinene, limonene, and carveol, respectively. The binding mode is illustrated in figures 4-6, while the 2D and 3D binding interactions are depicted in figures 7-12. The IC50 values are 0.12, 0.10, and 0.11 for α-pinene, limonene, and carveol, respectively. All chosen lead compounds had favorable

interactions with the selected ligand and demonstrated comparable drug-likeness against the NOS enzyme, with drug-likeness scores of 0.51, 0.60, and 0.50 for α-pinene, limonene, and carveol, respectively. The binding interactions of lead molecules are as follows:

Lead molecule	Vanderwaal's	CH bounding	π – π	π-Alkyl
α-pinene	Try 592 Pro570 Gly 591 Ser 590 Ala 463 Ser 462	----------	----------	Cys 420 Leu 429 Phe589 Try 414
Limonene	Ala 463 Ser 590 Ser 462 Gly 591	----------	Try 414	Phen 589 Phen 709 Ala 417 Cys 420 Leu 429
Carveol	Glu 597 Tyr 593 Gly 591 Ser 462	Trp 592	Try 414	Leu 429 Phen 589 Pro 570

The pharmacokinetic analysis of the ligands α-pinene, limonene, and carveol indicates a favorable pharmacokinetic profile, devoid of significant adverse consequences such as reproductive toxicity, irritating characteristics, tumorigenicity, and mutagenicity. The pharmacokinetic and toxicity profiling data of the lead compound are presented in Figures 13-15 and Tables 2-4.

Table 1: Results of docking of ligands like α-pinene, limonene and carveol against NOS enzyme

S. N.	Compound Name	Structure	B.E (Kcal/mole)	KI	IC 50
1	α-pinene		-5.2	8.77	0.12
2	Limonene		-5.75	9.704	0.10
3	Carveol		-5.5	9.283	0.11

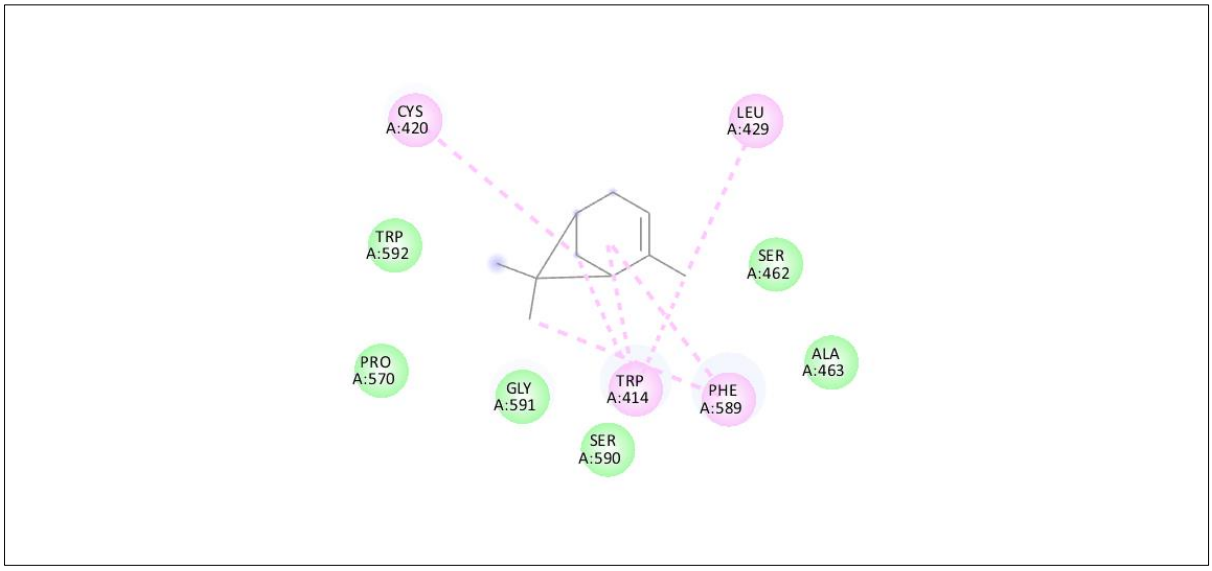


Figure 7: Two-dimensional binding mode of α-pinene within the active site of NOS enzyme

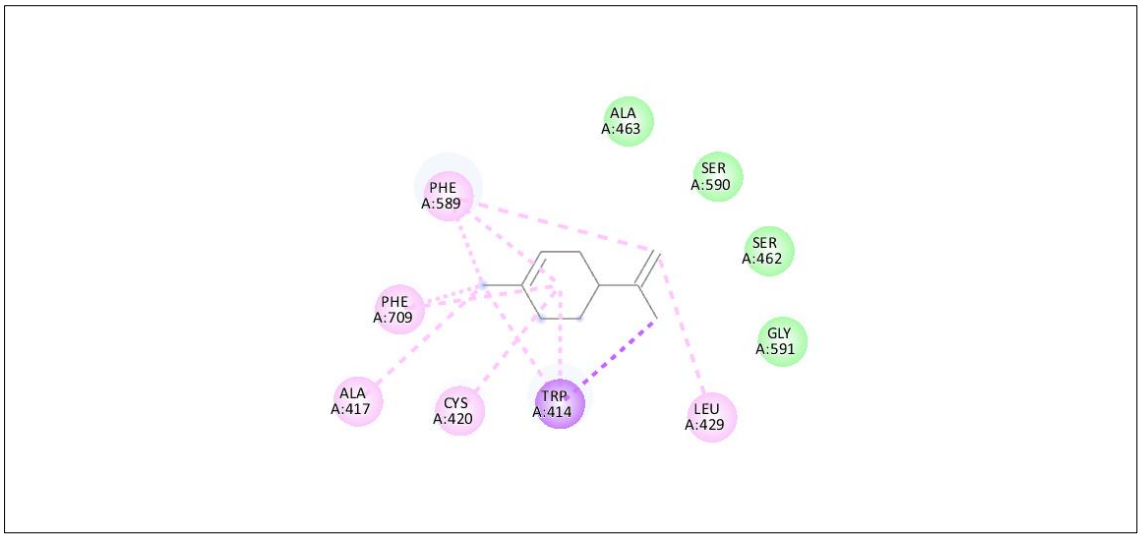


Figure 8: Two-dimensional binding mode of limonine within the active site of NOS enzyme

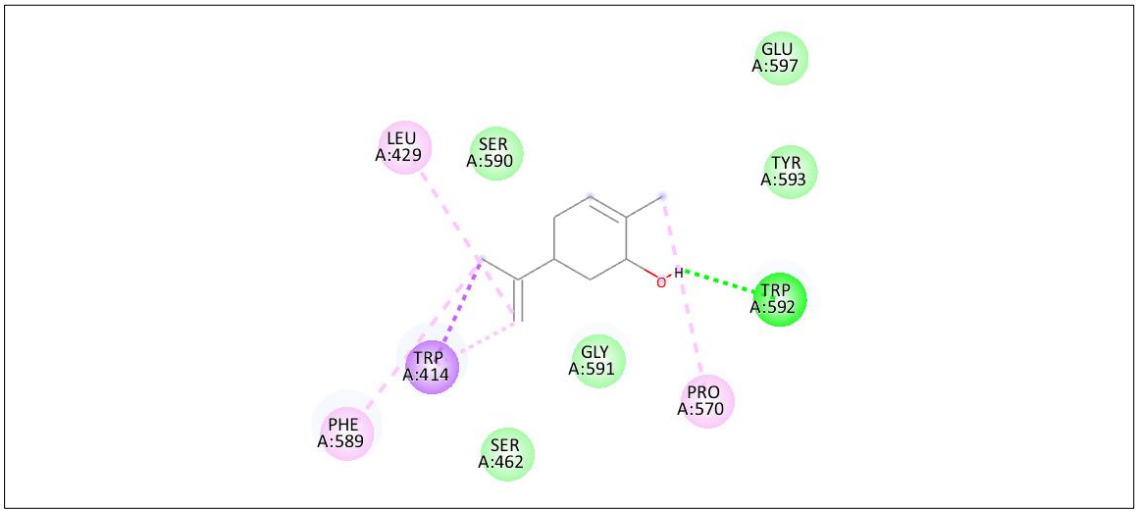


Figure 9: Two-dimensional binding mode of carveol within the active site of NOS enzyme

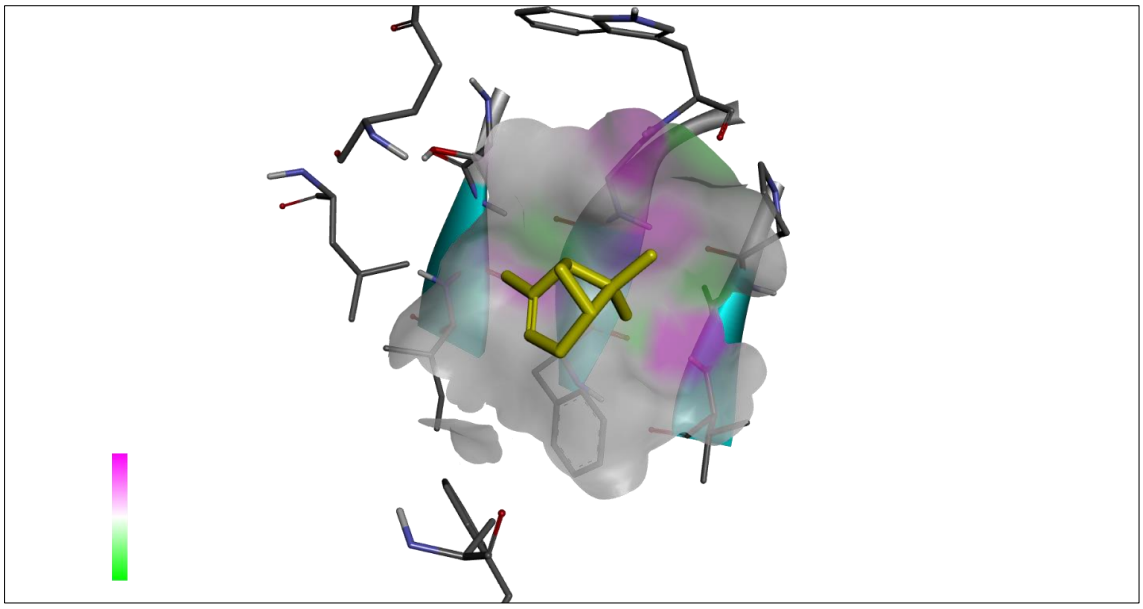


Figure 10: Three-dimensional binding conformation of α-pinene within the active site of NOS enzyme

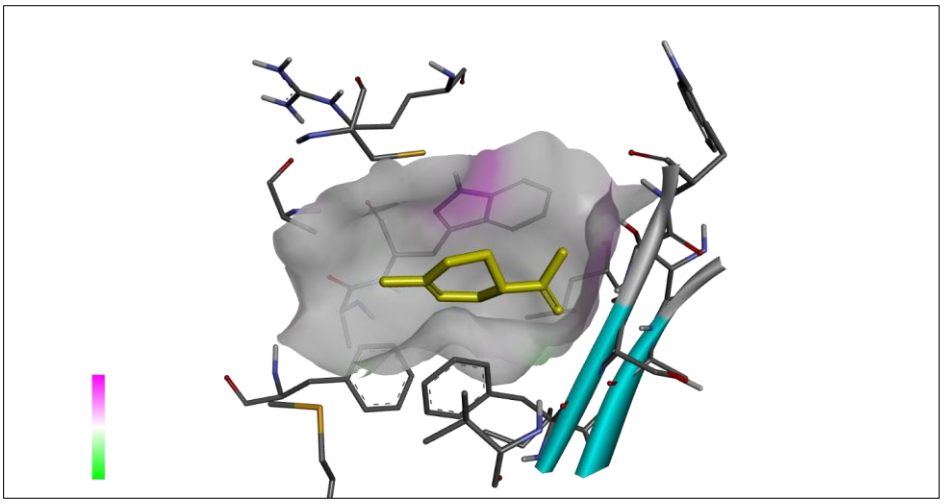


Figure 11: Three-dimensional binding conformation of limonene within the active site of NOS enzyme

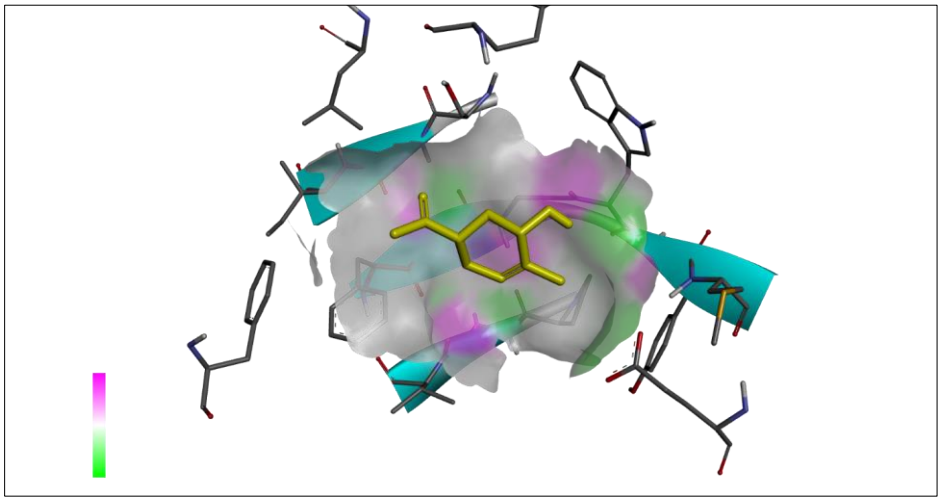


Figure 12: Three-dimensional binding conformation of carveol within the active site of NOS enzyme

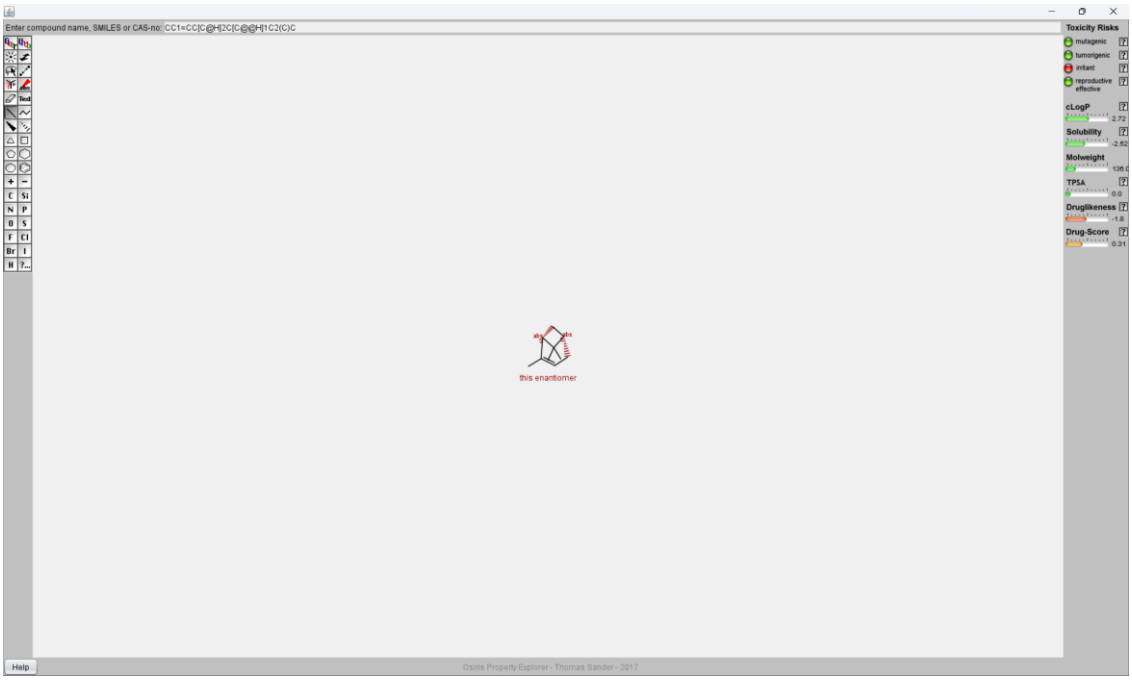


Figure 13: Pharmacokinetic and toxicity profiling of α-pinene

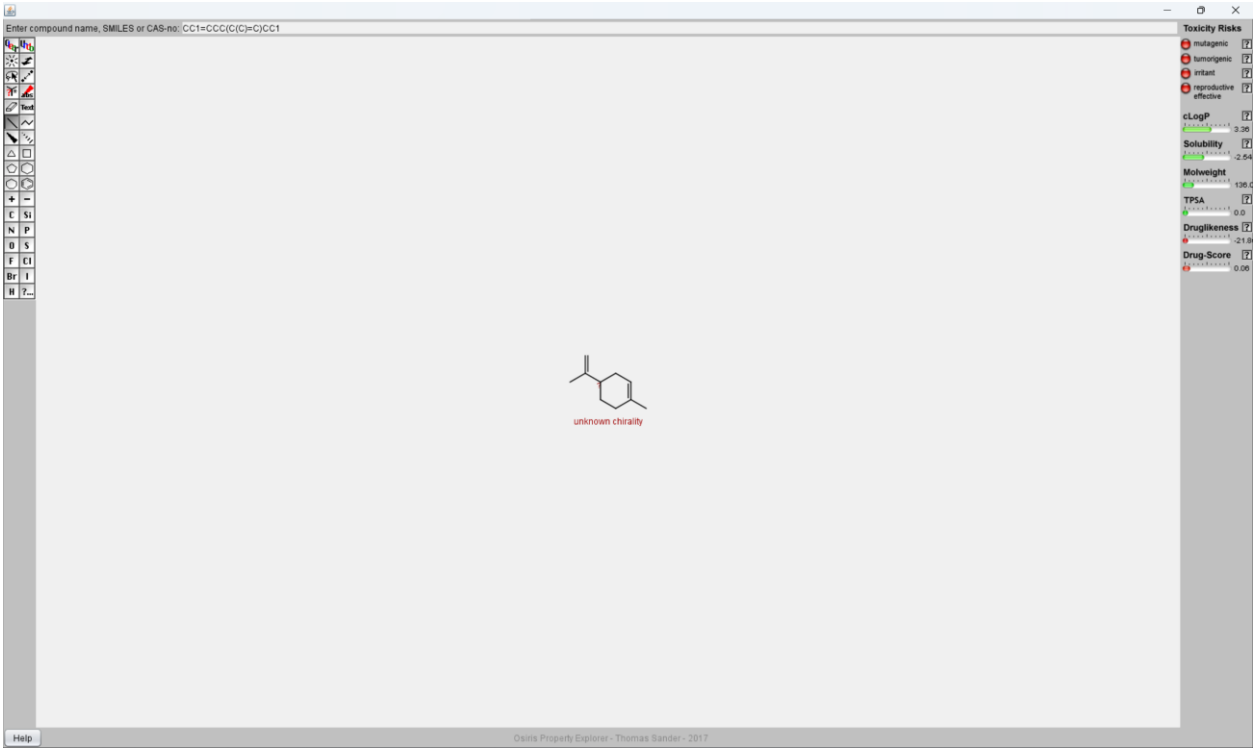


Figure 14: Pharmacokinetic and toxicity profiling of limonene

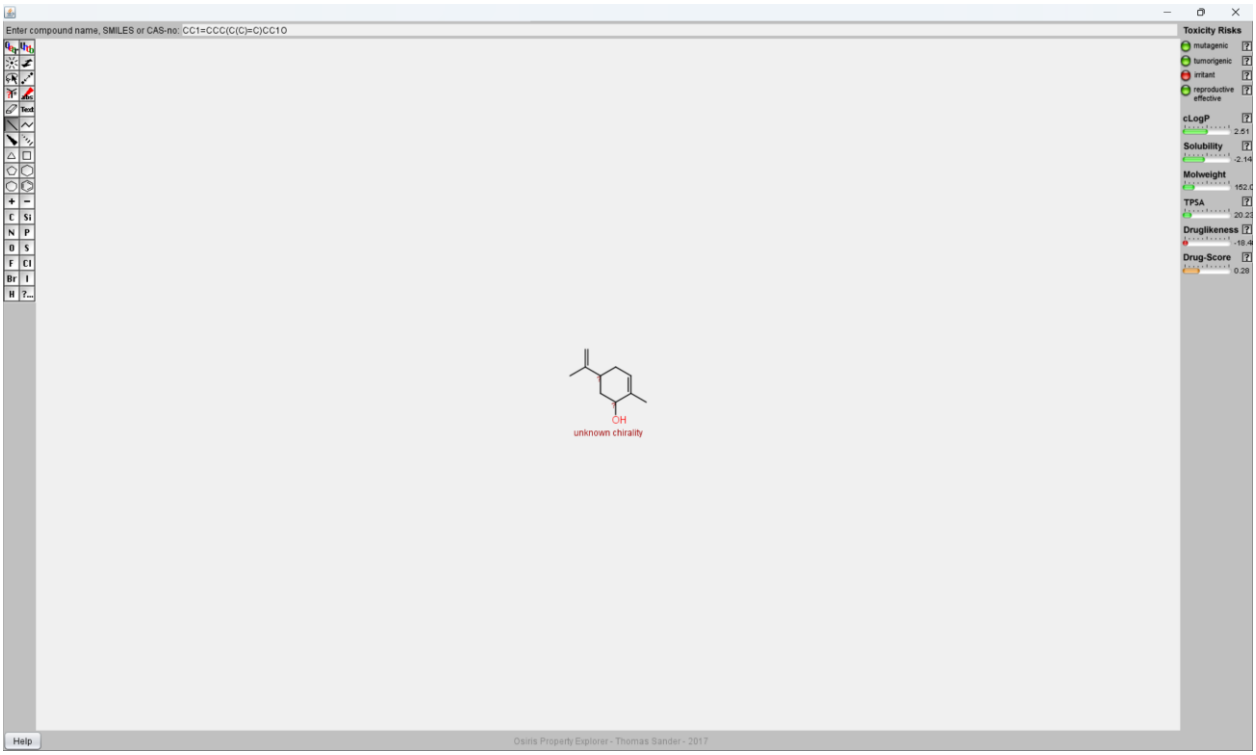


Figure 15: Pharmacokinetic and toxicity profiling of carveol

Table 2: Pharmacokinetic Profiling of lead molecules

Compound	ADMET			
	Mutagenic	Tumorigenic	Irritant	Reproductive effectivity
α-pinene	NO	NO	Yes	NO
Limonene	NO	NO	NO	No
Carveol	NO	NO	NO	No

Table 3: Lipinski Properties of lead molecules

Compound	*cLogP*	Solubility	Mol.wt.	TPSA	Drug likeness	Drug score
α-pinene	2.72	-2.52	136	0.1	-0.1	0.51
Limonene	3.30	-2.54	136.1	0.1	-2.18	0.60
Carveol	2.01	-2.14	152	20.2	-0.1	0.50

Table 4: Drug likeness of lead molecules

Compound	Lipinski rule of five	H bond donar(<5)	H bond acceptor (<10)
α-pinene	Yes	0	0
Limonene	Yes	0	0
Carveol	Yes	1	1

CONCLUSION

The molecular docking method facilitates the modelling of interactions between small molecules and proteins at the atomic scale, enabling the characterization of small molecule behaviour within the binding sites of target proteins and the elucidation of essential biochemical processes. The docking procedure comprises two fundamental steps: predicting the ligand shape, position, and orientation within the binding sites (often termed pose) and evaluating the binding affinity. The *in-silico* molecular docking of chosen monoterpenes with the NOS receptor was conducted in this study to elucidate the efficacy of antidepressants. The investigation's findings revealed that the interaction of all selected compounds exhibited inhibitory effects on the NOS receptor, resulting in a reduction in Ca^{2+} levels and cytokines, hence preventing tissue damage.

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